EPIZ⬡NE

Abstracts oral and poster presentations
4th Annual Meeting EPIZONE

"Bridges to the future"

7-10 June 2010
Saint-Malo, France
Hosted by AFSSA

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Content

EPIZONE

Welcome

Welcome

Dear participants,

We w elcome y ou all t o t he 4 [th] Annual Meeting of our   EU funded Network of Excellence, EPIZONE

"Bridges to the future"

The slogan "Bridges to the future" relates   to the ambi tion of EPIZONE to s upport the introduction of i nnovative, improved, fast, and acce ptable control m easures to combat animal d iseases in relation wi th fo od safety   and climat e change.   As platform and think-ta nk of   highly qua lified scientists EPIZON E develops new strategies and tools to face   n ew ch allenges in t he f uture an d r emains a so lid Network of Excellence in the coming years.

During this meeting y ou will meet scientists from many different fields of research, but all sh are their interests in animal disease control. This will give you the chance to sh are ideas an d thoughts, to learn f rom each ot her, t o bu ild n ew relat ionships and to strengthen the existing ones.

Special topic this year: Major epidemic threats

A main obj ective of E PIZONE is t o in teract w ith f ellow scien tists. Besides o ral presentations on thi s years topi c, we o ffer you the un ique possibility to express your opi nion on " the c urrent most threat ening viruses and the effects of c hanges e.g. in clim ate and the environment" and on "the current state of preparedness for the most threatening viruses: can their introduction be prevented, is early detection possible, and are tools availab le to contro l epidemic spread?" . We believe that by using int eractive opin ion met hods, we w ill be able t o bu ild bridges f or cooperation and collaboration in the future.

A word of   gratitude to Dr.   Philippe Vanni er, research director of AFSSA and   all involved members of AFSSA and   ISPAIA who have made this meeting possible for their time, w arm w elcome t o all part icipants and t he ent husiasm in organizing th is 4 [th] Annual Meeting of EPIZONE.

We wish you all a very successful and inspiring meeting

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

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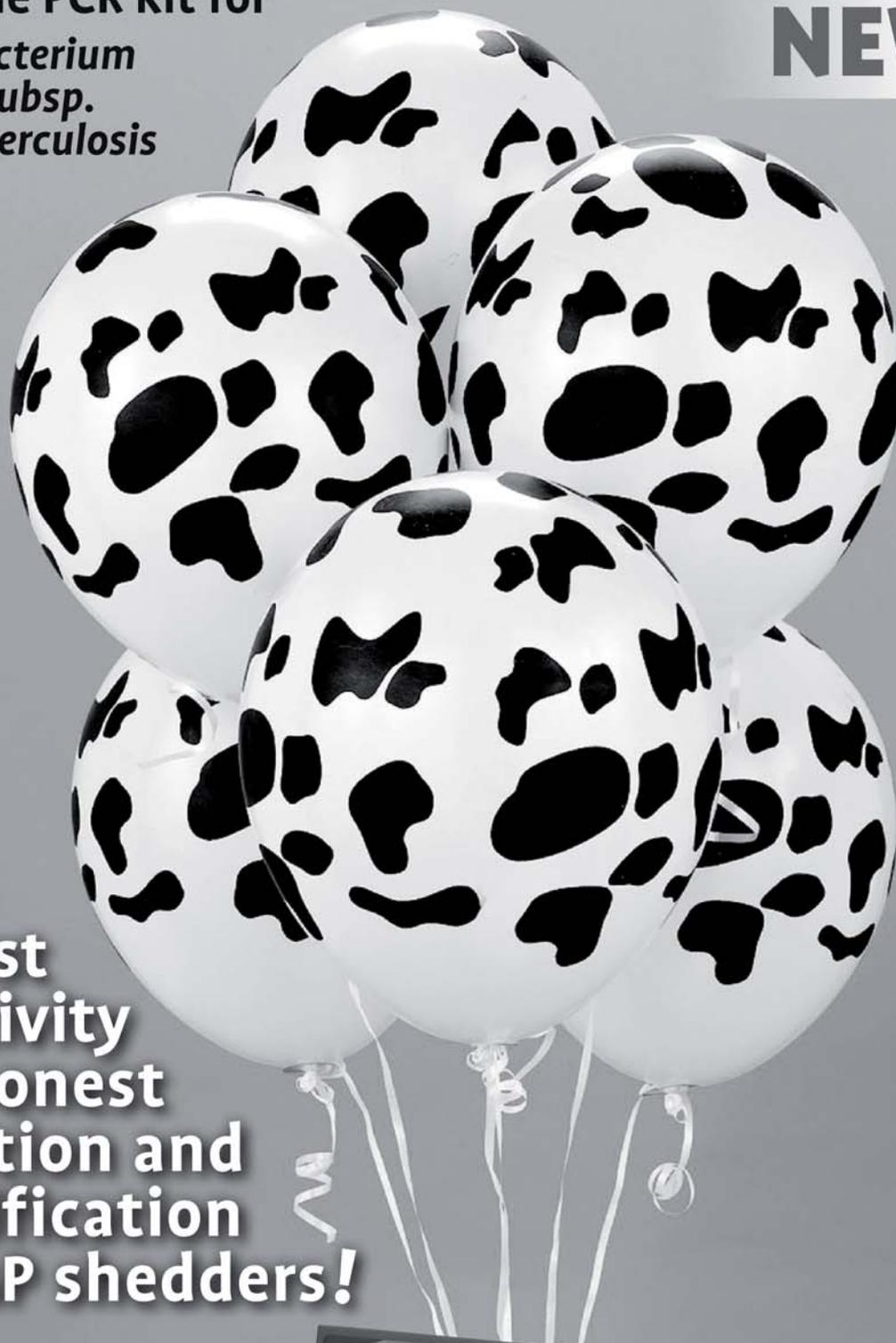
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Keynote
Rob Raynard

KEYNOTE: INFECTIOUS SALMON ANAEMIA: EMERGENCE, CONTROL AND SURVEILLANCE IN AQUACULTURE

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Key words: Infectious salmon anaemia

Infectious salmon anaemia (ISA) is a highly infectious orthomyxoviral disease of farmed Atlantic salmon (Salmo salar) caused by ISA virus (ISAV). Clinical ISA has caused major losses to salmon farmers in six countries. The disease was first reported from Norway in 1984, where it is still widespread. Subsequently it occurred in Canada, the USA, the Faeroe Islands and Chile. An outbreak occurred in Scotland in 1998/9 and this was eradicated at a cost then of over £20M.

The epidemiology of an outbreak of infectious salmon anaemia (ISA) in the Scottish Shetland Islands during 2008/9 is described. A total of six sites were confirmed ISA positive. Spread of the virus via movement of fish between marine sites, harvest vessels, movements of smolts and wild fish appear to have been of little or no importance. The spread is likely to be due to hydrodynamic currents, although local intra-company activity may have caused some spread. The use of management areas by Marine Scotland, defined by a simple but robust model partly derived from tidal excursions, appears to have been effective in restricting the spread of infection to a small area however spread within this area has been extensive. This localised water-borne spread is in contrast to a previous outbreak in 1998/9 which was spread over a wide geographic area by transport of fish and harvest vessels. The development following the 1998/9 outbreak of codes of practice that limited marine site-to-site movement of live fish and improved disinfection of vessels and processing plant waste may explain why the 2008/9 spread of infection was localised. Depopulation of confirmed sites is a key element of eradication and this has been achieved within 7 weeks of confirmation, although the last confirmed case suggests subclinical infection may persist undetected for months. The origin of the 2008/9 outbreak is unknown, it could be either local evolution from an avirulent strain of ISAV or with importation of ova or equipment; however the virus responsible for the 2008/9 outbreak belongs to a different genogroup (group 1) to the 1998/9 virus (group 3). Lack of synchronous fallowing of management areas increases the risk of ISA re-emergence. Movement of fish between sites in different management areas represents the greatest risk of regional-scale spread should this occur.

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Theme 4 Diagnostics
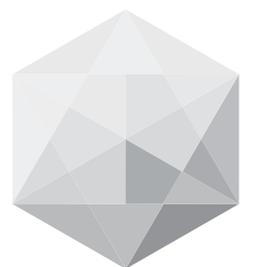
ORAL: IDENTIFICATION AND CHARACTERISATION OF BTV GENOME POSITIVE SINGLE MIDGES USING A HIGH THROUGHPUT SYSTEM

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Key words: BTV, DirectPCR, midges, genetic characterisation

Since the outbreak of BTV-8 in central Europe in 2006, an increased interest about the identification of Culicoides (C.) species responsible for virus transmission is existing. Therefore, a working group of entomologists was established for the analysis of midges in Germany, and an extensive entomological monitoring program was performed. Starting in April 2007, midges were collected with more than 90 traps in different regions of Germany. After a first characterization in midges of the C. obsoletus- and the C. pulicaris-group the collected midges were sent to the National Reference Laboratory for BTV at the Friedrich-Loeffler-Institut (FLI) for detailed molecular diagnostics. At the FLI the midges were pooled in portions of up to 50 and analyzed by real-time RT-PCR. Each BTV-genome-positive result was confirmed by using a second independent extraction procedure followed by a second different real-time RT-PCR analysis. In total 24513 pools of midges were investigated and the prevalence rate of BTV positive pools ranged from 0 to 17% depending from month and the location of catching. Thus, the identification and characterisation of single BTV positive Culicoides species in rests of un-analysed midges from catches with the highest BTV prevalence would be interesting.

For this high throughput analysis of single midges a real-time DirectPCR protocol was developed. First, the individual midges were homogenised in a special lysis buffer using 3mm steel beads. After an incubation step the homogenised midges were analysed for BTV genome by real-time DirectPCR. The useful application of this real-time DirectPCR in comparison to the classical way of RNA extraction was validated with BTV-8 spiked midges as well as by the analysis of housekeeping genes. No significant loss of sensitivity was observed using the real-time DirectPCR method for the genome detection of BTV in midges. Furthermore, the co-amplification of a Culicoides housekeeping gene as internal extraction and amplification control make it possible to identify the Culicoides species by sequencing.

Using these protocol more than 2600 midges of a catch with high BTV prevalence was investigated individually by two independent real-time RT-PCR assays. In summary, 285 single midges were positive in both real-time RT-PCR systems with Ct values between 27 and 40. For the single midges with a mean Ct values less than 35 (in total 54) a further genetic characterisation based on sequencing of the housekeeping gene was performed. Data of a molecular genotyping of these single midges will be presented and discussed together with the real-time PCR results.

ORAL: AFRICAN SWINE FEVER VIRUS: INTERLABORATORY VALIDATION OF A NEW REAL-TIME PCR ASSAY FOR IMPROVED MOLECULAR DIAGNOSIS

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VETERINARY AND AGROCHEMICAL RESEARCH CENTRE[1]; CENTER OF ANIMAL HEALTH, NATIONAL INSTITUTE FOR AGRICULTURE AND FOOD RESEARCH AND TECHNOLOGY[2]; AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES ALIMENTS[3]; IZSUM[4]

Key words: African Swine Fever virus; real-time PCR assay; molecular diagnosis; pig disease

A real-time polymerase chain reaction (rtPCR) assay has been developed for the simple and rapid diagnosis of African swine fever (ASF). The assay is multiplexed for simultaneously detection of swine beta-actin as an endogen control.
Primers and FAM-labeled TaqMan-probes specific for ASF virus (ASFV) were designed in the consensus nucleic acid sequence resulting from the alignment of more than 180 P72 gene sequences from the Genbank database. rtPCR protocol and linearised recombinant plasmid containing the P72 gene insert were distributed to three additional laboratories (CISA-INIA, AFSSA, IZSUM) for comparative validation of the assay.

The linearity and efficacy of the rtPCR assay was confirmed with recombinant plasmid and viral genomic DNA. The analytical sensitivity was estimated between 5 and 50 equivalent copies per reaction by titration of the control plasmid. At the intra-laboratory level, repeatability and intermediate precision CV remained below 3.0% and 3.3% respectively. Inter-laboratory reproducibility CV was 5.4%.

The absence of non-specific reaction was confirmed in negative blood or tissue samples from domestic pigs, as well as in the presence of genomic material from other swine viruses. The sensitivity and specificity of the assay were demonstrated using a panel of more than 40 isolates from different historical and geographical origins, including recent isolates originated from Sardinia, Caucasus and East Africa. The assay was also evaluated on field samples collected from domestic pigs or wild suidae during outbreaks or in endemic zones in Sardinia, Caucasus and Africa. The positive or negative status of the samples has previously been identified by PCR and rtPCR (Agüero et al., 2003; King et al., 2003). 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 rtPCR amplicons.

The new assay was evaluated in blood and tissue samples collected at different days post-infection from experimentally inoculated pigs. Infected animals were detected positive during the whole course of the infection, from early to late stages of infection (up to 42 dpi), even in subclinically infected animals. Compared to other PCR and rtPCR assays, improved detectability could be observed in samples collected at the very early and late stages of infection.

The robustness of this new multiplex real-time PCR for ASFV detection has been validated by the EU and three national Reference laboratories. The assay presents a high sensitivity and specificity, including the currently circulating isolates. Moreover, the positive early and long term detection observed on samples from experimentally infected animals highlighted the potential of the new rtPCR assay for improving ASF diagnosis of acute and subacute virulent strains, even in subclinically infected animals. The new rtPCR assay provides a rapid, sensitive and reliable molecular diagnostic tool for ASF diagnosis that can be useful both for surveillance in ASF-free areas and control in endemic areas.

ORAL: DEVELOPMENT OF AN IMMUNOENZYMATIC ASSAY BASED ON THE DOUBLE RECOGNITION ELISA TECHNIQUE USING PRRSV'S N RECOMBINANT PROTEIN AS CONJUGATE AND ANTIGEN.

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INGENASA[1]

Key words: PRRSV, diagnostic, ELISA DR

1. Introduction and Objectives

Porcine reproductive and respiratory syndrome (PRRS) is characterised by reproductive failure of sows and respiratory problems of piglets and growing pigs. The disease is caused by the PRRS virus, a virus currently classified as a member of the order Nidovirales, family Arteriviridae, genus Arterivirus. PRRS occurs in most major pig-producing areas throughout the world. The reproductive failure is characterised by infertility, late fetal mummification, abortions, stillbirths, and the birth of weak piglets that often die soon after birth from respiratory disease and secondary infections.

There are several ELISAs for serological testing, all of them based on indirect or blocking techniques. Due to the widely spreading of this disease and its economical importance it is very important to control it since very early states. INGENASA has developed a precocious, sensitive and highly specific new immunoenzimatic assay able to detect antibodies specific of N protein of the European type.

2. Materials and Methods

The new assay is based on the double recognition enzyme linked immunoassay technique using PRRSV's N recombinant protein both as conjugate and for coating.

For evaluation of the assay, different sets of samples have been used:

• 1002 sera previously characterized as negative by indirect ELISA.
• 48 sera previously characterized as positive by PCR.
• Sera from 70 animals experimentally infected with PRRSV, which were bled at different days post infection.

3. Results

For 997 of the 1002 sera previously characterized as negative by indirect ELISA, negative results were obtained using the DR assay. 42 of 48 sera confirmed as positive using PCR, yielded positive results using DR assay. 88.4% of experimental sera were detected at day 7 post infection. Moreover the detection of antibodies is maintained until late stages of infection.

4. Discussion and Conclusions

These results have indicated that the Double Recognition Immunoassay developed by INGENASA has a specificity of over 99% and detects antibodies specific to PRRSV in very early stages of the infection, which is 7 days post infection in 88.4% of cases.

Due to its early detection, the assay is a useful tool to control the serological status of new animals and for surveillance of negative herds. Moreover, the ability of detect late humeral response allows monitoring the vaccination status.

ORAL: DEVELOPMENT OF ISOTHERMAL AMPLIFICATION METHODS FOR RAPID DETECTION OF CLASSICAL RABIES VIRUS

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Key words: Loop Mediated Recombinase Polymerase Rabies

Isothermal nucleic acid amplification offers the opportunity to develop molecular tests for "in field" applications. WP4.4 working group have concluded that these technologies require investigation for pen-side testing. An isothermal amplification method where all genotype 1 (classical rabies) viruses can be detected would be of benefit to diagnosticians in situations where reverse transcription PCR following isolation and purification of viral RNA is not possible. Rapid and reliable detection of the virus is required for appropriate post-exposure treatment of infected humans and vaccination of "in contacts". In addition, in field testing of wild-life allows epidemiological study and determination of effectiveness of control strategies.

Loop-mediated amplification (LAMP) is a relatively simple method that is rapid, sensitive and apparently not as susceptible to the effects of inhibitory substances in the test material compared to PCR based assays (Notomi et al., 2000). No rabies LAMP assay that can be applied generally is available although an assay specific for virus circulating in the Philippines has been recently published (Boldbaatar et al., 2009). The distribution of classical rabies is worldwide with the exception of a few island countries and the continent of Antarctica, and human infection is prevalent in Africa, the Indian subcontinent and parts of south east Asia. We have developed a single tube assay that is capable of detecting virus in both the Cosmopolitan and Arctic-like clades of the virus which covers the majority of classical rabies strains. We have demonstrated that the amplification products can be visualised both in real-time using intercalating dyes in under 20 minutes and using commercially available lateral flow devices. In addition, viral RNA can be amplified directly from FTA cards which can be used to stably transport and inactivate rabies virus. We have also recently developed a method to detect rabies RNA using recombinase polymerase amplification methods (Piepenburg et al., 2006) for classical rabies virus and compared this method to LAMP with respect to ease of design of assay, ease of use, sensitivity, specificity and speed.

ORAL: AVIAN INFLUENZA (AI) RNA RING TRIAL TESTING BY REAL TIME RT-PCRS AND MOLECULAR PATHOTYPING

HANNA, AMANDA[1]; MAHMOOD, SAHAR[1]; COWARD, VIVIEN J[1]; PAVLIDIS, THEOFILAKTOS[1]; UNSAL BACA, AYSEL [2]; ÇÖVEN , FETHIYE [3]; FERNANDEZ-PINERO, JOVITA [4]; HARDER, TIMM [5]; SMIETANKA , KRZYSZTOF [6]; MINTA, ZENON [6]; CHERBONNEL, MARTINE [7]; BRIAND, FRANÇOIS-XAVIER [7]; JESTIN, VÉRONIQUE [7]; GIL, PATRICIA [8]; VAN BORM, STEVEN [9]; HANDBERG, KURT J[10]; JØRGENSEN, POUL H[10]; EJDERSUND, ANNELI [11]; CATTOLI, GIOVANNI [12]; MONNE, ISABELLA [12]; SAN MIGUEL, ELENA [13]; DAKMAN, ASIYE [14]; KOCH , GUUS [15]; HEUTINK, RÉNE[15]; BEER, MARTIN [5]; HOFFMANN, BERND [5]; BROWN, IAN H [1]; SLOMKA, MAREK J [1]

VETERINARY LABORATORIES AGENCY[1]; PENDIK VETERINARY CONTROL AND RESEARCH INSTITUTE[2]; BORNOVA VETERINARY CONTROL AND RESEARCH INSTITUTE[3]; CENTER OF ANIMAL HEALTH, NATIONAL INSTITUTE FOR AGRICULTURE AND FOOD RESEARCH AND TECHNOLOGY[4]; FRIEDRICH-LOEFFLER-INSTITUTE[5]; NATIONAL VETERINARY RESEARCH INSTITUTE[6]; AGENCE FRANÇAISE DE SECURITÉ SANITAIRE DES ALIMENTS[7]; CENTRE DE COOPÉRATION INTERNATIONALE EN RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT[8]; VETERINARY AND AGROCHEMICAL RESEARCH CENTRE[9]; TECHNICAL UNIVERSITY OF DENMARK, NATIONAL VETERINARY INSTITUTE[10]; STATENS VETERINARMEDICINSKA ANSTALT[11]; ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLE VENEZIE[12]; LABORATORIO CENTRAL DE VETERINARIA[13]; ETLIK VETERINARY CONTROL AND RESEARCH INSTITUTE[14]; CENTRAL VETERINARY INSTITUUT OF WAGENINGEN UR[15]

Introduction and Objectives: Since 2006, annual proficiency panel testing has been organised by the EU avian influenza (AI) Community Reference Laboratory (CRL) at the Veterinary Laboratories Agency (VLA, UK), and engages over 40 labs in Europe and third countries. This is a rigorous assessment of RNA extraction followed by AI real time RT-PCR (RRT PCR) and molecular pathotyping. The EPIZONE project is an opportunity to focus on a smaller number of labs (n=15) in an AI RNA RRT PCR ring trial, where stable distribution of RNA to participating labs circumvented variability in RNA extraction. This enabled AI RRT PCRs' analytical sensitivity and efficiency to be assessed directly. All EPIZONE AI labs were tasked to conduct AI RRT PCR testing by the EU-recommended methods for generic AI detection (M gene RRT PCR) and notifiable AI (NAI) detection (H5 and H7 RRT PCRs). Laboratories were also free to include AI RRT PCR approaches of their own choice. NAI samples were further investigated by molecular pathotyping. Material and Methods: A panel of 32 AI RNA samples was prepared in RNA Safe Buffer (RSB), and was distributed to all participating EPIZONE labs. Each sample was tested for AI and NAI (H5/H7) by appropriate RRT PCRs which included the corresponding EU-recommended protocols. Importantly, all 32 samples were prepared at titre ranges that typically occur in AI clinical specimens. The EPIZONE panel was divided into two groups of specimens, where the first included samples 1-20. These 20 included 15 AI specimens, ten of which were NAIs, ie five H5 and five H7 AIs. Identification of NAI by RRT PCR was followed by molecular pathotyping (amplicon sequencing). The second group (samples 21-32) were a ten-fold dilution series of one H5 isolate and one H7 isolate. These were tested by M gene and H5 or H7 RRT PCRs accordingly. These two dilution series served to address the analytical sensitivity of each RRT PCR in each laboratory.
Results: First, RSB was validated as a reliable means of preserving RNA integrity for stable transport of the EPIZONE AI RNA Panel at ambient temperature. In testing samples 1-20, 13 of 15 labs returned fully correct results for successful generic detection of all AI panel members (n=15) by M gene RRT PCR. Eleven and ten labs respectively successfully detected all H5 (n=5) and H7 (n=5) samples correctly. The efficiency values observed for each AI RRT PCR will also be presented. Twelve labs attempted molecular pathotyping, where four successfully sequenced and correctly identified the pathotype for all 10 NAIs. Analytical sensitivity results will be presented where the dilution series of H5 and H7 viral RNAs (samples 21-32) was investigated along with quantified in vitro transcripts for the corresponding AI RRT PCRs.

Data will be presented which demonstrate the detection limit (copies of RNA molecules) for each AI RRT PCR in each laboratory.

Discussion: The EU-recommended AI RRT PCRs had all been previously validated, and their successful use in this EPIZONE AI RNA ring trial affirmed their sensitivity and specificity. This has also been observed consistently in annual EU AI PCR proficiency testing since 2006. However, some variability in test performance that was unrelated to RNA extraction was noted. Several in-house AI RRT PCRs were found to be equally effective, but two commercial RRT PCRs for generic AI and H5 detection were poor. This ring trial also revealed some difficulties in the interpretation of NAI molecular pathotyping, mainly due to inclusion of one unusual NAI sample.

ORAL: DETECTION AND SUBTYPING OF INFLUENZA A USING A DEDICATED SUSPENSION MICROARRAY

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Key words: Influenza A, birds, humans, subtyping, multiplex assay

Rapid detection and subtyping of influenza as a surveillance tool for animals and humans would enable much earlier warning and comprehensive approach for the presence or introduction of dangerous new strains of the virus such as H5N1 and H1N1. Such an assay could be a key element in zoonotic threat surveillance.

A deeply multiplexed RT-PCR assay consisting of 38 primer pairs which utilizes Luminex xMap technology for detection has been developed at Lawrence Livermore National Laboratory and tested on 69 animal and human samples at the National Veterinary Institute (SVA) in Sweden. Luminex operates using a flow cytometer architecture modified with lasers to read color-coded beads covalently linked to oligonucleotide tags. The current format of the assay allows identification of the most common human H1, 2, 3 and many common avian H3, 5, 7, 9 subtypes. The assay used here consists of 38 primer pairs and 41 Luminex tags (3 H1, 6 H2, 5 H3, 5 H5, 9 H7, 6 H9, 2 matrix, 1 NS1 and 4 controls). Preliminary sensitivity of this assay for influenza A detection has been determined by measuring egg infectious doses of virus and by copy number has been found to be comparable to that of published TaqMan assays that are currently in use for H5, H7 and general influenza A surveillance in poultry. The capability of the assay to detect all of the target H subtypes has been demonstrated. Specificity has been shown by a lack of cross reactivity of the assay to H6 and H4 subtypes commonly found in poultry and wild birds respectively. Expansion of the assay capability to the sixteen hemagglutinin subtypes found in poultry as well as the addition of neuraminidase typing capability is envisioned.

The experiments at SVA were performed on 53 samples from birds and 16 samples from humans. Overall, of the known Influenza A positive samples there were 2 outright detection failures (2 cloacal swabs from birds) giving the assay a 96% detection rate (49/51). In detected samples, subtyping accuracy was 85% with 5 subtyping failures and 2 mistyped samples (41/48).

EPIZONE

Theme 6
Surveillance and epidemiology
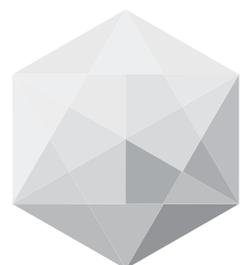
ORAL: FOOT-AND-MOUTH DISEASE IN THE MIDDLE EAST AND SOUTHERN ASIA: A CONTINUED THREAT TO EUROPE

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INSTITUTE FOR ANIMAL HEALTH[1]; CENTRAL VETERINARY LABORATORY[2]; FMD INSTITUTE ANKARA[3]

Foot-and-mouth disease (FMD) serotypes O and A are endemic in much of the Middle East with serotype Asia 1 occurring more sporadically. Periodically new virus strains arise in the eastern part of the region and spread westwards in an epidemic fashion. We have used phylogenetic analyses of complete VP1 nucleotide sequences to study the molecular epidemiology of FMD virus (FMDV) in the region in order to improve our understanding of virus spread.

In 2005, a sub-lineage of the O-PanAsia strain (Middle East-South Asia topotype) was identified in Pakistan and Iran. This strain was named O-PanAsia-2 and had its origins in north-western India (2001). It was subsequently identified in Bhutan (2003-2004, 2007-2008), Nepal (2003, 2007-2008), Pakistan (2005-2008), Iran (2005-2009), Afghanistan (2006-2007), Jordan (2006), Turkey (2007-2009), Saudi Arabia (2007-2009), United Arab Emirates (2007), Egypt (2007), Palestinian Autonomous Territories (2007) and Israel (2007-2008). The O-PanAsia-2 strain has become widespread in the Middle East, but has not completely supplanted earlier strains which continue to be isolated in, for example, Nepal (O-Ind-2001 in 2008-2009; unnamed lineages in 2009), Pakistan (O-PanAsia in 2006) and United Arab Emirates (O-Ind-2001 in 2008-2009).

In 2005, a new epidemic strain of FMDV type A was identified (A-Iran-05) which appeared to spread from the Iran/Afghanistan area into Turkey (2005), reaching European Thrace in January 2006, Saudi Arabia (2005), Pakistan (2006) and Jordan (2006) apparently supplanting the previous prevalent strains A-Iran-96 and A-Iran-99. Evolution of this virus strain in Turkey lead to the designation of two sub-lineages named A-Iran-05(ARD-07) and A-Iran-05(EZM-07); these sub-lineages have been restricted to Turkey. In 2007 a new A-Iran-05 lineage was identified in Afghanistan and named A-Iran-05(AFG-07); this sub-lineage was subsequently identified in Pakistan (2008-2009) and Iran (2009), but so far not elsewhere. In November 2008 another distinct lineage was identified in Bahrain and named A-Iran-05(BAR-08). This sub-lineage rapidly spread and was identified in Iran, Iraq, Kuwait and Lebanon in January 2009, Israel and Libya in February 2009, Pakistan (March 2009) and Turkey in September 2009. It is not clear why this particular lineage has spread so readily. In April/May 2009 another distinct lineage was detected in Pakistan but has not yet been named. To our knowledge the A-Iran-05 strain has not been detected in India.

In recent years, FMD type Asia 1 has isolated very infrequently from the Middle East, although it presumably continues to circulate in some countries within the region. In 2008 and 2009 Asia 1 was identified in Pakistan and was most closely related to earlier viruses from the region (Afghanistan (2001) and Iran (2001 and 2004). In 2009 Asia 1 also identified in Bahrain, however, this was found to be genetically distinct and information provided by the Project Directorate on FMD (Mukteswar, India) indicated this virus was closely related to 2009 Indian viruses.

Clearly FMD in the Middle East presents as a complicated epidemiological situation and genetic typing is required to identify the causal viruses and reveal how they have spread. Thus this work is critical to understanding the epidemiology of FMD.

ORAL: CIRCULATION OF CLOSELY RELATED LOW PATHOGENIC AIV H7N7 IN MALLARDS IN POLAND

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Key words: avian influenza, H7N7, wild birds

Four avian influenza viruses (AIV) of H7N7 subtype were isolated in 2007- 2009 from mallards (Anas platyrhynchos) in Poland. The whole haemagglutinin (HA) and neuraminidase (NA) genes were sequenced. Additionally, partial or full-length sequences of selected internal genes of these strains were also established.

All of the tested strains were of low pathogenicity with two different HA0 cleavage site amino acid sequence motifs: PEIPKGRGLF (isolates A/mallard/PL/1/08, A/mallard/PL/41/09 and A/mallard/PL/446/09) and PELPKGRGLF (isolate A/mallard/PL/16/09).

The A/mallard/PL/446/09 isolate was located on the same branch of the HA phylogenetic tree with A/mallard/PL/41/09 isolate (99.3% of nucleotide identity). On the other hand, the NA phylogenetic tree revealed a close relationship between A/mallard/PL/446/09 and A/mallard/PL/16/09 strains (nucleotide similarity of 98.6%). Interestingly, the latter isolates had been isolated at the same sampling site (a pond in the city of Gdansk, near the Baltic Sea) almost exactly one year apart (mid January - late December 2009). The sampling location of A/mallard/PL/41/09 isolate was about 100 km from Gdansk and the time interval was around 9 months. Moreover, the HA and NP genes of A/mallard/PL/16/09 isolate showed a very high degree of identity to A/goose/Czech Republic/2009(H7N9) strain (HA: 99.8%, NP: 99.5%, GenBank accession number GU060482-83) but significantly lower similarity in relation to the other internal genes (95-97%). The A/mallard/PL/1/08, isolated at the end of 2007 in eastern Poland fell into the same clade with A/mallard/PL/41/09 & A/mallard/PL/446/09 isolates (HA gene) and A/mallard/PL/16/09 & A/mallard/PL/446/09 isolates (NA gene) with sequence similarity up to 97%. Based on the results we suggest that the A/mallard/PL/446/09 isolate is a reassortant virus that acquired the surface protein gene segments from A/mallard/PL/41/09 (H7 segment) and A/mallard/PL/16/09 (N7 segment). Additionally, a close relationship of the aforementioned isolates with the HA and NA of A/mallard/PL/1/08 isolate suggests that both gene segments of the tested H7N7 strains had been derived from a common precursor virus.

Our results clearly indicate that the closely related AIV viruses had been perpetuating in a population of mallards in Poland for a relatively long period of time. On the other hand, an example of the A/mallard/PL/16/09 and a very high similarity of its HA and NP genes to A/goose/Czech Republic/2009 indicates an occasional spill over from wild bird populations to domestic birds. The ongoing molecular studies on internal genes may shed more light on the origin and evolution of AIV H7N7 isolated in Poland.

ORAL: THE REPRODUCTIVE PERFORMANCE AND THE PCR-STATUS OF CALVES OF CATTLE THAT SEROCONVERTED FOR BTV-8 IN THE NETHERLANDS IN 2008

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Key words: bluetongue; vertical transmission; fertility; cattle

In 2007, BTV-8 re-emerged for the second year in the Netherlands with an increase in morbidity and mortality. In addition, cattle farmers reported a reduced fertility performance in their cows. We have studied fifteen selected herds of which the farmer had decided not to vaccinate. These were matched to 10 vaccinated herds by geographic region. In July 2008, the start of the study, all cattle in the non-vaccinated herds of >1 year old were sampled. All seronegative cows entered the study program in which blood samples were tested by ELISA with intervals of three weeks. Sampling stopped for cows that tested seropositive and ceased in all remaining cows in December 2008. Seroconversion was associated with infection of BTV-8. Further, newborn calves originating from infected dams and from vaccinated dams were sampled shortly after birth, and tested by PCR for BTV-8. Fertility data were obtained between 2005 and August 2009 from the Royal Dutch Cattle Syndicate (CRV). Four reproductive parameters hypothesized to be associated with BTV-8 were defined. These were: Non return for a repeated insemination within 56 days after first insemination (NR), number of inseminations per cow per assumed pregnancy (Nins), time between first and last insemination (Δt_days) and abortion between 100 and 260 days after last insemination (ab). For the analysis of the association between BTV-8 seroconversion and fertility, all cows that were included in the study were assigned to one of three mutually exclusive BTV-8 categories; vaccinated, infected, or not vaccinated and not infected in 2008. Multi-level analyses were used to estimate the effect of BTV-8 infection on the fertility.

In the study period July-December 2008, 185 cows (17.2%) out of 1,074 initially seronegative cows in non-vaccinated herds seroconverted, indicating infection by BTV-8. Three out of four reproductive parameters, NR, Δt_days and Nins, showed an effect by BTV-8 infection. The chance of a return to service (NR=0) was 5.0 times higher (95% CI: 1.9-14.3), Nins was 1.7 times (95% CI: 1.4-2.0) higher, and Δt_days was 2.5 times (95% CI: 2.4-2.6) higher in cows infected by BTV-8 compared to cows remaining uninfected in the same study period. We found no significant effect of BTV-8 infection on the chance of abortion (ab).

From the 185 cows infected in the study period, 80 were infected during gestation.. This eventually resulted in 48 samples from their newborn calves. 10 out of 48 tested PCR-positive (20.8%; 95% CI: 9.3-32.3%). The PCR results of the newborn calves were significantly associated to the number of months in the dam was gestation at the moment of seroconversion. Cows infected in the second half of gestation had a significantly higher chance (15.5 times (95% CI: 1.3-190.4) on delivering a PCR-positive calf than cows infected in the first half of gestation. None of the 256 calves from vaccinated dams tested PCR-positive after birth (0%; 95% CI: 0-1.4).

No effect of BTV-8 infection on percentage of abortion was found, however, only late abortions were analyzed in this study (>100 days in gestation). On the other hand, more inseminations needed for pregnancy, and a longer time between first and last insemination, suggest a higher probability of (unnoticed) early abortions. We conclude that BTV-8 infection in 2008 negatively influenced fertility, the time of a BTV-8 infection in pregnant susceptible (seronegative non-vaccinated) dairy cows has strong influence on the BTV-8 status of the offspring.

ORAL: DEVELOPMENT AND ASSESSEMENT OF A PEN-SIDE TEST BASED ON THE USE OF VP73 PROTEIN FOR DETECTION OF ANTIBODIES AGAINST ASFV

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Key words: ASFV, pen-side test, Diagnostic

The epidemiological situation of ASF worldwide has highlighted the need for front-line/penside tests as good tools for disease control. It will therefore assist African countries to improve their diagnostic capacity and could improve the general knowledge about the epidemiology of the disease in various regions where it is currently not possible. A lack of front-line tests also negatively impacts on routine and post outbreak surveillance.

To this end, INGENASA together with the ASFRISK EU project, have developed a rapid, one-step immunochromatographic strip (pen-side test) capable of specifically detecting anti-ASF antibodies in serum specimens.

The qualitative assay is based on a direct immunoassay in which the detector reagent consisted of red super carboxyl latex micro particles covalently coated with VP73 purified protein. The capture reagent was the same protein adsorbed on the nitrocellulose membrane strip to form a test line. In addition, blue latex micro particles coated with a control protein and a second line created above the test line, by the immobilization of anti-control protein antibodies, was used as a control of test.

A serum specimen was then applied to the sample pad. The anti-VP73 antibodies present in the sample specifically bound to the labelled micro particles. The antibody-protein binding complex formed migrated until the nitrocellulose membrane by the flow caused by capillary action and reacted with the immobilized VP73 protein which generated a visible test line. The control latex micro particles continued to the next line, revealing a visible control line. Thus, a positive serum yielded a red test line and a blue control line, whereas a negative sample produced only a blue control line. The entire test procedure was completed in 10 minutes.

In order to check the sensitivity and specificity of the test, a collection of serum samples previous classified as positive or negative according OIE-prescribed serological tests were used.: i) A panel of 70 sera obtained at different days post infection from several in vivo experiments at CRL CISA-INIA and IZS-UM, with different isolates belonging to p72 genotype I and the p72 genotype X (the most variable and genotypically distant genotype), and ii) A panel of 375 field serum samples (208 typified as positive and 167 as negative by formal tests) from different origins (Kenya, Tanzania, Nigeria, Congo Brazzaville, Togo and Burkina Faso).

Regarding the analysis of the experimental sera, the assay showed specific antibody detection from day 10 post infection, which is slightly higher than that obtained with formal serological procedures described at the OIE Manual (ELISA and Immunoblotting). The analysis of field serum samples gave a 98, 5-100% correlation in sensitivity with the formal serological techniques.

This single-step assay can be performed rapidly and easily without special equipment. The immunochromatographic test provides a reliable method for detection of anti-ASF antibodies where laboratory support and skilled personnel are limited.

ORAL: PORCINE CIRCOVIRUS (PCV-2) VIRULENCE ATTENUATION BY MUTATIONS IN THE CAPSID MOTIF SPECIFIC OF GENOGROUP

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Key words: PCV-2, capsid and virulence

Post-weaning Multisytemic Wasting Syndrome (PMWS) is due to Porcine Circovirus of type 2 (PCV-2). Molecular epidemiology studies reported two main genogroups PCV-2a and PCV-2b and identified in the ORF2 protein, the unique constituent of the viral capsid, a pattern named genogroup motif. The latter is strongly conserved into each genogroup and is located between amino acids 86 and 91. In 2005, a PMWS outbreak occurred in North America. This event seems to be correlated with the emergence of new virus isolates which mainly belonged to the PCV-2b genogroup. The hypothesis that isolates of PCV-2b genogroup were more virulent than those of PCV-2a emerged. The aim of this study was to check if strains representative of each genogroup differ in virulence and if the capsid motif specific of genogroup is involved in PCV-2 strain virulence variability.

Constructions of two infectious clones representative of both PCV-2 genogroups were performed: PCV-2a and PCV-2b clones. Site-directed mutagenesis was carried out on the two clones to exchange the capsid motif. Two mutants were obtained: one with the PCV-2b motif in the backbone of PCV-2a (PCV-2ab) and the second with the PCV-2a motif in the backbone of PCV-2b (PCV-2ba). Production of capsid protein and viruses was assessed for the four constructions by immuno peroxydase monolayer assay (IPMA). An experimental trial was carried out to assess the virulence of the four constructions. Forty SPF piglets of six week-old were divided into 4 groups of eight piglets, two groups (4 piglets) were control groups with or without immunostimulation. In the four other groups, pigs were transfected with either one of the two parental or the two mutants and immunostimulated. Clinical signs were monitored daily. Viral genomic load in sera and seroconversion were evaluated weekly. All the pigs were euthanized between 35 and 40 days post-inoculation (dpi). Viral genomes and infectious virus were measured by IPMA and quantitative PCR in the organs. Viral antigens were detected in microscopic lesions by immuno histo-chemistry.

In vitro study demonstrated that all constructions were infectious. During the in vivo trial, no differences were found between clones and mutants for clinical signs, except that the pigs inoculated with parental clones presented more severe hypertrophy of tracheo-bronchial lymph nodes. In the PCV-2a group, the pigs were seropositive at 35 dpi. All the animals of the PCV-2b group seroconverted between 21 and 28 dpi like the PCV-2ba mutant inoculated pigs. No seroconversion was observed for the PCV-2ab group. Genomic viral load was detected since 14 dpi with 104.8 copies/ml on average in 5/8 PCV-2a pigs and at a mean of 106.8copies/ml in all PCV-2b pigs. For the PCV-2ba mutant, viral load was transient for all pigs (mean of 104.1copies/ml at 14dpi) while no viral genome was detected in the serum of the PCV-2ab inoculated pigs. Genomic loads and infectious virus were detected in organs of pigs inoculated with both parental clones and both mutants.

To conclude,

(i) Both clones and mutants were infectious in vitro.

(ii) In vivo experiment showed virulence difference between the two parental clones, PCV-2b being more virulent than PCV-2a.

(iii) The PCV-2ba mutant was moderately attenuated while the PCV-2ab mutant was highly attenuated. The work evidenced that exchange of four amino acids of the capsid motif specific of genogroup influences the virulence of PCV-2.

ORAL: PROTOTYPE DATABASE FOR STORING DATA OF BIOLOGICAL AGENTS AND THEIR SEQUENCES: NEW DEVELOPMENTS

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Key words: datbase/ sequence/ molecular epidemiology

Most veterinary diagnostic laboratories are routinely isolating and identifying pathogens. Very often they are also typing them with various tools which allow typing at much more precise level than simply the species level. These tools could also allow the identification of virulence markers (such as antibiotic resistance in bacteria). On the other hand dating and geo-localising the isolates that are typed open new avenues for molecular epidemiology studies that could help understanding trends in epidemics or even allow better emergency preparedness. To simplify storing and evaluation of the data, a prototype "generic" web-based molecular epidemiology database that is easy to customize for the different pathogens was created. The main objective of this database is to increase collaboration between European laboratories working in the same field, and provide national as well as international institutions a better picture of the circulation of pathogens worldwide. The corresponding work package was implemented in EPIZONE. To keep the costs low, open source programs were used. The database is simple to customize and to keep update.

The prototype database needs a Linux server (e.g. Debian or Ubuntu), with an Apache2 webserver and with MySQL installed. For customizing the scripts, skills in SQL, PERL and HTML are necessary. The software package consists of different PERL scripts, and a database configuration script for the basic table containing the biological and epidemiologic data of the organisms, the sequence table, and the table with the sequence types (genes or genomic fragments). The data type and the fields in the basic table are configured during setup. Depending on the field names and type of data to be stored, the scripts have to be configured. In addition, the field names and the types for the output of data and lists can be configured according to individual needs.

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

The prototype database (administrator mode) was supplemented with two new modules: one for geotagging (via google maps), and a tool that allows to retrieve data from GenBank. The data can be exported both as a text file and as an excel file. After checking if the downloaded sequences are correct and amending the remaining fields, the data can be imported into the database. Here, the module excludes GenBank numbers that are already stored.

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Presentations projects
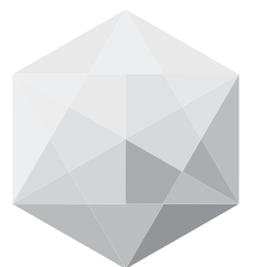
PROJECT: GLOBAL FOOT-AND-MOUTH DISEASE RESEARCH ALLIANCE (GFRA): FUTURE GOALS AND DIRECTION

GAY, CYRIL[1]

AGRICULTURAL RESEARCH SERVICE[1]

Key words: Foot-and-Mouth Disease, Research, Alliance, Workshop, Countermeasures

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

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

Next Steps: The GFRA will hold a workshop during the EPIZONE meeting in St. Malo, France, on June 10, 2010 to recruit new members, review current FMD research projects, solicit input on research priorities, and explore new strategic research collaborations needed to advance the progressive control and eradication of FMD.

PROJECT: IMPROVE TOOLS AND STRATEGIES FOR THE PREVENTION AND CONTROL OF CLASSICAL SWINE FEVER

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Key words: Classical swine fever,marker vaccine, epidemiology, DIVA diagnostics, back yard pigs

Although classical swine fever (CSF) has been eradicated in wide areas within the
EU the disease is endemic in some new member states particularly in back yard
pigs.
In order to improve the eradication strategies the CSFV_goDIVA project(EU FP7) aims are a) the final development and testing of a live marker vaccine candidate for the prevention and improved control of CSF, both orally and intramuscularly applicable; b) the development and optimisation of accompanying discriminatory diagnostic tests; c) the production of an effective, oral delivery system for the marker vaccine for use in wild boar and back yard pigs; d) the easy selection of diseased animals. The improved knowledge on immunological reactions and pathogenesis will support a more efficient vaccine application and provide data for the epidemiological models.
Epidemiological studies of CSF in domestic and back yard pigs and in wild boar including molecular epidemiology intend to increase the insight of CSF transmission and persistence.
Epidemiological models will be developed to support risk assessment as well for conventional eradication strategies as for new strategies using the new vaccines and diagnostic tools.
Expected results:

This project will result in the availability of methods allowing more ethical and environment friendly meat production from healthy and protected animals. This will be achieved by a better understanding of CSF epidemiology, by the availability of adapted vaccines and marker vaccines with discriminatory assays and sampling protocols applicable for specific emergency situations.

The expected outputs of the present project will be:

a.Better understanding of CSF epidemiology, including pathogenesis, transmission and reservoir
b.Evaluation of the third generation of live CSF marker vaccines
c.Development and validation of accompanying discriminatory tests detecting antibody response as well as the differentiating of viral antigens.
d.Scenario-analysis and cost-effort evaluation of control protocols in domestic pigs
e.Design of specific sampling protocols
f.Development and evaluation of optimised surveillance strategies
g.Better understanding of the mucosal immune response after vaccine oral delivery
h.Insight in the potential use of antivirals as a possible control strategy
i.Development and evaluation of new or adapted techniques usable under low-tech and on-site environments.

PROJECT: THE GLOBAL LABORATORY DIRECTORY (GLAD): BUILDING A FUNCTIONALLY EFFECTIVE LABORATORY COMMUNITY TO ADDRESS PUBLIC HEALTH CHALLENGES

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WORLD HEALTH ORGANIZATION[1]; NATIONAL MICROBIOLOGY LABORATORY[2]; IHRC, INC[3]; WORLD HEALTH ORGANIZATION [4]; NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES [5]; UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT[6]; CENTERS FOR DISEASE CONTROL AND PREVENTION[7]

Key words: Laboratory networks

Laboratories play a critical role in facilitating timely recognition and response to public health emergencies of international concern (PHEIC). However, capabilities and capacities vary widely between laboratories around the world.

During the past two decades, we have witnessed significant change in the way scientific communities share experiences, exchange methods and ideas, expertise and resources through use of emerging Internet and satellite technologies. The ease of travel, access to e-journals, rapid internet searches, daily outbreak updates and virtual meetings further create opportunities to meet and connect in ways never possible before. These electronic gateways are becoming more accessible to even those laboratories which have been the most remote or isolated. Scientists, laboratories and networks have embraced these developments, but the challenge is to capture and share this ever-expanding, vibrant, science-based connectivity and to find ways to support the viability of laboratory networks that serve as irreplaceable repositories of experience and knowledge.

Connecting laboratories through networks enables scientific communities to contribute their expertise in response to PHEIC while adding value and enhancing opportunities to enrich their own work.The Global Laboratory Directory (GLaD) is a support system designed for building, connecting and sustaining laboratory networks and their members. Its function is to provide new and existing networks a portal for more visibility, peer-to-peer support and access to successful solutions and templates. GLaD focuses on networks and their members as the entry point as they connect across multiple geographic sectors and disciplines. Networks that focus on human and animal infectious diseases, health-related environmental and non-biological hazards that may constitute PHEIC (described in Annex 2 of the International Health Regulations of the World Health Organization) are included. The International Health Regulations Coordination Department is committed to laboratory capacity support through both its arms in Lyon and Geneva. Established links with these networks provide access to more accurate and timely evidence-based information across the globe, in regions and in countries.

GLaD has 3 components: 1) GLaDMap, where laboratory networks and their members contribute information that is visualized through a dynamic, interactive relationship tool. A pilot study is being conducted to collect network and their member's profiles into database at www.GLaDMap.org; 2) GLaDSupport, where network operability activities are undertaken with focus on supporting network managers. The activities include meetings, teleconferences, providing a forum for communication and discussion; and 3) GLaDResource, where information on best relevant management practices are shared and where tools, documents and protocols are archived for ready access to networks and their members.

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Keynote
Ken McCullough
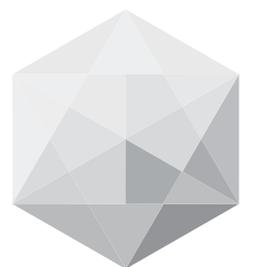
KEYNOTE: VIRUSES, VACCINES AND DENDRITIC CELLS: A QUESTION OF LIFE, THE UNIVERSE AND EVERYTHING?

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Key words: Dendritic cells

Dendritic cells (DC) play a critical role in immune defence development by mediating the interaction of various components within the innate and adaptive immune compartments. Of particular importance is the interaction between conventional DC (cDC) and plasmacytoid DC (pDC). During the last decade, the increase in our knowledge on the critical roles of DC has elaborated our understanding of how viruses evade and modulate immune defence processes, as well as promoted research and development of vaccines for targeting DC.

The efficacy of immune defence development is open to manipulation by viral pathogens, particularly when infecting DC. Classical swine fever virus (CSFV) and porcine circovirus type 2 (PCV2) offer insights into the diversity of virus-induced manipulation of immune responsiveness. CSFV infects and replicates in both cDC macrophages where it antagonises the type I interferon (IFN) induction pathway, by Npro-mediated proteasomal degradation of interferon production is induced and itsαregulatory factor (IRF)3. In pDC IFN overproduction during acute disease results in immunopathological consequences. As for PCV2, its main mode of immunomodulation appears to rely more on the viral nucleic acid, which has immunoregulatory characteristics by preventing "danger" recognition by cells of the innate defences. In addition, PCV2 may also divert immune responsiveness against another virus or vaccine into a more regulatory pathway, seen as a promotion of IL-10 production following immune stimulation by another antigen.

As can be seen from these examples, an important element in virus interaction with the immune system is the manner by which the DC respond to pathogen-associated molecular patterns. While viruses have mechanisms to interfere with this "danger" recognition, such processes of detection have permitted the development of vaccines targeting DC. Recent efforts in the latter field have focussed on synthetic vaccine carriers, often based on biodegradable polysaccharide particles or gel-like meshes. The surface of these particles can be decorated with ligands for DC receptors. Examples of the ligands tested to date include mannose for the C-type lectin mannose receptor and lipopeptides for the toll-like receptors TLR2/1 and TLR2/6. Others approaches have employed antibodies specific for structures including integrins, Siglecs, galectins, and other C-type lectins such as DC-SIGN and DEC205. There is also interesting comparison to be made with vector vaccines employing virus-like particles, whereby natural viral ligands for cell receptors such as heparan sulphate glycosaminoglycan structures and integrins may prove applicable.

In conclusion, the DC family represents a critical immune defence element open to modulation by virus infection, but also central for targeted, and therefore efficacious vaccine delivery. The characteristics of the immune modulation depend on how the virus or targeted vaccine interacts with the DC subsets, and the outcome offers either pathological problems for the host or efficient protection form the pathogen.

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Theme 5
Intervention strategies
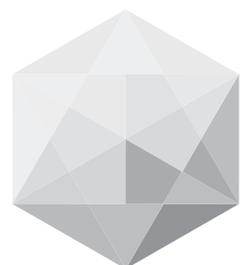
ORAL: USE OF A MOUSE MODEL TO TEST THE VACCINATION EFFICACY OF RECOMBINANT AFRICAN HORSE SICKNESS VACCINES

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Key words: African horse sickness, mouse model, vaccines

African horse sickness, which is trasmitted by the bite of AHS virus infected hematophagous insects of the genus Culicoides, is the most lethal disease known to horses. Live attenuated vaccines, manufactured and used routinely in South Africa, though efficacious, present a serious of safety concerns for their use in non-endemic countries. There is limited efficacy available for AHS inactivated vaccines. Thus, recombinant vaccines, using viral vectors or expressed viral proteins, have been investigated by some researchers. In this respect, we have recently shown the potential of modified vaccinia Ankara (MVA) encoding the VP2 gene of AHS virus to serve as vaccine vector for AHS since this construct induced virus neutralising antibodies in ponies (PLoS One 4, e5997).
However, research on improved AHS vaccines is hampered by the difficult and expensive experimental conditions derived from infecting horses with such a lethal virus. Recently, a model of bluetongue, closely related to AHS virus, has been developed using interferon alpha receptor knock-out (IFNAR - / -) mice (PLoS One 4, e5171). After validation of this experimental system for AHS virus we have tested the protective efficacy of MVA VP2 and VP7 crystals in a vaccination and challenge experiment.

ORAL: PSEUDOTYPING OF BACMAM RECOMBINANTS WITH THE G PROTEINS OF THE RHABDOVIRUSES VIRAL HEMORRHAGIC SEPTICAEMIA VIRUS AND VESICULAR STOMATITIS VIRUS TO IMPROVE GENE TRANSFER EFFICACY INTO VERTEBRATE CELLS.

KEIL, GUENTHER [1]; SCHIRRMEIER, HORST [1]; LORENZEN, NIELS [2]; GIESOW, KATRIN [1]

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Key words: BacMam technology, transduction, pseudotyping, rabbit hemorrhagic disease

Baculovirus Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) recombinants carrying expression cassettes with mammalian promotor elements are suitable for delivery into and expression of genes in many mammalian cell types but also in cells of avian and piscine origin. This transduction approach has been named BacMam method but is also designated BacMam technology. BacMam viruses are easy to generate, have a broad in vitro host range, are applicable for transient and stable gene transfer, and are cost-effective in comparison to chemical transfection procedures. In contrast to applications in vitro, so far reports on the use of this method for immunisation of vertebrates against pathogens are limited. We had reported previously the induction of specific B-cell and CD4+ and CD8+ T-cell immune responses against foot-and-mouth disease virus by BacMams expressing the structural proteins P1-2A together with 3C protease after intramuscular injection into mice and development of a protective immune response in rabbits immunized with purified BacMam viruses expressing VP60 of rabbit hemorrhagic disease virus (RHDV). Usability of BacMams expressing African swine fever antigens for immunization of pigs is analysed in an EPIZONE internal call project and available results will be communicated.

Current developments for next-generation BacMam vectors for vaccination purposes are aimed at enhancement of the transduction efficacies in vivo by increasing the antigen expression or by display of specific ligands on pseudotyped virus particles.

Towards this goal we constructed a series of novel baculovirus transfer vectors that contain vertebrate cell-active transcription regulation elements and genes encoding the envelope glycoproteins G of the rhabdoviruses vesicular stomatitis virus (VSV-G) and viral hemorrhagic septicaemia virus (VHSV-G) under control of the baculoviral p10 promoter.

Recombinant baculoviruses expressing p10 VSV-G or p10 VHSV-G in insect cells and which contained in addition a vertebrate cell-active green fluorescent protein expression cassette were pseudotyped with the respective G proteins in their envelopes which led to an increase in transduction efficiency in both mammalian and piscine cells, indicating that the cell entry-supporting activity of the rhabdoviral G proteins may be class independent. To analyse whether pseudotyping BacMam virions with VSV-G and VHSV-G improves vaccine properties in animals, respective recombinants expressing the RHDV VP60 were generated. In a pilote experiment, four rabbits were immunized with the VSV-G pseudotyped BACMam/ieVP60. All animals developed a specific antibody response against RHDV and all animals were protected against a challenge infection wit a lethal virus dose. Experiments to test whether also VHSV-G pseudotyped BACMam/ieVP60 is effective and to evaluate the pseudotyping-mediated augmentation in vaccination efficacy are initiated. The acquired results will be discussed.

ORAL: USING SMALL INTERFERING RNAS (SIRNAS) TO COMBAT A FISH PATHOGENIC VIRUS

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Key words: siRNAs; VHS virus; Fish, Aquaculture

RNA interference (RNAi) is an intracellular mechanism used by the cell to specifically inhibit gene expression through interaction at the mRNA level resulting in cleavage of the mRNA or repression of translation. The cell accomplishes this by the aid of a large protein complex called the RNA-induced silencing complex (RISC). This complex 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 small interfering RNAs (siRNAs) has received attention due their high degree of target specificity. These can guide RISC to bind specific mRNAs, defined by sequence complementarity to one of the strands in the siRNA, followed by cleavage of the mRNA. Because synthetic siRNAs can be designed to target a variety of 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 give examples of results on delivery and effect of siRNAs designed to target viral genes of the fish pathogenic rhabdovirus viral hemorrhagic septicemia virus (VHSV) in rainbow trout cells. The disease causes high mortalities in salmonid fish aquacultures why intervention strategies are highly in demand.

ORAL: EFFICACY OF TWO COMMERCIAL VACCINES (BTVPUR ALSAP 8 (MERIAL) AND BOVILIS BTV8 (INTERVET) AGAINST A BTV-8 VIRULENT CHALLENGE IN GOATS

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Key words: BTV8, goat, inactivated vaccines, efficacy

INTRODUCTION

Bluetongue is an infectious, non-contagious OIE listed arboviral disease, which naturally infects domestic and wild ruminants. There are 24 to 25 different Bluetongue virus (BTV) serotypes, with little cross-reactivity. Since 2006, BTV-8 has spread throughout Europe, causing severe disease and heavy financial losses.

BTV-8 vaccines have been developed rapidly by several companies and the first commercial vaccine batches were made available in 2008 and are used in the field since 2008. In the course of their development, these inactivated vaccines were first tested in sheep and cattle – the 2 major ruminant species in the EU. Goats also are susceptible to BTV-8 and represent a potentially important reservoir of virus, with an EU population close to 13 millions heads. While BTV-8 vaccines have been used off license in goats, little information was available on the efficacy in that species.

The data presented here are a collaborative research performed by AFSSA to assess the efficacy of two different BTV-8 vaccines in goats: BTVPUR AlSap 8 (MERIAL) and BOVILIS BTV8 (INTERVET). Both are inactivated vaccines.

MATERIAL AND METHODS

Thirty 3.5-months-old Saanen goats sero-negative to BTV were randomly allocated to 3 groups of 10. Two groups (A and C) were subcutaneously vaccinated twice (D0 and D28) with 1 mL of the two commercial vaccines BTVPUR AlSap 8 (group A) or BOVILIS BTV8 (group C). Another group (B, controls) was injected with 1 mL of physiological saline, at the same route and regimen. On D49, the groups were challenged with a virulent inoculum containing BTV-8. Goats were then monitored daily for rectal temperature and clinical signs for 25 days after challenge. Furthermore, goats were regularly monitored for viraemia (Pan-serotype qRT-PCR & BTV-8 specific qRT-PCR) for 28 days after challenge. Serological testings were regularly performed.

During the experiment it was found out that the BTV-8 challenge inoculum was contaminated with another BTV serotype. Because of this contamination, a BTV-8 specific qRT-PCR was used to assess viraemia in vaccinates and controls.

RESULTS

All vaccinated goats had sero-converted 14 days after the 2nd vaccination and had neutralizing antibodies against BTV 8. The kinetics of onset and levels of neutralizing antibodies are similar for both vaccines.

In the control group, clinical signs and increase of rectal temperatures were mild. All controls were viraemic (CT values range between 22 and 30) at all time points between D56 and D77, using the BTV-8 specific qRT-PCR. As compared to the control group, clinical expression of the disease was significantly reduced in both vaccinated groups. No BTV-8 viral RNA was detected in the blood of any of the
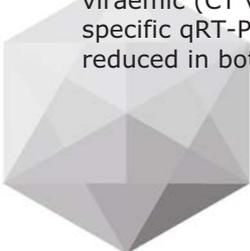
vaccinated animals between D56 and D77. The differences between vaccinated and control groups on viraemia were statistically highly significant.

CONCLUSION

In the present study, vaccination of goats with two injections of 1mL of BTVPUR AlSap 8 or 1mL BOVILIS BTV8 provided a significant clinical protection against a BTV-8 challenge and completely prevented BTV-8 viraemia in all animals. Qualitative data obtained in this experiment showed no difference in the kinetics and levels of the humoral response induced by these two inactivated vaccines.

ORAL: IMMUNOGENICITY AND PROTECTION INDUCED BY MODIFIED VACCINIA VIRUS ANKARA (MVA)-BASED VACCINES AGAINST RIFT VALLEY FEVER VIRUS (RVFV).

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Key words: Rift Valley fever virus, DNA immunization, recombinant MVA vector, prime boost

Rift Valley fever (RVF) is a viral zoonosis that affects domestic ruminants as well as humans. Infection with RVF virus (RVFV) causes abortion of pregnant animals along with a high mortality rate in newborn lambs and calves. In humans, it usually manifests itself with influenza-like symptoms, but occasionally leads to more serious complications like haemorrhagical fevers, encephalitis and retinitis with high morbidity and mortality. There is a need for improving the current vaccines against RVF and several strategies are being tested in different research laboratories. Recent work has shown the efficiency of DNA vaccines to confer protection against lethal RVFV in different mouse models.

In this work we have evaluated the immunogenicity and protection efficiency of different vaccination regimens upon lethal RVFV challenge in Balb/c mice using recombinant MVA (rMVA) expressing both Gn/Gc viral glycoproteins as well as the viral nucleoprotein N. We analyzed the induction of specific immune responses, either after a combined, prime boost (DNA-rMVA) approach or upon a single rMVA administration.

The level and efficiency of the induced humoral immune responses were tested by IFA, ELISA and western blot assays as well as by virus neutralization. Preliminary results indicate that prime-boost approaches enhances the immune responses against the viral nucleoprotein, and that mice immunized with this approach present higher survival rates than mice vaccinated with pCMV-N alone upon lethal challenge with a virulent, heterologous strain of RVFV. However these mice showed clinical signs long after challenge. In contrast, a pCMV-Gn/Gc + rMVA-Gn/Gc regimen did not improve the level of protection achieved by the pCMV-Gn/Gc vaccine alone, but none of the surviving mice displayed clinical signs. Surprisingly, a single dose of the rMVA-Gn/Gc was sufficient to induce protection in 100% of the mice upon viral challenge with very limited clinical display (1/7).

These data indicate that rMVA expressing RVFV antigens should be considered as potential vaccine candidates for the prevention of Rift Valley fever.

ORAL: PARAMYXOVIRUS VECTOR VACCINES FOR THE CONTROL OF RIFT VALLEY FEVER VIRUS

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Key words: Rift Valley fever virus, Newcastle disease virus, Vaccine, Vector vaccine

Rift Valley fever virus (RVFV) is a mosquito-borne RNA virus of the Bunyaviridae family. RVFV causes recurrent large outbreaks among humans and livestock with high mortality rates. The virus is endemic to the African continent and the Arabian Peninsula and there is a growing concern for spread of the virus towards new virgin soils, since mosquitoes capable of transmitting RVFV are not confined to its current habitat. In Africa, RVF is controlled by vaccination with a live-attenuated vaccine or a vaccine based on inactivated whole-virus. The live-attenuated vaccine is, however, not safe for unborn and young animals. The inactivated vaccine is safe for animals of all age groups, but is less effective than the live-attenuated vaccine, requiring multiple administrations for optimal immunogenicity. The RVFV structural glycoproteins Gn and Gc are preferred candidates for the development of subunit vaccines that optimally combine efficacy and safety. We study the feasibility of using Newcastle disease virus, an avian paramyxovirus, as a vaccine vector for the in vivo production of the RVFV glycoproteins. We show that NDV-based vector vaccines protect mice from a lethal RVFV challenge, and demonstrate their ability to induce neutralizing antibodies in calves and lambs, the two primary target species of RVFV.

EPIZ NE

Theme 7
Risk assessment
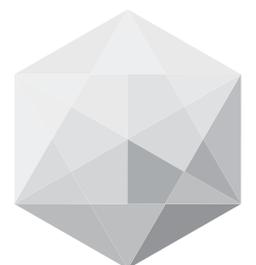
ORAL: LIVESTOCK MOVEMENT DATA FOR RISK ASSESSMENT AND MORE EFFICIENT TRACING IN CASE OF OUTBREAKS

NATALE, FABRIZIO[1]; SAVINI, LARA[2]; GIOVANNINI, ARMANDO[2]; CALISTRI, PAOLO[2]; POSSENTI, LUIGI[2]; PALMA, DIANA[2]; HOFHERR, JOHANN[1]; FIORE, GIANLUCA[1]

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Key words: social network analysis, traceability, livestock movements, epidemic model

Social Network Analysis (SNA) can be used to study networks built from livestock movements' data in order to evaluate the risk associated to farm holdings in spreading infectious diseases. In the case of diseases transmitted by direct contact, such as foot-and-mouth disease or hog cholera, the movement of animals between farms is an important way of spread. The use network based epidemic models on real-life livestock movement data (available from the national livestock databases) allows achieving more realistic simulations in respect of the classical assumption of random distribution of contacts.

SNA classical centrality measures are based on static networks. Their use for the characterization of the epidemiological risk of holding based on their trade patterns poses some challenges linked to the need of taking into account the temporal dynamics of the livestock trade. We propose a new centrality measure named Disease Flow Centrality (DFC), which reflects more realistically a disease flow processes within a dynamic network and seems able to solve some of these challenges. The new measure is based on a new network traversal algorithm which mirrors more realistically in respect of breadth first search and depth first search traversal algorithms the epidemic process of interest, the temporal dynamics of network relations based on animal trade and the specific role of markets in the livestock industry. The measure has been tested on large datasets represented by all the transport of bovines in Italy in the year 2007, 2008 and 2009.

In addition to the research on the new centrality measure, at a more operational level, we have developed an application which enables real time graphical exploration of livestock movement data in national databases in order to perform more efficient trace back and trace forward exercises in case of outbreaks. The system which has been put in place and tested since 2009 on the Italian Bovine Database gives the possibility of dynamically exploring forward and backwards contacts from a given holding and date of outbreak.

A further risk characterization of holdings can be performed using a network based susceptible-infected-removed (SIR) meta-population epidemic model. This simulates the most likely origin and destination of a disease at different moment of time in the past and in the future in respect of the detected outbreak date and the repartition of animals in the three compartments of susceptible, infected and removed in the infected holdings at the moment of detection.

This approach represents a concrete application of network theory in relation to the EU livestock traceability system. This tool would allow a quicker and more efficient intervention by local veterinary services in case of outbreaks making use of the large amount of data which is becoming more and more available in national livestock databases.

ORAL: RISK MODEL FOR THE DAILY INFECTION PROBABILITY OF CLASSICAL SWINE FEVER VIA EXCRETIONS AND SECRETIONS

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Key words: classical swine fever, transmission, stochastic model, infection probabilities, secretions and excretions

In recent classical swine fever (CSF) epidemics, for many infected herds, the route of virus introduction could not be identified. Several studies were conducted to elucidate the transmission routes resulting in introduction of the virus into these herds. These studies were only partly successful. Distance was indicated as a major risk factor, with the probability of infection decreasing with increasing distance to a primary infected herd. Measures to control CSF epidemics are largely based on this relationship and include preventive depopulation of herds and emergency vaccination. These control measures have, however, far-reaching economic and socio-ethical consequences. More insight into the contribution of different transmission routes to epidemic spread will enable tailor-made control measures during CSF epidemics, instead of rigorous distance-based control measures. The aim of this study was to obtain more insight into the relative importance of transmission routes using a bottom-up approach.

Experimental data on virus excretion by infected pigs, survival of virus in secretions and excretions, and dose-response relationships were used to model CSF virus transmission from an infectious to a susceptible pig. The basic steps of microbial risk assessment were applied, i.e., exposure assessment, hazard characterisation, and risk characterisation. Model results gave (1) the relative contribution of secretions and excretions (faeces, urine, air, blood, saliva, nasal fluid, conjunctival fluid) to spread of the disease, and (2) the daily probability of infection of a susceptible animal via these secretions and excretions. Calculations were performed for the low virulent strain Zoelen, the moderately virulent strain Paderborn, and the highly virulent strain Brescia.

Model results indicated that virus strains differ with respect to the time-dependent infection probabilities and the relative contribution of secretions and excretions to transmission. Based on median values, the probability of infection of a susceptible contact pig via all secretions and excretions, but blood, over the entire infectious period was 1 for Brescia and Paderborn-infected pigs and 0.08 for Zoelen-infected pigs. Maximum daily probabilities of infection were observed from 5-11 d.p.i. for Brescia-infected pigs, 7-8 d.p.i. for Paderborn-infected pigs that recover from the infection, 6-23 d.p.i. for Paderborn chronically infected pigs, and at d.p.i. 7 for Zoelen-infected pigs. For all strains, contact with blood from infected pigs was an important route for infection. For secretions and excretions, larger differences were observed between strains. For Brescia-infected pigs, nasal fluid contributed most to contact transmission probabilities, for Paderborn-infected pigs, faeces (pigs that recovered) and urine (chronically infected pigs) contributed most, whereas for Zoelen-infected pigs, saliva contributed most. Although air was of minor importance in comparison to other secretions and excretions, maximum median daily infection probabilities were 0.9-1 for pigs infected with the Brescia and Paderborn strain.

The difference between virus strains in relative contributions of secretions and excretions to transmission probabilities implies that during epidemics control measures should ideally be based on the characteristics of the specific virus strain involved. This model could be used to provide more insight into the effect of these tailor-made control measures and ultimately allow for quantitative evaluation of such measures.

ORAL: NUSAP METHOD TO EVALUATE ASSUMPTIONS IN A BELGIAN QMRA (METZOON): A LINK BETWEEN RISK ASSESSMENT AND RISK MANAGEMENT

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Key words: NUSAP, QMRA, RISK ASSESSMENT, METZOON

Quantitative microbial risk assessment (QMRA) is used to estimate the probability and severity of health risks following the ingestion of food-borne pathogens. The overall quality of QMRA largely depends on the quality of the model input parameters, the validity of the model but overall the assumptions made. According to the Codex Alimentarius, assumptions having an impact on the risk assessment, should be considered at each step in the risk assessment and documented in a transparent manner. A rigorous evaluation of the quality of data & assumptions is necessary since they are often treated as if they were well-established knowledge. The approach known as the Numeral Unit Spread Assessment Pedigree (NUSAP) notational system was chosen to evaluate assumptions made in the METZOON model, a QMRA farm-to-fork risk model that was developed to assess the risk of human salmonellosis through consumption of minced pork meat in Belgium. The assumptions in the METZOON model were identified, prioritized and critically reviewed during a workshop. The pedigree matrix, to assess the assumptions, contained four pedigree criteria: influence of situational limitations, plausibility, choice space and agreement among peers. An additional fifth criterion was defined to assess the influence of the assumptions on the outcome of the METZOON model. From a list of 39 assumptions identified by reviewing the risk model, 13 assumptions were prioritized. Low scores, given by the experts, in the pedigree matrix refer to a high potential value-ladenness or a high degree of subjectivity. The overall pedigree strength per assumption is obtained by averaging the mean scores for situational limitations, plausibility, choice space and the agreement among peers over the scoring group members. Finally, the assumptions were analysed in a diagnostic diagram. In this diagram, assumptions with low overall pedigree strengths and having a strong estimated influence on the outcome of the QMRA are considered as weak links in the model. The diagnostic diagram identified four assumptions located in the upper right quadrant and judged as problematic. With respect to the pedigree matrix, workshop participants found it was hard to score the criterion "agreement among peers" and argued it was difficult to imagine if peers would have made a different choice in assumptions as compared to the analysts' choices in the assumptions of the METZOON model. However, the NUSAP evaluation pinpoints the strengths and the weaknesses in the METZOON model, and showed that it is a useful tool to enhance the quality in the QMRA process which is essential for decision makers for their risk management. Indeed, a debate on data (gaps) and assumptions will be beneficial for the transparency and acceptance of management decisions based on a QMRA model.

ORAL: POTENTIAL APPLICATION OF GENOMIC APPROACHES TO ASSIST IN FUTURE RISK ASSESSMENTS FOR THE IMPACT OF CLIMATE CHANGE ON THE HOST-PATHOGEN-VECTOR INTERACTION

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Over the last 10 years there has been a huge increase in the amount of genomic information for arthropod vectors, vector-borne pathogens and livestock hosts. This presentation considers how the information available in the field of genome/transcriptome/proteome research may contribute to future risk assessments for the impact of environmental factors, including climate change, on vector-borne diseases of livestock. While genomes of mosquito vectors for human pathogens have been published, the larger size of tick genomes has restricted their sequencing, and little data are available for Culicoides genomes.

Changes in the ranges and abundances of pathogens, vectors and hosts (e.g. driven by climate change, globalization and/or habitat destruction) may create new combinations of host-pathogen-vector interactions. Functional genomic studies for vector-borne pathogens may be used to investigate:-
• the livestock (host) - pathogen interface
• the vector- pathogen interface
• the livestock (host) - vector interface.
It is anticipated here that genomic and related approaches may enable breakdown of the risk pathway into the individual biochemical steps for each of these three interfaces, such that future risk assessments could be based on looking for certain gene combinations in the vector, host and pathogen.

Information on vector competence is critical for risk assessment, but experimental measurement of competence is time-consuming and expensive. Through functional genomic studies, it may in the future become possible to estimate vector competence by screening for the genes and proteins required for its component steps (e.g. receptor binding of virus to vector midgut cells, infection of midgut cells, the arthropod antiviral response, infection of vector salivary glands) such that the efficiency of a new pathogen-vector interaction may be predicted in terms of certain genes in the absence of direct laboratory data. Such studies may also reflect the diversity present in real-world systems to a greater extent than experimental systems involving artificially inbred populations.

Identifying the genomic basis of differences in pathogen characteristics, such as host tissue tropisms or threshold temperatures for replication, may in turn allow potential epidemiological consequences of an outbreak to be identified and assessed. For example, identification of the capacity of BTV-8 for transplacental transmission in 2006, or the capacity of the US WNV strain to replicate at lower temperatures in 1998, would have significantly altered the conclusions of early risk assessments. Genetic variations within natural pathogen populations resulting in significant epidemiological differences do occur and can spread rapidly through mechanisms such as viral reassortment. Technologies allowing the genetic basis of such differences to be identified and tracked in populations could result in a paradigm shift for risk assessment in the future.

ORAL: RISK OF INTRODUCING AFRICAN HORSE SICKNESS INTO THE NETHERLANDS BY IMPORTATION OF EQUIDS

DE VOS, CLAZIEN[1]; HOEK, ALIES[1]; NODELIJK, GONNIE[1]

CENTRAL VETERINARY INSTITUUT OF WAGENINGEN UR[1]

African horse sickness (AHS) is a vector-borne viral disease of equids that is transmitted by Culicoides spp. (C. imicola and C. bolitinos). Mortality in horses can reach 95%. AHS virus (AHSV) is an orbivirus belonging to the family Reoviridae, which also comprises bluetongue virus (BTV). The emergence of BTV serotype 8 in North-western Europe in 2006 has demonstrated that also Palaearctic species of Culicoides can be competent vectors for these orbiviruses. This has resulted in increased awareness of the risk of AHS in the Netherlands. However, not much information and data are available to evaluate this risk. Risk modelling is a useful tool to provide insight into the major variables influencing the risk of disease introduction and can help in optimizing prevention and control strategies. To analyze the probability of release and establishment of AHSV in the Netherlands by importation of equids, an import risk analysis was conducted. Countries worldwide were grouped into three risk categories: (a) high risk, i.e., those countries in which the virus is circulating, (b) low risk, i.e., those countries that have experienced outbreaks of AHS in the past or where C. imicola is present, and (c) very low risk, i.e., all other countries. Imports of equids were grouped according to species: (a) horses, (b) donkeys, mules, and hinnies, and (c) zebras. A scenario tree was constructed to outline all steps required for successful release of AHSV in the Netherlands and subsequent establishment. This scenario tree was the basis for a risk model in Excel and @Risk to quantify the contribution of risk regions and groups of equids to the overall risk of AHSV introduction into the Netherlands. More insight into the relative risk of these risk regions and groups of equids will help decision-makers in prioritizing preventive measures. The model contained information on importations of equids into the Netherlands, occurrence of AHS in the risk regions, disease parameters in host species, vector biology, and import regulations. The probabilities of release and establishment were calculated taking into account seasonal effects.

First model calculations indicated that establishment of AHS in the Netherlands is C, i.e.,°only possible when average daily (24h) temperatures are greater than 10 in the months May up till October. The risk of introduction of AHSV is highest in July. Despite low numbers of equids imported from high risk regions and implementation of preventive measures including quarantine and testing, these regions constitute a higher average risk for AHSV introduction than low and very low risk regions. Zebras contribute most to the risk of AHSV introduction, although numbers imported are very low. The estimated risk per imported animal is, however, highest for zebras due to the virtual absence of clinical symptoms and a longer viraemic period.

Although the model returned an estimate of the probability of AHSV release and establishment in the Netherlands, absolute values are an underestimate of the real risk since only importations of equids as given by import statistics were taken into account. Furthermore, model calculations did not include the risk of introduction of AHSV by other pathways, such as vectors, live animal products and vaccines (modified live). Besides, the model contained many uncertain input parameters. Sensitivity analysis will be performed to indicate those parameters that are major sources of uncertainty in model output and those with the best options for prevention.

ORAL: MULTIVARIATE ANALYSIS TO IDENTIFY POTENTIAL RISK FACTORS FOR THE PRESENCE OF SALMONELLA IN LAYING HENS REARED IN DIFFERENT HOUSING SYSTEMS

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Key words: Salmonella, laying hens, risk factors

In 2007 a study was carried out in 6 countries (Italy, Belgium, Germany, Greece, Switzerland and Turkey) to gather data on the prevalence of Salmonella spp. in laying hens flocks farmed in different housing systems. In Italy 12 flocks reared in battery cages farms, 10 in floor-raised farms and 8 in free range farms were sampled. 40 cloacal swabs, 5 pooled fecal samples and red mites were collected from each flock within 4 weeks before moving the birds to the slaughterhouse. Moreover, a questionnaire was distributed to collect information on general management practises and other specific aspects of the sampled flocks such as veterinary treatments and results of previous Salmonella investigations carried out in the farm.

The presence of Salmonella was detected in 9 flocks and, among the 8 different serotypes detected, S. Enteritidis was the most common one.

In order to identify the risk factors for the presence of Salmonella in laying hens flocks a univariate analysis was carried out taking into account data collected by the questionnaire. Then, potential risk factors identified were examined by a multivariate logistic regression model including as a binary outcome variable the Salmonella status of the sampled flock. The variables considered in the multivariable analysis were: season of sampling, farm size, number of houses in the farm, presence of pets in the farm, type of housing, water supply and Salmonella vaccination status. All variables considered were discrete except for "number of houses" that was continuous.

Independently from the housing system, a unitary increase in the number of houses seems to double the risk of presence of Salmonella ($p < 0.05$) in the farm. This may be explained by the fact that multi-house farms usually rear flocks of different ages and connections between different houses/flocks are hardly avoidable. As a consequence, the potential risk of introduction and spread of Salmonella is not negligible. The second significant risk factor found by the analysis was "vaccination" ($p < 0.05$). Surprisingly the risk of infection was significantly higher in vaccinated flocks compared to unvaccinated ones. This finding suggests that vaccination alone is not enough to reduce the prevalence of Salmonella; other preventive and control strategies beside vaccination, such as application of strict biosecurity measures, must be properly adopted to succeed in the control of Salmonella. Similar findings were obtained during the baseline study previously carried out in our country in accordance with the Commission Decision 2004/665/EC.

Then, the analysis put in evidence that for the variable "type of housing" the risk of Salmonella is lower in floor raised farms compared to battery cage farms ($p < 0.10$). The other variables investigated were not significant.

The results obtained by this study represent useful findings that should be taken into account when considering intervention strategies to reach the target for the reduction of Salmonella as defined by Reg (EC) No 1168/2006.

EPIZ NE

Presentations projects
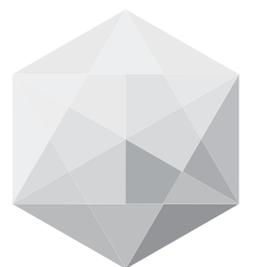
PROJECT: CAPACITY BUILDING FOR THE CONTROL OF AVIAN INFLUENZA THROUGH TECHNOLOGY TRANSFER AND TRAINING (CONFLUTECH)

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RESEARCH CENTER BORSTEL[1]

Key words: avian influenza, swine influenza, training, capacity building

ConFluTech is a Coordination Action funded by the European Union (EU) under FP6 and was launched in July 2007. The project seeks to facilitate technology transfer and training to promote capacity building in INCO (International Cooperation) target countries with particular emphasis on countries that border the EU, for better control of avian influenza and general outbreaks of infectious diseases in livestock.

Avian influenza (AI) or 'bird flu' is a highly contagious viral infection which can affect all species of birds and can manifest itself in different ways depending mainly on the pathogenicity of the virus involved and on the species affected. The highly pathogenic avian influenza (HPAI) virus causes serious disease with high mortality (up to 100%) - notifiable to the OIE.

A number of activities, including technical workshops, courses, training and collaborations were organized at both national and regional levels.

In the context of collaboration, links were established to

•Authorities in charge of Veterinary and Human Public Health, Research Centers and Universities in many countries

•A number of other EU-funded projects dealing with Avian influenza or other diseases

such as Flutrain, Lab-on-Site, Income, ASEM-Dialog and Epizone. In addition, ConFluTech has created links to FAO, Society for Tropical Veterinary Medicine (STVM) European Society for Veterinary Virology, CIRAD and the FGI-ARIAH in the Russian Federation

Through these links it was possible to extend the activities of ConFLuTech to Central and Eastern Europe, the Middle East, Central Asia and North Africa. Thus, seminars and technical workshops for veterinary staff from Lebanon, Palestinian Authority, Mauritania Macedonia, Serbia, Slovakia, Czech Republic, Slovenia, Croatia, Hungary, Lithuania, Estonia and Moldavia were successfully organized in the past.

The seminars and technical workshops held by the ConFluTech covered areas of great relevance for the control of the diseases.

These are:

•Biosecurity at farm and diagnostic laboratory level

•Epidemiology, monitoring and surveillance tools

•Sampling and transport of biological material

•Preparation of awareness booklets in different languages

In the year 2009 a total of seven national and regional workshops were held at national and regional basis in the following countries: Jordan, Iraq, Mauritania, Sweden, Austria, Georgia and Germany.

PROJECT: THE EU PROJECT FLUTRAIN – ACHIEVEMENTS TO DATE

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Key words: Training, technology transfer

The world wide avian influenza (AI) crisis has highlighted the need for comprehensive training and the transfer of technology to accession and INCO (International co-operation) countries with the clear goal of aiding these countries in combating AI with the most up-to-date diagnostic and disease management procedures. The FLUTRAIN project is aiming to fulfill this requirement at two levels. It has firstly approached the need for training by providing two workshops (Italy, January 2008 and Nigeria, November 2009) that called on experts in the field to pass on their valuable expertise in the diagnosis and management of AI to participants from accession and INCO countries. Training opportunities have also been provided in partner labs in order to consolidate the information and practical experience gained during the workshops. To add further to the training aspect of the project, an on-line course on the management and control of AI is being prepared which will be made available to veterinarians and diagnosticians who will work on AI.

The second goal of FLUTRAIN will be the transfer of technology to accession and INCO countries. This will include the provision of new, simplified and cost effective diagnostic methods and reagents. It will also involve the transfer of deliverables, both for serological and virological diagnosis that have been developed in three European projects namely AVIFLU, Lab-on-Site and FLUAID.

The consortium is made up of 11 members which includes 2 SMEs. Eight associated partners have also been identified who have been significant beneficiaries of the training provided by FLUTRAIN to date.

Finally, FLUTRAIN has identified and supplied funds, in a bi-lateral manner, for once-off specific support missions that have targeted specific AI problems in recipient countries.

In April 2009, the global outbreaks of pandemic (H1N1) 2009 has highlighted the unpreparedness of many laboratories to affront this crisis particularly if the virus infects pig populations. In order to face this emergency this the project has been amended (August 2009) so that important tools and information on the health status of pig populations to pandemic H1N1 will be generated and transferred to laboratories in developing countries.

PROJECT: NOVEL TECHNOLOGIES FOR SURVEILLANCE OF EMERGING AND RE-EMERGING INFECTIONS OF WILDLIFE (WILDTECH)

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UNIVERSITY OF NOTTINGHAM[1]

Key words: array technology, diagnosis, epidemiological surveillance, wildlife disease, European project

The WildTech FP7 project (2009-2013) addresses the problem of the increasing prevalence of new and emerging diseases arising from wildlife, which has important implications for disease spread to domestic animals and humans across Europe and globally. It is reported that 61% of known pathogens infect multiple animal species and 75% of all diseases which have emerged in the last few years are of wildlife origin. Therefore, disease surveillance in wildlife has important implications both for communities that rely on healthy domestic animals and also as an essential tool for the protection of human health.

The project consortium led by the University of Nottingham is composed of 12 partners from all over Europe and one in Canada, ensuring optimal coverage for designing surveillance methodology. In addition we have 27 Associate Partners from all over the world and a constantly growing pool of Collaborative Partners, who are contributing with additional samples and datasets and are involved in technology transfer programmes.

The work programme is subdivided into four key areas:

1. Establishment of array-based technologies for multiple screening of wildlife samples for pathogens.
2. Epidemiological analysis of historical and new field data to assess current wildlife disease incidence, prevalence and geographic distribution and determine the potential risk for the emergence of new diseases.
3. The development of a management system for accessing historical, published, and current laboratory and field data on wildlife disease.
4. Establishment of a surveillance network for emerging diseases of wildlife in Europe comprised of specialists in diagnostic technology, bioinformatics and wildlife health, with links to corresponding European and international networks.

The project has recently specified the priority pathogens and the species to be investigated. Research will focus on 21 pathogens in wild boar, deer, hares and urban rodents. The first pathogens to be screened on the nucleic acid and serology arrays are Bluetongue virus, Coxiella burnetti, Mycobacterium bovis, Toxoplasma and Trichinella spp.

The WildTech project represents the application and further development of existing diagnostic and informatic technologies and epidemiological approaches. Integration and successful application of these technologies will, in the long term, facilitate wildlife disease surveillance at the DNA/RNA and serological level leading to rapid and accurate diagnostic methods to monitor disease exposures in wild and domestic animals.

EPIZ●NE

Keynote
Hana Weingartl
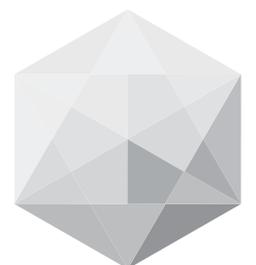
KEYNOTE: SUSCEPTIBILITY OF PIGS TO ZAIRE EBOLAVIRUS AND THEIR POTENTIAL AS AN INTERMEDIATE HOST SPECIES.

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Key words: Pigs, ebolavirus

Ebolaviruses, family Filoviridae, are one of the examples of re-emerging pathogens in humans and nonhuman primates. Swine was not suspected to be a potential host for these viruses, until detection of Reston ebolavirus in pigs in the Philippines. Subsequently, specific antibodies were found in pig farmers indicating exposure to the virus. This important observation raised the possibility that pigs may be an amplifying species and transmit Ebola virus also to humans. The current study investigated susceptibility of pigs to Zaire ebolavirus, a species commonly emerging in central Africa and far more virulent in humans the Reston ebolavirus.

Following mucosal exposure, pigs replicated Zaire ebolavirus to high titers (reaching 107 TCID50/ml), mainly in the respiratory tract and developed severe lung pathology, while transient low viremia was detected at 5 dpi. Shedding from the mucosa was detected for up to14 days post-infection and transmission was confirmed in all naïve pigs cohabiting with inoculated animals. These results confirm the susceptibility of pigs to ebolavirus infection, and identify an unexpected site of virus amplification and shedding linked to transmission of infectious virus.

EPIZ NE

Major epidemic threats
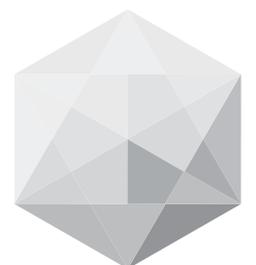
ORAL: RIFT VALLEY FEVER VIRUS SUBUNIT VACCINES CONFER COMPLETE PROTECTION AGAINST A LETHAL VIRUS CHALLENGE

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Key words: Rift Valley fever virus, Virus-like particle, VLP, Subunit vaccine, DIVA

Rift Valley fever virus (RVFV) is a mosquito-borne zoonotic virus that causes recurrent and massive outbreaks affecting humans and ruminants. The virus is endemic in Africa and emerged in Saudi Arabia and Yemen in 2000. Sheep, goat and cattle are the main species affected during a RVFV outbreak. Abortion storms and high newborn fatality rates, which can approach 100%, are typical features of such outbreaks. The virus can be transmitted to humans via direct contact with infected animal tissues and by the bites of infected mosquitoes. Disease in humans is generally mild consisting of fever, myalgia, headache and photophobia. A small percentage of infected individuals, however, develop more severe symptoms like retinitis, retinal lesions, hepatitis or hemorrhagic fever. Although the overall case-fatality rate is estimated at 0.5–1.0%, recent outbreaks show considerably higher numbers. The high case-fatality rates combined with the potential of rapid spread via its vector explains the recognition of RVFV as a potential bioterrorism agent by the United States government. Given the impact of RVF outbreaks on livestock, the human population, and the economy, there is an urgent need for a safe and effective vaccine.

We evaluated two vaccine candidates based on the viral Gn and Gc envelope glycoproteins of RVFV, both produced in a Drosophila insect cell expression system. Virus-like particles (VLPs) were generated by merely expressing the Gn and Gc glycoproteins. In addition, a soluble form of the Gn ectodomain was expressed and affinity-purified from the insect cell culture supernatant. Both vaccine candidates fully protected mice from a lethal challenge with RVFV. Importantly, absence of the nucleocapsid protein in either vaccine candidate facilitates the differentiation between infected and vaccinated animals using a commercial recombinant nucleocapsid protein-based indirect ELISA.

Additional studies must be performed to establish the DIVA property and minimal protective dose of these vaccine candidates in sheep.

ORAL: PECULIARITIES OF WEST NILE VIRUS EPIDEMIOLOGY IN THE WESTERN MEDITERRANEAN REGION: WILD BIRDS, EMERGING GENOTYPES AND VIRULENCE

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Key words: West Nile virus; Mediterranean; wild birds; phylogeny; virulence factors.

West Nile virus (WNV) is the paradigm of an emerging pathogen. In recent years this zoonotic arbovirus has expanded its geographic range dramatically. In parallel, significant changes have been observed in its epidemiology and virulence. In the past WNV was considered a pathogen of less importance, causing a mild disease in humans, during sporadic, small, self-limiting outbreaks resolving spontaneously. This notion is now changing as WNV is causing large, persistent epidemics, particularly in North America since its first occurrence there in 1999. Particularly noteworthy is the increase in pathogenicity for wild birds, the amplifying hosts, which once were considered non-susceptible to the disease, and now suffer high mortalities in some instances. Even in Europe significant changes have occurred recently, leading to increasing outbreak number and persistency, human affection, and virulence for wild birds. The reasons for this emergence are not known, but climate and global change on one hand, and newly arising genotypes of the virus on the other hand, have been claimed as driving factors.

In the last years we have undertaken comprehensive studies in order to better understand WNV epidemiology in the Western Mediterranean region. We focused on the phylogenetic relationships among WNV isolates from this region as well as on the characterization of their pathogenicity in different animal models. We found that all WNV isolates (whose complete nucleotide sequence is available) from this region since 1996 are genetically homogeneous, belonging to a single monophyletic group within lineage 1, which strongly suggest they derive from a single introduction event and further evolution and dispersion in the area. At this respect this situation closely resembles that of the New World, where a unique introduction occurred in 1999 and since then the virus has evolved as a monophyletic group characteristic of the American viruses. By opposite, in other European regions (and also outside Europe), co-circulation of viruses from different introductions, and even different lineages, takes place.

It is uncertain whether the higher incidence of WNV observed worldwide is due to new viral genotypes arising, with higher intrinsic pathogenicity. Among them, NS3-249Pro genotype has been claimed as a virulence marker for some strains. This genotype has arisen independently at least four times during WNV lineage 1 history, the last in the Western Mediterranean. We performed experimental infections in different animal models, including mammal and avian hosts, with a range of WNV isolates. From these studies we concluded that: 1) some Western Mediterranean WNV can be as pathogenic as the strongly pathogenic NY99 strain; 2) different but closely related Western Mediterranean WNV may significantly differ in their pathogenicity; 3) the newly arising NS3-249Pro genotype is not more pathogenic in the Western Mediterranean context; 4) Euro-Mediterranean wild bird species are susceptible to the disease upon inoculation with local viral variants.

In conclusion, the little wild bird affection caused by WNV in the Western Mediterranean demands explanations other than the low pathogenicity of the viruses circulating in this area, or the low susceptibility of local birds. Also, the frequent recurrence of outbreaks caused by phylogenetically homogeneous WNV is consistent with viral persistence in endemic foci in the area, rather than result from independent introductions from exogenous endemic foci such as those existing in Subsaharan Africa, which are highly heterogeneous.

ORAL: SURVEILLANCE OF WEST NILE VIRUS INCURSIONS IN THE NETHERLANDS: VALIDATION OF ANTIBODY DETECTING ELISAS IN HORSES

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Key words: WNV;ELISA;surveillance;sensitivity;specificity

West Nile virus (WNV) is an arthropod-borne flavivirus endemic in parts of Africa, North- and South America, Europe and East-Asia. During the last decades the disease was of minor importance as only a number of incidental outbreaks were reported from Africa and Europe. In 1999, however, this drastically changed after the virus was introduced in North America, and quickly spread through the USA, Canada, Latin America and South America and is since considered endemic in the American continents. This stimulated the discussion on a possible introduction and similar spread of WNV in Europe

In the enzootic cycle of WNV, birds are the virus' reservoir with ornithophilic mosquito species as transmitting vector. The virus has been isolated from over 100 mosquito species in the field. Several mosquito species serve as bridge vector, transmitting the virus from birds to mammals like humans and horses. Mammals are dead end hosts as their virus titres during the viremic phase are too low to infect mosquitoes. However, in both humans and horses neurological symptoms may occur. In US horses more than 25,000 cases of West Nile encephalomyelitis were reported with a high case-fatality rate of 30-40%. Horses can play an important function as sentinels in WNV surveillance.

Therefore, serological monitoring in combination with clinical surveillance of horses using WNV-ELISAs as screening test(s) is considered an important component of the early warning WNV surveillance programme in the Netherlands, and sensitivity and specificity (in the Dutch equine population) of available ELISAs had to be assessed.

Four ELISA kits and three in house ELISAs based on commercial reagents, were evaluated. These ELISAs were based on different test principles: four IgM-capture ELISAs, one indirect ELISA, and two species independent blocking ELISAs. For sensitivity evaluation several serum panels were made available from the USA (sequential sera after either mosquito challenge (n=20) or intrathecal challenge (n=29), sequential sera after WNV vaccination + challenge (n=11), and sera from WNV ELISA positive horses with neurological symptoms (n=86). For specificity evaluation different serum panels from the Netherlands were used (in total n=514, not all sera were investigated in all ELISAs).

In the initial experiments one IgM-capture ELISA and one blocking ELISA showed inferior test characteristics, whereas one commercial IgM-capture ELISA was very expensive, and therefore only its sensitivity was evaluated. Four ELISAs, however, were fully evaluated, and demonstrated good sensitivities and excellent specificities. For sera from horses with neurological symptoms, the sensitivity of the indirect ELISA detecting only IgG tended to be relatively low. Test characteristics with respectively sensitivity and specificity and their 95% confidence intervals were:

Commercial IgM capture ELISA A 0.96 (0.90-0.99); not analysed
In-house IgM capture ELISA 0.96 (0.90-0.99); 1.00 (0.99-1.00)
Commercial IgM-capture ELISA B 0.95 (0.88-0.99); 1.00 (0.99-1.00)
Commercial blocking ELISA 0.98 (0.92-1.00) ; 1.00 (0.98-1.00)
Commercial indirect ELISA 0.87 (0.78-0.93) ; 1.00 (0.97-1.00)

For a clinical surveillance program an in house WNV IgM-capture ELISA (MAC-ELISA) was the test of choice, while for serological monitoring a commercial WNV blocking ELISA was selected. Since these ELISAs detect flavivirus genus specific antibodies, positive results have to be confirmed by a WNV plaque reduction neutralisation test (PRNT) which is operational at the Institute for Public Health and the Environment, RIVM in Bilthoven.

ORAL: GENETIC EVOLUTION OF ITALIAN H1N2 SWINE INFLUENZA VIRUSES DURING 1998-2009: PRESENCE OF NEW REASSORTANT STRAINS

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Key words: Swine influenza viruses, genetic evolution, new reassortants, Italy

Influenza A viruses have a wide host range and have been isolated from avian and mammal species including humans and pigs. In swine three subtypes (H1N1, H1N2, and H3N2) are currently circulating worldwide. Unlike for human influenza, the origin and nature of swine influenza viruses (SIV) change in different continents. The H1N2 was introduced in the swine population in Europe and USA in different periods since the end of the 80s. The H1N2 SIVs currently circulating in Europe originated from those isolated in Great Britain in 1994 that subsequently spread to the swine population of continental Europe (4). These H1N2 viruses originally resulted from multiple reassortment events initially involving human H1N1 and H3N2 viruses, followed by reassortment with avian-like swine viruses (1). In order to better understand the epidemiology of H1N2 SIVs in Italy, the genomic characterization of the Italian H1N2 strains isolated during 1998-2009 was investigated.

In this period 421 SIVs were isolated and 50 were typed as H1N2. Partial sequencing of HA and NA genes (2) were performed on 32 H1N2 isolates. Fourteen H1N2 strains were then selected to get the complete sequences of HA and NA genes (3). Phylogenetic analysis was carried out by comparing sequences of Italian and European SIVs, human and avian influenza viruses retrieved from GenBank. HA gene of Italian H1N2 SIVs showed to be closely related to European H1N2 strains being located in a cluster clearly distinguishable from avian-like H1N1 SIVs forming a lineage branching off from the epidemic H1N1 human viruses circulating in the eighties. Italian strains isolated in 1999-2003 resulted to be closely related to contemporary European H1N2 strains that seem to be the most representative H1N2 SIVs circulating in Europe until now. Italian H1N2 strains isolated in 2003-2009 have established themselves as a distinct clade that branches off from the reference H1N2 strain A/Sw/It/1521/98 isolated in 1998. The deduced aa sequence of HA gene of the strains forming a new cluster showed a deletion of two aa at position 147, 148 within the HA1 region not observed in other European H1N2 strains. Phylogenetic analysis of NA gene demonstrated clustering of Italian H1N2 strains isolated until 2003 with European H1N2 SIVs. Interestingly, the recent Italian strains were located in a cluster clearly distinguishable from other H1N2 SIVs and closely related to the contemporary human H3N2 viruses. The sequence analysis suggests that a reassortment event between the Italian swine H1N2 and human H3N2 viruses may be occurred yielding the recent Italian H1N2 strains. In addition two reassortant H1N2 SIVs with avian-like HA and one with A/Port Chalmers/73 H3N2 like NA were isolated. The continuous virological surveillance showed that almost all H1N2 viruses characterized in Italy (29/32) in the last five years belongs to the new group. These results indicate the persistence of these reassortant strains in the Italian pig population and emphasize the importance of continuous SIV surveillance.

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ORAL: PANDEMIC INFLUENZA (A/H1N1) VIRUS IN PIGS.

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Key words: pandemic influenza virus, experimental infection, pigs

Introduction

The pandemic influenza A/H1N1 virus (H1N1v) is characterized by a unique combination of gene segments from both North American and Eurasian swine lineages. Swine plays an important role in the ecology of influenza A viruses being susceptible to viruses of both avian and mammalian lineages. This study provides preliminary information about pig susceptibility and potential ability to act as intermediate host for H1N1v viruses.

Material and Methods

Pandemic H1N1 Viruses. A/It/148/09 propagated in Caco-2 cells and kindly provided by Dr. Amendola, Faculty of Medicine of Milan.

A/Sw/It/290271/09 isolated in North Italy and propagated in 9-11 day-old SPF chicken embryonated eggs (CEE). GenBank accession numbers CY053616-CY053623.

Experimental infection. Two trials were conducted using SPF swine housed in BSL-3 laboratory facilities and infected intratracheally. Other SPF pigs were introduced for contact infection. The experimental infection designs are:

Trial1: H1N1v 148/09; TCID50:105,6; 2 infected pigs (IP) 30 days of age (DA); nasal swabs (NS: 1IP once 3 day post infection (DPI); tissue samples* (TS): 1IP/3DPI; serum sample (SS): 1IP/18DPI.

Trial2: H1N1v 290271/09; EID50:108; 5IP 150DA; 2 contact pigs (CP) introduced 0DPI; NS 2-4-7-9-11-14 DPI; SS 9-14-21-28 DPI

* trachea and lung

Nasal swabs and tissue samples were tested by influenza type A real time RT-PCR (rRT-PCR) (3). Positive samples of the first trial, nasal swabs 7 DPI in one infected and 9 DPI in one contact pig of the second trial were further inoculated into CEE. Antibody titers were determined by nucleoprotein A competitive ELISA (NPA-EL) and hemagglutination inhibition (HI) test using homologous antigens (1,2).

Results

First trial: animals showed respiratory signs and fever for 3 days. Lung lesions were multifocal areas of purple consolidation. Tissue samples and nasal swabs of a pig sacrificed 3 DPI resulted positive by rRT-PCR. Virus isolation was obtained from all these samples. Serum of other infected pig collected 18 DPI was positive by NPA-EL and HI tests.

Second trial: animals showed only an increase in body temperature for 3 days starting 1 DPI in infected animals and 1 day later in contact pigs. Nasal swabs of all infected pigs were positive by rRT-PCR 2, 4 and 7 DPI, and only two at 9 DPI. Contact pigs resulted positive at 4, 7, 9, 11 DPI. Virus isolation was obtained from both inoculated samples. Serological response was evidenced by NPA-EL and HI starting 9 DPI in infected and 14 DPI in contact pigs.

Discussion

These two trials show that pigs are susceptible to H1N1v and spread virus fast since contact pigs became infected and started to shed virus 4 DPI. Based on these results it could be assumed that H1N1v will spread fast and efficiently if introduced into pig farms increasing risk for establishing of endemic infections. So far, pigs have not been demonstrated to be involved in the epidemiology of the H1N1v.

Furthermore, one H1N1v was isolated from a pig farm in North Italy, the same area where several H5 and H7 avian influenza epidemics were recorded. The simultaneous circulation of swine, avian and human influenza viruses could generate potential changes in the virus characteristics through reassortment with co-circulating viruses and produce a significant impact on human health and global economy.

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ORAL: COMPARATIVE INVESTIGATIONS ON THREE DUCK SPECIES INFECTED WITH CLADE 1 AND CLADE 2.2 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUSES (HPAIV) H5N1

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Key words: HPAIV, duck species, immunohistochemistry, RT-PCR, tissue tropism

Introduction
Since 2001 the pathology of HPAIV H5N1 viruses in ducks changed to systemic infections and morphological lesions in particular in the brain. The main objective of this study was to investigate, whether 3-week-old muscovy ducks, peking ducks and mallards differ in susceptibility for infection with two different clades of HPAIV. The clinical outcome, the viral tissue tropism and viral shedding was analysed.

Material and Methods
We inoculated ten 3-week-old muscovy ducks, peking ducks and mallards oculo-oro-nasally with 10^6 50% egg infectious dose of Influenza A/duck/Vietnam/TG24-01/05 (TG24), Clade 1 and A/cygnus cygnus/Germany/R65/06 (R65), Clade 2.2, both of subtype H5N1. Using immunohistochemistry the distribution of influenza virus nucleoprotein was investigated. At days 4 and 8 post infection (p.i.) in 5 birds per virus-group the following tissues were sampled: beak, trachea, lung, heart, proventriculus, gizzard, duodenum, caecum, pancreas, liver, spleen, bursa, thymus, brain, eye, kidney, adrenal, gonads, skeletal muscle, and skin. Real-time-RT-PCR (RRT-PCR) was applied to quantify HPAIV load in cloacal and tracheal swab samples. The data obtained by RRT-PCR in the lung, the brain and the large intestine were compared to viral titers of calculated TCID50 per ml sample on MDCK cells.

Results
In principle TG24 was more virulent in the tested duck species compared to R65/06. Clinically, animals died peracutely or developed severe neurologic signs. The infection with TG24 led to 100% mortality in muscovy and peking ducks and two out of five mallards died. After R65/06 infection 4 out of 5 muscovy ducks died, but all 5 peking ducks and mallards survived until day 8 p.i.
Whereas the viral tissue tropism was widespread in muscovy ducks, it was more restricted in peking ducks and mallards. Predominantly, the brain, the lung, the heart and the pancreas were affected. Virus titres were higher in tracheal swabs than in cloacal swabs. The maximum viral load and titre was detected within the brain followed by the lung and the large intestine.

Discussion
In accordance with published data the virulence of HPAIV H5N1 viruses varies in ducks depending on the duck species and the virus strain used. Systemic infections occur and detection of the viral antigen in particular in the brain might be causative for the fatal outcome of the disease. Prospectively, our data enable a detailed analysis of host factors (genetic background e.g.) and virus strain factors (clade, sequence analysis e.g.) for pathogenetic investigations.

ORAL: AFRICAN SWINE FEVER: ARE WE READY?

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Key words: ASFV, diagnosis, epidemiology,

African swine fever (ASF) is one of the most complex and lethal disease of swine. The disease transmission involves domestic pigs, wildboars, warthogs and bushpigs. ASFV usually induces unapparent infection in a variety African wildboar population. Soft ticks (Ornithodoros spp) act as reservoir and biological transmission vectors.

ASF is endemic in the majority of sub-Saharan Africa. Within EU, it is confined to Sardinia. On June 2007, ASF was notified in the Caucasus region, in Georgia. It was associated to a new isolate, related to p72 genotype II, circulating in East Africa. Since then, ASF progressed to neighbouring countries Armenia, Azerbaijan and Russian Federation, reaching the near border with Ukraine, and, on October 2009, jumping to St Petersburg in north-western Russia, in the Baltic Sea. Current situation of ASF threatens the EU countries, Eastern Europe, the Black Sea basin countries and - in the worst case scenario – central Asia and even China, which has the largest pig population in the world.

There is no vaccine available and control strategies are based on rapid laboratory diagnosis. But, are we ready to combat with ASF? Would current diagnostic techniques be sensitive enough taking into consideration the new circulating viruses?

From 2000 to date, reliable, specific, and fast PCR tools have been developed and validated for virus detection. They have been shown to be highly sensitive for the detection of the new current circulating isolates in Europe and Africa.

However, serological surveillance studies performed in East Africa among 2004-2009, have shown low seroprevalence with, in contrast, a high incidence of virus in domestic pigs. This could be related to genome variability of antigenic ASF proteins in Eastern African isolates, the more variable and genotypically distant. To what extent current serological diagnostic techniques might be missing some of these new variants?.

To study these questions, different ASFV were selected on the basis of genome variability criteria and date collection. Particular emphasis was placed to those belonging to p72 genotype X, the most variable, and p72 genotype II, current European circulating genotype. After successfully adapted to grow in COS cells, new soluble cytoplasmic antigens were used in indirect ELISA tests for initial standardization. To evaluate the capability and competence of these new tests with regards to the formal ASF serological tests, a comparative study is carrying out using a wide panel of serum comprising: i) experimental sera from "in vivo" experiments with East Africa and Caucasus ASFV circulating isolates ii) field sera from East-West Africa and Sardinia from 2004 to 2009. The preliminary results using the new p72 genotype X-based antigen indicate correlation to those using OIE serological tests and INGEZIM PPA.K3 Compac®.

It's important to highlight the results achieved analysing negative serum samples from "apparently healthy" virus-positive pigs from East Africa. The capability of new others ASFV antigens from Africa and Caucasus to be used in ASF serological tests is under evaluation.

Theses results together with those obtained from in vivo experiments at CISA-INIA with recent isolates from East Africa and Caucasus regions, could be the first approach demonstrating the capability of the formal diagnostic techniques to perform a serological diagnosis with high sensitivity, specificity and confidence, adapted to current epidemiological situations.

ORAL: A STUDY ON AFRICAN SWINE FEVER EXCRETION AND SURVIVAL

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Key words: African Swine Fever, Excretion, Survival, Brazil78

Introduction

African Swine Fever poses a serious risk to European pig production with a mortality rate approaching 100%. Recently due to the outbreaks in Russia and in the Caucasus it re-emerged as a significant disease capable of threatening bordering countries. As a result, there is an impending need to understand and control its spread. Therefore, it is of extreme importance that all the parameters that have an influence on the spread of this disease are quantified so that preventive measures can be efficiently applied. A good understanding of excretion, virus survival and transmission efficiency is crucial to determine the best strategy to adopt for successful containment of this disease.

Materials and Methods

After inoculating 10 conventional pigs with a low virulence isolate (Brazil78) with an intranasal dose of $10^{4.5}$ TCID/50, several different excretion routes for the virus were assessed (including aerosol excretion) through virus isolation and quantitative PCR. Additionally, several lymph nodes and relevant tissues were collected at necropsy for virus isolation and quantitative PCR.

From a selected group of tissues (tonsil, spleen, retropharyngeal lymph node, parotid lymph node and kidney) a degradation experiment was undertaken. The tissues from the deceased animals were left to deteriorate at room temperature and sampled on different time points to understand the rate at which inactivation occurs within these tissues.

Urine and feces from animals presenting clinical signs was collected and exposed to temperatures of 5oC, 12oC, 20oC and 30oC in order to estimate virus survival.

Results

All the animals showed signs of acute disease from day 3: blood in the stools, increased respiratory rate, with pyrexia followed by a sharp decrease in body temperature. Although the Brazil strain was considered to be low virulent, mortality reached 100% on day 9 post-inoculation. The virus was isolated from oropharyngeal swabs, from blood (leucocyte fraction) and from feces from day 2 p.i., with maximum titers of approximately 10^5 TCID/50.

On vaginal swabs and nasal swabs, virus was present from day 4 p.i. No infectious virus was isolated from ocular swabs. Air samples were found positive from day 7 post inoculation. All the collected tissues were positive for virus. In the tissues that were left to deteriorate, infectious virus remained present for at least 21 days in spleen and liver and until day 4 in the remaining tissues.

Depending on temperature and initial virus load, feces samples remained virus positive for up to 9 days, while urine samples remained virus positive for up to 4 days.

Discussion and conclusions

The preliminary results for this study show that the excretion of the virus in different routes starts in a very early stage of the infection, with a very high titer. All relevant secreta and excreta contained infectious virus and may therefore play a role in the transmission of the virus to naïve pigs. Virus inactivation in feces and urine needs further studies to determine the inactivation rate and its natural variation at different temperatures. The detection and quantification of virus in the air will ultimately enable us to quantify transmission through the air over different distances. The stability of the virus in the tissues is also important in the maintenance of the viral cycle in connection to scavenging activities. Additionally, being able to detect virus in deteriorated tissue samples or feces samples that have been in the environment might enable detection and surveillance of ASF in wild boar in the field.

ORAL: OPTIMIZATION OF STIRRED-TANK BIOREACTOR OPERATIONAL PARAMETERS FOR BATCH PRODUCTION PROCESSES OF BHK 21 AN 30 CELLS IN FMD VIRUS PRODUCTION

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Key words: FMD virus, BHK 21 cell, Suspension cell culture, pH, DO

Batch culture modes are few advanced control systems. Bioprocess engineering approaches aim to improve animal cell culture. Limiting conditions can easily appear due to the complex and delicate nature of this system. Nutrient exhaustion, accumulation of metabolites that can be inhibitory or even toxic to the cells, lack of oxygen, low or high pH, shear stres and osmolarity are some of these limiting conditions. Optimization of bioreactor operational parameters for production processes targets the cells themselves to make robust, efficient and productive for FMD virus production.

The purpose of this study is to optimize the stirred tank bioreactor operational parameters for batch production processes of BHK 21 An 30 cells in FMD virus production. Determining the effects of bioreactor operational parameters on the BHK 21 suspension culture was aimed. Stirring speed, disolved oxygen, pH and inoculation rate were focused on as parameters in this study.

To understand and evaluate the interaction of the parameters a mathematical model was developed by an statistical program. Optimal operational values of parameters for the bioreactor maximizing the FMD antigen and the concentration of the cells determined by the statistical model

was evaluated by the validation experiments. The study revealed that operational parameters for the bioreactor were very important for the final cell concentration and the amount of FMD antigen. Optimal operational values of parameters are not the same for maximum final cell concentration and FMD antigen. Optimal operational values are %30±5, 7.00±0.1, 250 rpm and 0.55-0.60x106 cell/ml due to the dissolved oxygen (DO; air saturated), pH, stirring speed, inoculation cell number (Xo) respectively for maximum cell concentration and FMD antigen. Validation experiments showed that the model work properly for the maximal cell yield. Doubling time, and maximum net specific growth rate of the cell culture were calculated as $\zeta d = 11{,}50\pm0{,}85$ h, $\mu max = 0.0622\pm0.0046$ h-1.

ORAL: QUANTITATIVE ANALYSIS OF SPATIAL TRANSMISSION FOR BLUETONGUE VIRUS SEROTYPE 8 IN WESTERN EUROPE IN 2006

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Key words: bluetongue; transmission; spatial kernel; control measures

In 2006, NW-Europe was surprised by an introduction of a bluetongue virus serotype 8 (BTV-8), which appeared to be able to spread effectively between sheep and cattle herds, in the Netherlands, Belgium and Germany. To learn more about the dynamics of this epidemics, we analyzed the available data to quantify the spatial transmission via a spatial kernel. Because control measures were not uniform in the different countries in 2006, we have performed not only an estimation of the kernel for the total outbreak, but also for the different countries separately. It appeared that the transmission of BTV-8 in 2006 took place over larger distance compared with the Foot and Mouth Disease 2001 outbreak and the Avian Influenza 2003 outbreak in the Netherlands. Furthermore, the spatial kernel for Belgium showed quite different features between the period before and after the whole of Belgium was declared a risk zone by the Belgian government a few weeks after the start of the epidemic. It could be concluded that the more restrictive the spatial control measures are in the infected area the more transmission is kept at a local level. Therefore, transport restrictions seem to be capable of slowing the spread of the epidemic.

ORAL: EPIDEMIOLOGY OF HIGHLY PATHOGENIC PRRSV IN SOUTHERN CAMBODIA: PRELIMINARY FINDINGS OF A FIELD STUDY

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Key words: PRRS, Pig, Highly Pathogenic, Cambodia, Epidemiology

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most widespread endemic swine diseases in the world. After more than 20 years since the emergence of the disease, the understanding of PRRS is still not complete. Epidemiology, pathogenesis, management techniques have been studied and refined, but PRRS infection keeps coming back, posing a continuous and gripping challenge for researchers, producers and practitioners.

During the last three years, reports from China and Vietnam have alerted the world to a new and highly pathogenic variant of PRRS virus. The disease produced by this virus is characterized by high morbidity and significant mortality that has devastated the pig industries of the affected countries, and it has been classified by OIE as an extraordinary epidemiological event. Today new outbreaks have been detected in new areas of Vietnam and Philippines, confirming that the virus is quickly spreading throughout out South East Asia.

The long-term project objective is to look for the presence of new variant of PRRSV in the pig population of six Cambodian provinces to shed light on their relationship to other viruses in the region. The project also aims to identify risk pathways associated with introduction and to identify possible intervention strategies that take the epidemiological situation and pathways of introduction and maintenance into account.

The first field study within the project collected data on the Cambodian animal health system and the pig production system. Analysis of essential information gathered through meetings and a workshop with the Cambodian government, EU, USDA and private partners in Phnom Penh, the Cambodian capital, will be presented. Additional information from slaughterhouses, villages and farms in six provinces will be used to identify the role and importance of slaughter procedures, animal management, pig movements to the epidemiology of the disease in the region.

The findings of this analysis will be presented at the meeting.

ORAL: BUNGOWANNAH VIRUS – A NOVEL PESTIVIRUS AND RECENTLY EMERGED PATHOGEN. COULD IT BE A MAJOR EPIDEMIC THREAT?

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Key words: Pestivirus, myocarditis, stillbirths, preweaning death.

In June 2003 a syndrome of sudden death in sucker pigs, followed by a marked increase in stillborn foetuses and preweaning losses occurred on a large farm in NSW, Australia1. Cumulative losses in some weeks exceeded 50%, with total losses probably exceeding 50,000 pigs. Pathological changes consisted of a multifocal, non-suppurative myocarditis, consistent with a viral infection. The disease was described as the porcine mycocarditis syndrome (PMC)(1). Elevated IgG levels in body fluids of 50% of still born piglets indicated an intrauterine infection had occurred. Intensive studies involving the in utero inoculation of foetuses at different gestational ages confirmed that an infectious agent was present in the tissues of affected piglets(2). Following the use of sequence independent single primer amplification (SISPA) on nucleic extracts from inoculated foetuses, a novel pestivirus, now known as Bungowannah virus, was identified(3).

After the identification of viral sequence by SISPA, this pestivirus was isolated from the tissues of affected pigs and from the material used in the experimental transmissions. Viral replication was detected initially by RT-PCR and, later, a real time RT-PCR (qRT-PCR) was developed and viral loads quantified. Viral antigens were also detected in cell culture by immunoperoxidase (IPX) staining using convalescent pig serum. With the availability of an IPX staining procedure, a peroxidase linked assay (PLA) was developed to allow the detection of specific antibodies to this virus. Using a combination of the qRT-PCR and the PLA, a series of field and laboratory studies were completed. These data provided strong evidence that Bungowannah virus was most likely the aetiological agent in the PMC syndrome(4).

Analysis of the full length viral genome confirmed the classification of this novel pestivirus but have shown a low degree of identity with the currently recognised pestivirus species (BVDV-1, BVDV-2, BDV and CSFV) and with other pestiviruses ('pestivirus of giraffe', HoBi and the pronghorn antelope virus). The virus is also antigenically divergent. No IPX staining was observed with the pan-reactive monoclonal antibodies and there is little cross-neutralisation with high titred antisera to strains of BVDV, BDV, CSFV, Hobi and pronghorn.

Transmission studies in sows at different stages of gestation have confirmed that this virus is the cause of the PMC syndrome, resulting in high levels of loss and the birth of persistently infected piglets after infection in early gestation. Many aspects of the biology of this virus are similar to low virulence CSFV infection. Studies to examine the potential for transmission of this virus to sheep and cattle have also been initiated. Epidemiological studies have suggested that Bungowannah virus was probably introduced to Australia.

This presentation will review the clinical manifestations and impact of this virus, its potential for interspecies transmission, challenges in the diagnosis of Bungowannah virus infection and the potential threat that it may pose to countries in Europe.

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3. Kirkland, P.D. et al. (2007). Virus Res. 129: 26-34
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Presentations projects
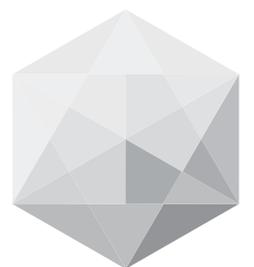
PROJECT: INTERNATIONAL NETWORK FOR CAPACITY BUILDING FOR THE CONTROL OF EMERGING VIRAL VECTOR BORNE ZOONOTIC DISEASES (ARBO-ZOONET)

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RESEARCH CENTER BORSTEL[1]; INSTITUT PASTEUR[2]; MARMARA UNIVERSITY MEDICAL SCHOOL HOSPITAL[3]; UNIVERSITY OF LIVERPOOL[4]; NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES[5]

Key words: Arbovirus, Rift Valley Fever, Crimean-Congo-Haemorrhagic Fever, West Nile Fever

The European Union Commission provided funds for a concerted action which aims at creating common knowledge on these diseases, sharing and exchanging data, expertise, experiences and scientific information regarding West Nile fever (WN), Crimean-Congo hemorrhagic fever (CCHF) and Rift Valley fever (RVF) are arthropod borne diseases of domestic and wild animals that can affect humans. The surveillance systems will be maintained and expanded, monitoring disease occurrence and vaccine use.

In the second year of the project the following activities interalia were organized:
1. Rift Valley Fever Diagnostic Workshop in South Africa, 2009
The workshop agenda included lectures on the epidemiology, clinical and pathological features and diagnosis (ELISA, RT_LAMP, Real-time PCR) of RVFV with a special focus on early detection and using a problem solving approach.
2. Regional Training Workshop on High Pathogenic Avian Influenza (HPAI) and Rift Valley Fever (RVF) in the Maghreb, 2009
A number of diagnostic tools (ELISA and PCR) were demonstrated and their value for field was discussed. In addition interactive field and laboratory activities were initiated, specifically for animal health professionals from different academic backgrounds and origins.
3. Intervention measures against WNV, RVFV and CCHFV: Where are we? in Turkey 2009
The symposium covered three areas: 1) Vaccines: new and existing ones, 2) Antiviral therapy and 3) Vector control. In addition, a satellite meeting was organized on epidemiological and diagnostic tools.
4. Risks of WNV, RVFV and CCHFV in Europe in France 2009
The workshop consisted of presentation on the epidemiology of WNF in Europe, risk factors and prediction of RVF and CCHF, ecological database for European birds and use of GIS for risk assessment.
5. Introduction to the Epidemiology Toolbox, Berlin, Germany 2009
The technical workshop covered the following areas: Background/Introduction to Epidemiology, epidemiological study design, analysing epidemiologic data, sampling strategies/sample sizes, serodiagnostic tests, risk analysis and quantification of animal health and disease

PROJECT: MED VET NET, NETWORK OF EXCELLENCE ON FOOD BORNE ZOONOSES

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Key words: Network of Excellence, food safety

Med-Vet-Net, a European Network of Excellence, has been primarily operated by and for scientists who belonged to 14 independent public health and veterinary institutes in 10 European countries. Over the past five years Med-Vet-Net developed a series of fully-integrated and interactive groups of scientists, including laboratory-based researchers, epidemiologists and risk assessors, all working towards the common objective of combating diseases in humans and animals throughout the European Union (EU). The Network's main activities have been targeted at food-borne zoonotic diseases, although other non-food-related zoonoses, such as bat lyssaviruses, have been investigated through Special Interest Groups (SIGs) operating outside the scientific workpackages but with seed-corn funding provided by Med-Vet-Net. The overall aims of Med-Vet-Net were to improve the understanding, prevention and control of zoonotic diseases in Europe through strategic and integrated high-quality collaborative research across the food chain; raise awareness among policy makers of zoonotic diseases, the general public and other stakeholders; and enhance the skills and knowledge base of European zoonotic disease researchers. A further objective was to take forward the concept of a 'virtual institute', thereby promoting the integration of veterinary and medical scientifi c activities within Europe in the field of food safety. The Communications Unit of Med-Vet-Net continued to produce regular newsletters with information about Network activities, profiles of key personnel and workpackages, and accounts of meetings held throughout the Network. The efforts to ensure the Network's long-term sustainability have borne fruit, with the formation of the self-funded Med-Vet-Net Association, officially launched in Brussels on 6 October 2009. The Med-Vet-Net Association aims to increase, capitalize and disseminate scientific knowledge on zoonoses with a main emphasis on food-borne zoonoses. The Association's statutes and internal regulation were registered on 1 June 2009 under the French Non-lucrative Association (1901) framework. It is noteworthy that all current scientific institutes have elected to join the Association as full members for an initial period of three years, on a self-funded basis. Durable integration has also been addressed at the scientific level with Med-Vet-Net pursuing opportunities for funding within FP7 and other areas, with the primary objective of maintaining and expanding multi-disciplinary research teams by incorporating external research groups and institutes into new proposals. In that respect, at least 10 collaborative projects involving Med-Vet-Net workpackages and scientists have been developed or started in Year 5, all of which will continue when Med-Vet-Net has formally concluded. It is important for the Association and the future of zoonoses research in Europe that activities do not stagnate. There are new challenges and new opportunities ahead. New collaborations will be forged, hopefully with the inclusion of new institutes and research groups, and with industry. Nevertheless, Med-Vet-Net is proud of its achievements over the past five years and, in particular, of laying the foundations for collaborative inter-disciplinary research in food-borne zoonosis in Europe.

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Keynote
Ian Brown
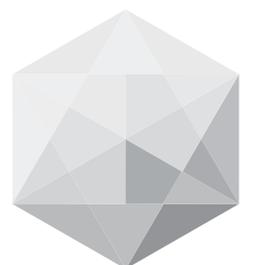
KEYNOTE: THREATS TO ANIMAL HEALTH FROM INFLUENZA, AN EU PERSPECTIVE.

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Key words: animal health, influenza, EU

During the last ten years there have been multiple threats to animal health in the EU from influenza. Primarily this has involved outbreaks in domestic poultry with highly pathogenic avian influenza (HPAI) of various subtypes. The cost to the industry and the community can be estimated in billions of Euros. Spread of low pathogenic avian influenza from wild bird reservoirs to domestic poultry with resulting mutation to virulence can have catastrophic consequences as was reported in Italy 1999-2000 (H7N1) and the Netherlands 2003 (H7N7). In addition, global spread of H5N1 HPAI has presented an additional threat since the virus appears to be maintained at least temporarily in wild bird populations and is influenced by complex dynamic interactions between domestic and wild birds. Furthermore, there have been frequent introductions of low pathogenicity notifiable avian influenza viruses to domestic poultry that have resulted in the imposition of control measures in accordance with the EU Directive 2005/94/EC. Other influenza viruses present significant disease threat to other hosts such as the recent pandemic (H1N1) 2009 virus whereby spread to animal populations provides additional challenges to the veterinary community and industry alike. The dynamic and long term persistence and evolution of this virus in domestic livestock, principally involving pigs remains to be defined, with potential consequences for veterinary public health. The presentation will include a synopsis of recent events, and look to preparedness and implications for EU Community animal health in the future.

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Keynote
Chris Olsen
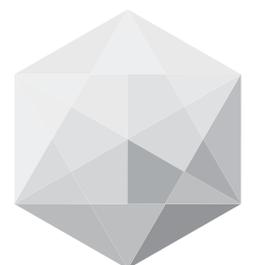
KEYNOTE: SWINE INFLUENZA IN NORTH AMERICA: VIRUS EVOLUTION, CONTROL AND PUBLIC HEALTH IMPLICATIONS.

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Key words: Influenza, virus, public health

Pigs have suffered the effects of influenza virus infection since at least the 1930s. For six decades, the primary cause of influenza in North American pigs was viruses of the classical swine influenza H1N1 lineage. However, in the late 1990s, a wide variety of novel reassortant viruses of multiple subtypes emerged within the North American swine population, with differing implications for both swine and human health. Some reassortants were recovered only from limited numbers of pigs (H3N1, H2N3), whereas others spread throughout the swine population (H3N2, H1N2 and H1N1) and included zoonotic infections of human beings. Finally, triple reassortant viruses from North America were one of the lineages of viruses from which the 2009-2010 pandemic H1N1 virus was derived. This presentation will review this course of virus evolution, as well as discuss methods and challenges to swine influenza surveillance and control among pigs, and past and on-going implications of swine influenza for human public health.

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Poster presentations
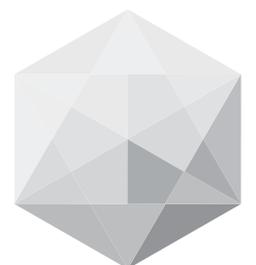
POSTER: MOTIVES OF LIVESTOCK FARMERS AND HOBBY HOLDERS TO VACCINATE AGAINST BLUETONGUE SEROTYPE 8 DURING A VOLUNTARY VACCINATION CAMPAIGN IN 2008 AND 2009 IN THE NETHERLANDS

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Key words: bluetongue, vaccination, motives, incentives, questionnaire

A major epidemic of Bluetongue virus serotype 8 (BTV-8) was diagnosed in the European Union (EU) in 2006 and established and spread within EU in 2007 and 2008. A vaccination campaign started in the Spring of 2008. In the Netherlands this was done on a voluntary basis, with financial support from the EU. At the start of the new voluntary vaccination campaign in 2009 (without financial support), we investigated the motives of livestock farmers and hobby holders to vaccinate using a postal questionnaire survey. In the framework of a voluntary vaccination campaign, it is important to know the motives why livestock farmers and hobby holders do - or do not - want to vaccinate their animals against BTV-8. When you know these motives, you can anticipate by using a communication campaign and/or using (economic) incentives in order to increase a possible low vaccine uptake. The questionnaire was sent to a random sample of 1,660 sheep farmers, 1,925 dairy farmers and to all 320 Dutch dairy goat farmers. Since there was not a sampling frame of hobby holders available, we developed an electronic survey, which was posted on 3 websites of hobby holder societies. Response rates were: 585 (35%) sheep farmers, 717 (37%) dairy cattle farmers, 42 (13%) goat farmers and 431 hobby holders. Mean vaccine uptake in 2008 was: 73% sheep farms, 71% dairy farms, 43% goat farms and 67% hobby holdings. Top-5 motives pro-vaccination were: prevention of production loss; subsidized vaccination; recommendation by practitioner; welfare reasons; contribute to the eradication campaign. Top-5 motives against vaccination were: high vaccination costs; no clinical BT-problems; presumed low infection risk; balance between vaccination costs and loss without vaccination; bad experience with earlier vaccination campaigns. Willingness to vaccinate was significantly lower in 2009 compared to 2008: 42% sheep farms, 58% dairy farms, 19% goat farms and 49% hobby holdings. Measures to stimulate vaccination among those that didn't want to vaccinate in 2009 were: subsidized vaccination; possibility to vaccinate their own animals; more information on efficacy/ safety of vaccine and why animals have to be vaccinated again; availability of a BT-vaccine combined with vaccine(s) against other diseases. About 30-50% of livestock farmers and hobby holders indicated to be willing to vaccinate against other BT serotypes in the future after introduction in the country. Subsidized vaccination is the most important requirement to do so. It is recommended to vaccinate newborns that will be used as replacement and breeding stock in order to prevent virus circulation, fertility problems and welfare problems. When the decrease in willingness to vaccinate will be structural in the coming years, more and more older animals with a protective immunity will leave the farm and the susceptible population will increase again, increasing the risk of infection and recirculation of BTV-8.

POSTER: A NOVEL BI-FUNCTIONAL DNA VACCINE EXPRESSING VP1 PROTEIN AND PRODUCING

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Key words: Bi-functional vaccine, DNA vaccine, Antisense RNA, Foot-and-mouth disease virus

To overcome the inability of a conventional vaccine against a virus to induce rapid protection against viral challenge, a novel strategy was performed to generate a novel bi-functional vector expressing antisense RNA targeted to 5'untranslated regions (UTR) and VP1 protein of foot-and-mouth disease virus (FMDV). FMDV 5'UTR containing the viral RNA replication start elementswas inserted inversely into the pIRES vector to produce antisense RNA, followed by insertion of FMDV VP1 gene to generate a recombinant plasmid pAS-IR-VP1. BHK-21 cells transfected with pAS-IR-VP1 plasmid showed a specific resistance against FMDV infection. In mice vaccinated with this plasmid, T cell proliferation was significantly higher than that in an unvaccinated control group. Anti-FMDV antibodies were detected up to 1:64 in the serum collected from mice boosted with pAS-IR-VP1 at 21 days after the first immunization. At 6 h post-vaccination 50–83% of the suckling mice survived a challenge with FMDV. The results demonstrated that a novel bi-functional DNA vaccine, producing antisense RNA targeted to FMDV 5'UTR and expressing VP1 protein, has been
successfully constructed and was able to induce a rapid inhibitory effect and immune response against FMDV infection in mice.

POSTER: REVERSE TRANSCRIPTION-LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR RAPID DETECTION OF HEPATITIS E VIRUS.

XI, LAN [1]

LANZHOU VETERINARY RESEARCH INSTITUTE[1]

The one-step single-tube betaine-free reverse transcription (RT)-loop-mediated isothermal amplification assay was developed for rapid diagnosis of hepatitis E virus. This assay amplified the target gene in less than 45 min (even as short as 20 min) under isothermal conditions at 63 degrees C, and the sensitivity of this assay was 100-fold greater than that of RT-PCR. This assay demonstrated a detection limit of 0.045 fg (nine copies/reaction).

POSTER: PREPARATION OF THE POLISH MAREK'S DISEASE FIELD STRAINS FOR THE CONSTRUCTION OF DELETIVE BAC CLONES.

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Key words: Marek's disease, bacterial artificial chromosome (BAC), deletive clones

Introduction. Marek's disease (MD) is a tumorous disease of poultry caused by herpesvirus called Marek's disease virus (MDV). Construction of the genetic recombinants and mutants of MDV in bacterial artificial chromosome (BAC) creates a possibility for development of new vaccine against MD. This technique facilitates construction of MDV clones lacking main oncogenes like meq, LAT or vTR. The aim of this study was the preparation of MDV strains for construction of MDV deletive BAC clones.

Materials and methods

Viruses. Two vvMDV (12/04 and 2/08) and three vv+MDV (31/07, 56/08, 73/08) strains isolated from liver and spleen of infected chickens were used then the homogenisates in PBS with 1% antibiotics mixture were prepared.

Chicken embryo fibroblasts (CEF). Primary CEF cultures were prepared from 9-11 day-old SPF chicken embryos according to standard procedure. Secondary CEFs were prepared from 48-hour primary CEFs. The growth medium was MEM with 10% foetal serum and 1% mixture of antibiotics (Gibco) while the maintaning medium was MEM with antibiotics. The cell cultures were incubated in 37.5ºC/5% CO_2.

Virus isolation. Isolation of the viruses was conducted in CEFs according to standard procedure. Three successive passages of each strain were conducted.

Immunofluoroescence assay (IFA). After 48-72 hours when CPE was observed, infected cells were fixed with cold 90% acetone. Primary monoclonal IgG antibodies anti-pp38 protein were used. After triple PBS wash the fixed cells were incubated with secondary anti-mouse IgG labelled with Alexa 488 fluor and read in fluorescent microscope (Olympus). As the positive result the presence of green florescent cells was observed.

DNA extraction. Third passage of the virus was used for DNA extraction by standard phenol-chlorophorm method and ethanol precipitation.

PCR. Reaction was done in 25 μl and contained DNA Taq polymerase (Finzyme), ICP4 or UL49.5 specific primers and other reagents according to manufacturer's procedure. The sequences of primers were: ICP4F: 5'GCAGCAAGGAGGAATGATAA3', ICP4R: 5'CATCTGAGGCATTTCACACA3',UL49.5F: 5'GGACTCATGGACATTCATAATGCAG3', UL49.5R 5'TTACCACTCCTCTTTAAACATATCT3'.

Restriction analysis. Digestion of total DNA was done by BamHI enzyme (New England Biolabs). Reaction was carried out in 30 μl in 37ºC for 2 hours then the DNA fragments were separated overnight (1.7V/1 cm2) in 0.5% agarose gel with ethidium bromide.

Transfection. Transfection of secondary CEFs with DNA of all strains was carried out according to a modified procedure of Morgan et al (1990). Transfected cells were incubated for 56-72 h until the presence of CPE.

Results and conclusions

After 56-72 hours of the 3rd passage of MDV strains an occurrence of CPE was observed. Until the 5th day p.i. the effect became well defined. The specific green fluorescence for pp38 protein was found in the case of all examined strains. After phenol-chlorophorm extraction viral DNA kept good integrity. After restriction digestion of the strains total DNA, the presence of restriction patterns specific to MDV was observed. By means of PCR for ICP4 gene the specific bands about 211 bp long were found in the case all examined strains. Similarly PCR for UL49.5 was positive for all strains. For testing the infectivity of total MDV DNA for BAC cloning all samples were used for transfection of secondary CEFs. All reconstituted strains have shown CPE identical with the native strains. The prepared MDV strains will be used for generation of deletive clones for construction of vaccine against MD.

POSTER: HOST RESPONSE TO FOOT- AND MOUTH DISEASE INFECTION IN CATTLE; POSSIBLE IMPLICATIONS FOR THE DEVELOPMENT OF "CARRIERS".

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Key words: FMDV, carrier, host-response

FMD is a viral disease with severe implications for agricultural trade in affected countries. Any cloven hoofed animal species may become infected, and ruminants, especially cattle and buffalo, may develop into "carriers" persistently shedding low amounts of virus for several years after exposure to the disease. The FMDV infection is defined as persistent when live virus can be detected for more than 28 days post infection.

FMD infection in ruminants involves initial viral replication in pharyngeal epithelia, from where the virus spreads systemically. Characteristic vesicular lesions develop in the cornified stratified squamous epithelia of the coronary bands and oral cavity within a few days of infection. Viremia occurs within 2-3 days of infection, but is rapidly cleared through the effect of circulating antibodies generated by the adaptive immune response. The host response involves initial activation of the innate immune response, with activation and recruitment of effector-cells, and subsequent activation of T- and B-cells, leading to the production of circulating antibodies, as well as activation of cytotoxic T-cells.
Previous experiments have indicated that the site of persistent replication of FMDV is located in pharyngeal lymphoid tissue, as well as the basal epithelia of the dorsal soft palate.

A series of animal experiments, with the aim of investigating the host immune response, and sites of viral replication at different time points during both acute and persistent phases of FMDV infection in cattle has been performed.

During these experiments, bull calves of 4-5 months of age were infected with FMDV O UKG 34/2001, and disease development was monitored for 32 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 qPCR of mouth swabs and oropharyngeal fluid (probang samples).
Tissue samples derived from endoscopical collection of biopsies of the dorsal soft palate from live animals at different times post infection, as well as samples of lymphoid tissue derived from staged post mortems were analysed for the presence of viral proteins through indirect immunoflourescence. These samples have also been analysed for the presence of specific populations of immune cells such as CD8+ T-cells and Dendritic cells. Biopsy samples are collected at different time points during acute and persistent infection in order to monitor the progress of viral replication, as well as the local cellular immune response, at specific sites over time.

In order to measure the systemic response to infection, serum concentrations of acute phase proteins Serum Amyloid A (SAA) and Haptoglobulin, as well as biologically active type 1 interferon (IFN 1) are being quantified. These markers of host immune response are also being used in order to detect any possible differences in host response throughout the infection in animals that become persistently infected compared to those that clear the infection effectively.

POSTER: COMPARISON OF THE SENSITIVITY OF NRT-PCR METHOD FOR DETECTION OF WEST NILE VIRUS GENETIC LINEAGE 1 AND 2

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Key words: West Nile virus, NRT-PCR, detection

Introduction. West Nile virus had caused mortality and morbidity of wild birds, mammals, especially horses and caused numerous neurological disorders. Isolates of the virus in the world are divided into two different major genetic lineages. Lineage 1 is found in North America, North Africa, Europe and Australia. Strains which belong to lineage 2 are endemic in Sub-Saharian Africa and Madagascar. The aim of the study was to determine occurrence of West Nile virus in Poland using NRT-PCR method (Nested Reverse Transcription PCR) and determination of the sensitivity of NRT-PCR method and also specificity with two genetic lineages: lineage 1 and lineage 2 of WNV strains.
Materials and methods
Field samples. 908 samples derived from wild birds were sent from all areas of Poland in 2009. 565 samples (62,22%) were sent by hunters, 16 (1,76%) were collected in the forest. 8 (0,88%) derived from ornithological stations. Wild Birds Rehabilitation Center provided 158 (17,48%) and Wild Birds Station provided 161 (17,7%) birds. The samples from the wild birds brain were collected.
Samples positive. 305-08 – homogenated sample of central nervous system of infected horse with WNV lineage 2; 328-08 – central nervous system sample from a horse naturally infected with WNV lineage 2; 4467.01 – brain sample from experimentally infected gull with a (102pfu) of lineage 1 strain IS-98-ST1; 882 - brain sample from experimentally infected horse with a viral doses in range (101pfu-105pfu). All extracts and RNA were obtained from UMR 1161 Virologie INRA AFSSA ENVA.
Primers for RT-PCR method. To develop primers we selected a conservative region of sequence 3'NCR (non-coding region of the genome of WNV Gene Bank Accesion Number: DQ211652). Primers for RT-PCR were WNV3 5'-GCC ACC GGA AGT TGA GTA GA-3', WNV4: 5'-CTG GTT GTG CAG AGC AGA AG-3'.
Primers for NRT PCR method. Primers for Nested-PCR were designed on the basis of RT-PCR amplicon sequence. The sequences were: NES5: 5' AAA GCC CAA TGT CAG ACC AC 3'', NES6: 5'TAGTCCTTTCGC CCT GGT TA 3'.
Virus RNA extraction. Genetic material of WNV extracted from the infected bird and horses brain homogenates with RNasy Mini Kit (Qiagen, Germany) following procedure.
Positive control. As a positive control in study we used control from WNV Kit (Prodesse, US) containing the 3'NCR region. The results of amplification with RT-PCR and Nested PCR were tested on the 2% agarose gel. Samples were visualized with an UV transilluminator and photographed.
NRT-PCR. Reaction RT-PCR was performed in 50 µl, Nested-PCR was performed in 25µl. Specific primers WNV3, WNV4, NES5, NES6, and other reagents were used according to standard procedure.
Results and conclusions
We tested sensitivity by performing amplification reaction with a 882 brain sample from experimentally infected horse as template containing a series of virus doses in range 105pfu-101pfu strain IS-98-ST1 of lineage 1. We were able to detect a genetic material in samples with viral doses 102pfu-105pfu.
Specificity of reaction for lineage 1 and 2 was determined with use of samples infected with lineage 1 and 2 viruses received from AFFSA LERPAZ. We were able to obtain positive results for both lineages.
After NRT-PCR reaction with 908 samples we didn't found any positive case of WNV infection neither genetic lineage 1 nor genetic lineage 2 in any of examined probes.

POSTER: VALIDATION ANALYSIS OF RISK FACTORS CONDITIONING THE PERSISTENCE AND THE DIFFUSION OF AFRICAN SWINE FEVER INFECTION IN SARDINIA

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Key words: African Swine Fever, risk factors, validation

Introduction. The African Swine Fever was introduced to Sardinia in 1978 and since then, despite the application of specific eradication plans, this infection has been considered endemic. After a long period characterized with low and stable values of incidence, there were two epidemic peaks in 2004 and 2005, thus causing many of the control strategies that had been applied to be questioned. For this reason a study was implemented to validate risk factors that are considered facilitators for the length and spreading of the infection from the ASF virus.
Methods. A retrospective type of study, based on the "case-control" model, was set up.
An equipe of experts arranged a scoring list of risk factors associated with the spread of the ASF infection, which was also used as the questionnaire used to collect data. Two large portions of territory from the Sardinia Region were considered, coinciding with the area covered by the Oristano ASL4 (Local Health Authority) and with the ASL1 area covering the Province of Sassari. The first area was evaluated as a "case" because of the very high occurrence of infection outbreaks during the 2005 epidemic; the second one was chosen instead as a "control" because it was only marginally involved in the recent epidemics. A randomized sample of pig farms was selected applying the ratio of 2 controls for each case.
Results. 61 pig farms were sampled in the Oristano ASL area and 112 in the Sassari ASL area. Based on the simple addition of the score attributed to the two territories, the result was a higher risk level for spreading ASF within the Sassari territory (83.6) compared to the Oristano territory (58.8). Actually, when breaking down the analytical data into narrower classification (type of farming, farmer practices, veterinary services and susceptible population), only the characteristics that are related to the type of farm seem to increase the Oristano ASL area risk level compared to the one in the Sassari ASL area.
Conclusions. The undertaken study has provided a clear picture of ASF infection concerns in Sardinia from a new point of view that had not been previously explored in depth. The information that was collected seems to authorize a general consideration that the parasite's epidemiologic role is more important compared to the host's: the viral pressure to which an area is subjected, expressed in terms of the number of primary outbreaks that generate an epidemic emergency, is confirmed to be the main risk factor in the spread of this infection.

POSTER: DEVELOPMENT OF A REAL-TIME-RT-PCR SUITABLE FOR DIAGNOSING VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS (VHSV)

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Key words: Viral Haemorrhagic Septicaemia Virus, Rhabdovirus, real-time-RT-PCR, PCR optimization

Viral Haemorrhagic Septicaemia Virus (VHSV) is an important fish virus that has caused several large scale fish kills in both fresh and saltwater fish. The virus is an enveloped negative-stranded RNA virus belonging to the family Rhabdoviridae and the genus Novirhabdovirus. Its genome consists of around 11000 bases and codes for six proteins. Several isolates of VHSV have been sequenced and four major genotypes of the virus are recognized (Genotypes I-IV) as well as several subgroups. Traditionally VHSV has been detected by cell culturization followed by ELISA. This method is sensitive and specific, but unfortunately quite time consuming. Diagnosing using PCR give an answer much faster. So far no real-time-RT-PCR has been published that recognize all 4 genotypes without giving false positives. Here we present a taqman based real-time-RT-PCR that recognizes all VHSV isolates in a panel of 79 VHSV isolates covering all known genotypes and subtypes and not 15 isolates of other fish pathogens. Through the development of the PCR we also learned some valuable lessons on real-time-PCR design which we would like to share as well.

POSTER: FISHPATHOGENS.EU: A DATABASE BASED ON FREEWARE SUITABLE FOR STORING ISOLATE AND SEQUENCE DATA OF PATHOGENS.

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Key words: Database, Viral Haemorrhagic Septicaemia Virus (VHSV), Infectious Haemorrhagic Necrosis Virus (IHNV), Spring Viraemia of Carp Virus (SVCV), Infectious Salmon Anemia Virus (ISAV)

We live in a world where the amount of information available is enormous. In order to keep track of the available knowledge, databases are needed to collect, store, and sort it. Www.fishpathogens.eu is a database developed and maintained by the European Community Reference Laboratory for Fish Diseases. The database was launched in June 2009 focusing on Viral Haemorrhagic Septicaemia Virus (VHSV)[1] and currently the database is being extended to include other fish pathogens e.g. Infectious Haemorrhagic Necrosis Virus (IHNV)[2], Spring Viraemia of Carp Virus (SVCV), and Infectious Salmon Anemia Virus (ISAV). The database design is based on freeware and could easily be implemented to include pathogens relevant for other species than fish. We will present a guided tour of the database using the data on the different fish pathogens as example. However if some are interested in the platform we are happy to cooperate and share the database structure with other Epizone members.

1: "FishPathogens.eu/vhsv: A user-friendly Viral Haemorrhagic Septicaemia Virus (VHSV) isolate and sequence database", Søren Peter Jonstrup, Tanya Gray, Søren Kahns, Helle Frank Skall, Mike Snow and Niels Jørgen Olesen, Journal of Fish Diseases, 2009

2: "An isolate and sequence database of infectious haematopoietic necrosis virus (IHNV)" Søren Peter Jonstrup, Heike Schuetze, Gael Kurath, Tanya Gray, Britt Bang Jensen, and Niels Jørgen Olesen, Journal of Fish Diseases, In press

POSTER: LOW-PATHOGENIC NOTIFIABLE AVIAN INFLUENZA SEROSURVEILLANCE AND THE RISK OF INFECTION IN POULTRY – A CRITICAL REVIEW OF THE EUROPEAN UNION ACTIVE SURVEILLANCE PROGRAMME (2005 – 2007)

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Key words: Avain Influenza, European Union, LPAI, Risk factors, Surveillance

Since 2003, Member States (MS) of the European Union (EU) have implemented serosurveillance programmes for Low Pathogenic Notifiable Avian Influenza (LPNAI) in poultry. To date, there is the need to evaluate the surveillance activity in order to optimise the programme's surveillance design. The objective of this study was to evaluate MS sampling operations (sample size and targeted Poultry Types (PTs)) and its relation with the probability of detection and to estimate the PTs relative risk (RR) of being infected. Reported data of the surveillance carried out from 2005 to 2007 were analysed using: a) descriptive indicators to characterise: MS sampling operations and its relation with the probability of detection and the LPNAI epidemiological situation; b) multivariable methods to estimate each PT's RR of being infected. It was observed that MS sampling a higher sample size than that recommended by the EU had a significantly higher probability of detection. PTs with ducks&geese, game-birds, ratites and "others" had a significant higher RR of being seropositive than chicken categories. The seroprevalence in duck&geese and game-bird holdings appears to be higher than 5%, which is the EU-recommended design prevalence, while in chicken and turkey categories the seroprevalence was considerably lower than 5% and with that there is the risk of missing LPNAI seropositive holdings. To conclude, it is recommended that the European Commission discusses with its MS whether the results of our evaluation calls for refinement of the surveillance characteristics such as sampling frequency, the between-holding design prevalence and MS sampling operation strategies.

POSTER: REUSING PUBLISHED DATA TO QUANTIFY FOOT-AND-MOUTH DISEASE TRANSMISSION PARAMETER B

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Key words: FMD; transmission; vaccination; meta-analysis

Foot-and-mouth is the disease most feared by livestock holders and veterinarians and since the 2001 outbreaks in Europe the need to more readily quantify viral transmission dynamics, prophylactic intervention and transmission parameters has been recognised as fundamental for decision makers and modellers. Often, compilation of such information traditionally relies on commissioning new experiments and reliance of disease affected cloven-hoofed animals. We report on a project designed to re-examine and re-utilise data from past FMD experiments which allow the effectiveness of intervention measures such as vaccination to be more accurately assessed.

An inventory of data from past experiments has been gathered and is presently undergoing analyses using state-of-the-art Generalised Linear Modelling to estimate transmission rates with and without vaccination.

Preliminary results indicate that meta-analysis of published data from 'old' experiments using newly developed techniques can provide useful data and help to replace, reduce and refine future foot-and-mouth disease transmission experiments, and as a consequence minimise animal suffering for research purposes.

POSTER: TRANSMISSION CHARACTERISTICS OF LOW PATHOGENIC AVIAN INFLUENZA VIRUS OF H7N7 AND H5N2 SUBTYPES IN CHICKEN LAYERS

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Key words: Avain Influenza, LPAI, Transmission, Transmission experiments

The purpose of this study was to investigate the transmission characteristics of H7N7 (chicken isolate) and H5N2 (wild bird isolate) low pathogenic avian influenza virus (LPAIv) in chicken layers. To this end, two transmission experiments were performed, each consisting of 30 pairs (divided in two groups) of conventional adult chicken layers: one chicken was inoculated with the experimental virus and the other remained as susceptible contact. Transmission was monitored by means of swab (cloaca and trachea) and blood samples. Egg production, virus shedding patterns and transmission parameters, such as latent period, length of infectious period (IP), the transmission parameter β, and the reproduction number R0, were estimated for H7N7 and H5N2 LPAIv. A drop in egg production was observed in infected layers. Transmission of these viruses was not associated with the amount of virus shed – measured by PCR equivalent titers – by an infectious chicken or the length of the IP. The R0 estimate for the H7N7 strain was above 1, which shows the ability of this virus to generate, though with a low probability, major outbreaks in chicken flocks. For the H5N2 strain R0 was below 1, which indicates that approximately all introductions of this virus would result in minor outbreaks. The implication of these findings for development of control measures such as surveillance will be discussed.

POSTER: SWINE INFLUENZA VIRUSES ISOLATED IN 1983, 2002 AND 2009 IN SWEDEN EXEMPLIFY DIFFERENT LINEAGES

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Key words: Influenza, Swine

Abstract
Swine influenza virus isolates originating from outbreaks in Sweden from 1983, 2002 and 2009 were subjected to nucleotide sequencing and phylogenetic analysis. The aim of the studies was to have an overview on their potential relatedness as well as to provide data for broader scale studies on swine influenza epidemiology. Nonetheless, analyzing archive isolates is justified by the efforts directed to the comprehension of the appearance of swine-origin influenza A (H1N1) virus. The findings happened to illustrate the evolution of swine influenza viruses in Europe, because the earliest isolate belonged to ´classical´ swine H1N1, the subsequent ones to Eurasian ´avian-like´ swine H1N1 and reassortant ˙avianlike˙ swine H1N2 lineages, respectively. The latter two showed close genetic relatedness regarding their PB2, HA, NP, and NS genes, suggesting common ancestry. The study substantiates the importance of molecular surveillance for swine influenza viruses.

POSTER: BOVINE TUBERCULOSIS IN LOWER SAXONY, GERMANY: MAKING A CASE FOR EFFECTIVE SURVEILLANCE

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Key words: Epidemiology, bovine tuberculosis, zoonosis

Bovine Tuberculosis (bTB) is a zoonosis caused by Mycobacterium bovis and M. caprae which can cause serious disease in cattle, sheep and humans. According to the EFSA Journal 2009, bTB prevalence in 2007 in Europe was highest in the United Kingdom and Ireland. Germany was declared officially bTB-free (Decision 97/76/EC) in 1997; bTB surveillance has since relied on official veterinary meat inspection as the primary surveillance method.

In 2008, a cow with remarkable gross lesions suspicious for bovine tuberculosis (bTB) was identified by meat inspection at home slaughtering in north-western Germany. Comparative tuberculin testing in the herd of origin of all animals older than six weeks (173 animals) revealed 101 (58%) reactors. Subsequent epidemiological investigations led to the identification of another 11 affected farms which had probably acquired bTB from the putative index farm; nine of them through animal trade and two through direct animal contact.

While the source of the initial introduction remained unknown, it was shown that all isolates tested shared the same molecular characteristics as determined by spoligotyping and analysing Variable Number of Tandem Repeats (VNTR), suggesting a common source of infection. The findings demonstrate that bTB can easily be transmitted via animal trade and may remain undetected for years in herds in the absence of tuberculin testing. Hence, we believe that bTB surveillance should not rely only on meat inspection, but on a combination of both meat inspection and intra vitam testing via tuberculin skin test.

POSTER: GENETIC MODIFICATION OF BLUETONGUE VIRUS; UPTAKE OF "SYNTHETIC" GENOME SEGMENTS

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Key words: Bluetongue, genetic modification, reassortant

Bluetongue virus (BTV), family Reoviridae, genus Orbivirus, contains ten double stranded RNA segments S1-S10. 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. The outbreak by BTV-8/net06 (IAH collection nr. BTV-8 NET2006/04, Maan et al., 2008) starting in 2006 have resulted in the largest BT-outbreak ever recorded. More recently, BTV-6/net08 (IAH collection nr. BTV-6 NET2008/05) was reported in The Netherlands and Germany. BTV6 is closely related to vaccine virus serotype 6, but harbors another segment S10. Remarkably, virus isolation on Culicoides cells also showed BTV6 with S7 of BTV8/net06 (Maan et al., 2010). BTV-research has already a long scientific record (reviewed by Roy 2005), including research on reassortments. Research desperately needs a method to modify genes to study all functions in more detail. Recently, reverse genetics for BTV has been published (see also van Gennip et al.). This genetic modification system needs cDNAs of all 10 genome segments to rescue (mutant) BTV.

We have developed a genetic modification of BTV based on the uptake of in vitro synthesized viral RNA. Genome segments S7 and S10 were reversely transcribed, PCR-amplified according to Potgieter et al. (2009), and sequenced with 454 technology (Roche GS FLX technology). cDNAs of both segments were synthesized, and cloned under control of the T7 RNA-polymerase promoter and an unique restriction site at the 3'-terminus. Capped positive stranded run-off RNAs were synthesized in vitro after digestion by the respective restriction enzyme. Monolayers of BSR-cells were infected by BTV6/net08, and subsequently transfected with purified in vitro synthesized RNA of S7 or S10. "Synthetic" reassortants were rescued by plaque purification and/or endpoint dilutions, and identified by serotype-specific PCR assays for serotypes 6 and 8, and serogroup-specific PCRs for S7 and S10 followed by sequencing. "Synthetic" reassortants of BTV6/net08 with S7 or S10 originating from BTV8/net06 were rescued. Natural reassortant BTV6/net08 with S7 from BTV8/net06 has also been found (Maan et al. 2010). However, BTV6/net08 with S10 from BTV8/net06 is a new reassortant not previously reported.

These results show the potency of this method to generate targeted single gene reassortants of BTV. Furthermore, with in vitro RNA synthesized from mutated cDNAs, it will be possible to rescue mutant BTVs in order to study viral functions in more detail. Moreover, subsequent experiments as here described will result in multiple-gene reassortants with more segments of BTV8/net06; e.g. S2 and S6 to change the serotype of the virus. One of the major drawbacks of this method is, however, the low rescue efficiency. The here described method can be improved by use of selective negative pressure to reduce the growth of BTV6/net08; e.g. in the presence of specific BTV6 neutralizing antibodies/serum, or by use of selective siRNAs (Stassen et al. 2007). Using these methods, molecular virological studies on all genome segments of interest become possible.

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POSTER: DEVELOPMENT OF REVERSE GENETICS FOR BLUETONGUE VIRUS SEROTYPE 8

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Key words: Bluetongue, reverse genetics, genetic modification, reassortant

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. 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). Molecular virology studies desperately needs methods to genetically modify BTV to study all the features of in more detail. Recently, a genetic modification system for bluetongue virus based on uptake of synthetic genome segments was developed (van Gennip et al., 2010). This method is very useful in research, but we expect that rescue of less viable BTV mutants will be hard. Recently, reverse genetics is published for BTV1 (Boyce et al., 2008). However, such a reverse genetic system is not available for virulent BTV8/net06 and other related orbiviruses.

In order to develop reverse genetics for BTV8/net06, extensive sequence studies have been performed on virus directly from blood and from cell culture. Therefore, 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 all available data (Maan et al., 2008) to determine the consensus sequences of all genome segments. cDNAs of all ten complete genome segments were synthesized based on the consensus sequences. These were cloned under control of the T7 RNA-polymerase promoter and an unique restriction site at the 3'-terminus. Capped positive stranded run-off RNAs were synthesized in vitro after digestion by the respective restriction enzyme. Monolayers of BSR cells 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. RNAs of segments encoding the "late" proteins were omitted. After 18 hours, transfected BSR monolayers were transfected again with a mixture of all ten RNAs.

Rescued "synthetic BTV" was studied and identified by several methods, like serotype-specific PCR assays, and serogroup-specific PCRs for S7 and S10 followed by sequencing of the amplicon. The method of rescuing "synthetic BTV" was conclusively proven by rescue of several unique single- and multi-gene reassortants with genome segments of attenuated BTV1. Surprisingly, most single-gene reassortants between BTV8 and BTV1 are viable. Based on isolated natural reassortants, it was generally believed that not all segments can be solely exchanged between viruses. For instance VP2 and VP5, representing the outer shell of the virion, were supposed to go along in reassortment events.

Reverse genetics will be used for genetic modification studies on BTV. Furthermore, the presented reverse genetics for BTV is very promising, and show the feasibility for the development of genetic modification systems for related orbiviruses, like African horse sickness virus and Enzootic hemorrhagic disease virus.

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POSTER: ISOLATION AND GENOMIC CHARACTERIZATION OF PANDEMIC H1N1 VIRUS FROM A PIG FARM IN ITALY

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Key words: pandemic H1N1 virus, genomic characterization, swine, Italy

Influenza viruses cause annual epidemics and occasional pandemics spreading worldwide and infecting a large proportion of the human population. In April 2009, a novel H1N1 influenza A virus (H1N1v) was identified as the cause of the present flu pandemic (1).

In November 2009, a 1264 swine breeder farm located in the province of Milan (Lombardia region, Northern Italy) experienced lower sow reproduction performances in farrowing units. Clinical signs in sows were fever, depression, anorexia, agalactia and in piglets diarrhoea and weight loss. The morbidity in sows was 30% and the accumulated mortality rate was <10%. Nine dead piglets and four nasal swabs from sows were submitted for diagnosis. Of these, 8 piglets (1-8) showed catarrhal enteritis whereas one (9) presented pneumonia with a purple area of consolidation in the apical lobes. Two pools of lung homogenates of 4 piglets each one and piglet 9 were tested for influenza A viruses and H1N1v by real time RT-PCRs (2,3). The first pool (animals 1-4) and piglet 9 resulted positive to the type A influenza as well as for H1N1v assay. Virus isolation was obtained by inoculation on SPF chicken embryonated eggs (A/Sw/It/290271/09, A/Sw/It/308288/09).

Complete genome sequencing was performed on A/Sw/It/290271/09 by a RT-PCR of 46 amplicons using a genome Primer Set (Applied Biosystems, Foster City, CA, USA). Maximun parsimony phylogenetic trees were created using MEGA4 upon multiple sequence alignments by ClustalW.

Genomic analysis confirmed a very high similarity to the genome of H1N1v circulating in humans. The comparison with H1N1v sequences available in GenBank revealed the presence in A/Sw/It/290271/09 of 3 unique amino-acid (aa) changes in PB2 (S405T), PB1 (K386R) and PA (K256Q), not yet associated to any well characterized phenotype markers of Influenza viruses. The M2 protein displayed C55F and the PA protein S409N substitutions, both corresponding to enhanced transmission phenotype markers already found in pandemic H1N1v sequences (4).

HA phylogenetic tree showed that A/Sw/It/290271/09 was placed in the H1N1v cluster clearly distinguishable from the Eurasian lineage of the Italian avian and swine H1N1 viruses. NA phylogenetic analysis revealed that SW/IT/290271/09 and H1N1v were related to "avian –like" sw/H1N1 viruses detected in Spain and Italy in the last decade.

Until November 2009, an enhanced monitoring program for swine influenza conducted in Italy, led to the isolation of 53 Swine influenza viruses belonging to the three subtypes currently circulating in Europe. Based on these results, it could be assumed that H1N1v was not circulating in the Italian pig population before this case. In Italy more than 70% of the swine industry and over 65% of the poultry farms are located in the Northern part of the country. Furthermore, in the last ten years, several epidemics of H5 and H7 avian influenza occurred in the same area involving either domestic or wild birds (5). The simultaneous circulation of human, swine and avian influenza viruses in this area increases the risk of reassortment events and could be considered of great concern for their implications in human health. Surveillance programmes should be implemented to undertake control measures for limiting the spread of H1N1v in farms and the transmission from animals to humans.

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POSTER: IDENTIFICATION OF POTENTIAL CRITICAL CONTROL POINTS IN SOUTHERN SOMALIA THROUGH THE INTEGRATED USE OF SPATIAL ANALYSIS AND SOCIAL NETWORK ANALYSIS (2008).

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Key words: Somalia, Spatial Analysis, Social Network Analysis, Critical Control Points

Introduction: in Somalia, despite the adverse environmental conditions, the livestock sector represents the backbone of the society contributing towards 60% of the total incomes. The pastoral system proved to be the most adequate strategy to sustain the livestock production that, despite its extensive nature, is an intensively market-oriented sector. In this scenario, the inefficiency of the public veterinary service and therefore of surveillance and control strategies is causing the Somali livestock industry to become extremely susceptible to restrictive measures imposed by importing countries that, in line with OIE regulations, are increasingly applying and demanding strict sanitary regulations and standards.

This project represents therefore an attempt to explore the integrated application of Spatial Analysis (SA) and Social Network Analysis (SNA) to contribute to the identification of potential critical control points aiming at supporting the establishment of cost-effective surveillance and control systems.

Methodology: the analysis is based on field-data collected in Somalia by the NGO "Terra Nuova" during the implementation of two E.C. funded projects. Specifically, data on Rinderpest (RP) prevalence, livestock population density, spatial features of randomly sampled locations, herds' movement patterns (home-ranges) and spatial data on major livestock trade-routes were available. A descriptive SA was undertaken (ArcMap™) to individuate areas with high interaction between nomadic and trade herds. These data coupled with information on the mobility of nomadic herds and RP prevalence allowed the selection of a specific region where subsequently a contact network was created (Ucinet™, NetDraw™) to assess the dynamics of aggregation of nomadic herds. Nodes of the network were considered all sampling sites visited during the RP sera-survey and a link between each pair of nodes was established when the herds' home-ranges were overlapping.

Existence of components, centrality measures (degree and betweenness) and network parameters were considered and calculated to identify more central and influential nodes within the network.

Results: the Lower Juba region was selected to build the contact network. In the identified region 13.8 % (9/65) of the locations were in contact with 2 trade-routes and 18.5% (12/65) of the locations were in contact with 3 trade-routes. The resulting 1-mode unvalued symmetric contact network, with 65 nodes and 958 links, proved to be highly cohesive and characterized by high average "Freeman degree" (14.73, range: 0 - 31) and "flow betweenness" (70.01, range 0-308.08) per node, a short average distance between reachable pairs (2.33), a small diameter (5), a high density (0.23) and a high clustering coefficient (0.76). Four (4) nodes were characterized by the higher degree and three (3) nodes by the higher beetweenness and as such are more influential. The analysis of components has identified one (1) major component constituted by 64 nodes.

Conclusions: the analysis highlighted the strength of the network parameters and centrality measures as added values in identifying those nodes potentially more influential within the network in the spread of infectious diseases. These added attributes might provide useful information for the identification of potential critical control points where to establish targeted animal health surveillance systems and control interventions. However the nodes considered to build the network represented a subset of all possible locations in Lower Juba. This limit is acknowledged.

POSTER: PORCINE TORQUE TENO VIRUS: DETERMINATION OF VIRAL GENOMIC LOADS BY GENOGROUP-SPECIFIC MULTIPLEX RT-PCR, DETECTION OF FREQUENT MULTIPLE INFECTIONS WITH GENOGROUPS 1 OR 2, AND ESTABLISHMENT OF VIRAL FULL-LENGTH SEQUENCES

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Key words: Torque teno virus (TTV); pig; real-time PCR; multiple infection; full-length sequence

Torque teno virus (TTV) is a non-enveloped virus with a circular, single-stranded DNA genome. TTV is currently classified in the unassigned genus Anellovirus, and distinct TTVs of tentative species-status infect a wide range of vertebrates. In domestic pigs and wild boars, porcine TTV occurs in two genogroups, TTV1 and TTV2, which are currently detected using only conventional PCR assays. To allow high-throughput testing, the present study describes development of a multiplex real-time (rt)-PCR assay for efficient simultaneous detection of TTV1 and TTV2. To demonstrate usefulness of this rt-PCR assay for large-scale testing, 203 serum samples from domestic pigs were screened for TTV infection. The detected rates of single TTV1, single TTV2, and double TTV1/TTV2 infections were 32%, 17%, and 32% and represent the first report on the occurrence of porcine TTV in Germany. In addition, 100 wild boar lung samples were tested that confirmed high prevalences of TTV infection. Moreover, establishment of genogroup-specific rt-PCR standards allowed the determination of mean viral genomic loads in sera from TTV-infected swine to about 104.5/ml, respectively. To verify the specificity of the rt-PCR assay, conventional PCR assays that amplify genogroup-specific, size-distinguishable products from the TTV untranslated regions were designed. In total, 50 clones derived from 24 PCR products obtained from 19 TTV1 and TTV2 single- or double-infected animals were sequenced. Phylogenetic analyses of these sequences demonstrated the frequent occurrence of multiple infections with distinct porcine TTVs of the same genogroup. Moreover, two porcine TTV full-length sequences were established, one for each genogroup.

POSTER: APPLICATION OF TOOLS FOR HIGH-RESOLUTION MOLECULAR EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE VIRUS IN WESTERN EURASIA

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Key words: Full genome sequencing, FMDV, A-Iran-05, O-PanAsia-2, Asia-1

1. Introduction and Objectives: Full-genome sequencing methods for FMDV provide molecular epidemiology data at greater resolution data than those generated by "conventional" methods such as VP1 analysis. This opens up the potential for using genome sequencing to reveal and identify the origin of un-clarified transmission events within epidemics. The aim of this study was to develop specific sequencing protocols for the predominant FMDV lineages (type A-Iran-05, type O-PanAsia-2 and Asia-1) currently circulating in western EurAsia.

2. Materials and Methods: PCR primers used for amplification and sequencing were designed for each of the 3 lineages of FMDV on basis of an alignment of full genome sequences (obtained from GenBank). RNA samples extracted from cell cultured viruses and clinical material causing recent outbreaks in the Middle East were used to develop and test the different sequencing protocols.

3. Results: The strategy adopted for the RT-PCR protocols generated a single S-fragment (~380 nt) and 20 overlapping fragments (of 500-700 nt) for the L-Fragment. Based on sequence identity, it was possible to use a universal set of primers for serotype O, A and Asia-1 viruses (11 RT-PCR assays in total) for the amplification of the 2A-3'UTR fragment (encoding non-structural proteins). In contrast, 10 lineage-specific primer pairs were required to amplify the 5'-end of genome containing the capsid genes. To date, 22 full genome sequences (13 serotype O, 5 serotype A and 4 serotype Asia-1) have been generated using these methods.

4. Discussion and Conclusion: The full genome sequencing protocols developed during this study can be used to investigate the evolutionary rates and mechanisms by which FMDV evolves within the Middle East region. In the future, further viruses will be sequenced and these data will be used to improve our understanding of the processes by which FMDV is maintained year-on-year in the Middle East and to assess the degree of undisclosed FMD infection that is present in Turkey and neighbouring countries.

POSTER: EFFICACY OF BTVPUR ALSAP® 1-8, AN INACTIVATED BIVALENT BTV-1 / BTV-8 VACCINE, AGAINST A BTV-1 OR A BTV-8 VIRULENT CHALLENGE IN SHEEP

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Key words: BTV-1, BTV-8, sheep, vaccine, efficacy

INTRODUCTION
Bluetongue is an infectious, OIE listed arboviral disease, which naturally infects domestic and wild ruminants. There are 24 to 25 different Bluetongue virus (BTV) serotypes, with little cross-reactivity. Since 2006, BTV-8 has spread throughout Europe, causing severe disease and heavy financial losses. Almost simultaneously, BTV-1, another devastating BTV serotype appeared in Southern Europe and progressed rapidly North. To date, several regions of Spain, Portugal and France are simultaneously affected with BTV-1 and BTV-8.
Vaccination campaigns have been demonstrated effective at controlling the disease and even in some case at eradicating BTV, provided that the vaccine was able to completely prevent viraemia and when vaccination coverage was high.
As there is little cross-protection between BTV serotypes, the availability of efficacious multivalent BTV vaccines, covering all serotypes circulating in one region, represents a significant improvement of convenience, as compared to monovalent vaccines, by reducing the number vaccination/handling of animals.
High convenience is a key element to achieve high vaccination coverage and thus increase the likelihood of eradicating BTV.
Here, we describe the clinical and virological evaluation of a commercial inactivated vaccine containing purified Bluetongue virus (BTV) serotypes 1 and 8. Vaccination / challenge experiments were conducted to determine the level of homologous protection induced by the vaccine in sheep.

MATERIAL AND METHODS
Twenty-two 4-months old, sero-negative, sheep were randomly allocated to 4 groups of 5 to 6 sheep. Two groups of 5 sheep were subcutaneously vaccinated twice (D0 + D21), with 1 mL of BTVPUR ALSAP® 1-8 (Merial S.A.S.). The two other groups served as unvaccinated controls. On day 42, one of the vaccinated groups and one of the control groups were challenged with a virulent BTV-8 while on D44, the two other groups were challenged with a virulent BTV-1. All sheep were then monitored 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, whatever the challenge, developed fever, clinical signs typical of BTV infection and were all viraemic at high titre, at all time points after the challenges.
Conversely, all vaccinates were protected from hyperthermia and clinical sign and none of them was ever viraemic.
Rectal temperatures at peak of hyperthermia (Max hyperthermia) and global clinical scores were highly significantly (p<0.01) reduced in the vaccinated groups as compared to their respective controls.

Complete prevention of viraemia was demonstrated in the two vaccinated groups through the absence of viral RNA the bloods of the vaccinated animals, while all control animals were detected positive. The differences between vaccinated and control groups were statistically highly significant (p<0.01).

CONCLUSION
In the present study, vaccination with the product tested provided complete and significant clinical and

virological protection against BTV-1 or BTV-8 challenges.
These results show that BTVPUR ALSAP® 1-8 may be used for bluetongue disease prevention (clinical protection) and for epidemiological control of BTV (virological protection).

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POSTER: THE HETEROCYCLIC COMPOUND BBP: A NOVEL IN VITRO INHIBITOR OF CSFV TARGETING THE VIRAL POLYMERASE

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Key words: antiviral, classical swine fever, pestivirus, polymerase inhibitor

Selective inhibitors of the replication of the classical swine fever virus (CSFV) may have the potential to control the spread of the infection in an epidemic situation. We here report a novel class of heterocyclic molecules that inhibits the in vitro replication of CSFV. BPP, a lead compound of this class displays a 50% effective concentration of 0.33 ± 0.25 µM and shows a dose dependent inhibition. Drug-resistant variants were selected in vitro and were found to carry either a I261N or a P262A mutation at the fingertip of viral RNA-dependent RNA polymerase (RdRp). BBP has reduced activity against variants that carry a T259S mutation responsible for resistance to the imidazopyridine 5-[(4-bromophenyl)methyl]-2-phenyl-5H-imidazo[4,5-c]pyridine (BPIP). Amino acids I261 and P262 are located in the immediate vincity of T259. It can thus be assumed that BBP, although structurally unrelated to BPIP may interact with NS5B at a site very close or identical to the binding-site of BPIP. Besides the imidazopyridines at least two unrelated classes of pestivirus inhibitors were earlier reported to select for drug-resistance mutations at the top of the fingerdomain of the BDVD RdRp. Our current observations provide further support for the hypothesis that the top of finger domain of the pestivirus RdRp is a hot-spot for inhibition of viral replication.

POSTER: PATHOGENICITY OF EPIZOOTIC HAEMORRHAGIC DISEASE VIRUS (EHDV) IN BRITISH CATTLE

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INSTITUTE FOR ANIMAL HEALTH[1]

Key words: Pathogenicity, EHDV, real time RT-PCR, ELISA

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 (IAH dsRNA virus reference collection MOR2006/14 EHDV-6 and ISR2006/07 EHDV-7) 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 and gel-based EHDV RT-PCR assays. Validation data assessing the sensitivity of these assays will be presented. In addition the sensitivity of available antibody detection EHDV ELISA tests will be assessed and the serological response of cattle to EHDV-6 and EHDV-7 will be measured and compared.

POSTER: DISTRIBUTION OF PATHOLOGICAL FORM OF PRION PROTEIN IN BRAINSTEM SAMPLES OF CLASSICAL AND ATYPICAL BSE

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Key words: bovine spongiform encephalopathy, BSE, PrP distribution, atypical BSE

When testing animals for TSEs with rapid tests, brainstem samples are the optimal tissues for analysis, due to the highest amount of proteolysis resistant form of prion protein in obex region. In this study we evaluated the distribution of PrPres in brainstem samples of cattle affected with classical and atypical BSE. Western blot was used to evaluate the relative amount of PrPres and its distribution cranially and caudally from obex region in brainstem samples collected for active surveillance. The signal strength from obex part of brainstem from a given case was used as the reference (100%) for other regions. Surprisingly in both classical and atypical BSE samples stronger signal in comparison to obex was observed in cranial parts of brainstem. While in classical and H-type BSE cases significant drop in PrPres amount was noticed in cerebellum in comparison to obex, L-type BSE was characterized by similar signal strength in both regions. Uniform distribution of PrPres especially in the section of brainstem located cranially to obex shows that when autolysed sample is tested for BSE, both classical and atypical BSE can be detected even when obex region is hard to identify or is missing.

POSTER: APPLICATION OF RT-PCR FOR DETECTION OF BOVINE RESPIRATORY SYNCYTIAL VIRUS (BRSV) INFECTIONS.

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Key words: BRSV, isolate, RT-PCR diagnostic , phylogenetic analysis

Bovine respiratory syncytial virus (BRSV) belongs to the Pneumovirinae subfamily of Paramyxoviridae family. It is one of the most important causative agents of respiratory tract disease in cattle and is responsible for high economical losses in cattle industry around the world.
Recent serological tests proved existence of antibodies directed against BRSV in high percentage of bulls from the Polish breeding herds. However, in Poland, BRSV haven't been isolated yet and the phylogenetic classification of Polish BRSV strains remained unknown.
This paper describes first characterization of BRSV virus isolated from nasal swabs from young heifers with clinical signs of lower respiratory tract illness, collected in some cattle herds from Northern Poland in winter 2008-2009 and
spring 2009.
RT-PCR products of the fragment of the BRSV gene encoding glycoprotein G were sequenced and subsequently compared with the reference nucleotide sequences of 19 BRSV isolates from all of the world and to human and ovine representatives of respiratory syncytial viruses belonging to the same Pneumovirus genus as BRSV. This comparison suggested close relationship of Polish strains to the strains isolated in Czech Republic in 2002-2003 and Danish strains from 80's and 90's.

POSTER: AN OVERVIEW OF THE EPIDEMIOLOGICAL SITUATION OF AVIAN INFLUENZA IN ITALY IN 2009

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Key words: Low pathogenic Avian Influenza, surveillance, control measures

Introduction–Italy has been affected by several epidemics of low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) caused by viruses of the H5 or H7 subtypes in the last ten years. These epidemics occurred mainly in a densely populated poultry area of the Veneto and Lombardy regions in North-Eastern Italy, inducing severe economic losses. From April to December of 2009 new LPAI outbreaks, both in the industrial and rural poultry sector, were identified. This paper describes the LPAI outbreaks detected during the surveillance activity implemented in Italy in 2009.

Methods–The application of the national monitoring programme implemented according to EU guidelines and legislation, allowed the identification of the LPAI infection in some industrial poultry farms of Lombardy Region. In order to asses the epidemiological situation, an extra-surveillance activity was set up in other Italian regions, both in the industrial and rural sector, mainly in areas at high risk of avian influenza introduction. These additional monitoring activities allowed the identification of some other infected farms. Moreover the epidemiological investigations revealed direct contacts (movement of live birds) between the outbreaks and other farms, resulting in secondary outbreaks. Restriction measures were applied in every outbreak according to 94/2005/CE EU Directive.

Results–From April to December of 2009 a total of 39 LPAI outbreaks caused by either H5 or H7 LPAI virus subtypes were confirmed by the National Reference Laboratory. Five outbreaks were detected in industrial meat turkey farms, four of them resulted serologically positive for H5 virus subtype and one for H7. The virus was isolated in 2 of them, resulted as an H5N7 and an H7N3 virus strain respectively. Furthermore, a breeder flock, located in Piemonte Region, was found serologically positive for LPAI virus of H7 subtype and two industrial game-hunting farm were found serologically positive for the H5 subtype. The other 31 outbreaks were detected in the rural sector. All of them resulted serologically positive for LPAI virus of H7 subtype and in two of them an H7N3 virus strain was isolated. Five of them were dealers, 8 retailers and 18 backyards/hobby flocks in different Italian Regions.

Phylogenetic analysis of the haemagglutinin gene of the H5N7 virus showed high homology at the nucleotide level with H5 isolates from wild birds in Italy and in Europe suggesting a recent introduction from the wild bird reservoir. In contrast the H7N3 isolates showed a high homology with the LPAI H7N3 viruses that caused outbreaks in Italy in 2007. In many cases, the epidemiological investigations identified uncontrolled animal movements as the principal risk factor in the spread of infection.

The regular application of national surveillance programme together with the tracing of animal movements allowed the prompt identification of the infected farms. Moreover the application of the eradication measures, as provided in for 94/2005/CE EU Directive, such as stamping out, restriction, bio security and additional monitoring activities allowed to contain the spread of infection.

Conclusions-The enforcement of surveillance and monitoring activities immediately after a new introduction of the infection in a free area, allow the prompt identification of secondary cases. The enforcement of restriction and eradication policies, together with an epidemiological data collection allow the assessment of the situation and the identification of risk factors involved , avoiding further spread of the infection.

POSTER: AN EXPERT WORKSHOP ON LABORATORY PREPAREDNESS AND CONTINGENCY PLANNING ORGANIZED WITHIN THE EPIZONE NETWORK OF EXCELLENCE

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Key words: Laboratory Contigency Planning, emergency

Introduction

The prompt identification of an infectious disease and the rapid onset of control measures is a prerequisite for the appropriate management of such emergency situations. In order to adequately and efficiently handle outbreaks of epizootic diseases, competent veterinary authorities, including veterinary laboratories, need to be prepared by having contingency plans available. Laboratory contingency planning (LCP) is designed to mitigate the risk of system breakdown and service unavailability in case of a crisis. It is meant to ensure that the laboratory is able to operate effectively and without excessive interruption or delay during an emergency situation. Furthermore it allows a laboratory to guarantee that the necessary quality standards will be met in a crisis and it also serves as a reference manual to all the (laboratory) personnel. Few experiences worldwide are available on this topic, and no specific EU guidelines have been implemented until now. The aim of this group was to outline the main objectives and activities started among EPIZONE Network of Excellence (Contract no Food-CT-2006-016236) related to laboratory contingency planning.

Methods

A working group of scientists, who are involved in contingency planning, was established and a workshop on this topic is to be conducted in Padua (Italy) at the Istituto Zooprofilattico Sperimentale delle Venezie in March 2010. The aim of this working group and of the workshop is to outline the relevant aspects having to be taken into consideration for the management of a crisis caused by "an exotic disease" at laboratory level. Steps for developing an LCP taking into account resource needs and procedures for recruitment will be discussed as well as quality assurance (QA) rules. The outcome will be generic as the animal host spectrum and the characteristics of each disease make careful adaptation of each LCP a necessity. In addition the use of laboratory exercises to provide training and to test the LCP "in practice" will be discussed.

Results

The workshop aims at producing a guideline on how to prepare a national LCP for "an exotic disease". This guideline should cover as many aspects as possible for the management of such a laboratory crisis and should also take into consideration QA, so that the outcomes are delivered in a way, from which it is easy to take action. Identification of needs such as personnel, equipment, facilities, diagnostic kits, reagents, operational protocols, finances, chain of command, information systems for emergency disease reporting is important. Experiences of participants with LCP and the use of such plans in crisis situations will be an important source of information and shall be taken into consideration. Suggestions for the exchange of personnel, availability of reagents and maybe inter-laboratory assistance, planned in advance (peace time) will be considered. The results of the working group activity and of the workshops will be presented at a poster.

Conclusions

Sharing experiences and spreading of excellence are main objectives of EPIZONE. This workshop aims at increasing knowledge on contingency planning and increasing laboratory preparedness. Sharing experiences on the organization and management of a crisis at laboratory level through an interactive work among participants is encouraged. The goal of the workshop is the harmonization and facilitation of the implementation of national LCPs for "exotic disease". This will be tackled by preparing a guideline document, which will be available at the EPIZONE website.

POSTER: DIAGNOSTIC EVALUATION OF A PRIMER-PROBE ENERGY TRANSFER REAL-TIME PCR ASSAY FOR DETECTION OF CLASSICAL SWINE FEVER VIRUS

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Key words: CSFV, HCLV vaccine, validation, real-time PCR

Real-time RT-PCR has become a powerful tool for molecular detection of classical swine fever virus (CSFV), the causative agent of classical swine fever (CSF), a contagious and devastating disease. This study describes diagnostic evaluation of a real-time PCR assay based on primer-probe energy transfer (PriProET) technology for detection of CSFV. The assay was first validated for use in a plate-format real-time instrument, e.g. Mx3005P. The PriProET assay was evaluated and compared with a TaqMan assay by testing a total of 203 samples including 175 clinical specimens and 28 batches of HCLV vaccine. The two assays had same results for 184 (91%) samples. Compared with the TaqMan assay, 19 more samples were found positive for CSFV by the PriProET assay, and confirmed by performing a melting curve analysis of the amplicons. An RNA mixture of both wild type CSFV and HCLV vaccine strain was not successfully differentiated by the melting curve analysis. The PriProET assay can be a routine molecular tool or a confirmative tool for diagnosis of CSF, especially in the case of samples that yield an inconclusive result in the TaqMan assay.

POSTER: APPLICATION OF FTA CARDS FOR SAMPLING LYSSAVIRUSES IN THE FIELD

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Key words: FTA cards, Lyssavirus, Rabies, EBLV.

FTA® cards comprise of filter paper, treated with a patented chaotropic agent that has been designed to preserve nucleic acids, whilst simultaneously inactivating any infectious agents by lysing cellular material. The validity of FTA cards has been proven using lyssavirus samples passaged and clarified before use in the laboratory. Here we describe the use of FTA cards for field samples of classical rabies virus and European bat lyssaviruses (EBLVs) as well as confirming inactivation of infectivity and stability of RNA with laboratory strains.

In total, 23 samples were applied to FTA cards; 19 brain samples from 'suspect' wildlife and domestic species (10 samples from Turkey, 9 samples from Mexico); 1 oral swab from a UK Daubenton's bat and 3 CVS infected mouse brain samples. RNA from all samples was extracted and tested by PCR. The CVS samples were tested for infectivity in cell culture and mice at one hour, and tested by PCR at days 0, 14, 28 and 42.

Nineteen samples tested positive for lyssavirus by real-time PCR and 13 tested positive by hnRT-PCR. PCR products produced were sufficient to be able to sequence and characterise the viral RNA, which is important when using samples collected in the field. The PCRs at different time points were positive for CVS on all days tested, with the exception of the 1:100 dilution on day 42. All RTCIT and MIT tests were negative for CVS.

The negative in-vitro and in-vivo inoculation tests confirm that CVS was inactivated after 1hr incubation at room temperature on FTA cards; indicating FTA cards are a safe way to transport samples in the post without the need for special handling restrictions. The positive results of the hnRT-PCRs show that even in samples with low levels of virus, viral RNA can be extracted from the cards after at least 28 days when stored at room temperature. The PCR and sequencing results undertaken on the field samples demonstrated that FTA cards are a reliable and convenient way to store saliva and brain samples, as well as allowing recovery of viral RNA of sufficient quality for diagnosis and characterisation. These results show FTA cards make sampling in surveillance schemes or epidemiological surveys safer and easier.

POSTER: MOLECULAR EPIDEMIOLOGY OF MOKOLA VIRUS SOUTH AFRICA

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Key words: Mokola, Lyssavirus, Rabies, Phylogenetics,

Mokola virus (MOKV) is a non-segmented, negative-sense RNA virus and is currently the only member of the Lyssavirus genus not to have been isolated in bats. MOKV is one of five lyssaviruses found in Africa (Classical Rabies Virus - RABV, Lagos Bat Virus - LBV, Mokola Virus, Duvenhage Virus - DUVV and the recently proposed Shimoni Bat Virus-SHIBV). MOKV is zoonotic, causes rabies like acute encephalitis, and was responsible for two human deaths. Several cross protection and cross neutralisation studies have demonstrated reduced protection against MOKV with the currently available rabies virus derived vaccines.

Due to limited surveillance, the epidemiology of MOKV is not well understood and the reservoir species is unknown. It was first isolated in Nigeria in 1968 from organ pools of shrews, since then more than 20 isolates have been reported in shrews (Nigeria & Cameroon), domestic cats (Zimbabwe, Ethiopia & S. Africa), domestic dogs (Zimbabwe & S. Africa), a rodent (Central African Republic) and humans (Nigeria) indicating a wide host and geographical range. Most isolations involved domestic cats with rabies like clinical signs and most of these animals had been previously vaccinated.

Limited phylogenetic analysis illustrates that MOKV is more heterogeneous than RABV genotype 1 viruses and it has been shown that a variety of lineages may be circulating in a relatively limited geographical area. In this multi-institute collaborative study, we draw upon our combined expertise and virus collections to collate and review historic samples, confirm their identity and utilise phylogenetic analysis to compare the complete genome sequences of all available Mokola viruses. Intra- and inter-genotypic phylogenetic comparisons will inform policy makers and those involved in the control, risk management, molecular epidemiology and evolutionary analysis of this diverse lyssavirus species.

POSTER: BASIC REPRODUCTION RATE (R0) OF BTV-8 BETWEEN DUTCH DAIRY CATTLE IN 2007

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Key words: Bluetongue; Culicoides; transmission; cattle

The basic reproduction ratio (R0) is an important parameter for the design of control programs for infectious diseases. The goal of the study was to determine the R0 of BTV-8 between Dutch dairy cattle in the 2007 BTV-8 epidemic.

In 2007, a sentinel network was initiated to monitor the circulation of BTV-8 in the Netherlands. In May, 275 dairy herds were selected and seronegative cows entered the sentinel network. From June on, each month 16 randomly selected cattle per herd were tested for antibodies in their milk. The percentage of seroconversions per herd per month was derived and with this information the R0 of the infection could be determined using a simple mathematical vector borne transmission model.

In the model, the cattle population per time t was divided into three subclasses of cows that were susceptible, infectious or recovered. Susceptible cattle could become infected after being bitten by an infectious vector. The recovery rate of an infectious cow was assumed to be 0.04 per day (infectious period of 25 days) in the default model and the recovered cows were assumed to have gained permanent immunity. The proportion recovered cows was derived from the field data as the proportion of seropositive cows for each dairy herd per measurement at time t. The value of the infection rate per day, which reflects the chance of being bitten by an infectious vector (Culicoides), V could be derived and R0 could be estimated. Parameter V is a multiplication of the Culicoides biting rate, the number of Culicoides on the cow, the proportion of infected Culicoides and the effectiveness of BTV-8 transmission from Culicoides to the cow. With this information the chance that a susceptible Culicoides became infected after biting an infectious cow was estimated. Assumptions based on literature were included in the model for the parameters recovery rate, the number of Culicoides on the cow, the Culicoides biting rate and the effectiveness of BTV-8 transmission from Cullicoides to the cow. The parameters, recovery rate of the cattle and the number of Culicoides on the cow were varied in a sensitivity analysis.

The model was able to determine an R0 for 302 herd months (88% of all herd months in the study). The median between-cattle R0 in the Netherlands in 2007 was 2.5 (5th %=1.4; 95th %=22.2).Overall median R0 values were highest in September (2.9) and October (2.8). However, per region (south, central or north) the month in which the R0 value was highest differed. The R0 estimates gave a good notion of the spread of BTV-8 across the Netherlands in time and space. The median rate of BTV-8 transmission from cow to Culicoides was 0.002 and ranged between 0 and 0.44. The model seemed more sensitive to the recovery rate than to the number of Culicoides on the cow. When the recovery rate was reduced, the median R0 value increased. For example, when the recovery rate was reduced to 0.017 (time to recovery increased to 60 days), the median value for R0 increased to 4.0. In the first months of the 2007 epidemic (August-October), BTV-8 behaved like a directly transmittable disease and transmission of BTV-8 only stopped when there were no or only a small number of susceptible cows left. In that period, Culicoides seemed not a limiting factor. In the months in which temperatures declined, Culicoides related parameters did become a limiting factor for BTV-8 transmission. The R0 represented the within-herd spread of BTV-8 in the field well and this transmission ratio could apply to other countries in which BTV-8 emerges, given a similar climate, grazing patterns and barn types as in the Netherlands.

POSTER: A UNIQUE ELISA KIT FOR DIFFERENT BIRD SPECIES: THE ROC CURVE TO DISCRIMINATE BETWEEN POSITIVE AND NEGATIVE BIRDS

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Key words: ROC curve, avian influenza, ELISA

Infection with influenza virus in different animal species lead to different serological reactions, and consequently a specific validation of serological tests is required. In our laboratory a competitive Elisa kit, designed for the analysis of sera from several animal species, has been developed following the production of a monoclonal antibody (Mab) against the nucleoprotein (NP) of avian influenza (AI) virus. A serum tests positive with this kit when if inhibits for more than 40% (PI> 40%) the activity of the enzyme linked to the Mab.

Therefore, the lower is the optical density (OD) value, the higher is the concentration of specific antibodies in the serum. 996 serum samples of 5 different species of birds, obtained from laboratory trials and natural outbreaks, were used in this study and were analyzed by the haemagglutination-inhibition (HI) (as gold standard) and ELISA tests. The OD measurement continue scale and the HI data obtained testing 547 positive and 449 negative serum samples were compared by the Receiver Operating Characteristics curve (ROC). The purpose is to calculate sensitivity (SE) and specificity (SP) of the new ELISA test and a cut-point value to discriminate between positive and negative animals. ROC curve is a graph which represents the 1-SP couples and SE corresponding to every possible threshold of diagnostic test result.

Serum samples of diverse bird species showed different ELISA values. As expected for an ELISA competitive test, high OD values corresponded to sero-negative animals and low OD values to sero-positive animals for all species except for quail sera which showed an abnormal (inverted) behaviour. The study shows that for turkey and chicken, a precise ELISA value perfectly discriminates between sero-positive and sero-negative animals with SE and SP values equal to 1. For ducks, quails and ostriches instead, the test leads to both some false positive and false negative results. In particular for quails, the abnormal behaviour of sera precludes the use of the Kit for this species. Although the ROC curve calculates SE and SP indeed, these results are not acceptable due to a percentage of inhibition lower than 40% in almost all cases as a consequence of the OD high values of positive sera.

The identification of the specific cut-point of the same ELISA kit according to the animal species allows the use of the same kit for different species with huge economic and practical advantages.

POSTER: CONSTITUTIVE AND VIRUS-INDUCED INTERFERON-ALPHA RESPONSES IN PIGS

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The constitutive expression of interferon (IFN)-alpha has been recently investigated in our lab in peripheral blood mononuclear cells (PBMC) of Specific Pathogen Free (SPF) pigs. IFN-alpha positive cells were both monocytes and plasmacytoid dendritic cells showing unusual molecular mass values of intracellular interferons (Amadori M. et al., 2010, Res.Vet. Sci. 88, 64–71). In a subsequent trial, constitutive expression of IFN-alpha1, alpha2, alpha5-6, alpha7-11 and alpha12 genes was demonstrated by RT Real Time PCR in PBMC of conventional pigs at 22 days of age (just before weaning) and 12 days later. 6 days after weaning most sera were IFN alpha-positive at low titres in the presence of a feed-back transcriptional block of all the IFN-alpha genes in PBMC. These results indicated that a non-infectious stressor (early weaning) has the potential to cause a release of low-titre IFN-alpha and a temporary inhibition of IFN-alpha gene transcription. Owing to the above, we were prompted to carry out a comparative evaluation of constitutive and in vitro virus-induced IFN-alpha responses. To this purpose, PBMC were recovered from venous blood of 80-day old Landrace x Large White pigs (way beyond the weaning stress). Cells at 2 x 106 cells ml-1 in RPMI 1640 + 5x10-4 M 2-mercaptoethanol + 5 % fetal calf serum were grown for 20 hours in 12-well microplates at 37 °C in a 5% CO2 incubator; they were either untreated (UN), or submitted to a priming treatment at 100 U ml-1 of recombinant porcine IFN alpha1 (PR), or to the same priming treatment + the addition of 30 Haemagglutinating Units ml-1 of infectious Newcastle Disease Virus (NDV) Lasota strain after 2 hours (V+PR), or the addition of NDV Lasota only after 2 hours (V). The expression of 11 IFN-alpha genes was investigated by RT Real Time PCR in the above PBMC samples and also in uncultured PBMC of the same pigs (T0). The release of IFN-alpha in tissue culture medium was investigated by a combination of a bio-assay on MDBK cells and a highly sensitive sandwich ELISA based on catcher monoclonal antibody (mAb) F17 and biotinylated K9 tracer mAb (Diaz de Arce H. et al., 1992, Vet. Immunol. Immunopathol. 30, 319-327). Results obtained on T0/UN/PR/V/V+PR samples indicated that IFN alpha 7/11 and (to a lesser extent) alpha5/6, alpha1 and alpha2 genes are involved in constitutive expression and no substantial increase of expression took place after virus stimulation. The same was also true of IFN alpha8 gene. Instead, the expression of IFN alpha3 was not evidenced under any experimental condition and that of IFN alpha12 was shown in one pig, only. The expression of IFN alpha4 was strictly priming-dependent. On the contrary, the expression of IFN alpha9 was only shown under V and V+PR conditions. Interestingly, virus-induced gene expression seemed to be inversely related to constitutive expression. On the whole the emerging picture points at a dynamic condition, whereby few IFN-alpha genes are constitutively expressed and multiple IFN subspecies can be expressed instead following viral induction. Also, IFN-alpha release by PBMC can be largely explained in terms of post-transcriptional control activities, since gene expression levels were often equal or greater in UN and PR, compared with V samples.

POSTER: COMPARISON OF THE EARLY TRANSCRIPTIONAL RESPONSE IN BLOOD CELLS BETWEEN FMDV AND CSFV INFECTED PIGS.

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Key words: Gene expression, blood, pigs, FMDV, CSFV

Viruses employ numerous strategies to evade and suppress the host innate immune system. Subtle differences in genetic background of viruses and hosts (e.g. breed) as well as the general health status of the host play a crucial role in how the host copes with a viral infection. This complex interplay between viral factors and host defence mechanism influences the adaptive immune response, and is decisive for the course of the viral disease. Ideally, these multiple and complex interactions can only be studied in vivo. We monitored the transcriptional response in whole blood of pigs infected with Foot-and-mouth disease virus (FMDV) and Classical swine fever virus (CSFV). Five pigs were infected oronasally with highly virulent CSFV strain Eystrup and 5 pigs were infected intradermally (in the bulbus of the heel) with highly virulent FMDV strain O Taiwan. Whole blood was collected in Paxgene-RNA tubes at day 0 (just before infection, dpi 0) and at dpi 1, 2 and 3. All infected pigs developed clinical signs of disease as reported earlier for these highly virulent strains, starting from 2.dpi, for both CSFV and FMDV. For both groups, RNA pools, consisting of equal amounts of RNA from each of the 5 pigs, were prepared for each day blood was collected. Messenger RNA expression in each pool was measured using the pig Agilent 44K oligonucleotide array. Time dependent changes in gene expression imposed by FMDV or by CSFV infection were extracted from array datasets. In addition, the level of gene expression in CSFV infected pigs was compared to that in FMDV pigs on the same day post infection. We detected common and markedly different changes in gene expression for both viruses. For instance, an unique expression profile of cytokines and chemokines was observed for FMDV and for CSFV. By this integrated approach we were able to identify host-processes in the blood of pigs specifically induced by FMDV-infection or by CSFV-infection. The impact of these processes on the pigs first line of defence towards both these highly contagious diseases will be discussed. Highly up-regulated genes detected in these experiments may be useful as biomarkers to distinguish between FMDV and CSFV.

POSTER: WEST NILE VIRUS SURVEILLANCE IN THE UK AND THE ASSESSMENT OF THREE PCR-BASED MOLECULAR TECHNIQUES FOR WEST NILE VIRUS DETECTION, INCLUDING A NOVEL REAL-TIME PAN-FLAVIVIRUS RT-PCR.

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Key words: West Nile Virus, Surveillance, PCR

West Nile virus (WNV) is an arthropod borne virus of the family Flaviviridae. Of all mosquito-borne flaviviruses, WNV has the most widespread geographical distribution and the largest vector and host range. The increase in geographic spread of the virus to novel regions over the last decade, and it's potential to cause disease in humans, horses and wild animals of economic and ecological importance, has led to WNV becoming a major veterinary and public health concern. The Veterinary Laboratories Agency (VLA) has been undertaking surveillance testing for WNV in wild birds in England, Wales and Scotland since 2001. Tests, undertaken on natural wild bird mortalities, have included virus isolation in Vero cells and RT-PCR. Approximately 2,500 wild birds, representing 130 species have been examined between 2001 and 2009. So far, there has not been any evidence for WNV infection during this surveillance study. Since 2008, the VLA has also offered serological testing, using the WNV Plaque Reduction Neutralisation Test (PRNT90), for the differential diagnosis of neurological disease in horses. No neutralising antibody to WNV has been detected in UK horses. The current increase in incidence of other flaviviruses in Europe has led us to develop a novel real-time pan-flavivirus RT-PCR for surveillance purposes. This assay has successfully detected a range of flaviviruses including WNV, Tick-Borne Encephalitis virus, Usutu virus, Japanese Encephalitis virus, Dengue virus and Louping ill virus (the only Flavivirus currently endemic in the UK). The nested RT-PCR, currently used for WNV surveillance, the pan-flavivirus qRT-PCR and a WNV-specific real-time RT-PCR are being comparatively assessed for sensitivity and specificity. The initial results suggest that the pan-flavivirus qRT-PCR detects a broad range of flaviviruses but is less sensitive for WNV detection compared to the WNV-specific techniques. Each method has substantial merits within different surveillance and diagnostic situations.

POSTER: SEQUENCE ANALYSIS OF PORCINE CIRCOVIRUS 2 GENOME FROM DIFFERENT PCVAD CLINICAL CONDITIONS

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Key words: pig, circovirus, sequence, PMWS, reproductive problems

Porcine circovirus 2 (PCV2) is the primary cause of several clinical conditions which are known as Porcine circovirus associated diseases (PCVAD). PCVAD affect pigs at different age and are responsible for important economic losses in the pig industry. Although the disease in pigs is known for more than ten years, the exact mechanisms of PCV2 pathogenesis are still to be determined. Several co-factors which could trigger clinical manifestation of circovirus diseases like concurrent viral or bacterial infection, stress, immunomodulation, were already identified.

Nonetheless recent research suggests the existence of at least two different PCV2 genotypes, which could explain differences in pathogenic properties of field virus strains.

The aim of this work was to sequence several field viruses from different clinical conditions (PMWS, reproductive problems) of affected pigs and detect markers responsible for development of particular clinical syndrome.

Ten different virus strains from PMWS cases and 10 strains from aborted piglets were analyzed in this study. Complete PCV2 genome was PCR amplified and cloned into plasmid vector. DNA construct was then sequenced and resulting genetic information analyzed. The comparison didn't reveal any significant genetic changes responsible for biological behavior of virus strains.

POSTER: ORF3 AND ORF2 ANTIBODY PROFILE OF PMWS AFFECTED HERDS

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Key words: circovirus, pig, serology, recombinant antigen

Porcine circovirus 2 (PCV2) is considered to be an important pathogen associated with a number of different syndromes and diseases in pigs. The genome of PCV2 is a single stranded circular DNA molecule encoding three open reading frames (ORFs). Nucleocapsid protein (ORF2) of the virus is highly antigenic and PCV2 infected pigs display high ORF2 specific antibody titres. Virus ORF3 protein is probably in some way responsible for pathogenic properties of the virus. Antibody profile based on ORF3 antigen still remains to be evaluated.

The aim of this work was to analyze serologic profile of PMWS affected herd using ELISA tests based on ORF3 and ORF2 antigens.

Antibody profile comparison was done in production pig farm in the Czech Republic on a panel of 30 piglets from 4 litters with subclinical form of circovirus disease. Piglets were sampled in two week interval and serum samples were examined by ELISA tests.

The titres of ORF2 specific IgG antibodies appeared 8 weeks after the birth and peaked at 16 weeks of age. ORF2 specific antibodies were detectable at relatively high and stable level even in the adult age (sows - 2 years).

ORF3 specific antibodies appeared sporadically for the first time at 12-16 weeks of age at moderate levels. The titres of ORF3 specific antibodies raised up mainly in adult animals. Although the concentration of ORF3 specific antibodies was considerably lower than the concentration of ORF2 antibodies, these antibodies were found in naturally infected as well as in vaccinated animals.

POSTER: RAPID DETECTION OF PORCINE CIRCOVIRUS TYPE 2 BY LOOP-MEDIATED

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Key words: Porcine circovirus type 2 (PCV2); Detection; Loop-mediated isothermal amplification (LAMP); Sensitivity; Specificity

Porcine circovirus type 2（PCV2）is now generally accepted as the major infectious agent involved in postweaning multisystemic wasting syndrome (PMWS). PCV2 infection has severe impacts on pig health and welfare, leading to weight loss, paleness, dyspnoea, diarrhoea and icterus. PCV2 is a small, non-enveloped, spherical single-stranded DNA virus. There are two major open reading frames (ORFs) in PCV2 genome. ORF1, called the rep gene, is involved in virus replication. ORF2, named the cap gene, encodes the major immunogenic capsid protein which has the type-specific epitopes and is considered of great value in PCV2 diagnosis.

The presence of PCV2 in tissues of pigs with PMWS has been proven by virus isolation, polymerase chain reaction (PCR), Real-time PCR, in-situ hybridization and immunohistochemistry. PCR-based detection methods are commonly accepted because of their high sensitivity and specificity. Loop-mediated isothermal amplification (LAMP) is a novel amplification method which was developed originally by Notomi et al in 2000. The most significant advantages of LAMP are the ability to amplify specific DNA sequences under isothermal conditions between 63-65∘C and a visible result within 30-60 min. The method has been applied successfully to the detection of human influenza A virus, severe acute respiratory syndrome coronavirus and Newcastle disease virus et al.

In our study, LAMP was employed to develop a rapid and simple detection system for PCV2. The amplification could be finished in 60 min under isothermal condition at 64∘C by employing a set of four primers targeting the cap gene of PCV2. The LAMP assay showed higher sensitivity than the conventional PCR, with a detection limit of five copies per tube of purified PCV2 genomic DNA. No cross-reactivity was observed from the samples of other related viruses including porcine circovirus type 1 (PCV1), porcine parvovirus (PPV), porcine pseudorabies virus (PRV) and porcine reproductive and respiratory syndrome virus (PRRSV). The detection rate of PCV2 LAMP for 86 clinical samples was 96.5% and appeared greater than that of the PCR method. The LAMP assay reported can provide a rapid yet simple test of PCV2 suitable for laboratory diagnosis and pen-side detection due to ease of operation and the requirement of only a regular water bath or heat block for the reaction.

POSTER: THE EFFICACY OF FMD VACCINE REDUCED NON-STRUCTURAL PROTEINS WITH A MAB AGAINST 3B PROTEIN

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Key words: Foot-and-mouth disease virus (FMDV); monoclonal antibody (mAb) against 3B protein; non-structural protein (NSP)

A monoclonal antibody, 3BIgG, against the prokaryotically expressed foot-and-mouth disease virus (FMDV) non-structural protein (NSP) 3B was obtained. The 3BIgG-sepharose conjugant (3BmAb-6BFF) was prepared by adding the purified 3BIgG into epoxy-activated sepharose 6BFF, incubating with the inactivated FMDV, then removing the sepharose by centrifugation. The vaccine was made from the supernatant emulsified with oil-adjuvant ISA206. Ten guinea pigs, 26 pigs and six cattle were vaccinated, and a vaccination control group was included without treatment with 3BmAb-6BFF. After 28 days, 9/10 pigs challenged with FMDV were protected, this result was the same as the control group, indicating that the vaccine potency was not reduced after treatment with 3BmAb-6BFF. The other animals were vaccinated weekly for nine weeks, and serum samples were collected to detect 3ABC-antibody titers. The results showed that 3ABC-antibody production was delayed and the positive antibody rates were lower when vaccination was carried out using vaccines treated with 3BmAb-6BFF compared with untreated vaccines. The findings of this study suggest that it is possible to reduce NSPs using a mAb-sepharose conjugant in FMD vaccines without reducing their efficacy.

POSTER: RECOMBINANT NONSTRUCTURAL NSP7 PROTEIN – SEROLOGICAL DETECTION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME INFECTION

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Key words: PRRS, recombinant antigen, Nsp 7,serology, antibodies

Porcine reproductive and respiratory syndrome (PRRS) is widespread disease of swine which causes respiratory problems in young pigs and reproduction problems like abortions, stillbirths and high mortality of weak piglets. The agent of this illness is porcine reproductive and respiratory syndrome virus (PRRSV) which belongs to the family Arteriviridae, the order Nidovirales. It is a small, enveloped virus with positive-strand RNA genome, which includes eight open reading frames (ORFs) – 1a, 1b, 2, 3, 4, 5, 6 and 7, from which ORFs 1a and 1b encode thirteen nonstructural proteins (Nsp) and ORFs 2-7 encode seven structural proteins (GP 2, GP 3, GP 4, GP 5, E, M and N).

Nucleocapsid protein N is extensively used in serological diagnostics of the disease. Nonetheless serological tests based on this antigen display nonspecific reactivity leading to inaccurate diagnostics. Furthermore diagnostic tests based on virus nucleoprotein can´t differentiate between postinfectious and postvaccination antibodies. On the other hand nonstructural proteins are produced in virus infected cells only and antibodies against Nsp are detected only in pigs infected with field virus or vaccinated using modified live virus vaccine.

The goal of this work was to express Nsp 7 protein of PRRSV. The gene coding for Nsp 7 protein was PCR amplified and cloned into pENTRY vector and then transferred into pDest17 vector. Expression of recombinant protein was tested in different Escherichia coli strains: BL21-CodonPlus (DE3)-RIPL, BL21(DE3)pLysS and BL21-AI. Induction was performed by IPTG or L-arabinose depending on used strain of bacteria cells for 3 hours at 37 °C. The best production of protein was observed in BL21(DE3)pLysS cells. Resulting protein was purified by metalochelating affinity chromatography from insoluble fraction. Purified Nsp 7 is serologically reactive and is suitable for usage as an antigen in indirect ELISA test for differentiation of postinfectious and postvaccination antibodies. From 176 examined samples 64,8 % of them gave identical results using both ORF 7 and Nsp 7 ELISA tests. 35,2 % samples gave discrepant results.

POSTER: DETECTION OF TTVIRUS IN THE CZECH REPUBLIC BY QUANTITATIVE PCR

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Key words: TT virus, pig, real-time, PCR, diagnostics

Torque teno sus viruses (TTSV) are small non-enveloped viruses with circular, single-stranded DNA genome. TT viruses belong to the family Anelloviridae and may infect humans and different animal species. Two different virus species have been identified to date in pigs, Torque teno sus virus 1 (TTSV1) and Torque teno virus sus 2 (TTSV2), which belong to the genus Iotatorquevirus. TTSV are widely prevalent in the pig population but TTSV infection is not directly responsible for any disease. At present the nested PCR with specific primers situated in the highly conserved region of the virus genome is used for TT viruses diagnostic.

The goal of this work was to to design and evaluate qPCR technique allowing specific detection of TTSV2 virus and to quantify the amount of virus particles in clinical samples. Samples and Taq-man probe for qPCR were situated into ORF1 part of virus genome. Ten- fold dilutions of plasmids containing ORF1 gene were used to construct calibration curve allowing virus quantization.

The total of 100 serum samples was used for virus detection. All samples were first checked by standard nested-PCR assay and then by qPCR test. TTSV2 was found in 50% of examined animals, virus titres ranged from 0 to 104 copies of virus genome per ml of blood serum.

POSTER: COEXPRESSION OF RABIES VIRUS GLYCOPROTEIN AND NUCLEOPROTEIN IN SILKWORM-BACULOVIRUS EXPRESSION SYSTEM AND ITS UTILIZATION AS A SUBUNIT VACCINE

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Key words: Silkworm-baculovirus expressionrabies, rabies virus, glycoprotein;nucleoprotein;subunit vaccine

Rabies is a fatal zoonotic infectious disease caused by rabies virus, being epidemic worldwide, especially in most Asian and African countries. Even though there are effective vaccines available, eradication of the rabies virus has remained problematic by the high cost of production and administration of the vaccines. Thus, There is a signiticant need for development of effective, safe, and economic rabies vaccines for animals.

The gene encoding rabies virus glycoprotein and nucleoprotein were modified by site-directed mutagenesis and linked by IRES sequence, then subcloned into baculovirus plasmid transfer vectors pVL1393, and inserted via homologous recombinations into baculovirus Bombyx mori nuclear polyhedrosis virus(rBmNPV). Expression of G and N proteins were detected both by immunofluorescence assays (IFAT) with recombinants virus that infected Bm-N cells and by western-blotting. Early fifth-instar silkworms were infected with the recombinant virus and silkworm haemolymph were obtained and detected by rabies virus antigen ELISA kit. Silkworm haemolymph was lysed ultrasonically and cell debris was removed by centrifugation. The suitable supernatant was used to prepare vaccine. Four to five-week-old BALB/c mice were immunized by intramuscular injection at hind limbs or orally perfusion. Rabies virus neutralizing antibodies were determined by the rapid flourescent focus inhibition test. Vaccinated mice were challenged with determined lethal doses of CVS rabies virus.

The expression of glycoprotein and nucleoprotein in Silkworm haemolymph was analyzed by sandwich-ELISA. The expression yield was about 60 fold more than the positive control, but was not detectable in the negative control. Before the challenge, the VNA of 10 injection vaccinated and randomly selected mice was assayed to be above 0.5 IU/ml while no VNA in 10 negative control mice could be detected. The high specific IgA antibody could be detected in orally administrated mice. Both of intramuscularly vaccinated mice and orally vaccinated mice survived after the CVS-24 challenge,while 9 out of 10 unvaccinated control mice died of the infection.

In this report, A rabies virus subunit vaccine consisting of the viral G protein and N protein was prepared from a Silkworm-baculovirus expression system. The G antigen and N antigen produced in BmN cells and silkworm haemolymph appeared to be authentic based on Western blotting. Our results showed that both intramuscularly and oral administration in mice conferred a protective immune response. I t is feasible to use this vaccine for preventing rabies virus infection in animals.

POSTER: COWS CAN BE PROTECTiON FROM HEN EMBRYO YOLK SAC MEMBRANE INACTIVATED VACCINE OF CHLAMYDOPHILA ABORTION

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Key words: chlamydophila abortion immunogenicity

Three strains of Chlamydophila abortion were isolated by hen embryo yolk membrane from dairy cows in Shanxi, Ningxia, Gansu province in China. The MOMP gene of three strains were amplification by PCR technique and there have 99.% homology among them. Virulence stabilities and immunogenicities of Chlamydophila abortion SX5, GS and NX strains were detected, and the virulence titers of SX5, GS and NX strains were 10-11 , 10-10 ELD50 and 10-10 ELD50 through 20 successive passages in hen embryos, respectively. SX5 strain was choose as a vaccine candidate strain.
The SX5 strain was inactivated by methanal after proliferation from hen embryo yolk sac membrane. Immunogenicity of SX5 isolation strain was examined using mouse model and cattle model. The result showed that 9/10 mice and 5/5 cattle of immunized group was protected from challenges, but 1/10 mice and 0/5 cattle of the control group. According to the virulence stability and immunogenicity tests, SX5 isolation strain may serves as vaccine strains.

POSTER: INVESTIGATION ON UNKNOWN VIRUS FOR GRAYLING (THYMALLUS THYMALLUS).

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

NATIONAL VETERINARY RESEARCH INSTITUTE[1]

Key words: Virus, Fish, Grayling

The abnormal mortality above 70% was detected in 150-200 g graylings reared in farms with other salmonid fish species for the purpose of river restocking.

The cythopatic effects were achieved after EPC, FHM, BF-2 and RTG-2 cell lines inoculations with diseased graylings internal organs supernatants and the unknown virus was isolated. The presence of VHSV, IHNV, IPNV, EHNV and SVCV in graylings were excluded using ELISA and PCR tests.

The unknown grayling virus isolate L01/07 was stored and its study has followed. It was found that the lipid virus membrane is chloroform sensitive and the 100 % membrane was lysed during the test. On the basis of the virus shape and structure analysis using TEM routine techniques, it could be classified to Rhabdoviridae.

Total RNA was extracted from virus infected cell lines using the commercial A&A Biotechnology kit.

Primers were designed to a highly conserved region of the viral RNA polymerase (L) gene sequences (Betts et al., 2003).

Probably the same virus was detected after six months in grayling survivors and brown trouts which were reared in pond neighboring the grayling ponds.

The pathogenicity of unknown virus isolated from grayling and brow trouts is discuses and its detailed molecular structure was analysed.

1. Betts A. M., Stone D. M., Way K., Torhy C., Chilmonczyk S., Benmansour A., de Kinkelin P.: Emerging vesiculo-type virus infections of freshwater fishes in Europe. Dis Aquat Org 57:201-212.2003.

POSTER: THE MONITORING OF VHSV, IHNV AND KHV IN POLAND IN 2009

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Key words: VHSV, IHNV, KHV, monitoring

In 2009 it was found that rainbow trouts in 23 farms were infected with VHS virus, in 7 farms with IHN virus and in carps originating from 46 farms KHV nucleic acid presence was detected. The number of clinical cases in 2009 considering the diseases caused by these viruses were the highest in the 10 years period.

In every case VHS or IHN viruses were being isolated at least in two of the following cell lines BF-2, EPC, RTG-2 or FHM and identify at least by two of the following methods i.e. ELISA, IFAT, PCR.

Koi herpesvirus (CyHV 3) nucleic acid was being identified using PCR method in Gilad modification and since 2009 also in Bercovier modification.

Pending that each year rainbow trouts originating from comparable fish farm numbers were checked on the presence of VHS, IHN, it could be concluded that these viruses are gradually spreading each year.

The growth of CyHV 3 nucleic acid presence cases in carp could be partly connected with the increase of carp farms which were monitored in consecutive years. It will be worth nothing that in last year relatively numerous cases of CyHV 3 nucleic acid presence in carp were noted which was not connected with abnormal mortality or clinical symptoms.

The results of monitoring of fish viral diseases confirmed that the realization of the official programmes of VHS and IHN control in all polish salmonid farms should be realized in all regions where the rainbow trout production and fish processing take place.

The realization of such a programmes is difficult in majority of traditional carp farms but the KHV should be still notifiable disease and some regulations concerning restriction of the movement of live carp so far unrestricted movement had to be applied.

POSTER: CONTRIBUTION TO THE SURVEILLANCE OF TICK-BORNE DISEASES IN NORTH-EASTERN ITALY: SPATIAL AND TEMPORAL DISTRIBUTION OF THE VECTOR TICK IXODES RICINUS AND ASSOCIATED PATHOGENS

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Key words: surveillance, tick-borne diseases, Ixodes ricinus, Italy

North-eastern Italy is a well known endemic area for Lyme borreliosis (LB) and tick-borne encephalitis (TBE) and account for more than 90% of human cases in the country. Recently, an other tick borne disease (TBD), the Human Granulocytic Anaplasmosis (HGA) has been diagnosed in this area. The aim of this work was to assess the spatial and temporal distribution of the main vector (the wood tick Ixodes ricinus) and agents of the above mentioned TBDs: Borrelia burgdorferi sl, TBE Flavivirus (TBEV) and Anaplasma phagocytophilum. Methods - The survey was conducted from 2006 through 2008 in 5 provinces of north-eastern Italy, namely Vicenza, Verona, Treviso (Veneto region), Pordenone and Udine (Friuli Venezia Giulia region). Tick sampling was performed by standard dragging in permanent sites, monitored monthly from March to November, and in itinerant sites, visited only once or twice. Each tick was identified and stored at -80°C. RNA/DNA were extracted from single adults, and from pools of 5 nymphs or 10 larvae. A one step real time RT-PCR was used for TBEV detection and positive results were confirmed by a nested RT-PCR. A multiplex real time PCR was used for the simultaneous detection of A.phagocytophilum and B.burgdorferi sl. Prevalence of infection in nymphs and larvae were adjusted for pooled samples and expressed as expected rates of infection.

Results – Overall, 66 sites were visited and 5484 ticks were collected in 6 permanent and 50 itinerant sites (11 were negative for ticks). Most of the of the ticks were larvae (2843), followed by nymphs (2444) and adults (197, sex ratio 1:1) and were found from the lower (120 masl) through the higher site (1308 masl). Tick density ranged from 315 ticks/100m2 to 5321/100m2. Agents of LB were found in all the monitored provinces and in about 50% of the sites, with an overall rate of infection of 9.7% in nymphs (range 9-11.4%) and 17.3% in adult ticks (range 13.5-29.8%). TBEV was found in 3 provinces (Pordenone, Udine and Treviso) but in 4 sites only, with an overall infection rate of 0.4% in nymphs (range 0-0.54%) and 2% in adults (range 0-6.7%). A.phagocytophilum was found in 4 provinces (except Vicenza) from 8 sites, with an overall infection rate of 1% in nymphs (range 0-1.77%) and 4.7% in adults (range 0-13.5%). In the permanent sites of Friuli Venezia Giulia region, the 3 pathogens were sympatric and 2 adult female ticks showed triple co-infection. Overall adults ticks were significantly more infected than nymphs, although several variations were found among provinces and sites. The pathogens were found through the 3 years of survey. However, B.burgdorferi sl was significantly more prevalent in 2006 and HGA agent in 2007.

Conclusions – TBDs pathogens showed different patterns of spatial and temporal distribution. The chance of LB transmission to humans is spread all over the area, and in some site up to 30% of the adult ticks were infected. HGA and TBE were more localized and showed hotspots of infection. The variety of tick density and infection rates highlights the need of assessing human TBDs risk on a local basis. Improved understanding of the distribution and population dynamics of the vectors and transmitted pathogens is a key element for assessing and managing the risks associated with vector-borne zoonotic diseases.

POSTER: OCCURRENCE OF BLUETONGUE VIRUS AND MIDGE VECTORS IN SAHARAWI TENT CITIES (ALGERIA)

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Key words: surveillance, vector-borne diseases, Bluetongue, Culicoides

Bluetongue (BT) is an orbiviral infection of domestic and wild ruminants, transmitted by many species of biting midges of the genus Culicoides. Several serotypes of BTV (BTV1, BTV2 and BTV4) have been recorded in different countries of north-western Africa in the last 10 years. The aim of this study was to investigate the occurrence of BTV infection and of Culicoides vectors in the Saharawi refugee camps in south-western Algeria, where sheep and goats production represent the main economic activity and the main source of protein, apart from foreign humanitarian aid. Methods - In 2006, blood samples were collected from goats and sheep from Saharawi refugee camps (group 1) and from neighbouring areas (group 2). Samples were tested by ELISA for antibodies to BTV and serotyped by serum neutralization test at the National Reference Centre for Exotic Diseases (CESME, Italy). Culicoides were captured in April from five sites by light traps (Goffredo and Meiswinkel, 2004) and stored in alcohol 70°. All Culicoides species were morphologically identified based on their wing pattern and mounted on microscopic slide when necessary (www.culicoides.net). Location, altitude, maximum and minimum temperature were recorded at each capture using data from weather station DAOF 60656 (www.wunderground.com).

Results – Overall, 369 sera were tested. Antibodies to BTV were found in 66 (36.9%) samples from group 1 and in 120 (63.1%) samples from group 2. Only antibodies to BTV-1 were found in group 1, whereas antibodies to BTV-1, BTV-2, BTV-4, BTV-8, and BTV-16 were found in group 2. Overall 95 Culicoides midges were captured by light traps in 8 sites. Maximum number of Culicoides was collected in El Aaiyoun (46 specimens) whereas in Rabuni and Smara no midge was found. Five species were identified: C. semimaculatus, C. sejfadinei, C. circumscriptus, C. schultzei group and C. obsoletus group. The most abundant was C. sejfadinei (50 specimens) collected in all sites, while only one C. schultzei and one C. obsoletus group were identified.

Conclusions – The results show that BTV infection is spread in the area confirming previous non published data gathered in 2004 in the refugee camps. Antibodies to BTV were found in a relevant number of Saharawi animals, whose previous movements could not be traced back certainly, but also in at least two autochthonous animals never moved from Saharawi refugees camps. The limited number of midges collected is consistent with the critical climatic conditions. However, the occurrence of BTV antibodies in autochthonous animals along with the presence of confirmed (C.obsoletus complex) or potential vectors (C.schultzei complex) support the hypothesis of a local viral circulation. Moreover, this is the first confirmation of the presence of C.obsoletus complex in Saharawi refugee camps.

POSTER: ANALYSIS OF THE INTERFERON ABROGATION ABILITY OF THE NON-STRUCTURAL I PROTEIN FROM INFLUENZA A

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Key words: Avian influenza, non-structural 1 protein, Interferon

Introduction. The Non-structural 1 protein (NS1) of influenza A viruses is a multifunctional protein that plays an important role in the virulence of the virus primarily by abrogating the synthesis of host type I interferon (IFN). A recent analysis of the carboxy terminal of NS1 from influenza viruses isolated from various species (human, avian, swine and equine) has revealed size variations caused by either truncations or elongations of the protein [Dundon and Capua, Viruses 1, 1057-72 (2009)].

Methods. NS1 genes (n=13) from a selection of influenza A viruses (e.g. avian origin H7N1, H7N3, H5N1 and human origin H5N1 and H1N1) with a variety of different truncations and elongations were cloned into the eukaryotic expression vector pCI-Neo (Promega). Mink lung and human carcinomic alveolar basal epithelial cells (A549) cells were then co-transfected with the pCI-Neo expressing the NS1 of interest and a second reporter plasmid expressing Firefly luciferase (pISRE-Luc) under the control of an interferon-stimulated response element (ISRE). The ability of each NS1 to abrogate IFN production in these cells was tested by measuring luciferase activity in relative light units (RLU) using a luminometer.

Results. Our results showed that there were differences between the NS1 from viruses of different origin and subtype in their ability to abrogate IFN production in both cell types. For example; the NS1 from H7N1 viruses showed a higher IFN-abrogation ability than the NS1 from the H7N3, H5N1 and H1N1 viruses respectively. The NS1 carboxy terminal length variations were not the cause of these differences.

Conclusions. The carboxy terminal length variations of the NS1 protein studied do not effect the protein's interferon abrogation ability. Future studies will include analysis of a larger number of NS1 proteins from different viral subtypes/species in an attempt to understand the differences in the IFN abrogation ability observed.

POSTER: GENETIC CHARACTERIZATION OF ITALIAN PARAMYXOVIRUSES SEROTYPE-9

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Key words: Paramyxovirus 9, sequencing, Italy

Introduction: Avian paramyxoviruses (APMV) are classified into nine serotypes (1 to 9) and infect both domestic and wild birds. APMV-1 (Newcastle disease), APMV-2, APMV-3, APMV-6 and APMV-7 are known to cause disease in poultry although the severity differs between the serotypes. Since it is not associated with clinical disease, Avian paramyxoviruses serotype-9 (APMV-9) has not been studied in any great depth and, as such, there is a paucity of genetic information available for this virus. With this in mind we have sequenced the complete F and HN gene and their flanking intergenic regions of four APMV-9 viruses isolated in Italy between 2004 and 2008 and have compared them to the prototype PMV-9/domestic Duck/New York/22/78 strain.

Material and methods: Four viruses (e.g. PMV-9/pintail/Italy/493/2004, PMV-9/Mallard/Italy/6226/08, PMV-9/Widgeon/Italy/6436/08 and PMV-9/Mallard/Italy/5709/07) isolated from cloacal swabs collected from hunted wild birds as part of a surveillance programme for avian influenza in North-Eastern Italy were used in this study. Viral RNA was extracted using the High Pure RNA isolation kit (Roche) and one step RT-PCR was performed using the extracted RNA and specific primers (sequences available on request). Amplicons were sequenced directly using the BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems).

Results: The nucleotide homology between the prototype American isolate and the Italian isolates ranged from 87.5% to 87.9% for the F gene and 88.1% to 88.6% for the HN gene while the percentage nucleotide homology between the four Italian isolates alone ranged from 98.6% to 99.9% for the F gene and 97.6% to 99.2% for the HN gene respectively. Isolate PMV-9/Mallard/Italy/6226/08 and PMV-9/Pintail/Italy/493/04 were 99.5% and 99.1% identical to each other for the F and HN genes respectively while isolate PMV-9/Widgeon/Italy/6436/08 and PMV-9/Mallard/Italy/5709/07 were 99.9% and 99.2% identical to each other for the F and HN genes. The cleavage site F) of the F protein is conserved in all isolates and identical to↓(I-R-E-G-R-I that described for PMV-9/domestic Duck/New York/22/78. Of particular interest was the 41 amino acid carboxy terminal extension of the HN protein that was identified among all the Italian isolates resulting in a full length protein of 620aa compared to 579aa for PMV-9/domestic Duck/New York/22/78. C-terminal length variations have previously been seen among the APMV-1 (NDV) viruses and has been used to categorize them into different lineages.

Conclusion: The present study has provided novel sequence data on a group of viruses for which there is a lack of such information, shows genetic variations in the APMV-9 genomes and indicates that APMV-9 can be classified into lineages based on their F and HN protein. It also begs the question whether other APMV serotypes (e.g. 2 to 8) can be similarly grouped. The generation of further genetic data from APMVs will answer this question. The findings of this study will need to be taken into consideration in any future development of molecular differential diagnostic procedures for APMVs.

POSTER: INFECTIOUS BLOOD OR CULTURE-GROWN VIRUS: A COMPARISON OF BLUETONGUE VIRUS challenge models

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Key words: bluetongue / vaccination / sheep / mice / challenge model

Bluetongue virus (BTV) is an emerging global threat for animal health. In Europe, vaccination against serotype 8 (BTV-8) recently has proven very effective, but the dynamic epidemiological situation requires a sustained research effort into new vaccines. Vaccine development and evaluation depend upon standardized challenge models using well-defined inocula of known infectivity. For bluetongue, this is only possible with culture-grown virus. Nevertheless, the World Organisation for Animal Health (OIE) recommends using infectious ruminant blood as challenge inoculum in BTV challenge experiments. The use of virus grown in cultured cells is discouraged because culture passages can lead to changes in virus phenotype including reduced virulence and replication efficiency in the host. Currently, the OIE considers clinical disease in control animals indispensable evidence of successful infection.

In the present study, two groups of five sheep were inoculated with either infectious calf blood lysate or culture-grown BTV-8 at a low passage number. There was no pronounced difference in the induction and progression of viraemia, which is the most objective parameter for the evaluation of vaccine efficacy in a challenge experiment. The equivalent virulence of both inocula was also confirmed by fatal infection of interferon receptor-deficient mice.

The recent availability of highly sensitive molecular methods for the detection of BTV can finally shift the focus away from clinical disease. For the sake of animal welfare and objective and repeatable BTV challenge experiments, the OIE should reconsider its policy on culture-grown virus.

POSTER: HUMORAL RESPONSE AND PROTECTIVE EFFICACY OF INACTIVATED VACCINES AGAINST BLUETONGUE VIRUS SEROTYPE 8 ONE YEAR AFTER VACCINATION OF SHEEP AND CATTLE

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Key words: bluetongue / vaccination / sheep / cattle / long-term efficacy

Bluetongue is a notifiable animal disease which is transmitted to ruminants by Culicoides spp. midges. Bluetongue virus (BTV) had previously been considered exotic to Central Europe; however, after its introduction in 2006, serotype 8 (BTV-8) has caused a major epizootic with considerable economic impact. Vaccinations with inactivated vaccines began in 2008, and have led to a dramatic reduction in the number of new cases in 2009.

In this study, the long-term efficacy of three commercially available inactivated vaccines against BTV-8 (BLUEVAC 8, CZ Veterinaria S.A.; Zulvac 8, Fort Dodge Animal Health; BTVPUR AlSap 8, Merial S.A.S.) was evaluated in a field survey and challenge experiments. A serological survey of 623 cattle and 223 sheep detected antibody prevalences between 75 and 100% one year after vaccination. All cattle had been vaccinated twice and there were no marked differences between vaccine groups. In sheep, twofold vaccination was only performed with one of the vaccines. This resulted in a significantly lower variance of ELISA-detectable antibodies compared to single vaccination with either of the other two vaccines.

Upon challenge with a European BTV-8 strain twelve months after vaccination, all three vaccines protected sheep and cattle against viral replication and clinical disease. Annual revaccination of susceptible ruminants is thus considered an adequate scheme for BTV-8 control in Europe. In the challenge experiment, virus replication was only detectable in two of 64 vaccinated animals. These animals had had the lowest antibody levels in their respective groups, and in one case, a pre-existing health problem was implicated in the vaccine failure.

POSTER: BLUETONGUE VIRUS SEROTYPE 8 IN SOUTH AMERICAN CAMELIDS IN GERMANY: EXPERIMENTAL INFECTION AND FIELD SURVEY

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Key words: bluetongue / experimental infection / South American camelids / alpaca / llama

Bluetongue (BT) is an infectious, non-contagious, multi-species disease caused by Bluetongue virus (BTV), an Orbivirus (Reoviridae) transmitted by biting midges (genus Culicoides). BT can have substantial economic impact and is considered a notifiable disease by the World Organisation for Animal Health. Historically, South American camelids were thought to be resistant to bluetongue disease, although there is serological evidence of apparently subclinical infection with BTV. Recently, fatalities related to BTV infection have been reported in captive llamas and alpacas. Their increasing popularity raises the question if South American camelids play a role in BTV epidemiology.

Accordingly, BTV prevalence in South American camelids in Germany was investigated by serological and virological analysis of over 1700 field samples. Reference material for the validation of different serological assays and data on the susceptibility of South American camelids to BTV infection were collected in an animal experiment. Three alpacas (Vicugna pacos) and three llamas (Lama glama) were obtained from breeders and experimentally infected with BTV serotype 8 by subcutaneous injection of $10^{5.6}$ TCID50 of a recent German isolate. Whole blood and serum samples were taken at regular intervals, and clinical data were recorded daily. Collected samples are analysed by full blood count, real-time quantitative RT-PCR, virus isolation, ELISA and serum neutralization tests. Experimental findings and conclusions will be presented at the meeting.

POSTER: PREVALENCE OF TURKEY RESPIRATORY PATOGENS IN THE FARMS OF CZECH REPUBLIC

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Key words: respiratory patogens, molecular method, turkey

Turkey rhinotracheitis virus (TRT), Ornithobacterium rhinotracheale (OR) and E. coli (infectious colibacillosis) are world-wide occurring pathogens of respiratory tract of poultry. Because of considerable economical impact of these pathogens on poultry industry, the routine diagnostics by sensitive molecular methods is necessary to limit their spread among turkey meat production flocks. The aim of this contribution is to evaluate the prevalence of these agents in turkey flock in the Czech Republic.
From 2008 to January 2010 field turkey samples from seven commercial flocks (two breeding farms, five production farms) in the Czech Republic were collected by veterinarians and sent to the laboratory for diagnostics of TRT, OR and E.coli infections. A total of 205 lung, tracheal and synovial fluid samples were collected from different age categories of turkeys displaying respiratory signs.
Conventional cultivation methods, PCR tests, or RT-PCR were used for the detection of bacterial and viral pathogens. After 24h incubation period and biochemical analysis using ENTERO test, isolates E. coli were obtained. Some of them (23%) were multiresistant to antibiotics after examination by Disk Diffusion Method (according NCCLS). These strains were isolated from breeding farms from animals with fibrinous inflammation of serous membranes and respiratory tract. Prevalence of O. rhinotracheale was 64%. The most frequent samples were tracheal smears (60%), with 30.3% of positive examination of O. rhinotracheale by PCR method. The highest prevalence was in lung tissue (45%) of turkey production farms. We noticed rather low occurrence of rhinotracheitis in both type of farms (15%). The occasionally positive results were from lung tissue (5.8%) only.

POSTER: IFNAR -/- MOUSE AS A LABORATORY MODEL TO STUDY THE HISTOPATHOLOGY OF AFRICAN HORSE SICKNESS VIRUS INFECTION.

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Key words: AHSV, IFNAR-/-, histopathology

African horse sickness virus (AHSV) causes a non-contagious, infectious disease in equids, with mortality rates that can exceed 90% in susceptible horse populations. Due to the similarities between BT and AHS viruses and their vectors, it has been suggested that should AHSV incur into Europe there is the potential for it to become as widespread as BTV. Recently, a model of bluetongue, closely related to AHS virus, has been developed using interferon alpha receptor knock-out (IFNAR-/-) mice (PLoS One 4, e5171). This experimental system has been validated for AHSV infection and the histopathology analyzed.

IFNAR -/- mice were infected with AHSV-4 and studied by conventional histopathological methods – paraffin smears and H&E stain- after the necropsy in natural death cases. The most striking changes were located in brain, lung, liver and lymphoid organs. In, brain all samples showed inflammatory infiltrated in the wirchow-Robbins space, focal gliosis and meningitis. The interalveolar spaces in the lung were severely infiltrated by monocuclear cells. The liver was focally necrotic and showed periportal inflammation areas. Apoptosis and lymphoid depletion were the most frequent lesions observed in spleen; in some cases acelular, eosinophilic, extracellular, and amorphous deposits affecting the white pull of the spleen were observed–spleen amyloidosis-.

The results clearly illustrate the pathogenicity of the AHSV-4 in IFNAR-/- mice. Further studies will be developed to analyse the presence of virus in the endothelial cells of the tissues and its participation in the pathogenesis of the lesions.

POSTER: AFRICAN SWINE FEVER VIRUS: A SELECTION OF NUCLEOSIDE AND NUCLEOTIDE ANALOGUES DISPLAYING IN VITRO ANTIVIRAL ACTIVITY

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Key words: African Swine Fever Virus; antiviral; alternative control strategies; pig disease

African swine fever (ASF) is a devastating disease with a high mortality rate in domestic pigs. Infection results in congestive-hemorrhagic alterations and functional disorders in the digestive and respiratory system. The disease is prevalent and endemic in the majority of the countries of Sub-Saharan Africa and has been recently introduced in the Caucasus region, increasing the risk of emergence in the EU. As no vaccine is available, an antiviral treatment could provide an acceptable alternative to the currently available control measures of stamping-out and pre-emptive culling. The objective of the study is the in vitro evaluation of selected classes of nucleoside and nucleotide analogues on the replication of African swine fever virus (ASFV).

Twenty-two chemical compounds, comprising nucleoside and nucleotides analogues, with inhibitory activity against DNA and retroviruses were evaluated. These included acyclovir (ACV) and cyclohexenyl guanine (CHeG), adefovir (PMEA), PMEDAP and various 3-hydroxy-2-phosphonomethoxypropyl (HPMP) nucleoside phosphonate analogues including cidofovir (HPMPC), HPMPA, HPMPDAP and HPMPO-DAPy. Serial 5-fold dilutions (100 µg/ml to 0.00128 µg/ml) of the compounds were added to Vero cell cultures following infection with Lisbon60, a Vero-adapted ASFV strain. The effect of the compounds on virus-induced cytopathogenic effects (CPE) was monitored at day four post infection as was the cytotoxic effect of on uninfected cells. The viral DNA content of cells was quantified by an in-house real-time PCR. Nucleotide analogues such as HPMPA, HPMPO-DAPy and HPMPDAP resulted in an efficient reduction of virus induced CPE, with low cytotoxicity and a selectivity index up to 1000. The most selective compounds were HPMPO-DAPy, HPMPA and most of HPMPA derivates [7-deaza-HPMPA, 3-deaza-HPMPA, cyclic HPMPA, cyclic 3-deaza-HPMPA and cyclic 7-deaza-HPMPA]. Reduction of viral DNA load was observed with low concentration (≤ 0.8 µg/ml) for HPMPO-DAPy, HPMPA and other HPMPA derivates [cyclic 3-deaza-HPMPA, cyclic HPMPA, 3-deaza-HPMPA, 7-deaza-HPMPA]. Both quantification of antiviral activity in CPE-based assay and viral DNA yield resulted in the same ranking in terms of antiviral activity. Nucleoside and nucleotide analogues with known inhibitory effect against DNA polymerases of DNA and retroviruses (De Clercq, 2007) have been evaluated against ASFV on Vero cell cultures. Consistent with previous study (Gil-Fernández et al, 1987), nucleotide analogues like HPMPA, HPMPO-DAPy and HPMPDAP, resulted in a potent and selective antiviral effect which was in contrast to the effect of antiherpes virus agents such as ACV and CHeG that require phosphorylation by a viral thymidine kinase before phosphorylation ot the active metabolite. The most effective compounds (in order of decreasing efficacy) were: HPMPO-DAPy and HPMPA, followed by 7-deaza-HPMPA, 3-deaza-HPMPA, cyclic HPMPA, HPMPDAP and cyclic 3-deaza-HPMPA. All resulted in EC50 below 0.8 µg/ml. The results of the nucleotide analogues were confirmed by real-time PCR where a reduction of viral DNA load was observed. Further studies are planned to determine the particular characteristics and mechanism of action of these molecules.

POSTER: FIRST COMPULSORY VACCINATION CAMPAIGN AGAINST BLUETONGUE VIRUS IN BELGIUM

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Key words: Bluetongue; Vaccination; Cross-sectional survey; Serology

Starting in August 2006, an epidemic of BTV serotype 8 (BTV-8) gradually disseminated throughout the North-Western European countries causing the most severe outbreak of this disease ever recorded. In order to control the devastating effect of BTV-8, several EU Member States decided to start vaccination before the following vector season. The campaign intended to reach a target of at least 80% of coverage. In Belgium, compulsory vaccination of all domestic cattle and sheep began in May 2008. The objective of this study was to explore the effect of livestock vaccination against BTV-8 in Belgium. Two cross-sectional surveys (serology and pcr) were undertaken during winter 2008 and winter 2009, hence before and after vaccination, respectively. The different results obtained from these surveys were considered as response variables in the analysis. Overall and individual patterns in serology evolution were studied. The effect of several vaccination predictors (ex: the time when the vaccination process started in the herd) on the evolution of serology and on the pcr response were analysed using linear and generalized mixed models. The results showed that 80% of cattle seronegative before the vaccination campaign seroconverted.

POSTER: WEST NILE DISEASE: EPIDEMIC UPDATE IN LOMBARDIA AND EMILIA-ROMAGNA (ITALY) IN 2009

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Key words: West Nile virus, surveillance, diagnosis

Introduction

In Italy the first outbreak of West Nile disease (WND) occurred in Tuscany during the late summer of 1998. At that time 14 horses had neurological symptoms and six of them died; no human cases were reported. Ten years after, in the summer 2008, WNV reappeared in northern Italy and occurred again in the same area in 2009 with significant mortality rates and neurological illness in horses and humans. The purpose of this paper is to update the WNV epidemic occurred in Emilia-Romagna and Lombardia showing data obtained through diagnostic activity performed by IZSLER.

Methods

The national plan for WNV monitoring has the aim of early detection of viral circulation through methods of active and passive veterinary surveillance based on: monitoring of neurological symptoms in horses; serological monitoring of sentinel horses; surveillance of wild birds; entomological monitoring.

Laboratory testing and diagnostic protocol. Blood, CNS, heart, liver and kidney samples of clinically affected horses and of susceptible birds as well as pools of mosquitoes were tested by PCR. Virus isolation was performed on Vero, Rk13 and C6/36 cells and the presence of WNV was confirmed by sandwich-ELISA using monoclonal antibodies (MAb). Sera samples were tested for the presence of anti-WNV antibodies by three distinct homemade ELISA tests: MAb-based competitive ELISA (which uses a neutralizing MAb against WNV); IgM-capture ELISA; IgG indirect ELISA. Positive sera were sent to the National Reference Center for exotic diseases (CESME) for confirmation by neutralisation tests.

Results

The first Italian case of WND in 2009 was confirmed in the second half of July in a horse with neurological symptoms. A little later, other positivities were found also in mosquitoes and wild birds. The provinces of territorial jurisdiction of IZSLER involved in the WND epidemic were: Reggio Emilia, Ferrara, Modena, Parma, Bologna and Mantova.

Passive surveillance on horses allowed to identify 32 clinical cases of WND including 11 fatal in 30 different farms. Active surveillance on horses based on serological monitoring allowed to identify 22 seroconversions in 18 farms during the period between the beginning of August and the end of September. PCR, carried out under a monitoring plan on wild birds led to identify 43 infected birds of 1270 tested: 27 european magpies (Pica pica), 5 carrion crows (Corvus corone cornix), 5 european starlings (sturnus vulgaris), 1 eurasian jay (Garrulus glandarius) 3 seagulls (Larus ridibundus), and 2 owls (Asio otus and Athene noctua).

Within the entomological surveillance 27 mosquito pools out of 1625 tested resulted WNV positive. All positive poolls, detected in 21 trapping sites located in Emilia Romagna, belonged to the species Culex pipiens.

In total 8 WNV strains were isolated. In detail: 4 viruses from birds, two captured (magpie, carrion crow) and two found dead (seagull, jay); 3 from horses (one from the brain of a dead animal and two from the blood of asymptomatic horses tested PCR positive and seronegative for WNV); one from a pool of mosquitoes.

Conclusions

The consecutive circulation in the Po valley for two years indicates that WND is probably becoming an endemic pathogen in northern Italy.

As in 2008, also in 2009 passive surveillance on equine has shown to be the most early and effective way to detect WNV activity however it will be less sensitive in the future, because of horse vaccination started in June 2009. There is a need to organize veterinary surveillance measures aimed at early detection of WNV activity.

POSTER: GENETIC CHARACTERISTIC OF BOVINE HERPESVIRUS 1 ISOLATES CIRCULATED IN CATTLE IN POLAND.

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Key words: BHV1, subtype, restriction enzyme analysis

Infectious bovine rhinotracheitis (IBR) is a highly contagious infectious disease that is caused by bovine herpesvirus type 1 (BHV1). Apart from respiratory disease, this virus can cause other clinical syndroms such as infectious pustular vulvovaginitis or balanoposthitis, conjunctivitis and generalized systemic infections in young calves. In cows infection is associated with decrease in milk yield, reduced fertility and abortions. Lately, more and more the infection runs as a subclinical. The purpose of the study was to determine subtype of BHV1 strains isolated from cattle in Poland. In total 5 different strains of BHV1 were isolated during last years. All isolates as well 4 archival and 2 reference BHV1 strains were analysed by PCR and restriction enzyme analysis. Specificity of the strains was confirmed by PCR with primers complementary to the nucleotide sequence of gD gene. Subsequently genomic DNA of the tested strains was digested with endonucleases Hind III, Hpa I and Pst I. Restriction enzyme analysis revealed that all BHV1 strains isolated in our studies belonged to the BHV1.1 subtype. Among archival viruses 2 strains had restriction pattern similar to the subtype BHV1.1 and 2 others to the subtype BHV1.2a. Presented study showed that currently subtype BHV1.1 is dominant in cattle population in Poland.

POSTER: DIFFERENCES IN THE ABILITY PRODUCTION BETWEEN ALLELE A AND ALLELE B NS1 PROTEINS βTO SUPPRESS INTERFERON FROM H10 INFLUENZA VIRUSES

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Key words: Avian influenz, NS1

Introduction:
One of the main strategies of the influenza A viruses expression or signalling toβ/αto avoid host immune responses is to inhibit IFN- the neighbouring cells, which induce their antiviral state by the stimulation of transcription from the ISRE promoter-containing genes (Stark et al.,1998). The viral NS1 of influenza A viruses is known to be an important regulator of innate and adaptive immunity on many levels (Hale et al., 2008). Our previous study indicated that the NS1 protein is a potential key factor for the different pathogenicity of the H10 avian influenza viruses in mink (Mustela vison). In this study, we applied an expression plasmid system carrying the ORF of NS1 of two avian H10 influenza viruses with different pathogenicity in Mink, to further investigate the the mechanisms by which the NS1 protein evades innate immunity and promotes the virulence of these H10 viruses.

Results and Discussion
Our results reveal that different non-structural protein 1 (NS1) of influenza viruses, one from allele A and another from allele B, show expression. Using a βdifferent abilities to suppress the type I interferon model system, we first demonstrated that NS1 from A/mink/Sweden/84 (H10N4) (allele A) could suppress an interferon-stimulated response element (ISRE) reporter system to about 85%. The other NS1 (allele B), from A/chicken/Germany/N/49 (H10N7), was also able to suppress the reporter system, but only to about 20%.
 promoter isβSince the induction of the IFN- , we next investigated the level ofβassociated with the production of IFN- secreted in the cell supernatant.β mRNA and the amount of IFN-βendogenous IFN- It has been observed that the NS1 protein of mink/84 but not chicken/49 strongly in the cellβ gene and secretion of IFN-βsuppressed the expression of the IFN- culture supernatant.
In summary, we provide evidence for the essential role for NS1 in the early stages of influenza virus infection in mammalian cells. The is,βresults demonstrate that the production of an important cytokine, IFN- affected by the function of NS1 protein.

POSTER: PATHOTYPING OF HPAIV STRAINS IN LABORATORY MICE

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Key words: Influenza, mice, H5N1

Interspecies transmission and pathogenicity are interrelated traits that involve multiple genes of both virus and host. Highly pathogenic avian influenza virus (HPAIV) of the subtype H5N1 were demonstrated to have clear differences in pathogenicity when inoculated into mice, and the virulence in mice generally correlated with their virulence in humans (Katz et al., 2000 Molecular correlates of influenza A H5N1 virus pathogenesis in mice, J. Virol. 74, pp. 10807–10810.). Thus, observing how additional isolates of HPAIV H5N1 are adapted to the restricted conditions in mice could provide important information on the requirements for efficient viral replication in mammals. And there is intense interest and urgency in understanding virulence factors of HPAIV in mammals.

Four recent HPAIV H5N1 strains from 2006 and 2007 isolated from wild birds and poultry species were characterized in the mouse model. The lethal dose 50 was calculated and a number of animals was killed at definitive time points to determine the genomic load from different organs as well as the virus titers. Three strains were demonstrated to be highly virulent for laboratory mice without any adaptation. Interestingly, the lethal dose 50 of one strain isolated from an outbreak in domestic ducks was increased by factor 10,000 compared to the other strains. Full genome sequencing revealed a distinction of only seven amino acids throughout six genome segments.

Subsequently, experimental infection of interferon receptor negative mice (IFNAR 0/0) showed surprisingly that the resistance to inoculation with the less virulent strain compared to one highly virulent strain remained in an only slightly reduced level (factor 1000). It was therefore concluded, that the observed substitution in the NS1 protein could not be the main reason for the different pathotype. Although different strains of H5N1 virus are known to have substantially different pathotypes, the effects of specific amino acid changes on the host ranges and virulences of H5N1 HPAIV remain largely unexplored. With the data from the closely related isolates we will be able to narrow down the relevant virulence markers at least in the mice model. Finally, with a reverse genetics system of the mouse-attenuated HPAIV H5N1, relevant reassortant viruses will be further investigated to identify the responsible genome segment(s).

POSTER: Q FEVER IN DAIRY CATTLE: IS COMPLEMENT FIXATION TEST USEFUL FOR DIAGNOSIS?

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Key words: Q Fever, shedder, CFT, ELISA, realtimePCR

The serological diagnosis for Q fever, traditionally based on Complement Fixation Test (CFT), has evolved during the last few years. Many ELISA tests have been implemented and are currently available in the market. ELISA tests are standardised, easily performed and more sensitive than CFT, nevertheless CFT is still largely used in many laboratories in many countries. Aim of this study is to understand if CFT is still useful for the diagnosis or if ELISA is preferable.

A cattle farm suffering from infertility/abortion problems was found infected by Coxiella burnetii. All the animals (n=266 sera) were twice tested for Q fever antibodies: with a manual CFT (based on a mix of Nine Mile and Henzerling strains, phase II, Dade Behring, cut-off value 1:10), and a commercial indirect ELISA (Checkit Q Fever, IDEXX, based on Nine Mile strain adsorbed in phase I and II).

All the lactating cows (n=138 individual milk samples) were also tested with Realtime PCR. The Coxiella DNA, extracted with a QIAamp DNA mini kit (QIAGEN) was detected by means of a real-time PCR (ADIAVET® COX REALTIME) on LightCycler 2.0. An internal control (IPC) is present in each reaction.

For the n=138 lactating cows, molecular results were compared with serological ones (CFT and ELISA) and statistical tests were performed (test of proportion on 2 independent samples), excluding doubtful ELISA results (n=5).

Serological tests revealed 51 out of 266 ELISA positive animals (19,17%) and 19 out of 266 CFT positive (7,14%), with titres ranging from 1:10 to 1:320. All the CFT positive samples were also ELISA positive. Among the 133 samples selected for statistical analysis, 38 tested positive with ELISA (28,57%) and 14 with CFT (10,53%).

Molecular analysis on milk revealed 26 out of 138 positive (18,84%), with cycle threshold (ct) values ranging from 26.75 to 35.58.

Objective of the study was to evaluate differences between group 1 (both negative with ELISA and CFT) and 2 (ELISA and/or CFT positive), and between group 3 (ELISA positive) and 4 (CFT positive), with respect to the shedder status. A statistical significant difference between groups 1 and 2 was found (p=0.008, CI 95%) and also between groups 3 and 4 (p=0.0171, CI 95%).

CFT is known to have low sensitivity, but high specificity. ELISA is more sensitive, easier to perform and to standardise but it detects antibodies for long time after the contact with Coxiella; on the other side, according to the literature, presence of ELISA negative shedders is observed.

The study shows that a serologically positive animal (group 2) has a significant higher probability to be a shedder with respect to a negative one (group 1). Considering the same comparison between ELISA positive (group 3) and CFT positive (group 4), the probability dramatically increases considering the CFT positive status.

We can conclude that CFT positivity is highly predictive of a shedder status. On the other side, not all the shedders (n=26) were found CFT-positive.

The complex epidemiology of this agent imply many problems, particularly the control/eradication at herd level, that require an appropriate animal infectious status definition and the implementation of measures in order to reduce the pathogen circulation and the exposure of animals and humans.

Due to the specific features of tests, that is: CFT mostly reveals IgM and phase II antibodies response while ELISA mostly records IgG and phase I antigen response, it is likely that different tests identify different classes of animals. This feature might represent a helpful and low cost tool in eradication programs.

POSTER: STABILITY STUDIES ON 146S PARTICLES OF INACTIVATED AND CONCENTRATED FMD VIRUS ANTIGENS

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Key words: Inactivated fmdv antigens, 146 S , Stability

The aim of this study is to evaluate the effect of non-specific proteins which were concentrated together with virus inactivated by BEI and concentrated by cross-flow method on 146S amount during storage at 4 ºC and -20 ºC.

At 12th month of study, 10 % antigen decrease was detected in concentrated type O vaccines stored at 4 oC, especially in group 3 and 4 (Group 3: Inactivated antigen 50% + Dulbecco PBS 48.5%+ Chloroform 3% + NaHCO3 1% Group 4: Inactivated antigen 50% + Dulbecco PBS 48.5%+ Chloroform 0.5% + NaHCO3 1%) and 30 % of antigen decrease was detected in group 6 which was control group.

All groups of concentrated type A vaccine antigens stored at 4 oC, after 3th month, 15 and 90 % of antigen decreases were detected after month 3 and at 12th month respectiveley. 95 % of antigen decrease was detected in group 6 which was control group of type A antigens. However group 3 and 4 showed slightly higher stability than others which showed 60-70 % of antigen decrease at month 6.

For all study groups of both type of antigens that stored -20 ºC, a sudden decline has been detected at month 3. For group 3, 4 and 6, relatively more stability have been detected.

Thirty percent decrease for those stored at 4 C and 60 % for those stored at -20 C were observed in total protein levels of all groups of both antigen types. Overall, antigens stored at 4 ºC were more stable than those stored at -20 ºC. Of course -70 ºC and -196 ºC would be better for antigens storage. Although we did not try them in this study because of the volume problems that caused by large scale production. In this case, antigen preparations described in group 3 and 4 will be more useful for routine use.

Today concentrated antigens are purified using PEG to get rid of irrelevant proteins. It was suggested that concentrated antigens may be stored at 4 o C up to two year by using further purification techniques.

POSTER: BIO-SEEQ PORTABLE VETERINARY DIAGNOSTICS SYSTEM: FIELD-BASED PCR TESTING WITH AUTOMATED SAMPLE PREPARATION

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Key words: Pen-Side, Sample Preparation, LATE-PCR,

Smiths Detection Diagnostics has developed the Bio-Seeq Portable Veterinary Diagnostics System, a portable Sample preparation and PCR instrument designed for field-based veterinary applications. The Bio-Seeq System has three core components; the instrument, the Sample Preparation Unit/Reagent Pack and Linear After The Exponential (LATE) PCR. The device has five independently controlled thermocyclers, each of which has four optical channels, allowing for highly multiplexed reactions, and the device is water-tight, which allows for decontamination via immersion. The Sample Preparation Unit (SPU) is a single-use consumable that is driven by the instrument and extracts nucleic acids from the sample. The assay-specific Reagent Pack contains lyophilized reagents that are stable for over one year at room temperature. The system also utilizes Linear After The Exponential (LATE) PCR, a novel PCR amplification methodology that has excellent sensitivity and enables the reliable multiplexing of 10 – 20 target organisms.

The Bio-Seeq System was designed to provide "sample in, answer out" PCR testing for field veterinarians. Knowledge of PCR and molecular biology are not required and the sample preparation unit has been designed to work on a wide range of samples. Operation of the Bio-Seeq System is simple. The user obtains the sample to be tested, inserts the Reagent Pack into the SPU, and loads the sample into the open port on the SPU. The barcode on the Reagent Pack is scanned on the instrument. The instrument will then direct the user to insert the SPU into one of the thermocyclers, after which, the user can then enter sample information and presses "start" on the screen. The Bio-Seeq System will then carry out an automated sample prep, PCR and endpoint analysis. Results are displayed as positive or negative and if the identity of the pathogen can be determined, such as sub type or strain, that will also be displayed. We have successfully developed a portable PCR system that provides true "sample in, answer out" capability. Each of the core components, the instrument, SPU and LATE-PCR, have been demonstrated to be equivalent or have superior performance compared to existing technologies that are commonly used for laboratory-based PCR testing. The first assay to be launched on the Bio-Seeq Portable Veterinary Diagnostics System was the pan-Foot and Mouth Disease Virus (FMDV) assay, which has been evaluated with several strains of each of the seven FMDV serotypes, other viruses that cause vesicular disease, and samples spiked with purified FMDV RNA. Performance of the pan-FMDV assay, on laboratory PCR instruments, shows very good equivalence to the gold standard PCR assay currently in use at the Institute for Animal Health, Pirbright. Testing on the Bio-Seeq Portable Veterinary Diagnostics System has begun with initial result looking positive. The results of the full validation will be presented if available. The Bio-Seeq System enables veterinarians to perform laboratory-quality PCR testing in the field. This capability will assist veterinarians in treating infected animals in a more efficient and timely manner, as well as providing the ability to rapidly respond in outbreak situations, resulting in better outbreak management and containment. We believe that the right technologies, designed to be used by veterinarians and animal health professionals in the field can significantly improve animal health and welfare by extending the labs diagnostics capabilities to remote areas. This is even more important in the face of an outbreak.

POSTER: WEST NILE VIRUS: CHARACTERIZATION OF MONOCLONAL ANTIBODIES AND POTENTIAL APPLICATION IN LABORATORY DIAGNOSIS

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Key words: West Nile virus, monoclonal antibodies, diagnosis

Introduction

West Nile virus (WNV) is a single-stranded RNA virus, member of the Japanese encephalitis virus (JEV) serocomplex in the genus Flavivirus. It is transmitted in natural cycles between mosquitoes and birds, whereas humans and horses are considered dead-end hosts.

The purpose of this work was to produce monoclonal antibodies (MAbs) against WNV and to characterize them for the subsequent use in laboratory diagnosis.

Methods

For MAbs production mice were immunised against the WNV reference strain E 101 and against the WNV isolate 203204/08, originated from magpie (Pica pica) during WN epidemic occurred in northern Italy in 2008.

Hybridomas were screened by indirect ELISA against the homologous virus and by immunofluorescence (IF) in infected Vero cells. Immunoperoxidase (IPMA) was used to evaluate the reactivity profile of MAbs with different WNV strains belonging to lineages 1 and 2 and Usutu virus. Capability of MAbs to neutralize virus infectivity was investigated by a virus-neutralization test (VNT) performed with 100 TCDI50 of the homologous virus in Vero cells. Some MAbs were tested in indirect ELISA at Pasteur Institute to assess the possible cross-reaction with members of JEV serocomplex, such as DEN1, DEN2, DEN3, DEN4, YF and JE. Each MAb was also examined in Western blotting (WB) and indirect ELISA with a recombinant E protein DIII (E DIII) produced in E.coli.

Six MAbs (three neutralizing and three non-neutralizing) were conjugated with peroxidase and evaluated in all possible combinations as capture and tracer antibodies, to develop sandwich ELISA assays for WNV antigen detection. Competitive ELISA assays were designed to evaluate the competition between MAbs and sera of experimentally infected SPF chickens and of horses immunized with inactivated vaccine. MAbs reciprocal competition was also studied in order to determine whether they bind to overlapping epitopes.

Results

During the screening phase 37 MAbs (raised against WNV E 101) were selected; among them 29 resulted specific and reactive with all WNV strains tested. The remaining eight MAbs showed cross-reaction between the JEV serocomplex, with MAbs 2A8 and 4G9 exhibiting high reactivity also against Usutu virus. Thirteen MAbs showed neutralizing activity: 12 of these recognized the recombinant viral protein E DIII and competed with each other. All MAbs were negative in WB, suggesting the conformational nature of target epitopes.

MAbs 3B2 and 3D6 provided the best performance when used in ELISA assays for antigen or antibody detection. The characterization of MAbs raised against the WNV 203204/08 isolate is still ongoing; results will be presented at the congress.

Conclusions

MAbs produced offer wide applicability in various analytical methods. MAbs 3B2 and 3D6 (neutralizing and reactive against WNV E DIII) can be used in sandwich ELISA, IF or IPMA for WNV identification and in competitive ELISA to reveal anti-WNV neutralizing antibodies in equine and avian sera. Moreover, conjugated with peroxidase they can be used as tracer in an IgM-capture ELISA for detection of early antibodies and diagnosis of recent infection in horses. Since 3B2 and 3D6 showed no cross-reactivity with JEV serocomplex, they are useful for the development of antigen and antibody detection tools for WNV surveillance in areas as the Mediterranean basin, where Usutu virus is present.

POSTER: PHYLOGENETIC ANALYSIS OF EUROPEAN BAT LYSSAVIRUSES ISOLATED FROM BATS IN POLAND

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Key words: bat, EBLV, phylogenetic analysis, Poland

In Europe bat rabies is caused by two genotypes of lyssaviruses that are related to, but genetically and antigenically distinct from rabies virus (genotype 1) that is presented in terrestrial animals. These are EBLV type 1 and EBLV type 2. EBLV1 is mainly detected in Serotine bats (Eptesicus serotinus) while EBLV2 is recorded in Myotis species (M. daubentonii, M. dasycneme). Within EBLV 1 and EBLV 2, two independent lineages a and b are presented which differs in geographical distribution. Infected bats are recorded in Europe mainly in Denmark, the Netherlands and Germany. Transmission of lyssavirus between bats and terrestrial animals is rare but it is possible. Human rabies cases due to EBLV1 and EBLV2 were previously recorded and described.

In Poland the first incident of bat rabies was reported in 1972 in Serotine bat in Krakow. After many years, next cases in bats were diagnosed in 1985 (Gdańsk), 1990 (Kętrzyn) and in 1995 (Warszawa). Generally, till 1998, four rabid bats were diagnosed.

From 1998 when rabies case was diagnosed in Serotine bat in Bydgoszcz city an increase of the number of bats sent for rabies diagnosis to regional laboratories was reported. It was the beginning of wide passive (re-active) surveillance of rabies in bats in Poland. All bats with contacts with human or pets, with strange behavior, unable to fly are send to laboratory and checked for the rabies virus with FAT. From 1972 to 2009, 72 rabies cases in bats were diagnosed in Poland.

The main goal of the study was the phylogenetic analysis of European bat lyssavirus isolates collected from bat in different region of Poland. Bat isolates have been propagated in mice to get more material for examination. RNA was extracted directly from the rabid animal brains or mouse brains had been inoculated previously with bat brain homogenates. Brain samples were homogenized in the water for injection (10% suspension) and RNA extraction was performed with the commercial kit QIAmp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instruction.

The fragments of nucleoprotein gene have been amplified by using RT-PCR and hnRT-PCR. The first 400bp sequences of nucleoprotein gene isolates were aligned using ClustalW program with 22 European bat lyssavirus nucleoprotein sequences available in GenBank. Philogenetic trees was constructed using DNA maximum likelihood method and demonstrated using TreView software. The phylogenetic analysis proved that EBLV-1 isolates were divided into two clades. 36 out of thirty seven isolates were similar to EBLV-1 a type whereas one of them was similar to EBLV-1b type and made a cluster with known EBLV-1b isolates.

POSTER: PHYLOGENETIC ANALYSIS OF AFSV STRAINS AND ISOLATES.

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Key words: ASF, PCR, genotyping, p72

In the current study, we present the results of genotyping of African Swine fever strains, deposited in NRIVVaM museum obtained from different countries diring 1960 – 1984: Kongo, Spain, Portugal, Angola, Sao Tome and Principe, Uganda, France etc., and also we have analysed ASFV isolates, obtained during the outbreaks of the disease in Caucasus republics in Russia (Dagestan, Rostov, Krasnodar, Orenburg, Kabardino-Balkaria, Kalmykia, Chechnia, North Osetia), whose activity was 6,0-7,5 HAD50 ml-1. With the purpose of estimation of phylogenetic resemblance, partial sequencing of gene encoding major viral protein p72 was performed, according to Bastos et al (2003). Multiple alignment and data analysis were carried out using BioEdit 7.0. (Tom Hall Isis Pharmaceuticals) and Mega 4.0.[Kumar et al.] software. Phylogenetic analysis by UPGMA method showed, that strains, obtained from Europe, Kongo, Angola, Sao Tome and Principe, refer to I Genotype, strain from Mozambique - to V Genotype, and strains obtained from Uganda and Tanzania – to X Genotype. All ASFV isolates, collected during the outbreaks of the disease in Russia, were referred to II Genotype.

Thus, during our investigation, strains, deposited in NRIVVaM museum and field isolates from Caucasus republics were divided into four different genotypes.

POSTER: PHYLOGENETIC ANALYSIS OF RHDV ISOLATES FOUND IN THE RUSSIAN FEDERATION

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Key words: RHDV; RT-PCR; real-time RT-PCR; VP60.

Rabbit haemorrhagic disease (RHD) is a highly contagious infection of domestic and wild rabbits, caused by a virus of the genus Lagovirus within the Caliciviridae family that has a positive-sense, single-stranded RNA genome.

In the period from 2003 to 2009 RHD outbreaks were registered in more than 13 regions of the Russian Federation. Consequently, development of a RHD diagnostic method using the genome analysis and determination of phylogenetic relationships among the isolates circulating in Russia is quite relevant.

To identify rabbit haemorrhagic disease using RT-PCR analysis we selected specific primers complementary to a RHDV genome VP60 gene conservative region. Furthermore, real-time RT-PCR was also used to identify RHD virus (A. Gall et al., 2007).

Application of these methods promoted RHD virus RNA detection in blood and a number of various organ samples like liver, lung, heart, spleen, kidney, muscles, skin and hair of infected animals.

The nucleotide sequences of VP60 gene fragments of the isolates found in the course of RHD outbreaks in the Russian Federation were compared with sequences of the gene VP60 sites published in the GenBank database. The RHD virus isolates detected during the researches were found to belong to some three genetic groups. The strains "Manikhino-09" and "V-87" showing the least homology observed (87%) belonged to different clusters. One more group was composed of isolates as found in the outbreak foci of the Moscow region in the period from 2003 to 2008 (namely, in Balashikha, 24.01.2008; Gzhel, 11.10.2004; Manikhino, 30.10.2003) and a strain "Belgorodsky-03".

POSTER: BLUETONGUE SURVEILLANCE IN BELGIUM: SURVEILLANCE SYSTEM EVALUATION

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Key words: Bluetongue, surveillance, risk, sensetivity

Bluetongue (BT) is an arthropod-borne viral disease of both wild and domestic ruminants. Thus, the distribution of the virus is dependant on environmental and climatic conditions which allows the vector to accomplish its transmission cycle (Mellor et al., 2008). Following the devastating epidemic which occurred in the passed few years, the EU Legislation 1266/2007 modified by 1108/2008 prescribed the implementation of passive clinical surveillance, sentinel surveillance and a combination of serological and/or virological surveillance, as well as a targeted risk based monitoring. In this context, each country is recommended to adapt its surveillance system in order to meet the objectives and prove the efficacy of its system. Four major components characterize the Belgian BTV surveillance:

• Monthly sentinel (sero)surveillance
• Cross sectional serological survey ('winter screening' (WS))
• Clinical passive surveillance
• Export testing

The aim of the present study was to evaluate major surveillance components for BTV in Belgium and to determine the relative importance of each component in the context of freedom from disease.

The scenario trees, as illustrated by Martin et al. (2007), were used to conduct this study. Each step, from infection to detection, were taken in account in the scenario tree model (Risk zone defined by risk maps based on outbreak data; Vector season period; Animal species (Bovine, Ovine); Laboratory test sensitivity). Relative risks (based on outbreak data, as well as on empirical data), reference and sample population parameters were fitted. These parameters were fitted with appropriate distributions to account for the uncertainty and variability around their estimation. Herd and group level sensitivity were then calculated on the hand of these input parameters. Appropriate methods taking in account the selection probability to estimate the sensitivity at herd and unit level were used.
In turn, each component's sensitivity as well as the whole system sensitivity was computed, thus their ability to detect the disease, if the disease was present at the legal design prevalence. Modeling the accumulation of data from the different surveillance components over time, given a constant disease introduction probability, provided insight on the most effective component with regard to early detection. Preliminary results show the passive clinical surveillance has high component sensitivity, providing the level of disease awareness is high, such as it was the case during the second epidemic in 2007 in Belgium. When disease awareness or the probability of showing clinical signs are low, such as in a context of vaccination, other components such as sentinel surveillance might be more appropriate. The final results will be presented at the meeting.
As a main conclusion, the present study provided insight on the important elements to take in account when quantifying the sensitivity of whole surveillance system. Also these methods, accounting for different components, as well as risk factors in a surveillance program, proved to be a useful tool, for policy makers and health authorities when implementing disease surveillance in a country in order to meet the international standards.

POSTER: NOVEL DIVA VACCINE CANDIDATES GENERATED BY RECOMBINATION-MEDIATED MUTAGENESIS

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Key words: Pestivirus, infectious cDNA clone, recombination, mutagenesis

Infectious cDNA clones are a prerequisite for directed genetic manipulation of pestivirus RNA genomes to obtain modified constructs specifically designed as new live vaccine candidates for example to combat classical swine fever (CSF). In this study, a novel strategy was used to facilitate manipulation and rescue of modified pestiviruses from infectious cDNA clones based on bacterial artificial chromosomes (BACs). The strategy involved targeted modification by Red/ET recombination-mediated mutagenesis of viral cDNA genomes, cloned within BACs obviating the use of internal restriction sites. For proof of concept, a new pestivirus BAC, pBeloRiems26, based on a CSF vaccine strain was generated and specifically altered by modifying the E2 coding sequence using homologous recombination within E.coli DH10B cells. The resulting cDNA clone, pBeloRiems26_E2gif, contained the complete E2 from a heterologous pestivirus and a novel chimeric virus could be rescued from full-length RNA transcripts. Our results demonstrate that recombination-mediated mutagenesis of pestivirus BACs provides a useful tool for expediting the construction of recombinant pestiviruses which may be the basis of new marker vaccine candidates against CSF.

POSTER: DEVELOPMENT OF MULTIPLEX PCR ASSAYS FOR MOLECULAR TYPING OF BRUCELLA SSP.

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Key words: diagnostic, brucellosis, PCR, Real Time, Bruce-ladder

Brucellosis is a very important infectious disease caused by bacteria of the Brucella genus.
The illness caused by these bacteria is endemic in caprine, ovine (B.melitensis, B.ovis), bovine (B.abortus) and porcine (B.suis) livestock. This disease is a Zoonosis; the geographic distribution varies according with the infecting brucella species. The most important infection sources are goat, sheep, cow and pig, there also being wild sources (hares, etc). The transmission mechanisms are also very diverse such as placenta, cutaneously, mucous membranes, insects, etc...
The importance of this pathology determines the application or eradication plans at an official level in most of the countries in which the disease occurs. As in any other case, one of the pillars on which the eradication plan of this disease is based is diagnosis.
INGENASA in collaboration with the Navarra University (Spain) has developed two Multiplex PCR assays (Bruce-ladder) to use in porcine and bovine species respectively and a Real Time PCR assay. All these products can be used for molecular typing of Brucella.

INgene Bruce-ladder V is based on the amplification of a Brucella's DNA fragment using specific oligonucleotides and combines different techniques of molecular biology in order to guarantee the reliability of the test:

1.DNA isolation techniques based on Chomczynski and Sacchi protolocol and amplification techniques provide a high degree of sensitivity and specificity.
2.Cloning techniques allowing introducing exhaustive controls into each phase of test.

The kit allows detecting and differentiating the Brucella affecting farm animals: B. melitensis, B. Abortus, B. suis and B. ovis and the RB51, B19 and Rev1 vaccines.

On the other hand, INgene Bruce-ladder suis is a fast method for molecular typing of Brucella, from purified DNA. The assay consists of three steps:
1.Extraction of the genetic material from the sample.
2.Amplification of a concrete region of specific DNA of the bacteria.
3.Evaluation of the amplification product.

The kit allows detecting and differentiating the 5 different brucellae Biotypes affecting animals: B. suis Biovar 1 B. suis Biovar 2, B. suis Biovar 3, B. suis Biovar 4 and B. suis Biovar 5.

Finally, a third assay which is based on Real Time PCR is being developed for the molecular detection of Brucella, from purified DNA or DNA from an isolated colony, in any liquid or colony Brucella culture.

Moreover, an additional method consisting on addition of magnetic beads bound to antibodies specific to LPS of Brucella has been evaluated to increase the sensitivity of all these assays when milk samples are used,

CONCLUSIONS

These multiplex PCR assay (Bruce-ladder) has been developed for rapid and simple one-step identification of
Brucella. The major advantage of this assay over previously described PCRs is that it can identify and differentiate in a single step most Brucella species as well as the vaccine strains B. abortus S19, B. abortus

RB51 and B melitensis Rev.1. In addition, B. suis biovars1, 2, 3,4, 5 can be identified by B.suis new multiplex PCR.

Regarding the Real Time PCR based assay, it can be a useful and sensitive tool to detect the brucellae affecting farm animals (B. melitensis, B. abortus, B. suis and B. ovis and the RB51, B19 and Rev1 vaccines) when rapid results are required.

Finally, the addition of magnetic beads has result to be a good tool for increasing of sensitivity in samples in which the low amount of antigen may cause detection problems.

POSTER: FORMULATION OF A DRY POWDER INACTIVATED FMD ANTIGENS/CHITOSAN COATED PLGA WITH DNA VACCINE OF FMDV FOR NASAL DELIVERY

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Key words: FMD; Chitosan-coated nanoparticles; Vaccine, nasal delivery

FMDV is a picornavirus that affects artiodactyls,especially cattle and swine. Control of the disease in endemic areas is carried out with inactivated whole virus vaccines that induce a serotype-restricted,short-lived protection, making frequent revaccination a prerequisite in control campaigns. The purpose of this study was to prepare a dry powder vaccine formulation containing whole inactivated FMDV and a mucoadhesive compound suitable for nasal delivery. Powders containing chitosan, sodium alginate(SA), trehalose, and PLGA（Poly(Lactic-co-Glycolic Acid)）were produced by sprying-drying.

Investigation and screening of nasal delivery system by the different powder prescription will be undertaken. Guinea Pigs are intranasally immunized delivery to either the nasal cavity or lungs by the dry powder delivery devices (the exit diffuser of the device designed for human use, like a syringe). Serum,nasal secretions and saliva was obtained, antibody response and T cell response will be measured. Methods have been developed for measuring secretory antibody responses to FMDV in nasal secretions and saliva. Our preliminary experiment show that guinea pigs vaccinated inactivated FMD antigens developed specific cellular and humoral immune responses with detectable serum antibody levels of IgA and IgG.

Chitosan-coated nanoparticles (cNPs) were found to be best suited for transfection. The addition of chitosan to the surface of PLGA nanoparticles was found to increase the penetration of the encapsulated macromolecules in mucosal surfaces. Base on above mentioned facts, a modification method were used to formulate DNA vaccine adsorbed on chitosan-coated PLGA nanoparticle for FMD. The purpose of these studies was to enhance antigen-specific mucosal and systemic immune responses (nasal secretions, saliva, serum) of intranasal/pulmonary delivery of DNA vaccine (P12A3C/IL6-pCDNA), which may provide a quick protection against the FMDV initial infection. Interleukin-6(IL6) of bovine has been cloned, and subcloned into PMD-18 vector. P12A3C/IL6-pCDNA plasmid has been constructed. Chitosan-coated PLGA nanoparticle are fabricated by spray drying technique. Plasmid are adsorbed on chitosan-coated PLGA nanoparticle. Particle characterization is in the process of detection. Our data show that FMDV capsid can be assembled in the transfect cells successfully and two days after transfection, the expression of FMDV proteins by the sandwich-ELISA in BHK-21 cells were detected. Next, pulmonary/intranasal immunization will be undertaken.

POSTER: DEVELOPMENT AND VALIDATION OF A TRIPLEX-REAL-TIME PCR ASSAY FOR THE DETECTION OF BOVINE HERPESVIRUS TYPE 1 GENOMES

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Key words: BHV-1 bovine Herpes respiratory real-time-PCR

Bovine herpesvirus 1 (BHV-1), classified as an alphaherpesvirus, is a major pathogen of cattle causing different syndromes such as infectious bovine rhinotracheitis (IBR), infectious pustular vulvo vaginitis (IPV) in cows and balanoposthitis (IBP) in bulls. BHV-1 control programmes involve vaccination with inactivated or attenuated glycoprotein E (gE) -deleted marker vaccines.

A triplex real-time polymerase chain reaction (PCR) assay for detection of BHV-1 was developed. One assay detects a glycoprotein D (gD) specific sequence. To differentiate between wildtype BHV-1 and gE-deleted vaccines a second real-time PCR system for the detection of gE specific sequence was established. Finally for controlling DNA extraction as well as inhibition-free amplification an internal control (IC) based on the beta-actin gene was introduced.

For the selection of primers and probes published sequence information of BHV-1 was used and alignment-based primer and probe selection was supported by the software package Beacon Designer 2.06. The design of the gE specific assay was optimized according to the gE-gene deletions in the different commercial BHV-1 marker vaccines.

Using a series of 10-fold dilutions of purified BHV-1 strain "Schoenboeken" standard DNA, it could be verified that the individual gD and gE real-time PCR systems as well as the combined assay with the beta-actin IC amplified the standard DNAs in a linear fashion from 10e7 copies per reaction down to less than 10 copies per reaction with an PCR efficiency of more than 95%. Identical high PCR-efficiencies were calculated using DNA dilution series of different BHV-1 strains. Furthermore the sensitivity of the newly developed triplex-BHV-1 real-time PCR was compared to the reference method "virus isolation". Both a 10-fold dilution series of BHV-1 in cell culture medium and a collection of semen samples were investigated using virus isolation and the triplex BHV-1 real-time PCR.

For validation of the diagnostic specificity of the BHV-1 realtime PCR assay nasal swabs, semen and different organ material from BHV-1 negative cattle were analysed. These data as well as the results of specificity tests with genetically related herpesviruses will be presented.

POSTER: DEVELOPMENT OF A NOVEL SEROLOGICAL ASSAY FOR THE DETECTION OF RABIES VIRUS NEUTRALISING ANTIBODIES USING LENTIVIRAL PSEUDOTYPES

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Key words: Rabies, Pseudotype, Lyssavirus

Rabies virus (RABV) causes an acute encephalitis that is almost invariably fatal. Whilst effective vaccines and post exposure prophylaxis are available it is estimated that over 55,000 human fatalities occur worldwide each year. This figure is, however, believed to be a gross underestimation of the actual number of human deaths, as a disproportionate number of fatalities occur across the developing world where reporting systems are mainly absent. The detection of virus neutralising antibodies (VNA) is key to the evaluation of vaccination status following pre-immunisation. Current tests for RABV VNAs require expensive reagents and equipment, along with biosafety containment level 3 facilities, something not easily achieved in rabies endemic areas.

The use of lentiviral pseudotypes as a vector for gene therapy is well documented. These have proven to be highly sensitive yet flexible platforms which can be adapted to allow the evaluation of vaccines and antiviral drugs against highly pathogenic viruses without the need for high level containment facilities or great expense. We have adapted this technology to allow the determination of serum neutralising antibody titres against highly pathogenic rabies and related lyssaviruses. The pseudotype assay only requires a small amount of serum in comparison to the standard fluorescent virus neutralisation assay (FAVN), and the use of different reporter genes, such as green fluorescent protein, luciferase, or β-galactosidase, makes it possible for the assay to be undertaken at low cost in laboratories throughout the world.

G-protein sequences from viral isolates representing each lyssavirus genotype and the newly classified Eurasian strains were cloned and co-expressed with lentiviral gag-pol and different reporter genes. The pseudotypes infected a number of target cell lines and produced titres almost equivalent to VSV-G protein pseudotypes. To date, over 500 sera have been evaluated concurrently using genotype 1, CVS-11 live virus (FAVN) and pseudotype neutralisation assays. Comparison of antibody titres reveals a 96.4% sensitivity and 100% specificity (r=0.83, p<0.001).

Neutralisation assays using pseudotypes with glycoproteins from other lyssavirus genotypes suggest that they provide a greater sensitivity compared to the current virus neutralization tests and will therefore allow a more accurate determination of serological response to these highly pathogenic infections. Importantly for the use of this assay in countries where the cold chain cannot be maintained, CVS-11 pseudotypes were highly stable during freeze-thaw cycles and storage at room temperature. These results suggest that the proposed pseudotype assay is a practical, effective and robust solution for rapid lyssavirus serosurveillance wherever it is needed.

POSTER: SURVEILLANCE ON TBEV AND CCHFV IN TICKS COLLECTED ON HUNTED WILD ANIMALS IN THE EMILIA ROMAGNA REGION (ITALY) - PRELIMINARY RESULTS

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IZS DELLA LOMBARDIA E DELL'EMILIA ROMAGNA[1]

Key words: ticks, wild animals, TBE, CCHF

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. In particular, novel tick species and associated tick-borne pathogens may be transported over long distances by migratory birds or the ecological features may change the epidemiology of these viruses. Crimean-Congo haemorrhagic fever (CCHFV, genus Nairovirus, family Bunyaviridae) is one of the most widely distributed tick-borne diseases in the world, affecting people in certain areas of Africa, Asia, Eastern Europe and the Middle East. CCHFV is transmitted by ticks of the genus Hyalomma, in particular Hyalomma marginatum. 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. The aim of this study is to investigate if TBEV and CCHFV could be detected by Real Time PCR in ticks collected on wilds animals in the Emilia-Romagna region (E-R).
Methods
Ixodid ticks were collected from roe deer (Capreolus capreolus), wild boar (Sus scrofa), red deer (Cervus elaphus) and the European brown hare (Lepus europaeus)in E-R. Animals were sampled after being hunter-killed during the hunting season, which is different for every host species. Ticks were removed and identified following taxonomic standard keys. Nucleic acids were analysed from pools of ticks collected on the same animal. 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. Primers and probe used in Real Time PCR for the CCHFV detection are described in Wölfel et al. (2007). The research of TBE virus was carried out by using Real Time PCR with primers and probe described by Schwaiger e Cassinotti (2003).
Results
A total of 288 I. ricinus ticks (249 adults and 39 nymphs) were collected from seven provinces of the E-R. I. ricinus ticks was the most abundant ticks detected.
None of the 95 pools analyzed reveled the presence of TBE and CCHF viruses by Real Time PCR.
Conclusions
I. ricinus is the dominant species in roe deer, red deer and hare in Emilia-Romagna. TBEV was not detected during this preliminary 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 E-R and more extensive survey has to be conducted to assess this risk. As to CCHFV E-R is currently at low risk of CCHF introduction even if the virus has to be introduced by any possible route, because the principal vector of the disease (Hyalomma marginatum) has a very low density in the region. Nevertheless CCHFV is now present in the Balkans and generally circulates in nature unnoticed in an enzootic tick-vertebrate-tick cycle. Surveillance on these diseases, even without evidences of the viral presence in E-R, is worth being considered and needs to be focused on tick-host-pathogen interactions involving both antibody research in wild animal (mainly hares) and viral research in ticks.

POSTER: SURVEILLANCE OF BLUETONGUE IN POLAND

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Key words: bluetongue, surveillance, poland

Bluetongue is an infectious but non-contagious viral diseases of domestic and wild ruminants, transmitted by certain species Culicoides. Until 2006 the Central and northern Europe was free from BTV infection. Since 2006 serotype 8 of BTV was recorded in the Netherlands, Belgium, Germany, Luxembourg and France. Epidemic situation of BT in Europe demonstrated tendency to spread in Northern and Eastern direction and represent an epidemiological risk for Poland.

Uniform guidelines for monitoring and surveillance system for bluetongue disease in EU was developed. In accordance with Community legislation disease surveillance is very important not only to assess the actual risk posed by animals movement but also to confirm the absence of the diseases and early detection the entry of virus into BT-free countries (zones) .

Poland establish according to Commission Regulation (EC) no 1108/2008 programme of bluetongue monitoring which consist passive clinical surveillance as well as serological survey in order to detect incidence of seroconversion of 20% with 95% confidence.

The minimum number of samples needed was calculated using the computer programme WIN EPISCOPE 2.0. As geographical unit adapted the territory of the polish administrative unit - powiat.

Sera from animals were screened using commercially available ELISA kit Ingezim BTV DR from Inmunologia y Genetica Aplicada- Ingenasa (Spain). All sample were assayed in duplicate. From seroconverted animals blood sample were taken on EDTA in order to detect of viral RNA with PCR methode.

In 2009 totally 32 304 blood samples were examined. 16 405 and 15 983 samples from cattle and sheep respectively were tested. Of the 32 304 samples tested in Poland using ELISA test 70 sera were recorded as positive and the remaining 32 234 were clearly negative. Among positive samples 41 originated from cattle and 29 from sheep.

All positive sample were checked for the presence of BT virus RNA by rt RT-PCR. All of examined samples were detected as negative.

Serological surveillance and virological investigation of blood samples from sero- positive animals in 2009 demonstrated that the Poland status for BTV is the free country. Although the presence of BTV vectors was confirmed in Poland. The risk of transboundary contamination by vectors or animals recommend maintaining of actual BT control measures in Poland according to EU legislation.

POSTER: PHYLOGENETIC ANALYSIS OF RABIES VIRUS FROM INFECTIONS OF THE ENDANGERED ETHIOPIAN WOLF (CANIS SIMENSIS)

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VETERINARY LABORATORIES AGENCY[1]; WILDLIFE CONSERVATION RESEARCH UNIT[2]; ETHIOPIAN WOLF CONSERVATION PROGRAMME[3]; ETHIOPIAN WILDLIFE CONSERVATION AUTHORITY[4]

Key words: Ethiopian wolf; Canis simensis; Rabies; Phylogeny.

Rabies in Ethiopia is dominated by an endemic infection of domestic dogs (Canis Familiaris) and continues to be a public health threat and an economic burden. The Ethiopian wolf or simien jackel (Canis simensis) is the rarest canid in the world and endemic to the Ethiopian highlands. With fewer than 500 surviving animals its vulnerability to severe loss or extinction from disease or other stochastic events necessitate the need for monitoring and diagnosis of rabies outbreaks. There have previously been two confirmed rabies outbreaks in the Ethiopian wolf population. In 1991/2, 41of 53 adult or sub adult wolves in five adjacent packs died or disappeared. Molecular epidemiological investigation supported the hypothesis that domestic dogs were the most likely source of infection and act as the principle reservoir for the disease. There was a further outbreak in 2003/4 with rabies virus being detected in 13 of 15 brain samples obtained. Phylogenetic analysis again demonstrated that the outbreak had resulted from a spill-over infection of the rabies viruses endemic to domestic Ethiopian dogs. This outbreak was controlled with a parenteral vaccination campaign organised by the Ethiopian Wolf Conservation Programme (EWCP-www.ethiopianwolf.org). In October 2008 the discovery of more wolf carcasses and disappearances prompted another investigation, brain material was sent to the Veterinary Laboratories Agency (VLA) from four wolf carcasses for diagnosis. RT-PCR confirmed the presence of rabies virus and a virus nucleoprotein-coding sequence was generated for each sample. Brain samples from a further wolf were obtained in May 2009. Rabies virus was also confirmed in these samples using nested RT-PCR and real-time RT-PCR, sequence data was also obtained. Phylogenetic comparison of the five wolves from the 2008/9 outbreak demonstrated that the sequence obtained from these samples were identical. Comparison of the 08/09 sequence obtained from the 2003/4 outbreak revealed a 1.2% divergence with wolf sample but only 0.4% divergence with rabies virus derived from Ethiopian dog. This analysis supports this being a further epizootic of rabies within these wolf packs with the likely source being the domestic dog reservoir. This work continues to provide valuable information to the organisations involved in protecting this species. Further vaccinations on key wolf packs and the local domestic dog population have been introduced since the 2008/9 outbreak. However further phylogenetic studies in Ethiopia may better define the rabies virus reservoir in this country.

POSTER: TRACKING THE INFECTION IN REAL TIME IN A FATAL CASE OF HUMAN RABIES.

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VETERINARY LABORATORIES AGENCY[1]; UNIVERSITY OF LIVERPOOL[2]; ROYAL VICTORIA HOSPITAL [3]; MEDICAL COLLEGEOF WISCONSIN[4]

Key words: Rabies, Lyssavirus, Milwaukee Protocol, Virus, Diagnostics

Rabies is endemic in many regions of the world and causes a fatal encephalomyelitis following infection of the central nervous system. The causative agent, rabies virus, is a member of the genus lyssavirus, family Rhabdoviridae. Many island states, have actively excluded the disease, (including the United Kingdom). Others have taken action to control and eliminate rabies in dogs (North America) and terrestrial wildlife (western Europe). Across the developing world, the burden of rabies continues to fall particularly heavily where dog rabies remains a notable public health problem. It is estimated by the World Health Organisation that over 50,000 rabies deaths occur annually as a result of dog bites, although this figure is considered to be a conservative estimate.

It is widely accepted that there is no proven effective treatment once clinical symptoms of rabies are observed. However, a recent report has described a form of induced-coma that is suggested to have contributed to the survival of a teenager infected with rabies in Milwaukee, US. This was in a child infected with bat rabies, who unusually had anti-rabies antibodies at the time of presentation. Although subsequent attempts to repeat this therapy have failed for a number of human presentations with rabies, there have been promising developments that have led to 3 survivors from approximately 30 attempts across the globe (2004,Wisconsin,USA), (2008, Brazil), (2009, Equatorial Guinea). Whilst this sample size is limited, it does present an encouraging trend. However, the absence of suitable animal models to develop this approach has limited investigation into its mode of action or its refinement.

Here we report on a 37-year-old woman who presented with a history of exposure to rabies virus whilst in South Africa. She was initially admitted to hospital with a progressive encephalitis. Nuchal skin biopsy, analysed using a Rabies TaqMan© PCR, demonstrated the presence of rabies virus RNA. The patient had not received pre- or post-exposure prophylaxis. She was treated according to the "Milwaukee protocol", which failed to prevent the death of the patient. Rabies virus was isolated from cerebral spinal fluid (CSF) and saliva, and rabies antibody was demonstrated in serum (from day 11 onwards) and CSF (day 13 onwards). She died 35-days after being hospitalized. Samples at autopsy demonstrated the presence of rabies antigen, viral RNA, and viable rabies virus in the central nervous system, displaying a heterogeneous distribution throughout the brain.

POSTER: SPATIAL AND TEMPORAL DISTRIBUTION OF TOGGENBURG ORBIVIRUS IN SWITZERLAND

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Key words: epidemiology, prevalence, goats, sheep, cattle

The Toggenburg Orbivirus (TOV) has been recently described in Swiss goats (1, 2). This virus is proposed to be a new member of the Bluetongue (BT) serogroup.

Until now, only little is known about the epidemiology and the affected species. Furthermore, spatial and temporal distribution of this virus remained unknown.

Goat serum samples were nationwide collected during winter and spring 2008, prior start of vaccination against BTV. They were serologically examined using commercial available BT ELISAs. In holdings with seropositive goats, closer investigations were performed. If possible, all the ruminants were sampled (EDTA blood) and tested for antibodies (Ab) against BTV. Seropositive samples were additionally examined for the presence of BTV and TOV genome using positive BT-specific (3) and TOV-specific RT-qPCR. Moreover, samples send for routine diagnostic at the IVI between 2008 and 2009 were also examined for the presence of TOV.

Although the TOV index cases had been identified in flocks located north of the Alps, no additional TOV-affected herd was observed in this part of Switzerland. In contrast, in the south of the Alps (Canton of Ticino), the seroprevalence in goats was 49% at animal and 60% at herd level. In the eastern (Canton of Grisons) and western (Canton of Valais) part of the Swiss Alps, 15.2% and 10% of tested goats were found Ab-positive respectively. Within affected flocks up to 100% of tested goats were serologically positive. In each region of Switzerland, TOV-infected (RT-qPCR-TOV positive) goats were detected. 3.8% of sheep in contact with TOV-positive goats but no cattle were seropositive and none of these animals was positive for TOV or BTV genome.

For a retrospective look into the goat population, serum samples from 1998 were analysed. As the most affected region was the Canton of Ticino, 200 samples from this canton were assayed. Although countries surrounding Switzerland were at that time completely free from BTV (4), 20% of the samples scored positive in ELISA and RT-qPCR confirmed the presence of TOV.

By testing the goats within routine diagnostics, TOV genome was detected in one goat with clinical symptoms from the Alps and in three healthy animals imported from Germany.

In conclusion, TOV is widespread in Switzerland. High seroprevalences are found mainly the southern part of the country and to a lesser extent within the Alps. In the field, only goats seem to be infected by the virus, other ruminant species do not support an efficient replication of TOV. The observations made on sera from 1998 sustain the hypothesis that TOV was already present 10 years prior to its detection.

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POSTER: AN EXPERIMENTAL INFECTION WITH SWINE VESICULAR DISEASE VIRUS IN PREGNANT SOWS TO DETERMINE THE DURATION OF PASSIVE IMMUNITY IN PIGLETS

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Key words: Swine Vesicular Disease, SVD, Passive Immunity, piglet

Swine Vesicular Disease (SVD) is a vesicular condition of pigs caused by an Enterovirus of the Picornaviridae family. In some circumstances SVD may resemble Foot-and-Mouth Disease (FMD) and member states which are known to have the disease must carry out surveillance and eradication programmes. In these programmes, the knowledge on the endurance of maternally derived antibodies is essential in case of an epidemic of SVD and may help to implementation proper control measures. The purpose of the study was to provide information on the duration of passive immunity in piglets born from SVDV infected sows.

To establish the duration of maternal immunity in piglets 4 pregnant SPF Large White x Landrace sows were infected with SVDV (0,5 ml/108.70). Pregnancy was established by progesterone detection (competitive chemiluminescent enzyme immunoassay). Sows at early pregnancy were inoculated in the bulbs of the heel, the isolate used for the experimental inoculation was originally obtained from a herd infected during the 2006 - 2007 SVD epidemic in Lombardy. After experimental inoculation, the sows were checked daily for clinical signs and blood was collected weekly for serological testing. Faeces samples were taken from the sows daily during the first 14 days post inoculation (dpi) and weekly afterwards.

At 3 dpi, all four sows were lame, anorexic and reluctant to move, oedema was observed at the coronary bands. Five dpi vesicular lesions were observed on the feet. All sows had vesicular lesions on the feet and on the snout, the animals recovered 22 dpi. Two of the 4 pregnant sows did not carry on gestation, the remaining 2 gave birth to 14 piglets, 1 of which died.

Blood samples were collected for serological testing from each piglet 3 days, 1 week and then weekly after farrowing. Before each blood sampling the weight of the piglets was recorded. Blood volume (BV) was estimated from the value of body weight (W) using the formulae of von Engelhardt (1966): up to 25 kg, BV=9.5xW -0.068 and above 25 kg, BV=17.9xW-0.27 . The antibody titers detected were then corrected for increases in blood volume according to the formula by Francis and Black (1984). Decay rates were estimated by linear regression after log transformation of the antibody titers. Mean and median antibody survival times were estimated by the Kaplan and Meier method. In piglets, neither clinical sigs of SVD nor evidence of infection were detected.

Virus isolation and PCR were performed to detect the presence of SVDV in faeces: all sows were positive at day 3 and negative 35 dpi. For antibody detection in sows and in piglets, competitive ELISA and isotyping ELISA were used, the sows were seropositive for IgM isotype antibodies 7 dpi. All piglets were seropositive for IgG isotype antibodies 3 days after farrowing and remained positive until a maximum of 193 for the first litter and 149 days for the second one. The half-lives of the maternally derived antibodies were estimated at 45 days (CI 95% 42-50) for the first litter and 36 (CI 95% 31-42) for the second one and the median survival times were respectively 176 and 161 days.

In order to establish maternal immunity duration in piglets 4 pregnant sows were infected with SVDV, the aim was to obtain at least 30 piglets to use in the experiment. Two of the infected sows did not carry on pregnancy and to reach the goal it was necessary to repeat the experiment. At present a second group of animal is under study.

POSTER: PANDEMIC H1N1 VIRUS OUTBREAK IN A CAT COLONY IN ITALY

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Key words: Pandemic H1N1 virus, cat colony, Italy

Influenza A viruses could infect a wide range of animal species but are typically host specific. However, whole viruses may occasionally be transmitted from one species to another and genetic reassortment between viruses from two different hosts can produce a virus capable of infecting a third host. During April 2009, a novel H1N1 (H1N1v) influenza A virus was identified as the cause of the present flu pandemic. The infection and transmission of the H1N1v have occurred primarily among humans but occasional transmission from infected people to susceptible animals has been documented. Some cases of H1N1v have been recently reported in cats but regarded domestic animals in close contact with family members. This paper report a confirmed outbreak of H1N1v in a cat colony.

From November 2009, a cat colony with approximately 90 cats experienced a high mortality rate. Seven adults and 18 young cats were found dead whereas other animals showed anorexia, depression, respiratory and gastrointestinal symptoms. Two adult cats were submitted to the diagnostic laboratory on 7th December. The observed macroscopic lesions were acute pneumonia with purple areas of consolidation and severe enteritis. Lung homogenates were screened for the presence of influenza A viruses by real time RT-PCR (3) and for the (H1N1)v by real time RT-PCR according CDC protocol. Two cats resulted positive to the type A influenza as well as for (H1N1)v assays. Virus isolation were obtained by inoculation onto SPF chicken embryonated eggs. To detect the most common feline viruses, samples were applied on CRFK, A-72 and VERO cells and gave negative results.

Partial sequencing of HA (1271bp) and complete sequencing of NA genes were performed on the two strains (A/Cat/It/304678/1/09 and /2) using primers described by CDC protocol and Hoffman(1) respectively. Maximun parsimony phylogenetic trees were created using MEGA4 upon multiple sequence alignments by ClustalW. Genomic analysis confirmed a very high similarity for HA and NA genes to the H1N1v circulating in humans.

Twenty sera were collected on 13th January from 18 adult cats and one dog living in the colony and one domestic cat living in the house of the colony owner. Serologic examinations were performed by a Mab based competitive ELISAs for detection of antibodies (Ab) against nucleoprotein A (NPA-EL) (2). Sera were further analyzed by HI test using the following antigens (Ag): A/Cat/It/304678/2/09 pandemic H1N1, A/Sw/Fin/2899/82 H1N1, A/Go/It/296426/03 H1N1, A/New Caledonia/20/99 H1N1. Sera were also tested for detection of Ab against FeLV and FIV by ELISA tests.

Of these 13 sera (11 from stray cats, one dog and one domestic cat) resulted positive to NPA-EL and to HI test with the homologous antigen (HI titres from 1/20 to 1/340). No cross reactions were observed by HI test.

Other tests preformed resulted negative.

This is the first case of pandemic H1N1v involving several animals. The family of the colony owner reported to have not clinical signs of influenza in this period, however in order to exclude an asymptomatic form, serological examination of family members is in progress (ISS, Rome). Based on these results and considering that the major part of positive animal are stray cats it could be suppose cat to cat transmission. This case emphasizes the need to reevaluate companion animals as potential reservoirs or intermediate hosts for reassortment of influenza virus.

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POSTER: APPLICATION OF GENOME ANALYSIS METHODS FOR IDENTIFICATION OF NAIROBI SHEEP DISEASE AGENT.

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Key words: Nairobi sheep disease, RT-PCR, sequencing

Nairobi sheep disease (hereafter NSD) is a transmissible acute infection affecting sheep, goats and humans, characterized by high temperature levels and hemorrhagic gastroenteritis, and occurs in Africa and India.

Our work was aimed at selection of oligonucleotide primers to be used to detect NSD virus genome with nested RT-PCR. To select the primer through a software program Bio Edit 7.0., we analyzed some nucleotide sequences of NSD virus (namely, strains NSD 708 and Ganjam G619) genome RNA S-segment conservative sites available in Gen Bank (the access codes are AF 504293.1 and AF 504294.1, respectively). Using a program Oligo 4.0., we calculated 2 primer pairs flanking fragments of 930 and 360 bp in size on the virus genomic RNA. The nucleotide sequence of the primers were as follows: NSD F1 5' - TaT gCT TCT gCC TTg gTT g -3', NSD R1 5' - ATC CgA TTg gCA gTg AAg -3', NSD F2 5' - AgA gCA CAT TgA CTg ggC -3', NSD R2 5' - gCC TTC CAA AgC CAg Tag -3'.

To test the primers selected, we carried out amplification of NSD virus genomic RNA, vaccine strain "MM/K-05". Thus, the reverse transcription was conducted at 50oC using an enzyme MMLV-reverse transcriptase, and the obtained cDNA was amplified in a two-round PCR with primer annealing temperature of 61oC. The PCR fragments obtained in the both rounds were sequenced by the dideoxy – mediated chain – termination method and sequences determination was performed, using genetic analyzer ABI PRISM 3130 (Applied Biosystems, USA). The analysis of the obtained sequences with a BLAST program available on site NSBI (http://ncbi.nlm.nih.gov) showed, that these fragments have 88 and 86% identities to genomic RNA of strains NSD 708 and Ganjam G619, respectively.

To determine the specificity of the primers selected, a nested RT-PCR was carried out, RNAs of a range of viruses like Rift valley fever, Crimean-Congo hemorrhagic fever and Akabane disease viruses being used as heterologous samples . The assay results were read for the PCR second round, with just a single band corresponding to the fragment of 360 bp being observed on the electrophoregram.

The assay sensitivity rate was determined through 10 fold serial of the virus strain "MM/ K-05", its initial titer of 5.8 lg TCID50/cm3. The highest dilution titer for the virus with RNA detectable in the assay second round was 1.8 lg TCID50/cm3.

Thus, the oligonucleotide primers to be used to detect NSD virus genome with nested RT-PCR were designed. Besides, the PCR conditions optimization, the determination of the specificity of the primers selected and the assay sensitivity rate were performed.

POSTER: DETECTION OF AKABANE VIRUS GENOME WITH REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

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Key words: Akabane virus, RT-PCR.

Akabane virus is a member of Simbu group in the Bunyavirus genus of the family Bunyaviridae, was first isolated from Aedes vexans and Culex triaeniorhynchus in 1960 and 1964, respectively, in Japan. The virus induces abortions, premature or still births and congenital diseases characterized by arthrogryposis, hydroencephaly or microencephaly in cattle, sheep and goat.

This work was aimed at development of a method for Akabane virus genome detection using a reverse transcription polymerase chain reaction (RT PCR) assay. Analysis of the segment nucleotide sequences available in GenBank showed that a nucleotide sequence of RNA S-segment might be suitable to differentiate the Akabane virus genome from other related bunyaviruses. Consequently, a pair of primers (F2-R2) complementary to a conservative site of a gene of a nucleocapside protein N located on the genomic S-segment, which was developed by J.K. Lee et al. in 2002, was selected. The primer nucleotide sequences were as follows: F2 - 5'-ACC AGA AGA AGG CCA AGA TG-3' and R2 - 5'-CAC ACG GTG CAT GTC GAT AA-3'.

As a positive control a 20% lyophilized brain suspension prepared from white mice infected with Akabane virus strain "B8935" was used. As negative controls some lyophilized brain suspensions from Nairobi disease or Rift valley fever virus-infected white mice were used.

The nucleic acid isolation was carried out using nucleosorbtions on a non-organic sorbent under the method described by Boom R. et al., 1990. The obtained RNA preparations were used to set up the reverse transcription assay. The reverse transcription was conducted at 50 oC for 30 minutes using MMLV-reverse transriptase and the primer R2. The resulted cDNA was amplified in 25 PCR cycles using Taq-polymerase and the primer pair F2-R2 at the following temperature regime: 20 sec at 94 oC (DNA denaturation), 30 sec at 58 oC (the primers annealing) and 30 sec at 72 oC (elongation).

Electrophoresis of the PCR products in 2% agarose gel showed that only a fragment of 365 bps in size was amplified on the band corresponding to Akabane virus. Sequencing of the fragment with a terminating triphosphate analogues method using a genetic analyzer ABI PRISM 3130 (Applied Biosystems) demonstrated its 99% homology to the strain "B8935" nucleocapside protein N gene sequence (AF AB000853.1)

Thus, the primers F2-R2 are quite suitable to differentiate Akabane virus from other bunyaviruses.

POSTER: RIFT VALLEY FEVER: A MULTI-AGENCY TEST OF FLORIDA'S RESPONSE TO AN HYPOTHETICAL INTRODUCTION TO THE USA.

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Key words: Rift Valley Fever; multi-agency exercise; Florida, USA

Rift Valley fever (RVF) is a zoonotic viral disease affecting ruminants and people. It was first recognized, as the name suggests, in the Rift Valley of East Africa, but it is now recognized to be an endemic disease affecting most of Sub-Saharan Africa and Madagascar. Since 1970, it has shown an ability to spread northwards to cause sporadic epidemics in Egypt, Yemen, and Saudi Arabia. It is considered an emerging pathogen. The disease in most humans is characterized by fever and malaise, but a small percentage of patients develop either fatal encephalitis and/or generalized hemorrhage. In ruminants, the disease is particularly severe in lambs and calves, which die of generalized hemorrhage; pregnant animals commonly abort. RVF virus is transmitted by several species of mosquito, but human infection is often associated with the slaughter of infected animals for food. Experimental studies have established that species of mosquito found in the USA can biologically transmit the virus, and the RVF virus is classified as a select agent. It is feared that RVF virus, if introduced accidentally or through bioterrorism, could have an even greater impact than West Nile virus on the animal and human populations of North America. There is also concern that RVF could spread to Europe.

Until this exercise, there had been no large scale exercise focused on RVF in the USA, nor apparently in Europe. In partnership with the State of Florida's Emergency Operations Center, a multi-agency exercise (State and Federal) was organized to test Florida's response to a simulated outbreak of RVF in both ruminants and humans. The 3 day exercise involved approximately 100 professionals in November, 2008. The outbreak was characterized by increased calf mortality and mild human cases on a large ranch in South Central Florida. A case of hemorrhagic fever in a child in Southern Florida was connected to the slaughter of goats. A case of retinitis diagnosed in a veterinary student in Northern Florida was connected to the initial introduction of the virus. The introduction of virus to Florida was linked to a bioterrorism event. The presentation will describe the scenario, the Incident Command System, and discuss the difficulties met by the different agencies in combating the spread of the virus and determining its origin.

POSTER: PHYLOGENETIC ANALYSIS OF SLOVAK AND CZECH PRRSV ISOLATES IN ORF7 REGION

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Key words: PRRS virus, phylogenetic analysis, ORF7, European genotype

Introduction and Objectives
Two genotypes of PRRSV, European (EU) and North American (NA) were recognized. The EU genotype was considered to be less variable. However, recently high genetic variability was found in Eastern European strains (Stadejek et al., 2006). As a result the division of EU genotype into three subtypes was proposed: a panEuropean subtype 1 and East European subtypes 2 and 3 (Stadejek et al., 2008). The Eastern European strains also exhibited extreme ORF7 size polymorphism, ranging 375 - 393 nt (Stadejek et al., 2006). All previous studies characterized nucleocapsid protein as one of the most conserved PRRSV proteins (Drew et al., 1997). The aim of this work was to genetically characterize PRRSV isolates from two countries of Central Europe (Slovakia and Czech Republic) by comparing ORF7 sequences and to analyze their relationship to the other European and American isolates.

Materials and Methods
Ten isolates were obtained from three different farms in Western Slovakia and thirteen isolates from ten different farms in Czech Republic. The nested RT-PCR was used to amplify ORF7 region with primers according to Drew et al. (1997). Purified PCR products were sequenced using automatic sequenser ABI PRISM. Entire ORF7 sequences were assembled using SeqMan and MegAlign program from DNASTAR package.

Results and Discussion
The nucleotide sequences of 10 Slovak and 13 Czech PRRSV isolates were aligned and compared to each other and to selected European and American strains deposited in GenBank. The phylogenetic analysis in ORF7 region revealed that Slovak isolates were clustered into three groups different phylogenetic groups, showing herd specific isolates. Czech isolates were clustered into several genetic groups. All Slovak and Czech isolates were typed as EU genotype, subtype 1, according to Stadejek et al. (2006). However, the extreme ORF7 size polymorphisms – 399 nt (132aa) was observed in two Slovak isolates – the longest described so far. Until now, the largest nucleocapsid protein described by Stadejek, et al. (2006) was composed of 130 amino acids (393 nt) in the Belarusian PRRSV sequences, belonging to subtype 2. The sequences of other Slovak and all Czech isolates had an ORF7 size 387 nt, identical with European reference strain Lelystad and other European strains.

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POSTER: CANINE INFLUENZA: AN EMERGING DISEASE OF INCREASING COMPLEXITY AND CONCERN

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Key words: canine influenza; H3N8 equine influenza;H3N2 avian influenza

In 2004, an H3N8 influenza virus was recognized in dogs in the USA as the cause of respiratory disease and, in some cases, death. The virus was capable of dog- to- dog transmission. Initially recognized in racing greyhounds, the virus subsequently spread into the pet dog population. Characterization of the virus revealed that it was closely related to the H3N8 equine influenza viruses of the Florida sublineage that had emerged in horses around 2002. Sequencing of the genes indicated that the virus had acquired several mutations that presumably had allowed it to adapt to dogs. How the virus jumped species from horses to dogs has not been established. Canine influenza is now endemic in many parts of the USA. Retrospective studies indicated that an H3N8 equine influenza virus had caused respiratory disease and death in hounds in the UK in 2002, but in this case sustained transmission between dogs was not established. In 2007, during an equine influenza epidemic in Australia, the H3N8 virus spread from horses to cause respiratory disease in dogs, but similar to the 2002 outbreak in the UK, the virus did not establish serial transmission in dogs.

Prior to the discovery of canine influenza in the USA in 2004, dogs were thought to be refractory to infection with influenza viruses. In 2007, a second type of canine influenza was confirmed in South Korea. This time the causal virus was an H3N2 avian influenza virus. This virus also has the ability to be serially transmitted in dogs.

In addition to H3N8 equine influenza viruses and H3N2 avian influenza viruses, infections of dogs with H5N1 avian influenza virus and pandemic H1N1 influenza virus have been reported, but serial dog-to dog transmission has not occurred with either virus.

This presentation will review the recent history of influenza in dogs and pose the question whether these recent reports signify that the dog may in the future emerge as a source of zoonotic influenza viruses.

POSTER: AFRICAN SWINE FEVER VIRUS - INFLUENCING THE DANCE OF LEUCOCYTES TO THE TUNE OF CHEMKINES?

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Key words: African swine fever virus chemokines

African swine fever (ASF) is caused by a large double stranded DNA virus (ASFV), which is capable of inducing a fatal haemorrhagic fever in domestic pigs and European wild boar. ASF is endemic in many African countries and in Sardinia and in 2007 was introduced to the Trans Caucasus region and Russian Federation.

The main target cells for ASFV replication in vivo are monocytes and macrophages which have a key role in activating and orchestrating the innate and acquired immune responses of the host, partly through the ordered expression and secretion of chemokines. An ASFV gene has already been identified that inhibits host signalling pathways and transcriptional activation of host immunomodulatory gene transcription and other genes of unknown function are likely to modulate the host response to infection.

In this study we examined the chemokine response in vitro and in vivo following infection with ASFV. Groups of pigs were infected with the highly virulent Benin 97/1 isolate which causes acute disease and high mortality and the low virulent OUR T88/3 isolate which causes unapparent persistent infections with low viraemia. Changes in transcript levels for selected chemokine ligand and receptor genes were measured in peripheral blood leucocytes by quantitative reverse transcriptase PCR. This identified genes which increased in transcription level by similar amounts following infection with both isolates and others which varied in amount. The amounts of chemokines in plasma and supernatants from infected cells were also measured using functional chemotaxis assays.

The results indicate differences in chemokine response induced by ASFV isolates of high and low virulence which may be important in pathogenesis and induction of an effective immune response.

POSTER: ANTIVIRAL PEPTIDES TARGETING DYNEIN DEPENDENT TRANSPORT EXPLOITED BY AFRICAN SWINE FEVER VIRUS

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Key words: Antivirals, African swine fever virus

Several viruses target the microtubular motor system at the early steps of the viral life cycle. African swine fever virus protein p54 hijacks the microtubular dependent transport in the cell by a high affinity interaction with a dynein light chain (DLC8). This is a 25 Kd protein externally exposed in the ASF virion. We investigated the potential of short peptides targeting the interaction motif to disrupt this protein-protein interaction. A set of inhibitor peptides targeting this sequence were shown to bind and compete for binding of the viral p54 protein with dynein by Nuclear Magnetic Resonance spectroscopy. Using this approach, we now report short peptides that inhibit viral growth. DYNBLK peptide sequences were found to inhibit viral reproduction at micromolar concentrations as shown by reduction of infected cell numbers, viral protein expression and virus production. Our results provide the first evidence that this binding site could be a valid target for antiviral therapy.

POSTER: VALIDATION OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR DETECTION OF SWINE VESICULAR DISEASE VIRUS IN EXPERIMENTAL AND FIELD SAMPLES

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Key words: Key words: RT-LAMP, Swine Vesicular Disease Virus, diagnosis, field samples

Loop mediated isothermal Amplification (LAMP) is a gene amplification technique characterized by the use of six different primers designed to recognize eight distinct regions on a target gene. The protocol is simple and rapid, being amplification completed in one hour under isothermal conditions. The product can be visualized by gel electrophoresis as a ladder-like pattern, by naked eye as turbidity or color change upon addition of a fluorescent intercalating dye.

In this study we report on the evaluation of the one step reverse-transcriptase LAMP (RT-LAMP) protocol, developed in the framework of the European Project LABONSITE, for the detection of Swine Vesicular Disease Virus (SVDV) in both experimental and field samples; the RT-LAMP target sequence is a portion of 163 bp of the 3D gene.

SVD is included in the list of diseases notifiable to OIE and a surveillance and eradication program is carried out in Italy where the disease is present. One requirement of the national plan is virological surveillance in faecal samples. Our laboratory, acting as the Italian Reference Centre, has a wide collection of SVDV isolates and field samples for a complete validation of new assays. RT-LAMP performances have been evaluated by comparison with the one-step reverse-transcriptase PCR (RT-PCR), routinely applied as screening test. In both methods the same viral RNA extracted by OIE standard protocol was used.

The assay specificity was evaluated on porcine enteric viruses representative of 10 serotypes and on 80 negative faecal samples from field, all scored negative with both methods. Concerning analytical sensitivity, the detection limit of LAMP corresponded to 180 TCID50, compared to 18-180 TCID50 of the one-step PCR.

Diagnostic sensitivity was initially studied using samples from 11 experimentally infected pigs. Faeces collected at different time points were analysed in parallel by RT-LAMP, RT-PCR and virus isolation (VI). From seven to twelve days post-infection (dpi) the three assays showed maximum concordance level with all or the majority of positive samples; at three and later than 14 (dpi) RT-LAMP showed to be less sensitive than RT-PCR but consistently more sensitive than VI.

Furthermore, diagnostic sensitivity was so far evaluated on 43 field faecal samples, positive by RT-PCR, from different SVD outbreaks occurred in Italy in the period 2002-2009. From 2004, members of two genomic sub-lineages have been present simultaneously in Italy: one comprises viruses typically evolved in Italy since 1992 (Italian sub-lineage) and the other includes viruses detected in Portugal in 2003/2004 and later also in Italy (Portuguese sub-lineage).

RT-LAMP showed a significant difference in ability to detect isolates of the two sub-lineages. In fact, RT-LAMP detected 28 out of 32 samples of the Italian sub-lineage, with a diagnostic sensitivity of 87.5% compared to one-step PCR, confirming the observation of a slightly lower sensitivity of RT-LAMP, as previously deduced from experimental samples. In contrast, only three out 11 samples (27%) of the Portuguese sub-lineage scored positive. This difference could be mainly due to more mismatching within primer sequences identified in isolates of the Portuguese sub-lineage compared to isolates of the Italian one.

Analysis of more field samples is in progress to further support and to confirm these preliminary results.

POSTER: TREATMENT WITH SPECIFIC IGY CONTROLS SEASONAL AND PANDEMIC INFLUENZA VIRUSES WITHOUT INTERFERING WITH THE DEVELOPMENT OF ADAPTIVE IMMUNITY

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Key words: Influenza, IgY, Protection, Immunological memory

Present strategies to deal with the threat of the flu pandemic include the quarantine of infected people and animals, the use of antiviral drugs as neuraminidase and ion channel inhibitors, and vaccines developed by pharmaceutical companies under government contracts. These approaches have met up with difficulties and we aim to develop a new form of oral immunotherapy using chicken IgY antibodies. Such antibodies are easy and cheap to produce, are well tolerated in humans, and one can utilize commercial laying hens that are available globally.

Laying hens immunized with H1N1, H3N2 or H5N1 inactivated flu viruses mount a strong immune response, and IgY purified from egg yolks had high titers against the homologous viral HA antigen. Intranasal administration of IgY anti- H5N1 to mice protected against lethal infection of highly pathogenic H5N1 in 100% of the animals if administered at the same time as or one hour prior to challenge. Interestingly, IgY against H5N1 also blocked viral invasion by H1N1 (PR8) during in vitro and in vivo challenges, demonstrating that IgY to H5N1 can indeed cross protect against infection with H1N1. Furthermore, mice initially protected by intranasal administration of influenza-specific IgY antibodies still developed protective immunological memory to the virus infection measured by serum antibodies and T cell responses.

Thus IgY anti flu can be used to control influenza viral infection without interfering with the development of adaptive immunological memory and the heterogeneity of the IgY response to viruses in chickens generates antibodies with broadly protective activity against influenza viruses.

POSTER: MONITORING AND SURVEILLANCE OF BLUETONGUE IN THE RUSSIAN FEDERATION

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Key words: bluetongue, monitoring, RT-PCR, ELISA, sequencing

In view of the complicated epizootic situation for bluetongue (BT) in EC countries, Ministry of Agriculture of the RF developed some special directives for order of cattle importation to the RF from EC countries. In accordance with the above directives NRIVVaM carried out examinations of the whole cattle imported. In this connection, the following scheme of cattle examination was developed that included biomolecular, serological and virological investigation methods aimed at bluetongue detection. Thus, on entering the territory of the RF, all the animals were subject to 30-day quarantine in the course of which materials for examination (namely, blood and sera) were collected on the quarantine days 1 to 4 and 26 to 30. All the samples were tested by PCR and also serologically for the presence of the antibodies in CFT and ELISA. In the case any positive result was obtained through one of these methods, the animal was removed from the other herd and directed for destruction, while the animal group where the sero- or PCR-positive animals had been detected was further examined using the above methods until a duplicate negative result is obtained.

The biomolecular methods as applied in NRIVVaM included the following: duplicate examination of every sample using one-tube PCR in-house kit (fragment 10 of a viral genome segment) and qRT-PCR in-house kit (fragment 5 of the genome segment) for routine investigations, and also a nested RT-PCR that provides bluetongue virus RNA segment fragment 7 detection (S. J. Anthony, 2004) and a qRT-PCR with the segment fragment 5 as a target (J.F. Thoussaint, 2007) were additionally applied to confirm the positive result. The serological methods included examination of every received sample in CFT with subsequent ELISA (namely, a kit for anti-VP7 antibody detection by competitive ELISA or a kit for early detection of anti-VP7 antibody in bovine, ovine and/or caprine sera or plasmas (ID VET)) if any positive result is observed.

Within the whole period of the researches this schedule of BT control among the imported livestock allowed detection of as many as 204 samples that were positive by PCR/ELISA. Later on, virus isolations with chicken embryos (A. Clavijo et al.) and cell cultures (like Vero, BHK or PSGK) were performed. The isolated viruses were investigated in serotype-specific RT-PCR (P.P.C. Mertens et al., 2007) and neutralization test, from which the isolates were classified to serotype 8. More detailed research of the obtained isolates aimed at BTV genotyping was carried out through determination of the primary nucleotide sequence of the virus genomic segment 2 and full-length segments 7 and 10, which confirmed that the found isolates were homologous to BTV serotype 8.

Thus, from the results of the monitoring and control for BT as conducted in the RF in the period from 2007 to 2009, BTV some carriers were detected among various livestock herds imported. We carried out virus isolation, identified the serotype of the found isolates and analysed the nucleotide sequences of BTV genome segments 2, 7 and 10.

POSTER: MOLECULAR CHARACTERIZATION OF ASF VIRUS STRAINS AND ISOLATES USING ANALYSIS OF KP86R AND B602L GENES VARIABLE SITES

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Key words: ASF, PCR, genotyping, B602L. KP86R

In the course of the work a range of ASF virus strains deposited in SRI NRIVVaM strain collection that had been received from various countries in 1963 to 1984 like Kongo, Spain, Portugal, Angola, Sao Tome and Principe, Uganda, France et al., and also some isolates detected during the outbreaks as occurred in the RF in 2007 to 2009 in Dagestan, Rostov, Krasnodar, Orenburg, Kabardino-Balkaria, Kalmykia, Chechnia and North Ossetia were used.

We carried out polymorphism analysis for amplified fragments of genes B602L and KP86R variable sites according to Gallardo et al (2006). As a result of the gene KP86R site amplification all the ASF virus isolates and strains examined were divided into three groups. The strains originating from Angola (1982) and Sao Tome and Principe (1979) had the amplified fragment of 250 bps in size, the strains from France (1964), Spain (1984), and Sao Tome and Principe (1979) had the amplified fragment of 320 in size, while on the matrices of strains from Mozambique (1987), Bartlett & Tanzania (1967), Uganda and the Russian isolates not any specific PCR product was amplified.

The gene B602L is the most variable ASFV genomic site that is due to inclusion or loss of some terminal inverted repeats within this fragment. Hence, its analysis provided more detailed differentiation of the ASF virus strains and isolates examined. The fragments obtained through gene B602L amplification were found to vary for each of the strains and isolates we have examined.

At the same time it should be noted that any investigations into phylogenetic relationships of various ASFV strains and isolates are to be primarily applied together with the data obtained through analysis of nucleotide sequences coding for basic proteins p72 and p54, and also amplified fragment size polymorphism for the genomic variable sites.

POSTER: DETECTION OF SWINEPOX IN A GERMAN TWO SIDE PRODUCTION UNIT - A MOLECLUAR BIOLOGICAL AND ULTRASTRUCTURAL STUDY

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Key words: Swinepox, PCR, ultrastructure

Swinepox virus infection results in an acute, mild or subclinical course and is characterised by typical poxvirus skin lesions in affected pigs (De Boer 1975: Arch. Virol. 49:141). Morbidity of Swinepox (SwP) may approach 100% in young stock up to 4 month of age whereas adults generally develop a mild, self-limiting form of the disease.

Between Nov. 2008 and March 2009 piglets in a German two side production unit showed red epidermal efflorescences. The lesions passing through a papula-, pustule- and finally crust-stage, were distributed uniformly over the entire bodies of the piglets. Vesicular stages were neither identified macroscopically nor microscopically. Nearly 50% of the nursery piglets were affected. Symptoms occured after 1 to 2 weeks after relocation to the nursery unit.

The macroscopical suspected diagnosis of a poxvirus infection was confirmed rapidly by electron microscopy. Diagnosis was performed within one hour by negative staining including ultracentrifugal and chemical enrichment technics. Poxviruses detected in the crust and epidermal suspension fluid are brick shaped, 250-300nm large, and exhibit a surface coat with irregular arranged filamentous structures. To exclude an orthopoxvirus infection by Cowpox virus or Vaccinia virus – the viruses have a similar brick shaped form - a molecular biological examination including PCR and sequence analysis of the Thymidinkinase-gene (TK-gene) fragment was performed (Feller et al. 1991: Virology 183:578; Schmoll et al. 2010: IPVS, abstract). The agent was finally identified as Swinepox virus; the isolates reveal a 100% nucleotide identity with other isolates from Germany published in the NCBI database (Acc.Nr. AF410153, M64000 und M59931). At least, tissue sectioning was accomplished. Sections of biopsy samples embedded in Epon were analysed in a TEM (Zeiss 906) at 80kv. Electron microscopical analysis resulted in the detection of large A-type inclusions filled with intracellular mature poxviruses. The inclusions are surrounded by local accumulations of immature viruses. A-type inclusions are found in cells in the upper part of the Stratum spinosum; in epidermal cells basal of these cells B-type inclusions ("virus factories") are frequent. Both inclusion body types are typical for suipoxviruses (Teppema & de Boer 1975: Arch. Virol. 49:151). In contrary to other poxviruses, e.g. orthopoxviruses or avipoxviruses, immature viruses found in the virus factories are rich in number and often of the same developmental stage. Crescents, immature viruses (IV stage) and immature viruses with already condensed viroplasm (INV stage) are detected in the granular cytoplasm beside accumulations of intracellular mature virus stages (IMV), which exhibit already the typical poxvirus structure. Bundles of filaments, sometimes with periodical cross striations, and crystall accumulations can be detected in the cytosol between the virions. The filaments are often in close contact to the immature virions exhibiting a role in viral cytosolic transport and virus assembly (Bearer & Satpute-Krishan 2002: Infectious Disorders 2:247; Radtke et al. 2006: Cellular Microbiol. 8(3):387; Wileman 2007: Ann. Rev. Microbiol. 61:149). Sometimes mature virions in the B-type inclusions are accumulated around small vesicles which, compared to fowlpox, may be of lipid character. Regarding the local arrangement of viruses and cytosolic character of virus factories, B-type inclusions seem to be the preliminary stage of A-type inclusions.

POSTER: THE ROLE OF SYLVATIC CYCLE INVOLVING WILD PIGS, TICKS AND DOMESTIC PIGS IN THE EPIDEMIOLOGY OF AFRICAN SWINE FEVER IN KENYA

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Key words: ASFV, sylvatic cycle, epidemiology, genotyping

African swine fever (ASF) is a lethal, hemorrhagic disease of domestic pigs for which animal slaughter and area quarantine are the only methods of control. Since first description in Kenya in 1921, the disease was maintained in African until the initial export in 1957 to Lisbon spreading between 1957 to 1990 throughout Europe and America. Currently ASF is present in Sardinia, in Caucasus Republics and in most than twenty Sub-Saharan Africa countries, where ASFV can infect hosts through either a domestic or a sylvatic cycle involving warthogs and bushpigs as well as ticks of the genus Ornithodoros.

In 2006, ASF broke out among commercial pigs in Kenya-Uganda border and spread in 2007 to Central Kenya around the city of Nairobi, after an apparent absence from the entire country since 2001. The outbreaks were traced to movements of domestic pigs and were not linked to warthogs or bushpigs. However, Kenya has had several outbreaks caused by regular reemergence of ASFV that could be result primarily from direct contact between infected domestic pigs with tick-involvement. In addition, experimental evidence suggests that pigs can exceptionally become infected from eating infected warthog tissues playing an important role in the transmission of the disease in the country.

We report the first extensive study to investigate the role of sylvatic cycle involving warthogs, O. porcinus porcinus ticks and domestic pigs in the epidemiology of ASF in Kenya. Samples from 83 ASF asymptomatic domestic pigs, 51 warthogs and a total of 1,576 Ornithodorus porcinus porcinus ticks from 26 independent warthog's burrow were collected from area of interface between domestic and wild pigs in Central Kenya, where ASF had been reported. Their infection status with ASFV was determined using combined p72-based PCRs, restriction analyses and virus isolation. In addition, sera from wild suids and domestic pigs were tested for anti-ASF antibodies using OIE serological prescribed methods. The presence of ASFV was determined in 62 of 285 (22%) O. porcinus pools, 3 of 51 (3.22%) warthogs serum samples and 41 of 83 (49%) domestic pigs serum samples. Interestingly, no seropositive response was found in any domestic pig in contrast with the warthog's results where all of them were seropositives. Nineteen O. porcinus, twelve domestic pigs and three warthog ASFV isolates were genotyped using a combination of full length p54-gene and p32-gene sequencing, partial p72-gene sequencing, and analysis of tetrameric amino acid repeat regions within the variable region of the B602L gene (CVR). Phylogenetic analysis indicated that the ASFV isolates obtained in this study from ticks and healthy domestic pigs clustered in a sylvatic cycle P72 genotype X, whose range encompasses viruses from Rwanda, Burundi, Tanzania and Kenya, whereas ASFV isolates collected from warthogs were placed into p72 genotype IX related to recent domestic pig outbreaks occurred in Kenya in 2007. This is the first demonstration of ASFV in ticks and warthogs from Kenya and the results obtained so far suggest that two different epidemiological cycles of ASF are coexisting in Kenya involving the warthog-domestic pig and tick-domestic cycle transmission.

POSTER: VIRUS QUASISPECIES ANALYSES USING THE GENOME SEQUENCER FLX

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Key words: Genome Sequencer FLX Virus quasispecies

RNA-viruses are assumed to exist as a so called quasispecies, not a clonal population. The quasispecies is characterised by a more or less diverse mixture of genome sequences and hence proteins. There is evidence that the diversity of genomes is essential for full infectivity of the virus or can influence the virulence of virus isolates.

Although attempts were undertaken to determine the sequence mixture of the quaisispecies using Sanger sequencing, only the advent of next generation sequencing technologies opened up the possibility to determine the sequence of individual nucleic acid molecules of the quasispecies with a reasonable effort and, more importantly, sufficient reliability. Due to the enormous depth of the sequence data that can now be achieved, statistical analyses of sequence mixtures gets possible. This is implemented in amplicon sequencing, but only in very narrow regions of a nucleic acid. Therefore, one must know the variable regions before starting analyses and will hardly ever get the complete picture.

In order to enable quasispecies analyses at the full genome level, the suitability of Genome Sequencer FLX data for and the feasibility of quasispecies analyses with this data was investigated. Moreover, the impact of preparation and quality of input data and of the software used for data preparation was dissected. Finally, a software pipeline was established to analyse the diversity of a virus isolate at the full genome level.

First analyses of sequences from influenza A virus isolates show a considerable variability in the diversity of the quasispecies underlying the consensus sequences.

POSTER: THE EFFECT OF INOCULATION DOSE ON DIRECT AND INDIRECT TRANSMISSION OF A HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS STRAIN H5N1 IN CHICKENS.

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Key words: transmission; avian influenza; H5N1; chickens

Highly pathogenic avian influenza is of major concern for the poultry industry, as the virus can spread rapidly in and between flocks, causing high mortality and severe economic losses. The aim of this study was to determine the probability of infection, determine dose-dependent virus transmission (direct transmission) and quantify spatial transmission (indirect transmission) for various inoculation doses.
We performed two transmission experiments with pair-wise housed layer type chickens, in which one bird per pair was inoculated with a HPAI H5N1 virus and the other contact-exposed. Various inoculation doses were used to determine the susceptibility (ID50), and possible relation between ID50, and infectiousness, expressed as the amount of virus shedding and the probability of contact birds becoming infected. Furthermore, we performed two transmission experiments with groups of layer type chickens placed in cages at different distances from a group of layer type chickens that were inoculated with a HPAI H5N1. Air samples and dust samples were taken to quantify virus load around the inoculated chickens.
The probability of infection increased with increasing inoculation dose but survival from infection did not depend on inoculation dose. The amount of virus shed in trachea and cloaca by the inoculated chickens in the time between inoculation and contact infection, differed between the various dose groups. Despite differences in latent period and virus shedding, the transmission rate parameter β and reproduction ratio R0 did not differ significantly between the various dose groups. This implies that in our experiment the amount of virus shedding is not a measure to predict direct transmission or the infectiousness of chickens. The rate of spatial transmission was limited even at a distance of 20cm between the inoculated and contact chickens. Furthermore, virus particles were not detected in air samples. This implies that airborne (indirect) transmission is not the main route of transmission of HPAI between individually housed chickens.

POSTER: A NOVEL COMBINATION OF TAQMAN RT-PCR AND SUSPENSION MICROARRAY TECHNOLOGY FOR THE DETECTION AND SPECIES IDENTIFICATION OF PESTIVIRUSES

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Key words: Real-Time PCR, Pestiviruses, Dedicated Microarray

Genus Pestivirus belongs to the family Flaviviridae, and contains four species which infect a wide variety of ungulates. The four species are classical swine fever virus, bovine viral diarrhoea virus 1 and 2 and border disease virus. Classical swine fever is considered the most serious and in many places, when an outbreak occurs, disease along with a stamping out policy results in heavy losses of animals. All these viruses (except CSFV) can cross-infect species and swine can be naturally infected with any of them. Therefore, differential diagnosis and the diagnosis of mixed infections is desirable.

The objective was to develop a through-put system which combines real-time PCR detection and the multiplex capability of a microarray format. The system allows for identification of a pestivirus using a "pan-pesti" real-time PCR followed by analysis of the PCR product on a suspension microarray platform for verification and species identification. Real-time detection and microarray identification were made on 100% of the twenty-six pestivirus strains tested with no cross-reactivity among the array probes. Other heterologous pathogens and clinical negatives did not produce a signal in real-time or on the microarray. Twenty-three pestivirus strains were tested and detected at 100 copies, with concordant real-time PCR and microarray results. Sensitivity was tested for three CSFV strains and found to be six viral genome copies/reaction. Correspondingly, the microarray signals at six genome copies were above the threshold of 500 MFI.

This study offers a novel analysis platform that combines real-time PCR with bead-based, suspension microarray technology. This approach suits many veterinary routine diagnostic laboratories by incorporating real-time PCR into the detection stage. It then allows for rapid further microarray analysis that can identify the particular pathogen species, as well as mixed infections. Due to cross-infections that occur with pestiviruses, this particular assay has relevance in many areas, including surveillance programs and in the QC of biological products. This study also acts as a validation which shows that for this assay, results for the real-time PCR and on the suspension microarray were identical. This assay has been published in Veterinary Microbiology (corrected proof,in press.) and further developed on an Epizone short term mission to Veterinary Laboratories Agency.

POSTER: SAFETY OF A BIVALENT BTV-4/BTV-8 INACTIVATED VACCINE IN PREGNANT AND LACTATING CATTLE

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MERIAL S.A.S.[1]; c/O MERIAL[2]

Key words: BTV, pregnant cattle, vaccine, safety, milk production

INTRODUCTION
Bluetongue is an infectious OIE listed disease of wild and domestic ruminants. Recent Bluetongue virus serotype 8 (BTV-8) outbreaks in Northern Europe have seriously affected the cattle and sheep industries. Vaccination of susceptible species is regarded as the method of choice for bluetongue control and several affected countries in Europe decided to implement large-scale compulsory vaccination campaigns with inactivated vaccines in domestic ruminant populations. Such vaccination strategy means that a large number of animals to be vaccinated are pregnant or in lactation, particularly in the cattle population. It is therefore of great importance to ensure that the vaccines used are safe in these categories of animals. The aim of the present study was to provide information regarding the safety in pregnant and lactating cows of vaccines of the BTVPUR AlSap® range (Merial).

MATERIAL AND METHODS
Ninety-six pregnant cows, at various stages of gestation and belonging to a BTV-free dairy farm were randomly allocated to 2 groups of 48 animals, on the basis of their pregnancy stage. One group was subcutaneously vaccinated twice (day 0 and 28) with 1 mL of an inactivated bivalent vaccine containing BTV-4 and BTV-8 (Merial). The other group was left unvaccinated and served as control.
The animals were monitored as follows:
- individual clinical observation and recording of rectal temperature during 4 days following each injection;
- recording of individual milk yield before vaccination and after vaccination for 4 months (official milk production control);
- monitoring of pregnancy and general health status of the offsprings at birth up to 15 days of age.

In relation to the BTV-8 epizootic that occurred in France in 2008, a natural BTV-8 infection of the herd was evidenced around 5 months after the first vaccination. The results obtained in the control cows showed that this infection had no marked effect on the reproductive performance of the cows or on the health status of the offsprings. The comparison of the vaccinated group to the control group was consequently judged reliable and relevant despite the natural infection.

RESULTS
The safety of two administrations of one dose of the bivalent BTV-4/BTV-8 inactivated vaccine tested was demonstrated in pregnant and lactating cows with:
- very limited and transient temperature increase following the first vaccination only,
- no treatment-related systemic reactions,
- no impact on the milk yield,
- no impairment of the reproductive performance of the vaccinated cows whatever the stage of pregnancy at which the vaccine was administered.

CONCLUSION
The results obtained in our study demonstrated the safety of a bivalent BTVPUR AlSap® inactivated vaccine administered in two injections in pregnant and lactating cows.

® BTVPUR Alsap is a registered trademark of Merial in the European Union and elsewhere.

POSTER: DEVELOPMENT OF A FOOT-AND-MOUTH DISEASE INFECTION MODEL IN SEVERE COMBINED IMMUNODEFICIENT MICE FOR THE PRELIMINARY EVALUATION OF ANTIVIRAL DRUGS

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Key words: FMD, antiviral drugs, SCID mice

Recently, European guidelines were amended (Council Directive 2003/85/EC) to facilitate the use of emergency vaccination in case of outbreaks of foot-and-mouth disease (FMD). However, emergency vaccines only confer complete clinical protection 7 days after vaccination (Golde et al, Vaccine, 2005). Moreover, FMD vaccines are serotype and to a lesser extent subtype specific, and thus have to be carefully selected prior to use (Paton et al, Rev Sci Tech OIE, 2005). Antiviral drugs could decrease this post vaccination immunity gap in a serotype non-specific manner and could be used as an adjunct to emergency FMD vaccination. Currently, we are optimizing the in vitro anti-FMDV activity of a promising class of non-nucleoside analogues that were originally identified in a screen against human enteroviruses. In a next step, the early antiviral leads will be evaluated in a small animal model. Preliminary titrations of Asia1 Shamir in 7- to 9-week-old Balb/c mice induced mortality at 2 to 4 days post inoculation (dpi). This is considered to be too fast to assess antiviral activity of early antiviral leads. Moreover, we were not able to induce clinical disease with O1 Manisa or C1 Noville. Therefore, we developed an FMDV infection model in 3- to 4-week-old severe combined immunodeficient (SCID) mice. SCID mice have an impaired adaptive immune response but a normal innate immune response. They may thus be suited for antiviral studies, because antiviral drugs would only be used before the onset of the adaptive immune response in emergency vaccinated livestock. Briefly, 10-fold serial dilutions of O1 Manisa, A22 Iraq, C1 Noville or Asia1 Shamir were inoculated intraperitoneally in 3 to 4 mice per dose and independently repeated 3 or 4 times. All PBS- or O1 Manisa-inoculated mice remained healthy until the end of the trial (14 dpi). Inoculations with A22 Iraq, C1 Noville or Asia1 Shamir evolved quickly into clinical disease and mice were euthanized because of ethical reasons. Hyperacute death at 1 or 2 dpi was observed in <5% of the mice. Between 2 to 4 dpi, 55% of the mice were less active or apathic and had moderate to severe respiratory problems. At necropsy, moderate to severe oedema of the lungs was observed. In mice that survived until 4 to 10 dpi, progressive weight loss was the most evident clinical sign. The lowest inoculation dose that resulted in 100% mortality was $10^{5.0}$ CCID50 per mouse. Mean times of euthanasia were 3 dpi for Asia1 Shamir, 4 dpi for C1 Noville and 6 dpi for A22 Iraq. Viral RNA levels in serum at 2 dpi were recorded with a semi-quantitative real time RT-PCR (data not shown) and will serve as a reference base in the future. In randomly selected animals, viral RNA was systematically detected in the lungs, heart, spleen, liver and kidneys. Histopathological analysis remains to be performed. In conclusion, these results confirm that FMDV can induce a severe generalized disease in mice, that the clinical outcome depends on several factors including age, mouse strain, FMDV serotype and strain and that high inoculation doses are required (Salguero et al, Virology, 2005; Kamstrup et al, Antiviral Res, 2006). Further, these experiments suggest that infections with Asia1 Shamir or C1 Noville in 3- to 4-week-old SCID mice evolve too quickly for an evaluation of antivirals against FMDV, taking into account that these antivirals would be administered during the one week post emergency vaccination immunity gap. FMDV infection of SCID mice with A22 Iraq may represent a valuable model for the preliminary evaluation of antiviral drugs.

POSTER: APPLICATION OF BAYESIAN TECHNIQUE FOR VALIDATION OF ELISA TEST FOR SEROLOGICAL DIAGNOSIS OF INFECTION WITH BOVINE FOAMY VIRUS

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Key words: Bayesian method, diagnostic test, ELISA, BFV

Introduction
The sensitivity and specificity of the serological diagnostic test are usually determined by comparison with a reference test often called a "gold standard", which is supposed to determine the true serological status of the animals. While ELISA technique is largely applied in serological survey a western blotting is often used as a reference test, but due to its imperfection in all interpretations of the ELISA results this method is not a suitable as a "gold standard". Therefore, the preferred method is to conduct a rigorous study to estimate the error probabilities of ELISA and western blotting test results simultaneously using Bayesian estimation method that recognizes the dual errors of the two assays. This estimation is based on the assumption that the classification errors in both tests are independent, conditional on the true infection status of animals. Bovine foamy virus (BFV) infections are commonly prevalent in cattle population worldwide, but the large-scale seroprevalence study of BFV infection is hampered by the lack of the validated ELISA technique. In presented study Bayesian technique was used for the estimation of the sensitivity and specificity of newly developed ELISA test, suitable for a large serological screening of BFV infection in dairy cattle from Poland.
Material and Methods
Serum samples collected from two independent populations (A n= 82 and B n= 120) of dairy cattle were tested for the presence of antibodies against BFV using western blotting test (WB) and ELISA test. Crude lysate of Cf2Th cells infected with BFV100 isolate and recombinant Gag protein of BFV100 were used as diagnostic antigen for western blotting and ELISA, respectively and the methodology of both tests were described elsewhere. Bayesian estimation was applied based on their cross-classified results, in regard to individual ones from two populations with different rate of BFV-infected cows. It involves proportions, using the prior distributions determined by modeling subjective probability about the unknown parameters through the use of beta distributions, and allows to obtain estimates and intervals, which are the true probability intervals (a 95% Bayesian interval contains the true parameter value with 95% certainty). Computations were performed by Gibbs sampling.
Results and Discussion
Two independent populations of bovine serum samples were tested by western blotting and ELISA test. These results were cross-classified in 2 x 2 table and obtained results were used for estimation of the prior distributions through the use of beta distribution. After eliciting prior distributions for each of the six unknown parameters (sensitivity and specificity of both tests and BFV prevalence in both populations), joint independence of these elicitations was assumed and the joint posterior distribution was obtained. The posterior medians of ELISA and WB sensitivity (Se = 97% for both) and specificity (Sp = 95% for ELISA and 94% for WB) were comparable, with nearly the same credible intervals, what indicated that both tests can be characterized as independent, conditional on infection status. Although the specificity of WB was slightly lower comparing to ELISA, the results clearly show that both tests have acceptable Se and Sp level, suitable for the detection of BFV infection in dairy cattle. Additionally, the range of prior probabilities of infection was compiled with positive and negative predictive values, what enabled us to determine the range of the prevalence of infection (27% – 80%) within which our ELISA test is the most reliable.

POSTER: NEW VACCINATION STRATEGY AGAINST EQUINE WEST NILE VIRUS INFECTION: USE OF RECOMBINANT CANINE ADENOVIRUS VECTORS

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Key words: West Nile; adenovirus vector; recombinant vaccine

Context: West Nile virus (WNV) is a widely spread arbovirus whose infection can lead to severe neurological conditions in humans and horses. On the American continent, WNV introduction and spread has been associated with more than 29000 human and 25000 horse cases. WNV has a long history of circulation in Europe and was recently the cause of two epidemics, in Romania in 1996 and in Russia in 1999. Few control and prevention methods are currently available and WNV inactivated vaccine has only recently been approved for use in equids in Europe. This vaccine does not allow for easy differentiation between vaccinated and naturally infected animals, on the contrary to recombinant vaccines bearing few selected viral genes.

Objectives: Recombinant vaccines derived from adenoviral vectors are known to induce strong and protective immune responses against a wide range of pathogens (Brun A et al, Vaccine, 2008). Our study has been designed to evaluate a new vaccine strategy against WNV infection, namely the use of recombinant canine adenovirus vectors (CAV) encoding a secreted form of the WNV envelop (sE WN) (strain IS-98-ST1, P. Desprès, Pasteur Institute).

Results: Canine DK cells were transduced with CAV-sE WN and expression of secreted envelop was confirmed by immunofluorescence and western blotting studies. Induction of humoral immune responses after intramuscular vaccination with CAV-sE WN was analysed in rodent model, i.e ICR mice. Neutralizing antibodies, directed against WNV E protein, were stimulated as soon as 15 days after vaccination. Moreover, vaccination protected mice against WNV challenge with the highly virulent IS-98-ST1 strain: 100% (6/6) vaccinated animals and none (0/8) of control animals survived WNV infection. Vaccinated mice were not viremic 3 days post infection and their brain samples displayed no WNV RNA, on the contrary to control mice. Vaccine efficacy was also investigated in naturally susceptible species, in horses.

Conclusions: A new vaccination strategy against equine WNV infection has been developed. This strategy appears to be promising in mice, with efficient and rapid induction of protective immune responses, and in horses, in which more advanced investigations are required in order to fully validate the proposed strategy.

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POSTER: THE PATHOLOGICAL ASPECTS OF RIFT VALLEY FEVER INFECTION IN AN EXPERIMENTAL TRANSGENIC MOUSE MODEL

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Key words: Rift Valley fever virus, IFNAR-/- mice, pathology,

Rift Valley fever (RVF) is a viral, mosquito-borne zoonosis that affects domestic ruminants as well as humans. Infection with RVF virus (RVFV) causes abortion of pregnant animals along with a high mortality rate in newborn lambs and calves. In humans, it usually manifests itself as a self-limiting disease, but occasionally leads to more serious complications like haemorrhagical fevers, encephalitis and retinitis with high morbidity and mortality. For many decades this disease has been confined to Sub-Saharan Africa but in recent years a steady increase in the number of outbreaks, including a more northward geographic spread, confirms that this disease has the potential to emerge in virtually any part of the world.

It has previously shown that the attenuated RVFV strains are fully pathogenic for mice lacking a functional IFN-alpha/beta system. As a consequence, these mice are highly permissive for virus infections. Since exposure to RVFV is hazardous for laboratory personal, these mice constitute a safer animal model to study RVF pathogenesis and/or vaccine efficiency studies.Taking advantage of IFNAR-/- deficient mice as a suitable animal model, we have evaluated the pathogenesis of a RVF virus infection in order to characterize the sequential pattern of lesions occurring in different tissues upon lethal virus challenge.

For this purpose, ten mice of 6-8 week old were inoculated i.p. with 2 x 10e4 p.f.u of the RVFV ZH548MP-12 attenuated strain and sacrificed at 12, 24, 48, 72 and 96 hours after virus inoculation. Samples from different tissues including liver, spleen, lung, heart, pancreas, brain, kidney, stomach, small and large intestine and lymph nodes were taken and processed for histopathology and immunohistochemistry. At the necropsy, the liver was friable and pale and the spleen showed small size.The main target of RVFV infection in IFNAR-/- mice was the liver. At 48 hours pi necrotic and apopototic hepatocytes were observed increasing in number at 72 hpi. Inclusion bodies were identified as early as 48hpi. Mixed inflammatory infiltrates were also found in the lesions. The use of a polyclonal anti-RVFV serum showed cytoplasmatic staining in a large number of necrotic hepatocytes. Other lesions observed were depletion and necrosis of lymphocytes and interstitial pneumonia.

The present findings indicate that these mice resemble the lesions and pathology described for other veterinary species such as sheep and cattle. Therefore, this model can be considered suitable for the initial screening of strategies and/or therapeutics to control the disease, such as vaccines or antivirals.

POSTER: CIRCULATION OF GROUP 2 CORONAVIRUSES IN A BAT SPECIES COMMON TO URBAN AREAS IN WESTERN EUROPE.

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Key words: Bats—Coronavirus— Field study—Public health—Zoonosis.

Introduction and objectives.
The majority of emerging infectious diseases in humans are zoonotic and more then 70% of these diseases are emerging from wildlive reservoir hosts.
Bat species constitute around 20% of all mammalian species and are geographically widely dispersed. Bats have a number of characteristics that increase the likelihood of intra- and interspecies transmission of viruses. In general bats have a long life span, they fly daily and sometimes long distances in pursuit of food or during seasonal migrations. Roost sites include locations in proximity to humans and frequent contact with intermediate hosts like domestic cats enlarge the possibility of transmission of virus from bats.
More then 30 viruses have been isolated from bats. Zoonotic transmission however has only been shown for rabies virus and related lyssaviruses (except for Lagos bat virus), Melaka reovirus, Ebola and Marburg virus, Nipah and Hendra virus and SARS-like coronavirus.
Coronaviruses (CoVs) are considered as a group of viruses with a high potential for interspecies transmission. They are found in a wide variety of hosts, including wildlife, livestock, poultry, pets, and humans. The genus coronavirus (CoV) belongs to the family of the Coronaviridae in the order of the Nidovirales. CoVs comprise three genetically and serologically distinct groups. Group 1 and 2 viruses are pathogenic for mammals. Group 3 viruses are foremost pathogenic for poultry. Due to their long genomes, high recombination frequency and high mutation rate, CoVs have the potential to adapt to new host species. Recent studies indicate that bats are the natural hosts for all mammalian CoVs and that the circulation of CoVs in other mammals is the result of occasional introductions from bats.
To assess the putative risks for public health of bats as reservoirs for CoVs with potential for interspecies transmission, we investigated to what extent CoVs circulate in a variety of bat species and populations in the Netherlands.

Material and Methods.
Bats were caught in several regions of the Netherlands. After catching, each bat was kept in a disposable cotton bag for the collection of faecal pellets. Species, sex, age, and reproduction status were determined. All bats were released at their capture site. The faeces were stored at – 20°C till processing. Faeces was resuspended in HBSS HANKS medium (Invitrogen) (10%, 5%, or 1%). The faecal suspensions were centrifuged at 3000 g for 20 min, and 100 μl supernatant was used for extraction of RNA. A nested CoV genus-wide RT-polymerase chain reaction (PCR) was performed.
Nucleotide sequences were analyzed using Bionumerics vs 5.1 (Applied Maths). Evolutionary trees were drawn using the maximum parsimony method. 1000 bootstrap analysis were performed.

Results.
Faecal samples of 211 bats representing 13 different species from 31 locations in the Netherlands were analyzed. Four species were found positive for CoV, viz. Myotis daubentonii, M. dascyneme, Nyctalus noctula and Pipistrellus pipistrellus. The overall presence of CoVs was estimated at 16.9%. At 15 of the 31 locations (48.4%) bats were found positive. No predictive factors for CoV positivity were found.

Discussion and conclusions.
To our knowledge this is the first report of group 2 CoV circulation in bats in Europe. We show that group

1 CoVs are commonly present in a variety of bat species at multiple locations in the Netherlands. This includes the identification of a new reservoir species. The circulating group 1 CoV lineages were rather species associated than location associated.

POSTER: PASSIVE SURVEILLANCE OF EUROPEAN BAT LYSSAVIRUSES IN THE UK (1987-2009)

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Key words: Rabies, European bat lyssaviruses, passive surveillance

European bat lyssaviruses type-1 and -2 (EBLV-1 and EBLV-2) belong to the genus Lyssavirus, family Rhabdoviridae. There are currently seven established lyssavirus genotypes, with EBLV-1 and EBLV-2 belonging to genotypes 5 and 6 respectively. Four additional bats from Asia and Eastern Europe have recently been ratified as new lyssavirus species. Lyssaviruses cause rabies, resulting in an inevitably fatal encephalitis. In 2002, a Scottish bat conservationist became the second person reported to die of EBLV-2 infection and there have been three records of EBLV-1 related deaths in Europe. Since 1987, over 10 000 dead bats have been submitted for EBLV surveillance in the UK. These submissions, by members of the public and in collaboration with bat conservation groups, have included all 17 native UK species and 7 non-UK species. Over 70% of all submissions were pipistrelle species.

Samples of brain were initially tested for lyssavirus antigen by the fluorescence antibody test (FAT). Confirmatory tests including, virus culture, RT-PCR and the mouse inoculation were undertaken where FAT results were inconclusive, if there was recorded human or animal contact, or the species was a known lyssavirus reservoir. All nine EBLV-2 positive bats recorded since 1987 have been Daubenton's bats. The total number of Daubenton's bats submitted accounts for 2.4% of all submissions, making the incidence 0.09% overall and 3.5% among Daubenton's submissions. There have been no records of EBLV-1 positive bats in the UK.

The number of submissions varied geographically and seasonally, with most bats received during the summer months. There were also variations in number of individual bat species, sex and age. Approximately 20% of all bats have had contact with animals and ~3% with humans.

To support bat surveillance for lyssaviruses in the UK we have developed a set of primers for the identification of bat species where morphological examination is unable to speciate a specimen, e.g. in cases of cryptic species. Primers based on a mitochondrial DNA, cytochrome b, and a genomic gene, β-actin, have been developed that successfully detect the range of bat species indigenous to the UK.

This extensive passive surveillance scheme has repeatedly shown EBLV-2 in native UK bats, suggesting that Daubenton's bats are a natural reservoir for the virus, with low level endemic infection. These data have helped inform public health decisions and provided insights into the dynamics of lyssavirus infections in reservoir hosts. Continued human and animal contacts with positive cases confirm the need for continued EBLV surveillance, education and awareness amongst general public and bat handlers throughout Europe.

POSTER: EVIDENCE OF WEST NILE VIRUS AND USUTU VIRUS CIRCULATION THROUGHOUT MOSQUITO SURVEILLANCE IN EMILIA-ROMAGNA REGION IN 2009.

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Key words: arbovirus, mosquito, West Nile virus, Usutu virus

Background. West Nile virus (WNV) and Usutu virus (USUV) are mosquito-borne flaviviruses of the Japanese encephalitis antigenic complex, phylogenetically closely related. USUV cycle is not well known as WNV, but the two viruses seem to show ecological similarities. While the WNV risks for human health was well recognized, medical importance of USUV is not fully understood. In summer 2009 USUV-related illness were reported in two patients in Emilia-Romagna region (E-R). Recently after detections of different human disease due to mosquito-borne virus in E-R (chikungunya virus in 2007, WNV in 2008 and USUV in 2009), an extensive entomological survey, to determine the presence and distribution of arboviruses in mosquitoes, was implemented starting from late summer 2007. Results obtained in 2009 summer were described, with particular reference to the circulation of WNV and USUV.
Methods. The survey area was portion of the Pianura Padana, near the River Po. The Eastern part of the area is located on the Adriatic Sea and characterized by the presence of a large wetland areas (Valli di Comacchio and Po River Delta).
Mosquito collection was performed mainly by modified CDC traps baited with CO_2 and by gravid traps in little part, for a total of 148 trap activated from May to October. All stations were geo-referenced and land use were obtained by spatial join.
Mosquitoes were pooled according to date, location and species, grinded and submitted to bio-molecular analysis. Pool aliquots were analyzed using 3 different PCR reactions: 1) traditional PCR for the detection of flavivirus-genus according to Scaramozzino et al. (2001), 2) traditional PCR for the detection of USUV (Weissenböck et al. 2004) and 3) Real Time PCR for the detection WNV according to the method of Tang et al. 2006.
Results. From May 6 to October 31, a total of 190,516 specimens were sampled and analyzed. The mosquitoes belonged to 3 genera of the family Culicidae: *Anopheles*, *Aedes*, and *Culex* (*Ochlerotatus* taxon was considered an *Aedes* sub-genus). The 50.5 % of the *Cx. pipiens* pools sampled came from urban areas and 20.4% from rural ones. Mosquitoes were grouped in 1,789 pools and submitted to PCRs, giving subsequent results: 19 WNV positive pools, 48 USUV positive pools and 8 positive pools for both the viruses.
Most part of positive PCR pools were composed by *Cx. pipiens* mosquitoes. Unexpectedly two pools of Ae. albopictus give positive results for presence of USUV RNA.
The first USUV positive pool were sampled two weeks before the first WNV positive pool was detected. The number of WNV-positive pools were significantly higher in rural areas (p<0,01) while USUV-positive pools were equally distributed.
Discussion. During 2009 WNV was detected only in *Cx. pipiens* pools, as observed in the 2008 survey performed in the same area, confirming this species as the principle vectors of WNV in Northern Italy. As to USUV, all but two positive pools involved *Cx. pipiens*, suggesting this species as the main vector of the virus. Interestingly, USUV were detected in two pools of Ae. albopictus, indicating this species as possible USUV vectors.
The PCR analysis showed a strongly and longer USUV circulation in mosquitoes respect WNV, as displayed by the higher number of USUV positive pools detected and by the presence of USUV positive pools two weeks earlier than WNV.
Despite the affinity between the two viruses, the data obtained didn't highlighted any obvious relation between them, indeed the 2 viruses appear differently distributed, at least in rural areas.
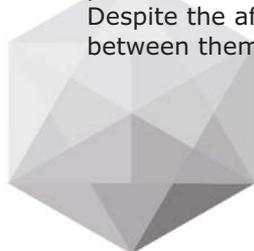
POSTER: NON-STRUCTURAL PROTEIN 1 OF INFLUENZA A VIRUSES DIFFERENTIALLY REGULATE INTERFERON-B IN HUMAN ALVEOLAR EPITHELIAL CELLS

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Key words: NS1, Influenza A, Interferon,

Introduction:

NS1 of influenza A viruses is a multifunctional protein that contains both an N-terminal RNA binding domain and a C-terminal effector domain, mediating the interaction with various cellular proteins. Although several other functions have been described for NS1, its IFN-antagonistic action is the most well-characterized activity of this protein. NS1 can continentally been divided into allele A and B based on its complete nucleotide analysis. This study aims to figure out the allele dependent functional differences in antagonizing IFN-β.

Methods
NS1 gene from H4N6 and H6N8 carrying both allele A and B were cloned into pcDNA3.1+. A549 cells and Mink lung cells were co-transfected with NS clones together with pISRE reporter plasmid expressing Firefly luciferase (pISRE-Luc) under the control of an interferon-stimulated response element (ISRE). Cells after 24h of transfection were stimulated with synthetic analogue of dsRNA, poly I:C which is a potent immunostimulant. To measure the level of IFN inhibition by allele A and B, cells were lysed 24h post-stimulation and luciferase signals were recorded. The produced IFN-β was measured using ELISA.

Results
It has been noticed that allele A of both strains (H4N6, H6N8) was more potent in abrogating the IFN-β production than allele B. A corresponding trend was also observed with produced IFN-β as measured by ELISA.

Conclusions:
There is about 34% putative amino acid difference between NS1 allele A and allele B of both strains. This might contribute in the difference of IFN-β inhibitory activity of respective alleles.

POSTER: GENOME ANALYSIS OF APMV-1 ISOLATED FROM A WILD BIRD AND SEVERAL OUTBREAKS IN SWEDEN

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Key words: APMV-1, F gene, Sweden

Introduction.
Newcastle disease is a highly contagious disease affecting domestic poultry, caged pet birds and wild birds with large economical consequences. Out of total eight genes in APMV-1 genome, comparison of the nucleotide sequences of F gene among the different strains of NDV is convenent and relaible to devide APMV-1 into groups, which could be further sub-divided into three lineages or eight genotypes. There have been several outbreaks of Newcastle disease in both commercial and backyard poultry in Sweden since 1995. The purpose of this study was to overview the genome characteristics of APMV-1 circulating in Sweden.

Methods.
Viruses were isolated in Sweden 1994 (asymptomatic black-headed gull; Öland), 1995 (Breeding hens, Skåne), 1997 (Layers and broilers, Skåne), 2008 (Pigeons, Skåne) and 2009 (Breeding chicken, Skåne). RNA from all the viruses were reverse transcribed using random primers and F gene was amplified using overlapping gene specific primer pairs. The PCR products were purified and sequenced using the same primers as for the PCR.
Results.
The resultant sequences were aligned and a phylogenetic tree was constructed using BioEdit software, MEGA4 and DNA star. Phylogenetic analyses showed that the isolates belonged to three different genotypes: I (1994), VI (1995) and VII (1997, 2008, 2009). The sequences of the 2008 and 2009 are highly similar (approx, 14 nt difference/1000bases in the F gene). All the isolates, except 1994, were virulent based on their F protein cleavage sites as expected.

Conclusions.
Viruses circulating in Europe also affected Sweden at several time points. The virus from 1994 was isolated from black-headed gull, which is the unique isolate from asymptomatic wild birds in Europe. The sequence similarity of the viruses isolated in 2008 and 2009 indicate the common ancestor for both isolated viruses.

POSTER: IN NATURA MONITORING OF CONSTANT AND COMPLEX EVOLUTION OF AVIAN INFLUENZA VIRUSES IN WILD MALLARDS: OBSERVATIONS OF GENETIC DRIFT, REASSORTMENT AND RECOMBINATION.

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Key words: Avian influenza, surveillance, evolution, sequencing

As part of a long term wild bird monitoring program using a funnel trap system installed at La Hulpe just south east of Brussels, three different cocirculating avian influenza viruses were isolated from wild mallards during the period November –December 2008. An H1N1 (A/Anas platyrhynchos/Belgium/09-762/2008) and an H5N1 virus (A/Anas platyrhynchos/Belgium/09-762-P1/2008) were isolated in November 2008, then an H5N3 virus (A/Anas platyrhynchos/09-884/2008) in December 2008.

The complete genome of these 3 isolates was sequenced, showing the complex evolution of these LPAI viruses. All genes shared common ancestors with recent (2001-2007) European wild waterfowl influenza viruses. The H5N1 virus is sharing genome segments with both the H1N1 (PB1, NA, M) and the H5N3 (PB2, HA) viruses. Most probably, the precursors of these viruses have reassorted, resulting in the observed gene constellations. All 3 viruses share the same NS sequence. Moreover, the HA sequence that is shared between both H5 viruses is showing 3 non-synonymous nucleotide substitutions, indicating a certain amount of genetic drift since the reassortment event, resulting in H5 gene segment in both the H5N1 and the H5N3 progeny viruses. To a lesser degree, some drift could also be observed for the N1 sequences: one non-synonymous substitution was observed when comparing the H1N1 and H5N1 NA sequences. The H5N1 PA sequence seems to result from contact with at least one other virus as it is different from the PA that is shared between the H1N1 and H5N3 virus. Another evolutionary mechanism, recombination, is seen in the NP : the H1N1 NP is largely identical to the H5N1 NP, except for a short 40 bp stretch of sequence information (position 1200-1240) that is completely different, and is identical with the H5N3 NP sequence at this position. The rest of the H5N3 NP sequence is unrelated to the H1N1 NP sequence. Most likely, both the H1N1 and H5N3 viruses have NP genes resulting from genetic recombination of ancestor NP genes resulting in the exchange of a short 40 bp region.

These data show that the natural reservoir of LPAI is maintained, diversified and selected following constant and complex evolutionary mechanisms including genetic drift (accumulation of mutations), reassortment, and recombination. Long term monitoring of AIV in wild fauna is important not only to study these dynamics but also to detect the emergence of new subtypes and pathotypes.

POSTER: PANDEMIC H1N1 EXPERIMENTAL INFECTION IN TURKEYS OF DIFFERENT AGE GROUPS AND THROUGH DIFFERENT ROUTES

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Key words: pandemic H1N, turkey breeders, commercial turkeys, experimental infection

Turkeys are very susceptible to type A influenza infections and could therefore represent a reservoir for the pandemic influenza virus (H1N1pv). So far 5 H1N1pv outbreaks have been reported in turkeys (Chile, Canada, Virginia, California, France).

Curiously all these 5 outbreaks occurred in breeder flocks while so far no cases have been reported in commercial turkeys. The lower number of breeder farms along with the stricter bio-security, the lower stocking density and the location of breeder premises in lower densely poultry populated areas, compared to commercial farms, are all reasons that lead to suppose that breeder farms run a lower risk of infection in contrast with what has been reported so far. The only described H1N1pv infection symptom in turkeys is a drop in egg production. Since AI monitoring programs have been implemented in most of the turkey rearing countries it is unlikely that infections in commercial groups could have simply not been detected. Susceptibility to H1N1pv infection could therefore be linked to different bird age or farm management procedures that lead to more and stricter contacts of infected stockmen with breeders (artificial insemination, egg collection, etc.). Attempts to infect turkeys of different ages with H1N1pv have been recently done (1-2) but no age related difference in susceptibility was reported. Following these considerations we tried to infect birds of different ages and categories as well as through different routes.

In the first part of our experiment we inoculated H1N1 pandemic virus in five 84 day old and five 84 week old turkey hens, keeping 2 control birds per group. Purpose of the study was to investigate age related susceptibility to virus infection. Animals were tested for clinical symptoms, virus shedding and antibody production.

In the second part of the trial the same animals were re-infected 4 weeks after the first infection. Route of administration was intra trachea in the younger 5 previously infected birds, intrauterine in the 5 older previously infected birds and again 2 birds were kept per group as controls. Furthermore seven younger birds (42 days old) were introduced in the group and infected intra trachea. No symptoms were observed following the first infection, viral RNA was evidenced in some of the birds up to 9 DPI, the same number of birds evidenced antibody production while contact birds remained negative. Following the second infection other birds previously AI negative turned positive, while contacts remained once again negative. The results of this trial confirm previous studies in that turkeys do not represent a risk for virus propagation since control birds in strict contact with infected ones resulted negative throughout the entire trial. In contrast with previous trials birds were susceptible to infection by both routes of inoculation as confirmed by PCR detection and antibody response. This fact is most likely due to the high infection dose applied and to the deeper inoculation in the case of the respiratory tract infection. No difference in infection susceptibility linked to bird age was evidenced thus suggesting that the occurrence of outbreaks exclusively in breeder flocks could be linked to farm management differences probably related to the practice of artificial insemination.

1 Terregino et al. Euro surveill. 2009 ;14: 19360

2 Swayne et al. EID 2009 Dec. 15 2061-63

POSTER: DEVELOPMENT OF A FOOT-AND-MOUTH DISEASE DIAGNOSTIC MULTIPLEX IMMUNOASSAY

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Key words: FMD, luminex, multiplex, immunoassay, diagnostic

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed livestock (including cattle, sheep, goats and pigs) which can also affect a large number of wildlife species. The causal agent of the disease is a small non-enveloped RNA virus classified within Aphtovirus genus, as a member of the Picornaviridae. FMD virus shows a high genetic and antigenic variability. Seven serotypes (A, O, C, Asia 1, and SAT 1, 2, and 3) have been identified serologically, and multiple subtypes occur within each serotype. FMD is widely distributed throughout the world and has a great potential for causing severe economic losses and trade disruptions in animals and animal products. Therefore, rapid and accurate detection of all FMDV serotypes is essential for immediate implementation of outbreak control measures and development of an effective vaccination strategy. This is of particular importance for FMD since other vesicular diseases, including swine vesicular disease, vesicular stomatitis and vesicular exanthema cause vesicular lesions in swine and cattle that cannot be distinguished from those caused by FMD. The objective of this project is the development of a multiplex immunoassay for FMD diagnosis by using the Luminex liquid array technology. The luminex technology utilizes polystyrene microbeads embedded with unique ratios of two fluorophores (red and infrared). Each unique dye ratio results in a distinct emission profile. This system will allow in the same analysis the identification of different proteins by the separation of the beads according to their endogenous fluorescence and the detection of fluorescence signal corresponding to the antibodies fixed on the beads. FMDV VP1 proteins of all serotypes, FMDV non structural proteins (3A, 3B, 3C, 3D and 3ABC) and antigenic proteins of other viruses inducing vesicular diseases will be coupled to microspheres labeled with different proportions of fluorescent dye. Proteins not available will be produced in E.Coli and/or in insect cells as his-tagged recombinant proteins. This assay will allow, in a single reaction and from a single sample, simultaneous detection of antibodies against vesicular disease viruses and FMDV structural and non structural proteins, coupled to microspheres labeled with different proportions of fluorescent dye. Such a test will thus allow differential diagnosis of FMD but also differentiation between infected and vaccinated animals. Preliminary experiments have been performed, using EMCV VP1 structural protein as model, in order to optimize both antigen purification and antigen-coupling conditions. For this purpose EMCV VP1 was coupled to two different bead sets: carboxy beads (covalent binding) or Ni-NTA beads (Ni-NTA-6xHis-tag interaction). For each bead set different parameters were tested and the different resulting VP1-bead complexes were incubated with anti-VP1 monoclonal antibody (mAb), anti-HisTag mAb or immune and non-immune Pig serum. The VP1 coupled microspheres reacting against serum samples were then incubated with biotinylated anti-species antibodies and then stained with streptavidin-phycoerythrin. Reactions were then analyzed using the Bioplex Luminex system. The results obtained allowed to define the optimal coupling conditions. In addition results obtained using pig sera are encouraging even if additional work needs to be done in order to decrease background and thus increase the specificity of this immunoassay. Analog experiments will be performed using FMDV VP1 (serotype O) and sera from naïve, infected or vaccinated animals.

POSTER: AT LEAST TWO DISTINCT VESICULOVIRUSES ASSOCIATED WITH OUTBREAKS ON PERCH IN FRANCE

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AGENCE FRANÇAISE DE SECURITÉ SANITAIRE DES ALIMENTS[1]

Key words: percids, aquaculture, PCR, rhabdovirus, epidemiology

Perch (Perca fluviatilis) an pike-perch (Sander lucioperca) are increasingly used in aquaculture in Europe because they represent an interesting alternative to diversify the market of fish products. Consequently, infectious diseases should have an increasing impact on the production, unless strategies for control are urgently developed. Among the pathogens of importance, rhabdoviruses are already responsible for serious losses in farms, for instance in France, Ireland and Danemark. Knowledge of the viral populations is a prerequisite to design optimal diagnostic tools in order to prevent dissemination. However, poor knowledge is available concerning percids rhabdoviruses 2, 3. To initiate the characterization of the viral populations on percids in France, we have obtained by PCR the sequence of the complete glycoprotein g gene of eight isolates (seven from perch and one from pike-perch) associated with outbreaks in French farms between 1980 and 2009.

Of the 8 isolates, 7 were clustered within the genogroup IV of fish vesiculoviruses, with various European freshwater vesiculoviruses from pike, grayling and largemouth bass 1. Three isolates collected between 2004 and 2009 exhibited 99% identity one to another while four other isolates collected between 1980 and 2002 had an identity superior to 95% one to another, including the isolate 4890 from pike-perch. Both groups were similar at about 89% in average. Surprisingly, the 8th isolate, from perch (R6146), clustered with brown trout isolates from Sweden and Finland in the genogroup I 4, 5. It exhibited only about 68% of identity with the other French isolates.

These first sequences of the g gene of percids French vesiculoviruses open the gate to the design of probes for developing sensitive and specific PCR tools for surveillance and selection of genitors free of virus. A future test should be able to detect isolates with very different sequences (groups I and IV). Such a test is urgently needed for preventing exchange of infected material between regions. More isolates from Europe should be studied for a more comprehensive inventory of the viral populations, both in farms and in the wild.

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POSTER: PREPARATION OF ACTIVE MATTER DELIVERY SYSTEMS BASED ON CHITOSAN

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Key words: Chitosan, Polyelectrolyte complex, Adjuvant

Vaccines are effective tools for controlling infectious diseases and adjuvants help for increasing their efficacy. Besides promoting suitable immun response an ideal adjuvant must be non-toxic, without side-effects, immunologically inert, biocompatible and biodegradable, with long shelf life and low production cost. Chitosan is a non-toxic, biocompatible, biodegradable, natural polysaccharide derived from the exoskeletons of crustaceans and insects. Chitosan's biodegradability and immunological activity make it a candidate for vaccine adjuvant. In this study ionotropic gelation behaviors of chitosan and its polyelectrolyte complexes have been investigated, with the aim of developing active matter carrying systems for use in controlled release of peptide and protein drugs as well as foot and mouth disease antigen (FMDA). The dispersions were obtained by sonication and the stability of these systems were studied by turbidimetry. Polyelectrolyte complexes (PECs) of chitosan with some natural polysaccarides have been prepared and among them chitosan-alginate PEC has been found to undergo gelation in the most favorable way. PECs also were prepared at presence of additional ionic crosslinker. Depending on the type of the ionic crosslinker PEC formation is favoured or unfovoured. Obtained results can further be discussed and served as the basis of design of FMD antigen carrier systems.

POSTER: AN ELECTRONIC LEARNING COURSE ON AVIAN INFLUENZA IN ITALY (2008)

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Key words: e-learning; avian influenza

Introduction

The prompt identification of an infectious disease is a pre-requisite for appropriate emergency outbreak management. In order to allow the spread of knowledge and management skills related to Avian Influenza (AI) preparedness and intervention in the case of AI outbreaks, the Italian Ministry of Health, in cooperation with the National Reference Centres for Epidemiology and Avian Influenza, implemented an e-Learning course using new web-based information and communication technologies to enrich learning and provide new models for lifelong learning and continuing professional development (CPD).

Methods

This course was designed to train veterinary officers involved in disease outbreak management, laboratory diagnosis and policy making.

The "blended learning model" was applied, involving participants in tutor-supported self-learning and collaborative learning activities, as well as virtual classes. The supporting system was based on the following key elements: online tutor, content experts, peer community, and training materials. In order to facilitate dynamics, classes had no more than 25 students. Participants were able to interact in different ways with course contents, with the experts and among peers.

Interaction with content was implemented through the use of videos and animations, incorporating audio and "drag and drop" quizzes, in order to engage learners in course content.

Collaborative learning was enhanced through project work activities, used also for group evaluation, and asynchronous forum sessions among peers, animated by the online tutor.

Interactions with experts were implemented by synchronous means, such as chat, carried out in each edition of the course.

The course was evaluated by different means, selected according to the learning objectives of each module: a multiple-choice questionnaire for the first module, group project work for the second, a case study for the third, and the production of a press release for the last module.

A pre- and post-assessment was performed through questionnaires administered at the start and end of the course.

Results

The course duration was 16 hours spread over a four-week period. Six editions were implemented, from February to October 2008; 705 public veterinary officers attended the course. Only 4% dropped out after application and before starting the learning path, while 100% of students who followed this course completed all the evaluation steps.

Each edition consisted of 4 different learning modules with theoretical and practical sessions aimed at developing skills on aetiology, epidemiology, surveillance, disease outbreak management, and AI risk communication.

With reference to evaluation of the course, none of the participants considered it to be poor, while 96.2% considered it good or excellent and 93% reported to have achieved the course goals.

Conclusions

The results obtained may be considered significant indicators of the course quality and success. Training resources resulted very satisfactory. Moreover these results strongly encourage further developments of this learning methodology, as an effective means for quickly spreading knowledge and practical and managerial skills. One of the major opportunities offered by e-Learning is the possibility of delivering the same contents to a huge number of beneficiaries in a short time.

POSTER: MOLECULAR EPIDEMIOLOGY OF AVIAN INFLUENZA VIRUSES CIRCULATING AMONG HEALTHY POULTRY BRED IN FARMS IN NORTHERN VIETNAM

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Key words: epidemiology, avian influenza virus, 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. In order to determine constellation of avian influenza virus subtypes including H5N1 circulating among domestic poultry in northern Vietnam, surveillance was conducted during year 2006-2009.

In the period between 2006 and 2009, throat and cloacal secretion specimens were obtained from a total of 2,904 healthy ducks, 240 Muscovy ducks and 2,850 chickens on farms and inoculated into embryonated eggs for virus isolation. Subtype H3N8 were isolated from 13 ducks raised in 2 farms in 2006, and subtype H6N2 were isolated from 7 Muscovy ducks bred in one farm in 2007. In year 2008, strains of H3N8 and H11N9 were isolated from 3 chickens and 2 ducks, respectively. Of note, subtype H5N1 was isolated from one duck in one farm in the same year. Subtype H12N5 was isolated from 2 ducks in a farm in year 2009. Phylogenic analysis revealed that the HA gene of one of the H3N8 strains isolated from chickens did not cluster together with those of any reported H3N8 strains, showing only 93% homology to that of a H3 strain isolated from a wild bird in Alaska, the closest counterpart. The H5N1 (A/duck/Vietnam/G12/2008) strain isolated from a duck in the study killed all six-week-old chickens, indicating that the strain used was highly pathogenic. In addition, sequence analysis identified a polybasic amino acid sequence at the cleavage site of the hemagglutinin (HA) gene of the strain, which was a characteristic found typically in HPAI strains. Subsequent phylogenetic analyses indicated that A/duck/Vietnam/G12/2008 carried HA gene clustered together with those (categorized as clade 2.3.4) of reported H5N1 strains isolated in Vietnam from March through May 2007. Phylogenetic analyses on PB2, PB1, PA, NP, NA and M genes were consistent with that of HA gene. Contrary to those 7 genes, NS of A/duck/Vietnam/G12/2008 did not converge with those of the reported H5N1 strains in the phylogenetic analysis, indicating that the NS was transmitted from a strain of different origin to emerge strain A/duck/Vietnam/G12/2008. Similar to other Southeast Asian regions, ducks may play a significant role as a reservoir for avian influenza viruses including H5N1 subtype, facilitating their reassortant to emerge strains with new lineage, in northern Vietnam.

POSTER: DEVELOPMENT OF KOI HERPESVIRUS DISEASE (KHVD) IN COMMON CARP (CYPRINUS CARPIO) INFECTED BY AN EUROPEAN KHV ISOLATE (KHV-E) IN CORRELATION TO THE IMMUNE RESPONSE

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Key words: KHV, KHVD, PCR, SNT

Common carp (Cyprinus carpio) were infected with a European koi herpesvirus (KHV) isolate (KHV-E) obtained from Dr. K. Way (CEFAS, UK) by immersion at 20°C water temperature (+/- 1 °C). After swimming in water with a KHV titre of 103 TCID50 / ml for two hours, carp were distributed among different aquaria (4 x 10 carp). Additionally, 20 carps were kept in the same room as direct contact controls and further 20 animals under water disinfection using "Huwa San ®" (70 ppm in water, Hungerbach GmbH, A. Pfeffer, Magdeburg, Germany,). The animal keepers were requested not to do a personal disinfection between the different aquaria to achieve "natural conditions" for transmission of a KHV.

Carp were observed daily for the occurrence of KHV disease (KHVD) up to day 92 p.i.. From day 10 – 14 post infection (p.i.), infected carps started to display severe clinical signs of KHVD: bleedings and petechia in fins and on skin, small and large circular patches on skin, massive skin mucus secretion, gasping at the water surface or staying in a corner. Depending on the aquarium, two to five fish died with severe KHVD signs. In all KHV infected fish, dead or surviving, KHV was detected by PCR, nested PCR, semi-nested PCR and quantitative realtime PCR. At weekly intervals non-lethal samples were collected from all fish in one aquarium and three fish from the control aquaria: fish were bled individually (1 ml each) for serum or plasma samples and a gill swab sample was taken using a Q-tip. In addition, droppings from all aquaria were taken. While the serum was used for neutralization assay and / or antibody ELISA, the swabs and droppings, stored in different media (cell culture medium, isopropanole, lysis buffer with proteinase K), were used for PCRs.

After four weeks p.i., the majority of infected fish recovered from KHVD but in each aquarium one or two fish still displayed disease signs such as cachexy, a darker skin compared to the other fish or even an abnormal swimming behaviour. Most of these fish died at weeks four to nine p.i..

In contact control aquaria, no disease signs were observed over the whole experiment but KHV was identified in fish which had not undergone disinfection, but not in fish kept under water disinfection. To ensure the effect of "Huwa San®", water disinfection was stopped for three week. After this period, KHV was also detected in these fish but without any disease sign. At the end of the experiment samples (blood, swabs, organs) were collected from all surviving fish and from 20 contact controls and were tested serologically and virologically.

The remaining 10, now KHV infected, fish from contact control tanks without and again with disinfection were placed in new aquaria with 10 naïve carps, respectively. After 14 days samples were collected from single individual fish.

POSTER: VETERINARY AND PUBLIC HEALTH INFORMATION SYSTEM IN VENETO REGION: INFECTIOUS DISEASES OUTBREAKS DATA MANAGEMENT

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Key words: EPIDEMIOLOGICAL SURVEILLANCE; NOTIFIABLE DISEASES; REGIONAL INFORMATION SYSTEM

Introduction. From 1994, Veneto Region has been developing a Regional Information System (RIS), which involves different Public Health Organizations: the Veterinary Services at regional and local level and the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE), which is the institute for animal and public health in the north eastern part of Italy. IZSVE represents the core of the informative network of the RIS, initiated through the creation of the Regional Data Bank (RDB) of animal farm and related industries, and subsequently integrated with the Veterinary Geographical Information System (GIS) for spatial data management. RDB is now connected with GESVET, a specific procedure for the registration and management of activities performed by the Veterinary Services of the Local Health Units (LHUs). At the same time IZSVE has developed an information system for diagnostic data management of laboratories activities (IZILAB). To optimize data collection and their analysis, an integration of these archives (farms and their production characteristics, their geographical location and the sanitary status of livestock) is going to be implemented. This paper illustrates this activity and its future development.

Methods. Data integration involved two specific programs (GESVET and IZILAB). GESVET is organized in different sections; one of them is dedicated to data management of outbreaks of notifiable infectious diseases. It allows to LHUs the recording of all the information related to the detection of suspicion, notification and eradication of an outbreak. The above mentioned procedures have been consolidated during the last two years and now their harmonisation is in progress, with the aim of automating the exchange of data between IZSVE laboratories and LHUs. The development of all the system was based on the methodology of the process analysis.

Results. Following a brief trial with a pilot group, each β release was tested, revised and put into production. Since February 2009, two LHUs are testing the "outbreaks section" in GESVET, and specific training meetings are routinely carried out. As GESVET and IZILAB have been developed with different purposes and in a different period during the last five years, there are relevant differences in their vocabularies, and databases are sometimes inconsistent; the integration activity will result in the configuration of the procedures in order to connect the kind of samples, collected by veterinarians, loaded in GESVET and in IZILAB.

Conclusions. To develop an integrated system of data recording on the sanitary status of livestock production in Veneto Region, future activities will include the analysis, comparison and harmonization of data set; moreover the sharing of information between these programs, in order to allow the best management of animal infectious diseases and related data availability for the users, will be implemented. RIS represents the basis for the establishment of epidemiological surveillance networks and a modern management of resources in veterinary public health.

POSTER: SWINE VESICULAR DISEASE BETWEEN-FARM TRANSMISSION PARAMETERS DURING THE 2006 – 2007 EPIDEMIC IN LOMBARDY REGION

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Key words: transmission, epidemic

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. Lombardy, with a densely populated pig area was most affected: 53 outbreaks were detected and some 150,000 pigs were stamped out. 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.
In the first epidemic period, SVD spread among the farms according to its typical routes of transmission. Instead, the second epidemic period may be further separated in two phases: the first could be considered a tail of the first epidemic period, while the second was characterized by an endemic trend in a small densely populated area and to achieve eradication it was necessary to depopulate a group of pig farms. No other SVD outbreak arose afterwards.
The purpose of this study was to estimate the between-farm transmission parameters in the different phases of the 2006 – 2007 SVD epidemic in Lombardy.

For each outbreak the date of virus introduction was established according to the available serological results. However, in case of contact with previous infected herd, introduction was traced back to the contact date. A temporal risk window (TRW) was defined as the time-interval in which the infected herd was supposed to transmit the infection: in this study TRW boundaries were delimited by the estimated virus introduction time and the application of the cleaning and disinfection measures after the elimination of the infected animals. For each week the number of newly infected farms, infected farms in their TRW and susceptible farms were computed. All the pig farms in Lombardy region that were active in the TRW of at least one infected farm were considered as susceptible. The transmission rates β and the respective 95% CI were estimated for each epidemic phase by fitting a generalized linear model. The average infectious periods T were estimated by Kaplan Meier method. The Reproductive Ratio (Rh), the average number of newly infected farms caused by an infected farm, was calculated for each phase multiplying β by T.

The transmission rate β, the average number of susceptible herds infected by each infected farm during a week, was 0.136 (95% CI: 0.095-0.187) in the first period, 0.231 (95% CI: 0.057- 0.598) in the first phase of the second period and 0.286 (95% CI: 0.131- 0.532) in the second phase. The average durations of the infectious periods were 54.4 (CI 95%: 48.66-60.14), 23.17 (CI 95%: 15.39- 30.95) and 20.42 (CI 95%: 16.40- 24.44). The Rh were respectively 7.4, 5.3 and 5.8.

The higher Rh value during the first epidemic period could be explained by the fact that SVD has often a sub-clinical course. In fact, in the first epidemic period the disease spread undetected for at least one month, while in the second one disease was reported earlier. Also, the choice of depopulating the small, densely populated area, seems to be confirmed by the persistently high Rh value of the last epidemic phase, indicative of a major disease outbreak.

POSTER: STUDY OF THE POSSIBILITIES OF PRECLINICAL DIAGNOSIS OF OVINE PULMONARY ADENOCARCINOMA

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Key words: jaagsiekte, adenocarcinoma, sheep, proteomics

Ovine pulmonary adenocarcinoma (OPA, jaagsiekte) is a disease caused by Jaagsiekte Sheep Retrovirus (JSRV). The virus induces neoplastic transformation of alveolar type II pneumocytes and Clara cells. The virus replicates in these cells only, but may be detected in the blood leukocytes using PCR techniques. Two-dimensional electrophoresis (2DE) is one of proteomic methods used in cancer biomarker discovery. Both with mass spectrometry it allows for identification of the protein changes occurring during the transformation of healthy cells into neoplastic cells.

Aim: The purpose of the study was:

- to determine the possibility of the early diagnostics of the ovine pulmonary adenocarcinoma (OPA) using PCR based methods.

- to find differentially expressed proteins in neoplastic lung tissue by comparison with healthy lung tissue using 2DE.

Material and methods: In the first part of the study, the application of RT PCR, PCR, hemi-nested PCR and real-time PCR methods were tested for the possibility of JSRV genome (LTR region) detection in blood and respiratory tract fluid of OPA affected sheep. The experimental group of 9 animals was used for the studies including 5 lambs infected with jaagsiekte sheep retrovirus (JSRV) by intratracheal inoculation at the age of two weeks. The samples taken pre mortem included blood and respiratory tract fluid (collected from infected animals). Lung tissue was collected and examined post mortem. The field studies included samples of blood taken from sheep from Polish flocks and lung tissue samples obtained from abbatoir, as well as DNA isolated from blood samples from abroad located flocks with history of OPA. Lung tissue samples were histologically examined for the presence of OPA. In the second part of the study, the expression of proteins in tumor and nontumor tissues were compared using two dimensional electrophoresis (2DE) for the identification of potential protein markers of OPA. The spots showing higher expression in 2D gels of neoplastic tissues were submitted to the mass spectrometry (MS)analysis for protein identification.

Results: Both proviral DNA and RNA were detected in the lung fluid taken from JSRV infected sheep showing clinical sings of OPA and in all neoplastic tissues. 5 of 66 DNA samples from the abroad located farms were positive for the presence of JSRV LTR. All the blood samples and lung tissue samples collected from Polish sheep were negative for the presence of JSRV LTR. The characteristic neoplastic lesions were found in all lung tissue sections of experimentaly infected sheep. As a result of 2DE and MS analysis, 38 proteins were identified in spots which were overexpressed in 2DE gels from neoplastic tissues. The proteins are known to be overexpressed in human neoplastic tissues and 10 of them are overexpressed in human non-small lung cancer – both in cancer cells and blood: cytokeratines CK8, CK18, CK19, HSP70, hnRNP A2/B1, enolase 1, pyruvate kinase M2, TPI-1, cathepsin H, aldolase A.

Conclusion: PCR based methods used in the study allow for JSRV LTR detection in lung fluid collected pre mortem from JSRV infected animals, but the methods are not sensitive enough for the virus detection in blood of all infected animals. The preliminary results of 2D electrophoresis are the basis for more detailed investigations of the neoplastic tissue proteome, e.g.: isoelectric focusing in narrow pH range to obtain better resolution of complex protein samples and proteomic analysis of serum proteins for OPA biomarkers discovery.

POSTER: RAPID DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS BY RT-PCR USING THE PORTABLE ENIGMA FL® PLATFORM

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Key words: RT-PCR; foot-and-mouth disease virus; diagnostics; field detection

Foot-and-Mouth Disease Virus (FMDV) is a positive sense single stranded RNA virus from the Picornaviridae family which infects cloven-hoofed animals including cattle, sheep, goats and pigs. In the event of FMD outbreaks, rapid diagnosis and timely implementation of control measures are critical to limit the spread of disease. In this pilot study, we evaluated a mobile PCR platform (Enigma FL®, Enigma Diagnostics) which could be potentially used for detection of FMDV in the field. This new platform performs automated RNA extraction, real-time RT-PCR and interpretation of the results in the device without the need for operator intervention. Initial studies focussed on the ability of the Enigma FL® to extract template RNA and used a lab-based real-time PCR machine (Mx3005p, Stratagene) to test the RNA samples. Using a dilution series of FMDV O1-Manisa, the overall detection limit and the individual CT values generated by the FMDV-specific real-time RT-PCR were not significantly different to that produced by preparing RNA samples in parallel using a MagNA Pure LC system (Roche). In subsequent studies, validated lab-based FMDV-specific TaqMan® real-time RT-PCRs were transferred onto this platform and all assay steps (RT incubation and the PCR amplification) was performed with non-lyophilised reagents on the Enigma FL® using an optimised amplification protocol of <60 minutes. Comparative studies showed that results from the Enigma FL® were similar to diagnostic real-time RT-PCRs used in the WRLFMD (IAH, Pirbright). Moreover, the limit of detection between the Enigma FL® assay and the WRLFMD diagnostic assays were identical over a range of virus dilutions. This pilot study provides data to indicate that it is possible to transfer a validated TaqMan® real-time RT-PCR assay onto the Enigma FL®, and that the results generated on this platform are concordant with results produced by lab-based equipment. Future studies will be directed at the development of a commercial consumable using lyophilised PCR reagents for FMDV and the evaluation of this technology in FMD endemic countries using field samples.

POSTER: SURVEILLANCE FOR CIRCULATING BLUETONGUE VIRUS ACROSS GREAT BRITAIN

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Key words: bluetongue surveillance cattle

Bluetongue virus serotype 8 (BTV-8) has been present in Northern Europe since August 2006, and at least 9 further serotypes are now also present in parts of Europe, with BTV-1 most notably present on the northern coast of France. BTV-8 was first identified in Great Britain in August 2007, and has since been controlled through a wide spread vaccination campaign. As part of its bluetongue control strategy the UK government has undertaken to define the extent of populations of ruminants infected with BTV, in order to comply with EU regulation (EC No 1266/2007). The aim of this survey was to test whether the cattle population in Great Britain was free of circulating virus at the end of the active vector season, in November.

The requirement for the survey was to provide 95% confidence that less than 2% of the national herd was infected with BTV. The country was split into five surveillance regions considered to be of similar epidemiological risk, previously defined by Defra based on risk of windborne incursions from the European Continent and the level of vaccine uptake: Western Midlands of England; Northern England; Wales; Scotland; and Southern and South Eastern England. As the latter had the first incursion of BTV in 2007 additional sampling was carried out in this region, further splitting it into nine areas, defined by Animal Health Office (AHO). Within each of the 13 geographical units the sample size required to attain the specified level of confidence was calculated; a within herd prevalence of 10% was assumed, based on data from the 2007 outbreak (Defra, 2008; Velthuis et al. 2009), and thus calculations were based upon a between herd prevalence of 20%, to give an overall disease prevalence of 2%. A two stage sample size calculation (Cameron and Baldock, 1998) was used to calculate the numbers of individual animals and herds required. Overall the sample required comprised 210 herds (3502 animals).

Samples were tested using a pooled panBTV-qPCR test, with the aim of detecting any serotype of the virus, but not antibodies present as a result of vaccination or prior exposure.

The final sample comprised 214 herd submissions (3553 animals) of which 46% contained only vaccinated animals and a further 8% contained some vaccinated animals. All samples tested negative for all serotypes of BTV. Thus within each of the specified geographical areas we are 95% confident that there is a less than 2% prevalence of circulating virus in animals, including those vaccinated.

POSTER: REAL-TIME PCR DETECTION OF VETERINARY PATHOGENS - SOLUTIONS THAT CAN ADAPT TO CHANGING NEEDS

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Key words: Real-time PCR, Detection, Molecular Diagnostics, Automation, Pathogen

Introduction:
Veterinary pathogen detection by nucleic acid (NA)-based analysis is widely replacing immunological and culture based methods due to higher sensitivity and speed. A key factor for successful amplification and detection of pathogenic NAs is an effective and reproducible sample preparation methodology providing reliable purification and maximum recovery of DNA and RNA. A way to achieve standardization and reproducibility of nucleic acid isolation is the employment of automated procedures. Real-time PCR techniques further improved work efficiency in veterinary molecular diagnostics. For various pathogenic targets, premixed and validated real-time PCR assays are commercially available, further decreasing hands-on time and error risk. Alternatively, self-designed or literature-derived real-time PCR systems can be used for pathogen identification. In both cases, methods need to be based on high-performing and reliable PCR reagents. However, once established such methodology may need to be adapted to changing requirements. For example the type and number of samples used to detect a certain pathogen may change over time, as well as the requirements related to the type and nature of pathogen itself, like in an outbreak situation. Sample types described below were taken as a case for testing of the adaptability of different sample preparation and PCR-based detection methods.
Materials and Methods:
Viral NA and bacterial DNA were isolated from veterinary samples including whole blood, serum and bacterial culture using automated, high-throughput sample preparation with the One-For-All Vet Kit on the BioSprint 96 instrument, and additionally using the QIAGEN instruments QIAsymphony SP and QIAcube in combination with the appropriate kits. The purified NAs were evaluated by real-time PCR targeting Bovine Viral Diarrhea Virus (BVDV) and Taylorella equigenitalis, using premixed commercial PCR reagents (QIAGEN cador reagents), or for Porcine Circovirus (PCV-2), using the PCR primers and probe described by Brunborg et al., J. of Virological Methods 122 (2004), p.171 in combination with a generic real-time PCR master mix chemistry following the supplied protocols.
Results:
Viral RNA was purified from individual blood samples derived from BVDV infected cattle and was detected using the cador BVDV RT-PCR Reagent. Reliable and linear detection of BVDV RNA was achieved after pooling of one BVDV positive and 10 or 50 negative blood samples. Taylorella equigenitalis DNA was isolated from bacterial culture and detected with the cador T. equigenitalis PCR Reagent over a 4-log range with high linearity (R2 > 0.996) and precision. The PCV-2 DNA was isolated from porcine serum with high repeatability and precision. Straightforward establishment of the PCV-2 assay was accomplished using the generic real-time master mix. Comparable results were obtained for different sample preparation procedures using the BioSprint 96, QIAsymphony SP and QIAcube platforms.
Discussion/Conclusion:
- Three automation platforms addressing different throughput show comparable results – demonstrating adaptability to changing requirements
- cador PCR reagents provide easy-to-use and validated premixed reagents for reliable veterinary pathogen detection and guarantee immediate results
- For upcoming and self designed pathogen tests, generic preoptimized master mixes offer a high-performing chemistry, allowing establishment of new tests with minimal optimization.

POSTER: DETERMINATION OF BOVINE DENDRITIC CELLS PHENOTYPE AND CYTOKINE EXPRESSION IN CATTLE INFECTED WITH BLV

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Key words: bovine leukemia, dendritic cells, cytokines, flow cytometry

Introduction: Dendritic cells (DCs) were first described in the spleen of mouse by Steinman and Cohn. DCs are leukocytes, that derived from bone marrow and they are the only cells able to present antigen to naïve T cells. They initiate primary immune response, exist in all lymphoid tissues, and express both MHC class I and class II antigens. DCs are specialized and most potent antigen presenting cells (APCs) with unique ability to prime effective immune responses. They express higher levels of MHC class II and accessory molecules on their surface, than other professional APCs. These cells are the aim of investigation in many laboratories due to their role as adjuvants for vaccines that prevent microbial infections and treat cancer.

Materials and Methods: The investigations were performed on peripheral blood mononuclear cells isolated by density gradient centrifugation from blood and on cells isolated from bone marrow, spleen and lymph nodes of leukemic cows. With the use of paramagnetic microbeads labelled with antibodies to CD14 (Miltenyi Biotech) monocytes were isolated and cultured in the presence of IL-4 and GM-CSF for dendritic cells generation. The panel of monoclonal antibodies was used for dendritic cells phenotyping in flow cytometer. Cytokines: IL-6, IL-10, IL-12p40 and IL-12p70 were determined in culture fluids using ELISA tests. The FITC-conjugated anti-BLV gp51 monoclonal antibodies were used in immunofluorescence (IF) and flow cytometry for detection of gp51 expression on dendritic cells. Morphology of dendritic cells was determined in scaning electron microscope (SEM) and in light microscope after staining according to the May Grünwald-Giemsa method.

Results: In cell cultures many types of DCs were observed on different level of maturity. The first step of DCs formation was observed after 24 h of monocytes culture. The veiled DCs were present in the cell culture after 7 days. We found that expression of CD11b, CD11c, MHC-I class and MHC-II on dendritic cells from leukemic cattle was much higher than in healthy cattle, but expression of CD11a molecules was stronger in dendritic cells of healthy animals. Expression of cytokines: IL-6, Il-10, IL-12p40 and IL-12p70 was stronger in experimental animals than in healthy control. The presence of BLV-gp51 was detected in dendritic cells in IF and flow cytometry.

Conclusion: Infection with BLV caused increase of cytokines expression and changes in the percentage of surface molecules on dendritic cells. The leukemic DCs morphologically differed from DCs of healthy cows: they had many vacuoles and cytoplasm had delicate structure with many granules. DCs generated from blood, bone marrow, spleen and lymph nodes had different morphology, shape, size and structure. Infection with BLV caused higher expression of cytokines and molecules CD11b, CD11c, MHC-I and MHC-II on dendritic cells.

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POSTER: USE OF LATERAL-FLOW DEVICES TO TRANSPORT AND ARCHIVE FMDV-POSITIVE MATERIAL

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Key words: FMDV, LFD, rRT-PCR, detection

Transport of samples over long distances and in high ambient temperatures currently poses a great challenge in developing countries, where maintenance of a cold chain can be problematic. This study describes the application of lateral-flow devices (LFDs) for transportation and archiving of foot-and-mouth disease virus (FMDV)-positive clinical material. RNA was recovered from positive LFDs (SVANODIP® FMDV-Ag, Svanova) following addition of suspensions prepared from vesicular epithelia (ES). Using a simple protocol, sections of these devices were tested for the presence of FMDV by one-step real-time RT-PCR (rRT-PCR) and virus isolation in cell culture. Although no viruses were propagated in cell culture, positive FMDV rRT-PCR results were obtained which suggested that positive LFD tests could be a viable source of viral RNA for molecular analyses. Interestingly, similar FMDV rRT-PCR results were generated for samples recovered and tested from different parts of the LFD (loading pad, wicking strip, as well as all sections taken from the test membrane). The stability of the RNA on the membrane was demonstrated during time-course studies for representative serotype O and SAT 1 FMD isolates. These experiments showed that positive devices could be incubated for at least 42 days (at 37oC and room temperature) without affecting the signal produced by rRT-PCR. Subsequent analyses also showed that recovery of larger fragments of RNA of up to 2 kbp was possible. Although further work is required to improve the reliability of the extraction protocols, these larger fragments were suitable for sequence analysis of the VP1 gene of FMDV. In conclusion, this study shows that an LFD test used to detect FMDV antigen could also be potentially used for safe transport and archiving of clinical material, and for subsequent molecular-based diagnosis of the disease. Ultimately, these findings should provide opportunities for improving existing diagnostic tools and sampling strategies in endemic countries.

POSTER: DIAGNOSTIC PERFORMANCE OF TWO COMMERCIALLY AVAILABLE CELL VIABILITY ASSAYS IN FOOT-AND-MOUTH DISEASE RESEARCH.

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Key words: FMD, cell viability assay, MTS, resazurin

The diagnostic performance of two cell viability assays was evaluated for their capability of detecting cytopathogenic effect (CPE) caused by foot-and-mouth disease virus (FMDV) on cell-based assays. One was a commercially available MTS based assay (CellTiter 96® Aqueous, Promega) and the other was a commercially available resazurin based assay (CellTiter Blue® (CTB), Promega). Both cell viability assays are based on redox dye's, meaning that they both distinguish living from dead cells based on their capacity to reduce a substrate to a different coloured reduction product.
Standard virus neutralisation test protocols where used on BHK-21 and SK-6 cell lines. Results were first microscopically read for CPE, after which the plates where examined by one of two cell viability assays. After fine-tuning the assay protocols, both cell viability assays proved successful. With the microscopically determined results as gold standard, both redox dye's had comparable sensitivity, specificity and precision (>90%) towards identifying CPE.
First the MTS assay was evaluated, here the absorbance was read at 490 nm. When the cut-off level for CPE was set at 75% of maximum discolouring (CPE<75%), the MTS based cell viability assay had a sensitivity and specificity of 96% and 97% respectively, with a precision of 96%.
Secondly the CTB assay was examined by fluorescence (Ex:560nm,Em:580nm), whit the cut-off level set at 75% of the maximum fluorescence. Comparable sensitivity specificity and precision were found for the CTB assay (99%, 98% and 98% resp.)
Equally promising results were achieved when measuring the absorbance values for the CTB assay (570nm-600nm). When the CPE cut-off level was set at 70%, assay sensitivity and specificity levels were 98% and 99% with a precision of 99%. The latter method is now used for serological test reading and the feasibility of this absorbance-read CTB viability assay for screening of antiviral compounds is currently under investigation, yet preliminary data are promising, as indicated by Z'-factor values ≥ 0,5 for the control samples. The conclusions are that both cell viability assays achieve comparable results when screening for CPE caused by FMDV. The CTB assay measured by absorbance seems to be the more cost effective assay and combines precision with easy handling.

POSTER: PHYLOGENETIC EVOLUTION OF POLISH ISOLATES OF SWINE INFLUENZA VIRUS

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Key words: pig, swine influenza virus, phylogenesis

Background. Influenza viruses (IV) infect a large variety of species, including pigs who are an important host in virus ecology. Among European pigs population viruses of H1N1, H1N2 and H3N2 subtypes are dominated. Knowledge about the genes constellation of IV isolated from different countries and hosts is valuable for monitoring and understanding of their evolution.

Materials and methods. Forty one SIV strains isolated from pigs with pneumonia, raised in 20 Polish farms, were identified and characterised. All 8 genes were sequenced and subjected into phylogenetic analysis together with the sequences of IV available in GISAID and NCBI websites.

Results and Discussion. Hemagglutynin (HA) gene of Polish isolates have been derived from contemporary strains of "avian-like" SIV. They were located in separate cluster from classical SIV, where pandemic H1N1 2009 viruses were evidenced. Phylogenetic analyses showed that the HA genes were most closely related to the HA genes of H1N1 viruses circulating in European pigs during last 10 years, such as Sw/Cotesd'Armor/1488/99 (99.5% homology with Sw/Pol/RU1/02), Spain/53207/2004 (99.5% homology with Sw/Pol/J2/04) and Sw/Switzerland/8808/2002 (97.9% homology with Sw/Pol/RU1/02). Phylogenetic distance between Polish Sw/Pol/KPR1/04 strain and pandemic H1N1 2009 virus was approximately 70%.

Sequence similarity between the N1 genes of Sw/Pol/KPR1/04 and H1N1 viruses isolated from pigs in England (e.g. Sw/Eng/195852/92) was the highest and equals 95.7%. Pandemic H1N1 strains were located in the same cluster, with 91.2% similarity to Sw/Pol/KPR1/04.

Phylogenetic relationships between the six internal genes of all H1N1 and H3N2 isolates indicate close relationship to the genes of European swine viruses circulating since the 1980s and posses the highest similarity with H1N1 and H3N2 subtypes isolated from 2004 (96–98% similarity). In contrast the M1 gene may have originated by two different ways, depending on the year of isolation. Phylogenetic analysis of Sw/Pol/Bal7/2009 virus representing H3N2 subtype showed that it was grouped in a distinct subcluster together with the newly avian-like H1N1 viruses from Poland, and it was the most similar to Sw/Spain/53207/2004 (99.1 %) and other H1N1 and H1N2 viruses isolated within the last 8 years. In this subcluster pandemic H1N1 viruses were found. Another strain A/Sw/Pol/T2/08 of H3N2 subtype was located separately form remaining Polish strains in other subcluster, together with avian and human isolates obtained since the 90s. In general, H3N2 strains were located in swine cluster, in the main prevalent European group of H3N2 isolates called A/PortChalmers/1/73-like lineage which has evolved separately from the human H3N2 virus lineage around 1973. The sequence identities between human and swine isolates were lower (94.4–95.5%) than those among human (99.5–100%) or among swine isolates (98.4–99.8%). Pandemic H1N1 virus was characterized as reassortant of three lineages: classical SIV prevalent in North America, avian-like SIV from Europe and avian-like related SIV from Asia.

Summarizing, phylogenetic study of currently circulated H1N1 and H3N2 Polish isolates indicated a distinctive evolutionary trait for European SIV. Two H3N2 isolates have external genes of human origin with A/PortChalmers/1/73 as a ancestor and internal genes present in "avian-like" SIV mostly of H1N1 subtypes.

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POSTER: IDENTIFICATION AND CHARACTERISATION OF SEQUENCES OF THE INTERFERON REGULATED MX GENES FROM WILD AFRICAN SUIDS.

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Key words: ASFV virus reservoir host resistance

African swine fever virus (ASFV) is the causative agent of an important trans-boundary disease of domestic swine. Disease primarily manifests as an acute haemorraghic disease with death occurring 7-10 days post infection and mortality rates approaching 100%. Serial passage of the virus through tissue culture as well as directed gene knock-out by recombination can generate attenuated viruses that are potential vaccine candidates. Importantly the virus does not cause disease in wild African suids such as the bushpig and warthog, showing that mechanisms of effective host resistance to ASFV exist. The interferon response is an important part of the host response to viral infection and ASFV is sensitive to inteferon. Mx gene products are effectors of the type-I interferon response and have anti-viral activity against a range of different viruses. We have shown that Mx gene products can inhibit ASFV replication in vitro. Interactions between ASFV and MxA were similar to those seen between MxA and ssRNA viruses suggesting a common inhibitory mechanism.

Due to the clear difference in disease progression between domestic pigs and wild African suids infected with ASFV, we were particularly interested in comparing the interferon response of the different species. As a starting point we indentified Mx promoters and gene product from genomic DNA purified from warthog and bushpig tissues. A genomic and functional comparison between our novel sequences and similar ones from domestic swine is presented.

POSTER: POLITICS OF HEALTH: ANIMAL DISEASES AND GLOBAL PUBLIC THREATS

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FOOD AND AGRICULTURE ORGANIZATION[1]

Key words: Epidemics; Animal Diseases; Global Public Health; International Politics

Given that the last twelve years have seen a resurgence of epizootic diseases such as Nipah Virus in 1998 in Malaysia, Severe Acute Respiratory Syndrome in early 2003 in China, and Highly Pathogenic Avian Influenza from 2004 to 2010 mainly in Southeast Asia but also in Europe and Africa, there is worldwide fear that more animal-borne diseases will strike in the future.

Old and new animal diseases that can cross over to humans and have the potential to rapidly spread in human populations need to be factored in equations that shape domestic and international policies, especially those concerned with safeguarding the global commons of public health. The implications of epizootic diseases in international economic affairs now span far beyond their potential impacts on commerce and trade. Attention now focuses on the very basic drivers of change in global landscapes, and how these changes affect ecosystems, including the health of species living within these. Additionally, more attention is paid to how public health threats, and their accompanying fears, impact economic progress, human development, and national security. In our increasingly interconnected world, understanding the close relationships between micro-organisms harboured by animals and human health are becoming more relevant, and these, coupled with the global interdependence of health issues, are critically important in designing comprehensive national, foreign and security policies.

Novel and major epidemic threats are rapidly flourishing against a background of security and economic challenges –climate change, the proliferation of weapons of mass destruction, international terrorism, energy security, unemployment and deepening recession. It is therefore now clear that many factors contribute to the re-emergence and intensification of animal diseases, including economic, social, cultural, environmental, evolutionary, and demographic factors; all of which seem to be in a constant state of flux in rapidly evolving multidimensional contexts.

Despite our desire to find some clarity and reduce uncertainty, there is currently very little solace to offer. Much of the disease intelligence so far developed has proved to be of minimal benefit in foresight and early detection of epizootic diseases. Faced with imperfect information in many areas, much of the more recent efforts are focused primarily on prediction, identification, and prevention of emerging epizootic diseases followed by contingency preparations and emergency responses to what is actually found. However, much needs to be done in improving the cost-effectiveness of disease intelligence investments. In response, the United States and the European Union are now cautiously investing in initiatives to close the gap between what is known and what lies in the black box filled with unknowns, keeping in mind the abovementioned funding and sustainability issues.

It is argued in socio-political circles that the starting point is to improve surveillance systems at the human-animal interface, without forgetting the importance of livestock keeping to the livelihoods of rural majorities in developing countries. Networks of scientists must not forget the political and economical dimensions of epizootic diseases. Either way, stakeholders should advocate preparedness, prevention, detection, and control of epizootic diseases to uphold global public health. This is without doubt a timely moment to reflect on the critical relationships between livestock production, animal diseases, food security, poverty reduction and global public health.

POSTER: OCCURRENCE AND EXPRESSION OF TOXIN GENES IN CLOSTRIDIUM PERFRINGENS ISOLATES FROM HEALTHY BOVINE

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Key words: Clostridium perfringens, toxin genes, bovine

Introduction

Clostridium perfringens is the most important animal pathogen among anaerobic sporulating bacteria responsible for enterotoxemia of many warm-blooded animals (birds and mammals). The production of the major toxins is a base for classification into one of the five toxotypes (A – E). All type A strains produce α toxin, type B α, β, and ε toxins, type C α and β toxins, type D α and ε toxins, and type E α and ι toxins. The acquisition or loss of toxin genes (cpb, etx, iap, cpb2, cpe, cpa) by C. perfringens isolates showed panmictic nature of this species. For these reasons, detection of toxin genes at any isolated strain, despite his origin, is recognized like potentialy source of toxin genes for environment. Taking into account that pathogenicity of C. perfringens is conditioned by presence and activity of toxin genes, the study were undertaken for assessment of toxin genes occurrence and expression of most often detected toxin genes.

Material and Methods

Isolates for the present study were obtained from 10 bovine farms from 5 to 70 cows in the herd. One gram of faeces was plated for counting C. perfringens. Twelve obtained isolates from positive samples were tested for the presence of cpa, cpb, cpb2, etx, iap and cpe toxin genes which encode respectively for alpha toxin, beta toxin, beta2 toxin, epsilon toxin, iota toxin and enterotoxin by using a mPCR method. Afterwards, randomly choosen isolates classified by mPCR for type A subtype beta2 were tested for cpa and cpb2 expression by reverse transcriptase polymerase chain reaction (RT-PCR) method on the base of mRNA presence.

Results and Discussion

Among 525 analyzed isolates suspected of belonging to C. perfringens species, 286 strains were confirmed. Toxin type and its subtype identification revealed that 37.8% of the isolates belong to type A, 57% isolates belong to type A subtype beta2. Enterotoxin gene was detected in 1.7% strains. Three from these isolates had additionally cpb2 toxin gene. There were detected 3 isolates possessed cpb2 gene only. Among 286 analysed C. perfringens isolates, 3 of them (1%) belonged to type E subtype beta2 and one isolate besides cpa and cpb2 toxin genes contained additionally cpb and iap, occurred respectively in type C and E.

Analysis of type A subtype beta2 isolates (n = 30) for cpa and cpb2 toxin gene expression showed that majority of isolates expressed both genes.

Similar to results of other studies there were noted dominance of isolates type A and among them more than half of isolated strains possessed cpb2 gene. Relative low percentage of isolates with enterotoxin, beta and iota gene in our study maybe, according to some authors, sufficient reservoir of these genes, taking into account a panmictic nature of C. perfringens species. Really interesting is detection of isolate, which may be classified like type C and also like type E. Similar situation was noted among Clostridium botulinum isolates analyzed in our laboratory.

POSTER: ESTIMATING WILD BOAR DENSITY AND SPATIAL EXPLORATION OF THE INTERFACE WITH IBERIAN PIGS

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Key words: wild boar, spatial, African swine fever

As part of the risk assessment of the introduction of African swine fever (ASF) in the European Union under the EC ASFRISK project, we explore the spatial relationship between wild boars and pigs reared in an outdoor system, like the Iberian pigs in Spain.

Free from ASF since 15 years ago, the disease was endemic in Spain for more than 30 years (1960-1995), causing underdevelopment of a sector that has turned into the main livestock industry in Spain after ASF eradication. The disease was controlled after a joint effort between farmers, government and laboratories to early detect and slaughter the positive animals and by increasing the biosecurity measures. Still nowadays, vaccine is not available. The current epidemiological situation of ASF in Europe requires the identification of areas at higher risk of exposure in "times of peace" in order to prevent the economic losses if an incursion occurs.

During the ASF epidemic in Spain, the main transmission route was direct contact between infected and susceptible swine. Despite the industrialisation of the pig production and high biosecurity measures together with an, Iberian pigs, an excellent representation of a sustainable production system, are reared in extensive fenced pastures that occasionally establish contact with wild boars. The increase in the wild boar population in the last decade, both in Spain and in other European countries, has favoured the chances of such contacts occurring. Still, the estimation of the wild boar population is based on incomplete data, mainly on hunting records, which underestimates the impact that the disease would have on other susceptible swine.

With the application of geospatial techniques, we estimated the population of wild boars based on hunting bags (2007 data), adjusted by survey results, and on habitat distribution characteristics based on land-use with trees from the Fagaceae family or wet/dry crops. Next, we considered their proximity to high density areas of Iberian pigs, taking into account possible movements of wild boars.

The study aims to offer a specific approach starting from a general point of view at the country level and focusing on a limited area where ASF was endemic and where the availability and representativeness of data can amplify the utility of the method.

Results will be compared to the historical ASF outbreak distribution in that region, especially after the disease was controlled for in the intensive pig industry.

The estimation of wild boar population could be extrapolated to other regions and the methods employed can be used for the assessment of the domestic-wildlife interface for other diseases too.

POSTER: DEVELOPMENT OF A COMMERCIAL REAL TIME RT-PCR KIT FOR TYPING THE EUROPEAN BLUETONGUE VIRUSES (1, 2, 4, 6, 8, 9, 11 AND 16) AND A SEROGROUP ASSAY FOR EHDV DETECTION

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Key words: Bluetongue EHDV rtRT-PCR "European-BTV"

Since 1998, 12 distinct incursions of bluetongue virus (BTV) from eight different BTV serotypes have resulted in outbreaks of bluetongue disease throughout Europe. The presence of nine BTV types (BTV-1, 2, 4, 6, 8, 9, 11, 16 and 25) in Europe, as well as EHDV in Algeria, Morocco, Israel and Turkey, has highlighted a need for more rapid and reliable diagnostic assays to detect and type these viruses from clinically affected and suspect animals. Initially, several BTV group specific assays targeting the genome segment 1 (encoding the highly conserved viral RNA polymerase – VP1) were developed by LSI (Laboratoire Service International) or the Institute of Animal Health (IAH) (Shaw et al 2007). IAH and LSI have subsequently worked in partnership to develop and produce assays for the detection and typing of BTV, and detection of EHDV. This paper reports the validation of these assays with different BTV and EHDV strains.

RNA, isolated from a range of distinct BTV isolates from different locations world-wide (including both Eastern and Western strains/topotypes) and nine different EHDV genotypes, were used to check the specificity and sensitivity of these assays. The RNAS were isolated from field samples or tissue cultured viruses and were amplified for BTV typing in real time (rt) RT-PCR assays using primer sets and a probe labelled with FAM, that target genome segment 2 (encoding the serotype-specific outer capsid protein VP2) of the different BTV types. Primers and a probe targeting genome segment 9 (encoding the conserved inner-core-protein VP6) were also used for detection of EHDV. These tests were designed as "duplex" assays, with an "Endogenous Internal Positive Control" consisting of a set of primers and a probe labelled with VIC, targeting the Beta-Actin gene. An External Positive Control (EPC) was also used, consisting of a plasmid obtained by cloning. These nucleic acids were purified, quantified and diluted in storage buffer.
The sensitivity and amplification-efficiency of the rtRT-PCR assays were determined by testing a 10-fold dilution series of BTV or EHDV RNA, diluted in TE Buffer. The limit of detection of each rtRT-PCR was determined by testing this quantified plasmid.

All the BTV strains were detected by the homologous type-specific BTV assays. All of the EHDV strains were detected by the EHDV specific assay. No cross reaction was identified between the different BTV typing assays and the EHDV assay. The BTV-typing and EHDV specific assays were shown to have amplification-efficiencies of 100% and 101% respectively.

In conclusion, the rtRT-PCR assays for BTV typing and EHDV detection were all shown to be specific, highly sensitive and capable of identifying different virus strains from around the world. The detection limit of each PCR was shown to be 3 to 10 copies of plasmid, confirming their ability to detect very few amounts of virus. These rtRT-PCRs are available as part of a single kit, which simplifies BTV typing and EHDV detection.

Shaw A.E. et. al., (2007) Development and validation of a real-time RT-PCR assay to detect genome bluetongue virus segment 1 Journal of Virological Methods. 145, 115-26.

POSTER: A SEQUENCE DATABASE FOR RABIES VIRUS

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Key words: Sequence Database Rabies

Rabies is a fatal viral disease. Reports from the World Health Organization estimate that in excess of 55,000 rabies deaths in humans occur worldwide each year. With relatively few exceptions, terrestrial rabies has been eliminated from Western Europe, however rabies remains endemic in most of Eastern Europe. Oral rabies vaccination control campaigns involve the costly and intensive use of aerial-dropped baits targeted at the major vector, the red fox (Vulpes vulpes).

The success of any control policy would depend heavily on the rapid and accurate diagnosis and epidemiological analysis of the initial cases, e.g. to confirm the source of an imported case or exclude a vaccine related case. Genetic sequences of confirmed diagnostic isolates can be used in association with other sequence data from simultaneous outbreaks or from existing databases to produce accurate epidemiological information. Published virus sequences are available on Genbank for rabies isolates. However, there are often several partial gene sequences for each virus isolate, duplication of data by reference labs and limited or absent submission details, resulting in extensive searches of the literature to collate the relevant data. We describe a newly developed sequence database for rabies virus isolates which combines the virus, sequence and laboratory information necessary to assist authorities to deal with rabies epizootics, incursions and importations.

POSTER: MOLECULAR CHARACTERISTIC OF CLOSTRIDIUM BOTULINUM STRAINS BY USING PCR BASED METHODS

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Key words: Clostridium botulinum, PCR, real - time PCR

Bacteria from the species of Clostridium botulinum are spore – forming anaerobic rods which are able to produce the most dangerous biological toxins in environment. These toxins are etiological agents of botulism in human and animals. Among Clostridium botulinum there are isolates which have ability to produce two kind of toxins. There were also observed possibility of acquisition of toxin genes and loss of toxic properties among Clostridium botulinum strains.

The aim of this work was molecular characteristic of 26 isolates which were previously described and classified into the species of Clostridium botulinum in aspect of loss and acquisition genes which determine their toxigenicity.

Material and methods

Twenty six strains which were previously classified into Clostridium botulinum species were used in this investigation (8 strains of toxinotype A, 12 strains of toxinotype B and 6 strains of toxinotype E) and additionally reference strains types A, B, E (NCTC 887, NCTC 3815, NCTC 8266).

Incubation. Strains were inoculated into the TPGY broth and incubated for 48 hours at 37oC under anaerobic conditions. The next step was inoculation several drops of liquid culture on FAA (Fastidious Anaerobic Agar) plates and incubation for 48 hours at 37oC under anaerobic conditions. After incubation biochemical properties of analyzed strains were estimated.

DNA isolation. Isolation of DNA from single colonies of each analyzed strain which was grown on FAA was performed by using kit for DNA isolation – "Genomic Mini AX Bacteria" (A&A Biotechnology). Isolation of DNA was conducted according to the protocol supplied by producer.

Real – time PCR analysis for classification into the species Clostridium botulinum.

For classification into Clostridium botulinum species the real – time PCR based method was used for detection of gene which determine nonhemaglutinin component (NTNH) production in botulinum protoxins and which is common in all strains of this pathogen.

Multiplex PCR for determination of toxinotypes. For determination of toxinotypes multiplex PCR with detection on agarose gel was used. This method enabled detection of region which determine production of active form of botulinum toxins (BoNT) types A, B, E.

Results and summary

Out of 26 analyzed strains only 13 were possessed genes which determine production of botulinum toxins. From 8 strains which were previously recognized as toxinotype A only 3 had ability to produce botulinum toxin type A and in one case was observed presence of genes which determine production of A and B toxins. From 12 strains which were previously recognized as toxinotype B, only in 7 of them was detected gene which determine production of toxin type B, while in one case was observed presence of genes determine production of A and B toxins. Among 6 strains, which were previously recognized as toxinotype E, was observed absence of gene which determine production toxin of this type, while in one case was observed presence of gene which determine production of toxin type B. All investigated strains demonstrated characteristic biochemical properties for Clostridium botulinum species.

Conducted analyzes showed possibility of loss and acquisition of genes which determine production of botulinum toxins among Clostridium botulinum strains. Mechanism of this phenomena is not clearly explained. In spite of similar biochemical properties, there is a possibility of changing or loss of toxigenicity of Clostridium botulinum strains during isolation process, that could cause difficulties in diagnostic of botulism.

POSTER: DEVELOPMENT OF AN AUTOMATED REAL-TIME MULTIPLEX PCR FOR SIMULTANEOUS DETECTION OF CLASSICAL AND AFRICAN SWINE FEVER VIRUSES

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Key words: Swine fever diagnosis, real-time PCR, automated extraction

Background. Classical swine fever (CSF) and African swine fever (ASF) are highly contagious diseases of pigs for which differential diagnosis on clinical and post-mortem signs is impossible. To improve capacity for molecular detection of these pathogens, automated extraction of viral nucleic acid from porcine blood has been optimised and a multiplex RT-PCR developed for detection of both CSFV RNA and ASFV DNA. The assay includes an internal control to monitor for false negative results.

Methods. Automated extraction of viral nucleic acid from porcine blood was optimised using the QIAGEN QIACube and BioRobot Universal extraction systems. A multiplex RT-PCR was developed using the Invitrogen Superscript Platinum III one-step RT-PCR kit. The automated multiplex assay was compared to existing assays for detection of CSFV RNA and ASFV DNA in porcine blood and tissues.

Results. Successful extraction of viral nucleic acid from porcine blood using the BioRobot Universal was achieved using 50µl of diluted sample and a proteinase K treatment. The QIACube permitted extraction of 140µl EDTA blood with no need for dilution or protease treatment permitting a greater yield of CSFV from blood samples. The automated extraction methods and multiplex RT-PCR was marginally less sensitive for CSFV detection than the existing manual RNA extraction and CSFV RT-PCR method. Inhibition of RT-PCR amplification of both viral and IC templates was occasionally observed.

Conclusions. The automated extraction and multiplex RT-PCR is suitable for detection of both CSF and ASF with increased throughput capacity. The inhibition of RT-PCR amplification in some samples highlights the importance of including internal controls

POSTER: INVESTIGATION ON THE ROLE OF PIG MANURE FOR INDIRECT TRANSMISSION OF CLASSICAL SWINE FEVER BY TRANSPORT VEHICLES

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Key words: CSFV; pig-manure ;spreading of disease

Transport of live pigs poses a huge risk for spreading disease. Special concern is tied to the exotic viral infections such as classical swine fever (CSF) which is eradicated in the majority of the European Community but still constitutes a risk following transport of pigs of unknown disease status. In many cases a combination of illegal import or swill feeding and transportation of live pigs have resulted in large epidemics.

During transportation of pigs, manure will be left on the floor and the walls of the transport vehicle. In case of suboptimal cleaning and disinfection, infectious manure may still be present. This is especially a risk during the winter season where the temperature does not allow for proper hot-water cleaning.

In collaboration with the Danish Pig Breeders we have started a study to examine the variability of virus contents in pig manure from recently infected pigs. Feces from pigs infected by CSF virus strains of high to low virulence will be analysed.
Feces collected from the isolation facilities housing experimentally infected pigs was tested by PCR and by virus isolation using methods adapted from Weesendorp et al. 2009 (1). In bulk manure from pens of pigs infected with highly virulent CSFV Kozlov strain the presence of viral RNA was detected in feces from 4 to at least 11 days post infection (dpi) when the experiment was terminated.

Results will be compared to virus analyses of serum from the same groups of pigs.

Furthermore we will analyse how representative a sample of manure is. This is done by dividing a larger sample of known CSFV positive manure into 10 samples to be analysed individually.

The poster will include presentation of further results from daily manure samplings from piglets infected by CSFV Kozlov, CSFV Eystrup, CSFV Bergen and CSFV Lithuania representing both older and more recent strains.

1. Weesendorp E, Stegeman A, Loeffen WL (2009) Quantification of classical swine fever virus in aerosols originating from pigs infected with strains of high, moderate or low virulence. Vet Microbiol 135: 222-230

POSTER: DEVELOPMENT OF ELISA TEST FOR DETECTION OF INFECTION WITH GENETIC VARIANTS OF BOVINE LEUKEMIA VIRUS

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Key words: BLV, env gene, variants, ELISA

Bovine Leukemia Virus (BLV) like other retroviruses has developed several approaches to survive in the infected host. Its potential to generate the genetic variability of env gene is one of the most effective strategies. In the previous study in naturally BLV infected cows we identified several point mutations in 5' end of env gene, leading to subsequent amino acids substitutions on epitopes G and H of surface glycoprotein gp51. For this study we selected three genetic mutants representing the following phenotypes : no 18 – G'-H-GG', no 306 – G-H'-GG', no 317 – G'-H'-GG' as well as fourth – wild type – G-H-GG derived from gp51 FLK. Firstly 903bp sequence of whole gp51 protein with signal peptide was cloned into pCRBlunt vector and subsequently FLK-ATG-gp51-pCRBlunt construct was obtained. Next the 146bp region of env gene encoding G and H epitopes of all these mutants was amplified by PCR and cloned into AvrII and BglII sites in env gene of FLK-ATG-gp51-pCRBlunt. Finally four constructs were generated – three representing all mutants FLK-ATG-gp51/18/pCRBlunt, FLK-ATG-gp51/306/pCRBlunt, FLK-ATG-gp51/317/pCRBlunt and one – wild type FLK-ATG-gp51-pCRBlunt and used for construction of recombinant baculovirus transfer vectors. The presence of correct sequence in recombinant bakmid DNA were confirmed by PCR and sequencing. In next step Sf9 insect cells were infected with recombinant baculoviruses and expression of chimeric proteins were detected by IPMA with monoclonal antibody directed against not mutated epitope D. Localization of recombinant proteins in insect cells done by Western Blot showed that both proteins represented mutants and wild type gp51 were secreted as a soluble protein to culture media. Next ELISA tests were designed and each of antigen were tested firstly with homologous serum from cattle no 18, 306, 317 and also BLV positive serum, adequatively and all with negative control serum too. In ELISA with recombinant wild type gp51 protein and one out of three mutated one – no 18 there was noted a clear discrimination between the reactivity of negative sample versus positive serum and homologous, respectively (twofold difference in OD values). Then 436 field sera which were identified as negative in commercial blocking ELISA test were screened by ELISA with recombinant FLK/18/gp51 protein. Cut of value was set up as the mean value obtained for homologous serum no 18 plus one standard deviation. In this conditions 20 (4,5%) serum samples recognized previously as negative highly reacted with the antigen representing gp51 protein showing phenotype G'-H-GG'. In conclusion, newly developed ELISA test allow to find as BLV positive serum samples which were not detected in ELISA test with antigen based on wild type gp51 BLV protein.

POSTER: EVALUATION OF PRIPROET PCR FOR DETECTION OF SWINE INFLUENZA VIRUS IN CLINICAL MATERIAL

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Key words: swien influenza virus, Real-Time PCR

Introduction
Fluorescence Resonance Energy Transfer (FRET) is powerful technique for characterizing distance-dependent interactions between dyes. The PriProET technology works through FRET and combines probe-based real-time monitoring of PCR amplification with confirmation of probe hybridization using probe-melting profiles (1). In one diagnostic step it's possible to measure fluorescence of amplicon's increase and determine the specificity of PCR product according to melting curve. This method was evaluated to detect swine influenza virus (SIV) in clinical material.

Materials and methods
Primers and probe sequences for Pri-ProET PCR were designed according to the most conservative region in matrix (M) gene of SIV. Sequences for alignment were taken from GeneBank in NCBI website and from SIV strains isolated from clinical material in Poland. Forward primer was labeled with FAM and OregonGreen as a donor dyes. As acceptor dyes for probe labeling dyes with different absorption maximum, like: HEX, TAMRA, Cy3 and Cy5 were selected.
Briefly, the M genes of H1N1 (A/Sw/Bel/1/98) strains was amplified by RT-PCR and the amplification products were cloned into the pCR 2.1 vector using a dual-promoter TOPO TA cloning kit (Invitrogen). The number of RNA copies was calculated. Tenfold dilutions of the RNA transcripts, ranging from 1 to 1010 copies/µl, were prepared. Pri-ProET PCR was analyzed for every pairs of dyes with the same RNA dilutions of RNA transcripts. Other subtypes, like H1N2 and H3N2, represented by A/Sw/Eng/96 and A/Sw/Fl/1/98 strains and by the field isolates were also included.

Results
All primer-probe sets used permitted the amplification of the expected fragment size (170bp) in electrophoresis assay. Specific melting curves, with temperature around 74ºC, were observed only in sets with Cy5, Cy3 and TAMRA dyes with FAM or OregonGreen as donors. Four different dyes were tested with the same target RNA transcripts dilutions. Using Cy5 probe dye and FAM or OregonGreen-pimer dye, the limit of detection in terms of M gene copy number for the H1N1 subtype was 101 gene copies/µl of in vitro transcribed RNA. For variants with TAMRA and Cy3 dyes sensitivity decreased to 103 gene copies/µl. There were no positive results from Pri-ProET using HEX dyes. Levels of fluorescence energy were 4000dR, 3000dR, 2500dR with using Cy5, Cy3 and TAMRA, respectively. No excitation was obtained with HEX labeling probe. Fluorescence was increased using OregonGreen as a donor in relation to Cy5. There was no correlation between particular subtype and specificity of melting curve. All results obtained in conventional SyberGreen-PCR have been confirmed in Pro-ProET PCR using OregonGreen-Cy5 pair of dyes with comparable sensitivity to detect influenza virus RNA in clinical material.

Discussions
Pri-ProET method was found as a tool for detection, quantification and variability checking of SIV genetic material. In this report it is shown that Cy5 is the most suitable acceptor for Pri-ProET assay. Cy5 and OregonGreen are the best pair-dyes in selected group of acceptors with non-overlapping spectrum. OregonGreen dye was confirmed as more stable then FAM dye. Interspecies and intersubtypes reassortation in influenza viruses world can limit subtyping based on internal genes amplification in this method. Internal genes, as M gen, can posses the same genetic information in several subtypes of SIV. As it was shown the same sequence can be detected in H1N2 and H3N2 subtypes.

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POSTER: STUDIES ON THE INVOLVEMENT OF INTEGRINS TO THE BINDING AND ENTRY OF WEST NILE VIRUS INTO HOST CELLS

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Key words: West Nile Virus, Receptor, Integrin

West Nile Virus (WNV; Flaviviridae) was initially isolated in Uganda in 1937 and gained worldwide attention when it was introduced to the USA in 1999. In the natural transmission cycle WNV is carried between mosquitoes and birds but humans and horses get consistently infected with possible fatal outcome (dead end hosts). A growing number of WNV outbreaks have recently been reported for Europe. The worldwide travel and trade together with the climate change may allow WNV to spread further in so far unaffected areas. Differences in the transmission efficiency may originate in the differences in the molecular interactions of the viral infection. Advanced studies on the prevalence and transmission of WNV are therefore necessary.

The aim of our studies is to identify factors determining the susceptibility of selected vertebrate species to WNV, starting with the role of the cellular receptor, and the receptor-virus-interactions, respectively. The search for possible cellular binding partners in viruses of the Flaviviridae familiy has been intensified in recent years. The integrin alpha v beta 3 was first isolated and characterised by Chu and Ng (2004), J Biol Chem. 279(52):54533-41, as a putative receptor for WNV. By contrast, Medigeshi et al. (2008), J Virol. 82(11):5212-9, using another strain and different approaches, demonstrated that WNV entry is independent of integrin alpha v beta 3.

To elucidate the potential role of the integrin as a receptor/co-receptor or within a receptor complex for virus entry, we established cell culture models. Embryonic mouse fibroblasts lacking either the integrin subunits alpha v, beta 3 or beta 1, and wildtype cells were isolated. In a first assay the mentioned cell lines were infected with four WNV strains, NY99 (Lineage 1), Uganda (Lineage 2), Dakar (Lineage 2), and Sarafend (Lineage 2), to investigate the WNV replication ability after five days of incubation. In a following binding experiment infected cells and cell culture supernatant were harvested 1 hr, 24 hrs and 48 hrs after incubation with the virus. Viral genomes representing virus particles in cells and supernatant was measured with quantitative real-time RT-PCR, and normalised to cell numbers using the cellular house keeping marker gene GTF2i, and virus titers representing infectious virus were determinated. Our infection experiments showed that (i) all strains are capable to replicate in all cell lines used, (ii) titers and ct data are different among the four WNV strains, (iii) binding and replication efficiency regarding the deficient cell lines can be compared. Therefore, we postulate that integrins do in fact play a role for virus binding and entry into cells but they are not the exclusive receptor for WNV.

POSTER: PATHOGENESIS OF PANDEMIC AND ENDMIC INFLUENZA VIRUS A/H1N1 IN PIGS

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Key words: pigs, influenzavirus H1N1

Porcine influenza virus strains of subtype H1N1 are endemic and cause clinical disease in pig farms worldwide. The pandemic virus A/H1N1/09 contains gene segments with ancestors in the classical North American (HA, NP and NS) and Eurasian (NA, M) swine influenza lineages. In the course of the pandemic spread of influenza virus A/H1N1/09 in humans, infections of pigs with this virus are reported from 10 countries in Europe, Asia, America and Australia. To compare the pathogenesis of pandemic and endemic influenza viruses of subtype H1N1 in pigs, we performed experimental studies using influenza virus A/Regensburg/D6/09/H1N1 derived from a human patient and three antigenetically distinct endemic porcine influenza viruses of subtype H1N1 isolated in Germany. In addition pigs who had overcome an infection with an endemic H1N1 strain were exposed to the pandemic virus A/H1N1/09.
Experimental infections were performed intranasally in 10-week-old pigs. Animals were investigated for clinical signs. Nasal and rectal swab samples were taken daily to monitor virus excretion using real-time RT-PCR and virus isolation. Immune responses were analysed using a commercial H1N1 ELISA and hemagglutination inhibition (HI) assays were performed with different porcine influenza viruses of subtype H1N1 as well as H1N2 and H3N2 in order to investigate serological cross-reactions. Three weeks after endemic H1N1 virus infection, three pigs were exposed to the pandemic A/H1N1/virus and analysed similarly. Necropsies were performed on all animals. Lung, trachea, lymph nodes, tonsil, brain, heart, liver, and spleen were subsequently analysed immunohistochemically and by real-time RT-PCR. The results will be presented.

POSTER: RE-EMERGENCE OF WEST NILE DISEASE IN THE HORSE POPULATION OF CENTRAL ITALY

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Key words: West Nile Virus, surveillance, horses, birds, mosquitoes

West Nile virus (WNV) disease is an important, zoonotic emerging arbovirus infection, with surveillance of viral activity in animals being the key factor for its control. In 1998, Italy reported the first equine outbreaks of WNV disease (WNVD) in Tuscany. From 2001, a national veterinary surveillance system was adopted, based on the reporting of avian mortality and on the repeated serological testing of sentinel chickens and horses, in areas considered at risk of WNV introduction. The criteria for case definition was that proposed by the CDC. Until 2008, no further WNVD cases occurred. During this year, the circulation of WNV was reported in horses, birds and mosquitoes, in an area involving eight provinces of the regions Emilia-Romagna, Veneto and Lombardia. Subsequently, in these regions, animal and human active and passive surveillance was strengthened, essentially based on horse syndromic surveillance, on serological and virological monitoring of horses, wild (death or captured) and domestic birds, on bird mortality monitoring and on mosquitoes surveillance. During 2009, in Latium and Tuscany, the regions under our jurisdiction, both clinical and serological cases of WNVD were reported only in horses. Cases occurred in the surveillance areas and in a Tuscan province outside the control area. The latter outbreaks were identified through the syndromic surveillance. As in the regions which had registered WND cases in 2008, we also adopted the extended surveillance plan in 4 km radius area, having as centre the stables registering an outbreak. In Latium, 650 equine whole blood samples and 500 bird tissue or swab samples were virologically tested and resulted all negative. Of the 1500 equine serum samples serologically examined, positive or doubtful results were obtained for a total of 29 horses of 17 different stables, all located the province of Latina. Specifically, 10 animals tested positive/doubtful towards IgM antibodies and/or 19 towards IgG antibodies. None of the serologically positive animals presented clinical signs referable to WNVD or were virologically positive. Confirmatory analyses, performed at the WNV National Reference Centre (CESME), identified 5 WNVD cases. In Tuscany, the 350 equine whole blood samples and 950 birds tissue or swab samples virologically examined, also resulted all negative. Of about 1250 serologically tested equine serum samples, positive or doubtful results were however obtained in a total of 29 horses, with the involvement of 19 stables of 3 different provinces (Arezzo, Lucca and Pisa). Specifically, 15 animals tested positive/doubtful towards IgM antibodies and/or 19 towards IgG antibodies. The only WNVD cases confirmed by the CESME were in one province (Arezzo) outside the surveillance area. In particular, 9 WNVD cases were confirmed and 5 new stable outbreaks were identified. In 2 of these outbreaks, horses (N=3) with suspect clinical signs referable to WNV infection were present, but no mortality cases were reported. The results of the 2009 surveillance activity in Latium and Tuscany demonstrate that WNV has actively circulated not only in the north of the country, but also in at least 2 provinces of central Italy. Despite these results, in both regions WNV was not detected in trapped mosquitoes, nor were humans WNVD cases reported. Also because viral isolation was not successful, no hypothesis can be advanced regarding the origin of the infection and the lineages. Future surveillance will allow to better understand if the infection has became endemic also in Central Italy.

POSTER: EXPRESSION AND CHARACTERISATION OF CSFV ERNS PROTEIN IN PICHIA PASTORIS.

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Key words: DIVA diagnostic, CSFV, Pichia pastoris

Although outbreaks of CSFV still occur in Europe, the disease has been successfully eradicated in domestic pigs in most of the member countries of the European Union due to strict eradication programmes. However, the disease still persists in several wild boar populations around Europe, which acts as a reservoir for CSF. Efficient live vaccines against CSFV are available for emergency vaccination or prophylactic use, but they do unfortunately elicit the same antibody pattern as those observed in naturally infected animals. This means that infected animals cannot be distinguished serologically from vaccinated animals. Alternatives to these currently used live vaccines are the new DIVA (Differentiating Infected from Vaccinated Animals) vaccines that in combination with suitable DIVA diagnostic tests, can be used to monitor and control infections(1).

The purpose of this study was to gain more knowledge about Pichia pastoris as expression system and whether it is possible to produce active CSFV structural proteins in a quality good enough for further application in DIVA diagnostic assays.
Current cDNA from CSFV Paderborn encoding the Erns gene has been successfully amplified by PCR, TOPO cloned and after restriction enzyme digestion and sequencing inserted into the expression vector pGAPZαC.
Future work will involve transformation of the recombinant plasmid into Pichia host strain X-33 by electroporation and selection of zeocin resistant transformants containing the Erns gene confirmed by PCR. The transformants will be induced with glycerol for expression and the cultural supernatant subjected to purification using the epitopes His-tag and c-myc, both incorporated into the expressed protein, to evaluate which method that gives the highest yield. Characterisation of the purified proteins will be carried out using SDS-PAGE and Western blotting before developing an ELISA or a Luminex assay using the produced Erns protein as coating antigen.

The hope is that the Pichia pastoris expression system will turn out to be a very useful tool for production of CSFV proteins for diagnostic purposes as indicated in previous studies(2). One big advantage using Pichia is the secreted expression but the system has also many of the same advantages seen in higher eukaryotic expressions systems while being as easy to manipulate as E. coli. In addition, it should be fast, easy and less expensive to use this system compared to other eukaryotic expression systems and the yield much higher.

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POSTER: INTERNAL VALIDATION OF REAL-TIME RT-PCR METHODS FOR THE DETECTION OF BLUETONGUE VIRUS IN DIFFERENT SAMPLES

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Key words: bluetongue virus, real-time RT-PCR, validation

Bluetongue virus (BTV), an arthropode-borne Orbivirus with a segmented double-stranded RNA, is a major pathogen of ruminants. At least 24 serotypes have been well characterised. BTV serotypes -1, -2, -4, -9 and -16 caused disease outbreaks from 1998 to 2005 in some Mediterranean countries. Starting with 2006, several new bluetongue virus serotypes, in particular BTV-8 but also -1, -6 and -11, have emerged in temperate regions of North-Western and Central Europe, including Austria.

For rapid and reliable laboratory diagnosis, real-time RT-PCR (RT-qPCR) methods are most commonly used by European National Reference Laboratories. Several RT-qPCR assays, targeting different regions of the BTV genome, have been recently published and have been demonstrated to detect BTV of all 24 serotypes – so called pan-BTV assays. Additionally, some commercially available BTV RT-qPCR kits have been validated. However, since laboratory-specific equipment and sample preparation may well influence assay performance, each laboratory is well advised to carry out additional internal validation to determine the method that allows optimal diagnostic sensitivity and specificity.

Six different pan-BTV RT-qPCR assays (a-f) were selected for internal validation: (a-d) are commercially available one-step RT-qPCR kits, whereas (e) and (f) are modified versions of 2 published RT-qPCR reference methods.RNA isolation was performed either manually using the QIAmp® Viral RNA kit (Qiagen), or automated with the Nucleospin® RNA Virus Kit (Macherey&Nagel, Germany). For all experiments, RNA samples (5 to 10 μl) were heat denatured at 95°C for 2 min prior to RT-qPCR. For sensitivity testing, a dilution series of BTV-8 positive field blood samples was analysed. To test for assay specificity, eight different BTV serotypes and several common viral and bacterial pathogens, as well as a set of BTV-negative blood samples were tested by all six methods. Ring trial samples distributed by the EU CRL for Bluetongue at IAH Pirbright (UK) were also included. The applicability of the assays for testing EDTA-blood and tissue samples from different animal species, based on the co-amplification of reference genes like GAPDH or ß-actin mRNA, or internal RNA controls such as EGFP, was also studied. All data were statistically analysed.

We observed major differences in the assay performance of the 6 kits. Reaction efficiencies varied from 51% to 130% and only 3 assays showed acceptable correlation coefficients and reached the detection limit defined by the reference method. In two cases, the sensitivity of the tests was lower than 100% (96.15%), indicating that they may fail to detect BTV-positive samples in the field. In addition, one of three commercial kits featuring co-amplification of reference gene mRNA (GAPDH, ß-actin) failed to detect mRNA from Lama, which can also serve as host for BTV infection.

In summary, we have determined the applicability of several BTV RT-qPCR methods for BTV screening of EDTA-blood and tissues from different animal species. Clear differences in assay sensitivity as well as specificity emphasise the importance of internal laboratory validation of commercial BTV RT-qPCR kits despite previous validation by the manufacturer.

POSTER: CONVENIENCE OF THE USE OF FTA CARDS FOR SIMPLE COLLECTION/STORAGE OF SAMPLES AND FURTHER DETECTION OF ASFV DNA

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Key words: African Swine Fever, FTA cards, molecular diagnosis

African Swine Fever (ASF) is a highly contagious disease that remains endemic in large parts of Sub-Saharan Africa and in Sardinia, producing great economic losses. In 2007, ASF emerged in the Caucasus for the first time, and has since spread to several countries in the region, being a major epidemic threat to EU. As there is no vaccine available, rapid diagnosis of the disease is of outmost importance to contain its spread. Diagnosis is usually performed in equipped labs, although new tools for the on-site ASF virus (ASFV) detection are being developed. Key points of an accurate diagnosis include: appropriate collection and rapid convenient transport of suspicious samples to the lab, storage of samples in adequate conditions, and execution of suitable diagnostic techniques.

FTA cards (Whatman) are made of special filter paper that contains a chemically treated fiber matrix. It is described that once a biological sample is applied to the FTA card, chemicals rapidly lyse cells, inactivate proteins and stabilize the DNA. This kind of cards are described for the simple collection and storage of clinical samples at room temperature, allowing long-term stability and further detection of present DNA even for years.

Fresh EDTA-blood and serum samples collected at different days from experimentally ASFV infected pigs were applied to FTA cards. They were allowed to completely dry and subsequently stored at room temperature. Small disks were punched from sample area and put in a PCR plate well. Optimisation of the fast procedure for viral DNA purification on FTA cards was carried out to avoid cross contamination and to afterwards incorporate the PCR mix directly to the washed and dried disks. Conventional and real-time PCR were run according to SOPs established in the lab (www.asf-referencelab.info). For a comparative study of the system convenience, aliquots of fresh collected blood and serum samples were also processed as usual to determine the presence of ASFV DNA following the reference PCR SOPs. Most of infected samples collected on FTA cards were properly detected, though higher Ct values were observed when compared real-time PCR results.

Analysing daily collected samples, positive results were obtained in fresh blood and serum samples from 1st and 3rd day post-inoculation (dpi), respectively. Samples collected on FTA cards were ASFV positive from 2nd dpi in blood and 3rd dpi in serum after three years of room temperature storage.

Two ASFV positive blood and serum samples were serially diluted in negative porcine blood and sera, respectively, and analysed for sensitivity studies, being detected as positive until 10-4 and 10-3, respectively. When FTA cards impregnated with these samples were analysed, 10-3 (real-time PCR) or 10-4 (conventional PCR) was the detection limit for blood sample, whereas 10-1 was the lowest positive dilution for sera.

When clinical signs appear in ASFV infected animals and urgency for a rapid diagnosis is needed, the viraemia titre is sufficiently high to be detected by using FTA cards. The advantages of simple collection, transport and storage at room temperature, together with the non-infectious basis of samples collected on FTA cards, the easy treatment procedure and the potential combination with newly developed pen-side tests, show FTA cards as a possible convenient way to make real the ASFV DNA detection in basic/regional labs as a first line tool for the rapid on site diagnosis.

POSTER: AVIAN INFLUENZA IN ITALY. THE ROLE OF NON-INDUSTRIAL (RURAL) SECTORS.

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Key words: avian influenza, rural sector, free range, wild bird, phylogenetic analysis

Since 1997, the Italian poultry production system has experienced several outbreaks of avian influenza (AI). In 1997 a highly pathogenic (HP) AI H5N2 epidemic occurred in the rural poultry sector, without the involvement of any industrial poultry farm.

From 1999 to 2005, HP and low pathogenic (LP) AI involved mainly the industrial sector and only sporadic outbreaks affected non-industrial farms. In these years, the industrial poultry sector appeared to play a major role in the spread of AI. Consequently, AI monitoring plans were implemented to allow the rapid detection of AI introduction and spread mainly in industrial poultry farms located in densely populated poultry areas (DPPAs).

Aim of this study was to delineate, on the basis of new epidemiological findings, the role of these two poultry producing sectors in the epidemiology of AI in Italy.

In 2007, 11 backyard/hobby or dealer flocks and 6 industrial meat turkey farms were found positive to LPAI H7N3 virus. Moreover an LPAI H5N2 virus sub-type was found in a duck and geese free-range farm in Emilia Romagna region and in 2008, an LPAI H7N1 virus subtype was isolated in a dealer flock in the same area.

During 2009, the LPAI H7N3 virus sub-type re-emerged in non-industrial farms and, from May to December 2009, a total of 31 outbreaks (18 backyard and 13 dealer/grower flocks) were identified in 9 Italian regions. The epidemiological investigation identified several links among rural flocks located in different geographical areas. This confirms that the non-industrial sector has a wide-ranging network of at-risk contacts within the national territory and represents a non-negligible part of the Italian poultry production system (in Italy, about 55 million birds are traded per year in this sector).

The phylogenetic tree of the HA genes of 2009 LPAI H7N3 viruses revealed the existence of a close genetic relationship among them (similarity ranged between 98.6% and 99.9%). Detailed analysis highlighted that the sequences of 2009 H7N3 viruses formed a monophyletic cluster together with the sequences of the LPAI H7N3 viruses identified in 2007. These results suggest that the 2007 and 2009 H7N3 epidemics were caused by viruses originating from a common progenitor and underline the importance of the rural poultry sector in the persistence and evolution of AI viruses.

Taking into account that rural poultry are mainly kept in free-range, multi-species, multi age farms with low biosecurity levels and exposed to a great number of contacts at-risk, it is possible that these farms act as the epidemiological link between the wild reservoir of AI viruses and intensively reared poultry. This role can be inferred from the phylogenetic analysis carried out on the HA genes of LPAI H5N2 and LPAI H7N1 viruses isolated in 2007 and 2008. respectively. This analysis showed that these viruses had the highest nucleotide similarity (99.8% and 99.7%, respectively) with strains isolated from wild birds in the same period and geographical area.

The 2007-2009 H7N3 epidemics highlighted the need to implement a new set of AI control and bio-security measures, in the rural sector mainly at the top of the production chain (commercial farms such as dealers and growers). In this production sector, monitoring activities should be strengthened to rapidly detect the introduction of AI viruses from the wild reservoir, with the aim of limiting both the possible spread of AI infections to industrial poultry and the risk of emergence of HPAI virus due to the uncontrolled circulation of H5 and H7 LPAI viruses in domestic poultry.

POSTER: REAL-TIME PCR DETECTION OF EQUINE HERPESVIRUS TYPE-1 AND TYPE-4 IN DIFFERENT TYPES OF SAMPLES OF ABORTED FOALS IN TURKEY

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Key words: Equine Herpesvirus, Real-Time PCR, Foals, Turkey

There has been a growing interest on horse racing in Turkey and therefore racing horses has an economical impact on horse industry. Infectious diseases like equine herpes virus (EHV-1 and EHV-4) affects equine health all over the world.and decreases the performance and production of racing horses by causing reproductive and respiratory disorders. Therefore, this study was planned to detect EHV-1 and EHV-4 in different types of samples collected from aborted foals in Turkey using real-time PCR.This would help in rapid diagnosis and differentiation of herpesviruses in horses. For this, 11 aborted foals from different horse studs were analysed. Samples from 11 lungs, 11 spleens, 11 livers, 8 kidneys, 2 brains and 1 lung exudate were taken. Blood from 7 mares aborted were also taken. DNA was extracted from the tissues and lung exudate using QIAamp DNA Mini Kit (Qiagen), and from blood using High Pure PCR Template Preparation Kit (Roche). DNA samples were then analysed for the presence of EHV-1 and EHV-4 using spesific primers by real-time PCR (SYBR Green). EHV-1 DNA was detected in 4 of 11 lungs, 3 of 11 spleens, 3 of 11 livers, 2 of 8 kidneys, 1 of 2 brains and 1 lung exudate sample. No EHV-4 DNA was detected in any of the samples taken from aborted fetuses. Also, neither EHV-1 DNA nor EHV-4 DNA was detected in the blood of mares aborted. The sequencing of positive samples is going on to determine EHV strains circulating in Turkish horses.

POSTER: DEVELOPMENT AND EVALUATION OF A NEW EPITOPE-BLOCKING ELISA FOR THE UNIVERSAL DETECTION OF ANTIBODIES TO WEST NILE VIRUS

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Key words: West Nile virus; ELISA; diagnostic; serology; surveillance

West Nile virus (WNV) is an arthropod-borne flavivirus belonging to the Japanese Encephalitis serogroup. It cycles between mosquitoes, acting as vectors, and birds, acting as amplifying hosts. WNV causes clinical disease in birds, horses and humans. Most severe clinical outcome includes neurological signs, leading to meningitis, encephalitis, and death in a limited number of cases.
In the last 15 years the virus has spread relentlessly in many parts of the world. In Europe, large-scale outbreaks have occurred recently in Italy, Central and Eastern Europe, some of them still ongoing. Serological surveillance in birds and other animal populations at risk (horses) informs about exposure to WNV infection in a given territory, and helps establishing the epidemiology of the disease locally as well as to adopt control measures adapted to risks. Serological analyses of WNV-specific antibodies rely mainly on ELISA or IFA techniques for screening, and virus-neutralization tests for confirmation. However, few commercial sources are currently available fulfilling the needs for an optimal serological screening test: 1) rapid test amenable for high throughput screening and automation; 2) unique test valid for the range of WNV host species, including many species of wild birds as well as mammals; 2) low consumption of serum: some bird species subjected to surveillance are small birds which can be bled only for limited amounts of serum, often very few l serum);μmicroliters (some commercially available ELISAs require as much as 50 4) affordable prices for large-scale surveillance.
We have undertaken a project leading to the development of an ELISA technique valid as screening test for WNV antibody detection in the range of vertebrate species susceptible to WNV infection, useful not only for WNV diagnostic but also for WNV surveillance, overcoming the problems currently affecting commercially available ELISA tests. For that we raised a panel of monoclonal antibodies (MAbs), of which, after different immunochemical analyses, one MAb (1D11) was selected with optimal characteristics for this test. The MAb 1D11 was able to bind whole virus particles and to neutralize virus infection in vitro, recognizing a neutralizing epitope within the envelope (E) glycoprotein of the virus. This MAb, labeled with horseradish peroxidase, was used to compete with WNV-specific serum antibodies for virus-binding in vitro. We optimized this epitope-blocking ELISA in a way that enabled its validation with a number of experimental and field sera, from a wide range of wild bird species, as well as of susceptible mammals. The ELISA detected the presence of antibodies to WNV (confirmed by VNT) in field samples from horses and from a wide range of avian species, including representatives of the following Orders: Anseriformes, Ciconiiformes, Charadriiformes, Galliformes, Passeriformes and Phoenicopteriformes. In addition, the new ELISA detected antibodies to WNV in sera from experimentally infected red-legged partridges, before the outcome of neutralizing antibodies, possibly because this ELISA is sensitive to a wider antibody repertoire than VNT. In comparison with other commercially available ELISA tests, the new ELISA was advantageous since it not only displayed high sensitivity and specificity but also required much lower amount of sample to be accomplished: 10 microliters per analysis.

POSTER: GENETIC CHARACTERIZATION OF PESTE DES PETITS RUMINANTS VIRUSES ISOLATED FROM SHEEP, GOATS AND CAMELS IN SUDAN

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Peste des petits ruminants virus (PPRV) is a Morbillivirus (Paramyxoviridae) known as mostly affecting sheep and goats. It causes high morbidity and mortality (90-100 %) when occurring in naive populations. It is widespread in Africa, the Middle East, and Asia (1). It has reached the Thrace region of Turkey and has recently spread in Morocco (2008) and Tanzania (2009). Genotypic classification of PPRV according to the 3' end of the nucleoprotein (N) gene can discriminate four lineages of the virus, and is an efficient tool for a worldwide survey of PPRV spread (2).

Serological surveys have shown that camels are receptive to PPRV infection. Clinical suspicions have been reported in this species, with respiratory distress and mortality. However, PPRV had never been isolated from samples collected in suspect animals. In Sudan, 130 samples collected from camels, sheep and goats between 2000 and 2009 were examined. Eighty-two samples were positive for the antigen-detection ELISA, 62 (76%) of them being also positive for the RT-PCR test. Five PPRV have been isolated from these samples. Sequencing of 255 nucleotides of the 3' end of the N gene from the 5 isolates, and 23 RNA, samples extracted from lung tissues revealed that the vast majority of the viral sequences belonged to lineage IV, usually found in Asia, instead of lineage III, formerly widespread in East Africa. Two clusters were further defined within lineage–IV PPRV, both of them showing little within-cluster variation over the study time (overall nucleotide divergence of 1.2% to 3.9%). Species distribution in samples (camels and sheep) was perfectly fitted by the 2 clusters, thus suggesting a possible genetic bias of PPRV according to the host species. These findings provide important epidemiologic information on PPRV host distribution which had never been described before, and may be useful to track PPRV transmission.

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POSTER: RISK MAPS FOR WEST NILE DISEASE PRESENCE IN THE VENETO REGION (NORTH-EASTERN ITALY)

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Key words: vector borne disease; environment; risk maps; GIS; remote sensing

In August 2008, West Nile Disease (WND) was diagnosed in a horse with neurological symptoms and in a magpie (Pica pica) in the Emilia Romagna region of Italy. In 2008-2009, an intensive WND monitoring program involving public health and veterinary services of the Veneto, Lombardy and Emilia-Romagna regions was implemented.

In this two-year period, in the Veneto region, 171 horse premises, 10 wild birds (8 magpies and 2 hooded crows) and 23 other animal species (19 stray dogs and 4 sentinel cattle) tested positive. After the disease was found in animals, passive surveillance in the infected area detected 25 seropositive humans (12 in Veneto, 11 in Emilia Romagna and 2 in Lombardy). Phylogenetic analyses demonstrated that the viruses circulating during 2008 and 2009 were closely related.

The appearance of WND in the Po Valley (which spans the Veneto, Lombardy, and Emilia Romagna regions) in 2009 raised particular interest in its epidemiology and spatial distribution, with the aim of mapping possible risk areas. However, the creation of such risk maps is very complex, given that the ecology of the arthropod vectors depends on a variety of landscape and environmental factors (e.g., temperature, humidity, precipitation and vegetation), and reliable maps can only be obtained by using suitable mathematical models and spatial analyses algorithms that include such variables.

In this study, risk maps for the distribution of WND in the Veneto region were produced based on the 2008-2009 distribution of infected horse farms to assess the presence of the disease. For each of the presence points, temporal Fourier transformed NASA MODIS data were extracted (original resolution of c. 1km in the Sinusoidal projection, re-projected to WGS84 latitude/longitude system at a resolution of 1/120th degree). The data were available at intervals of 8-16 days, in the following "channels":
• Middle Infrared (MIR);
• Day-time and night-time Land Surface Temperature (dLST and nLST);
• Normalised Difference Vegetation Index (NDVI);
• Enhanced Vegetation Index (EVI)

Other satellite and ground-collected data on precipitation were available from CMORPH and WORLDCLIM, as monthly composites or averages. Data on elevation were extracted from a digital elevation surface derived from MODIS v5 dataset.

Data were processed using ESRI ArcGIS and IDRISI software. Since no "absence" points were available, a set of "pseudo-absence" points was selected within a defined distance from the presence points and within the regional boundary.

Risk maps were produced by establishing the relationship between the satellite and disease/vector data for points where records of the disease were available. Mathematical algorithms based on non-linear (maximum-likelihood) discriminant analysis, developed by the EDEN LRRS team, were applied. 100 bootstrap models were performed for each disease, and satellite and other variables were selected in a step-wise inclusion manner to maximize the corrected Akaike Information Criterion (AIC).

The models identified the likely key predictor variables for the presence of disease in horse farms in the Veneto region. It would be possible to monitor the changes in these variables in the territory of interest to define the areas where the disease would be likely to be observed, predicting changing levels of risk over time. However, much work is still necessary to explain the results of the presented risk maps in biological terms. Moreover, data on WND vectors and hosts have yet not been included and need to be accurately processed in order to obtain more reliable risk maps.

POSTER: CAN EUROPEAN DOMESTIC PIGS BE PROTECTED FROM VIRULENT AFRICAN ISOLATES OF AFRICAN SWINE FEVER VIRUS BY EXPERIMENTAL IMMUNISATION?

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Key words: ASFV pig vaccination

In 2007 African swine fever (ASF) was introduced into Georgia and from there spread rapidly to neighbouring countries in the Caucasus, including Southern European Russia. A subsequent, and no doubt related, outbreak is currently underway in Russia with the virus apparently moving steadily north- and westwards towards the borders of the European Union. ASF has been reported in the town of Gukovo close to the Russian-Ukrainian border, and more worryingly, several thousand kilometres to the north in Mga, Leningradskaya. Mga is approximately 165 and 190 km from the Russian borders with Estonia and Finland respectively. In the same year that ASF was introduced into the Caucasus the virus appeared on the island of Mauritius in the Indian Ocean, thus the threat of ASF introduction to Europe from Africa or Russia is unfortunately not remote.

ASF is a highly contagious, acute haemorrhagic disease of pigs caused by a large (~ 200 kbp) icosahedral DNA virus (ASFV). Virulent isolates kill domestic pigs within 7 - 10 days of infection. Currently there is no vaccine or prophylactic available for ASF. Domestic pigs can survive infection with less virulent isolates of virus and in doing so can gain immunity to subsequent challenge with viruses from related, but not distinct, geographical areas (Mebus & Dardiri, 1980; Ruiz Gonzalvo et. al., 1983; Hess., 1987, Oura, 1995). As the current ASF outbreak in the Caucasus and Southern Russia stresses the risk of trans-boundary introduction of the disease from Africa, we tested an experimental immunisation protocol to see whether it was possible to protect European domestic pigs from virulent African isolates of ASF virus. Animals were sequentially inoculated with a non-pathogenic strain of ASF virus (OURT88/3; genotype I) and then a closely related virulent virus OURT88/1 strain (genotype I) as a boost. These pigs were then challenged with two different virulent African isolates; Benin 97/1 (genotype I) or Uganda 1965 (genotype X). This immunisation strategy protected most of the pigs challenged with either Benin or Uganda from both disease and viraemia. These two African isolates were selected as they were recognised by ASFV specific IFN-γ ELISPOT assay using lymphocytes isolated from OURT immune pigs and have entire genome sequence to compare with OURT isolates. Interestingly the Georgia isolate (genotype II) demonstrated a reasonably high level of cross-reactivity in vitro. As the whole genome sequence of this virus has been recently completed, a cross-protection experiment with Georgia isolate is our next objective. Here we show that the combination of modern molecular biological and immunological techniques enabled us to predict cross-protection efficacy and will hopefully lead toward the development of an effective ASFV vaccine.

POSTER: DEVELOPMENT OF ISOTHERMAL AMPLIFICATION ASSAYS FOR THE DETECTION OF TAYLORELLA EQUIGENITALIS.

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Key words: isothermal, RPA, lamp, equine, taylorella

Contagious equine metritis (CEM) is an equine venereal disease caused by infection with the bacterium Taylorella equigenitalis (T. equigenitalis). Our aim was to compare two rapid isothermal amplification methods, Recombinase Polymerase Amplification (RPA) (Piepenburg et al., 2006) and LAMP, by designing assays to detect T. equigenitalis, directly from equine genital swabs.

In both assays, primers targeted the 18S rRNA sequence of T. equigenitalis. These primers were labelled using biotin and fluorescein, which enabled rapid downstream detection of the amplification products with a lateral flow device. Both assays were tested against a panel of bacterial species, including the closely related T. asinigenitalis, to determine diagnostic specificity and sensitivity. The analytical sensitivity and efficiency of the PCR was calculated using a dilution series of target DNA.

In conclusion, to isothermal assays were successfully developed to detect T. equigenitalis straight from equine genital swabs. The assays were specific and did not cross react with the closely related T. asinigenitalis. These tests provide a rapid and accurate means of identifying the causative agent of CEM directly from equine genital swabs.

POSTER: CRIMEAN-CONGO HAEMORRHAGIC FEVER, TICKS AND SWEDISH MIGRATORY BIRDS

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A number of migratory bird species migrate to the North from winter quarters in Africa, Middle East and the Mediterranean countries. Some of these species are, due to their behavior, more prone to pick up ticks than other species. This can be a problem if the ticks are carriers of pathogens. One such emerging pathogen is Crime-Congo Hemorrhagic Fever virus (CCHFV). The primary vector of CCHFV is the tick Hyalomma marginatum (Acari: Ixodida). One of the characteristics of H. marginatum is that it attaches to its blood meal host for long periods up to 26 days. If the host is a migratory bird this gives ample time to transport the tick to northern countries like Sweden. Questing H. marginatum ticks have been observed in Denmark and ticks found in Germany and Netherlands are suspected to have been carried there by migratory birds, far north of the usual range of the species. The ongoing climate change is expected to change the climate in Sweden in such a way that it will probably be suitable for H. marginatum in the future. Further the climate change may also change migratory patterns and the species of birds that transport ticks to Sweden. If H. marginatum establish populations in Central Europe a whole new range of birds, including the larger thrushes and finches, will be available as transport hosts to the ticks. Sixteen bird species have been caught in Scandinavia carrying H. marginatum. The most common is the willow warbler (Phylloscopus trochilus) with an estimated population size of 10-16 million breeding pairs. The others are listed in descending order of abundance (numbers are the estimated number of breeding pairs in Sweden): Tree pipit (Anthus trivialis) 3,5-7 million, White wagtail (Motacilla alba), 0,5-1 million, Garden warbler (Sylvia borin) 1-3 million, Whitethroat (Sylvia communis) 0,5-1 million, Blackcap (Sylvia atricapilla) 0,4-1 million, Reed warbler (Acrocephalus scirpaceus) 500 000 - 600 000, Wheatear (Oenanthe oenanthe) 350 000, Common redstart (Phoenicurus phoenicurus) 100 000 – 300 000, Sedge warbler (Acrocephalus schoenobaenus) 50 000 – 200 000, Bluethroat (Luscinia svecica) 140 000 – 180 000, Yellow wagtail (Motacilla flava) 170 000, Thrush nightingale (Luscinia luscinia) 20 000 – 50 000, Red-backed shrike (Lanius collurio) 26 000 - 34 000, Ortolan bunting (Emberiza hortulana) 7000, Collared flycatcher (Ficedula albicollis) 4600 - 5700.

POSTER: GENETIC VARIABILITY OF RNA VIRUSES: BIOLOGICAL SIGNIFICANCE AND MECHANISTIC ASPECTS

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Key words: RNA viruses, pestiviruses, evolution, RNA recombination

Genetic changes of viruses can lead to dramatic alterations of their biological properties including changes of virulence and adaptation to new hosts, and may even result in the emergence of new infectious diseases. Examples for the latter are diseases such as acquired immunodeficiency syndrome(AIDS), severe acute respiratory syndrome (SARS), rabbit hemorrhagic disease, porcine respiratory and reproductive syndrome, and avian influenza. Accordingly, rapid evolution of viruses is a major concern to human and animal health and economic welfare. Four of seven main virus classes are represented by RNA viruses including a number of important animal pathogens like foot and mouth disease virus, classical swine fever virus, bluetongue virus, and influenza virus. While it is well known that the high mutation rate of RNA genomes is mainly due to incorporation of non-complementary bases during RNA synthesis, the molecular basis of RNA recombination is poorly understood.

To study fundamental aspects of RNA recombination, we have established an RNA recombination system which allows the efficient generation of recombinant pestiviruses (Family Flaviviridae) after transfection of cells with synthetic nonreplicable subgenomic transcripts derived from bovine viral diarrhea virus and classical swine fever virus. Using this system we have proven the existence of a replication-independent mechanism of RNA recombination in addition to the commonly accepted replicative copy choice recombination. To identify RNA signals involved in efficient non-replicative joining of RNA molecules, RNA recombination was targeted to the 3' nontranslated region. Determination of the recombination sites of more than one hundred independently emerged recombinant viruses resulted in the identification of preferred recombination sites. Furthermore, the results of our study showed that the frequency of RNA recombination directly correlated with the RNA amounts of both recombination partners and can be strongly increased by defined modifications of the 5' and 3' ends of the recombining RNA molecules. Implications of the obtained results for a better understanding of the mechanism of RNA recombination will be discussed.

POSTER: TOWARDS A QUALITATIVE SPATIAL MODEL FOR RISK OF INCURSION OF CRIMEAN-CONGO HAEMORRHAGIC FEVER IN LIVESTOCK IN EUROPE

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Key words: : Climate change, risk assessment, spatial, Crimean-Congo haemorrhagic fever

1.Introduction and Objectives
Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne virus which infects livestock and causes serious illness in humans. It may be transmitted to humans through handling meat from infected livestock and tick bites. It is currently emerging in Turkey and the Balkans. This presentation sets out progress in building a qualitative spatial risk assessment for CCHFV infection in livestock under current conditions in Europe.

2.Material and methods
The approach is based on six layers which describe the risk factors for release and exposure to livestock within Europe. The release focuses on CCHFV-infected immature ticks carried into Europe by migrating birds (Layer 1) although other routes of tick entry including importation of wildlife and livestock should be considered too. The exposure includes temperature and humidity that are limiting factors for survival and moult of the immature ticks (Layer 2), distribution of the tick vectors (Layer 3), vertebrate host reservoirs (Layer 4), livestock hosts (Layer 5) and proximity of livestock to the tick-vertebrate-tick host interaction (Layer 6).

3.Progress
Simulations of the current climatic suitability for the main tick vector Hyalomma marginatum in southern Europe have been provided for Layer 3. Data have also been collected for Layer 4 (obtained from the Atlas of European Mammals (EMMA Database)) and Layer 5 (obtained from FAO). The release focuses on migratory birds' carrying CCHFV-infected ticks into the country (Layer 1) from those countries where CCHFV cases were reported in 2008 (e.g. South Africa, Turkey, Iran, Russia) and countries where there is virological or serological evidence for CCHF (and vector presence) including sub-Saharan Africa. Bird species which migrate from sub-Saharan Africa and have contact with the ground in arid habitats have been listed for those countries across Europe represented by participants on the work package.

4.Discussion and Conclusions
Geographical Information Systems have been used to combine the climate suitability distribution for H. marginatum (Layer 3) in Europe with FAO cattle density data (Layer 5). This could be used to identify likely hotspots of tick bite prevalence in cattle and other livestock. Given the catholic feeding habits of the tick vector, the distribution of vertebrate hosts (Layer 4) may not be a limiting factor. The focus now is consolidating breeding range maps, abundance and habitat preference for the main bird species. A case can be made for the current risk of CCHFV incursions (through release from infected ticks attached to migrating birds) into northern Europe being lower than for southern Europe.

POSTER: CELL CULTURE AND NEW TECHNOLOGIES FOR IN-VITRO PROPAGATION OF HEV VIRUS

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Key words: HEV 3D in-vitro propagation

Hepatitis E virus (HEV) is a small, non-enveloped, single-stranded, positive-sense RNA virus and causes acute hepatitis in human. It is commonly spread via the faecal-oral route through contaminated water supplies occurring most often in developing countries.

HEV transmission may be zoonotic in developed regions with swine and other animal species serving as viral reservoirs. The zoonotic transmission routes are poorly understood, largely because HEV is extremely difficult to propagate in vitro.

Twenty one cell lines, in a recent study, were assessed for their ability to support HEV replication and only one, PLC/PRF/5, was reported to be permissive in in vitro culture. The same study was reproduced in earlier trials at the VLA without success. The Rotary Cell Culture System (RCCS) was investigated since it allows the cells to grow in 3D and to more realistically resemble the in vivo structure of their tissues of origin. A successful infection was established in 3D cells and this system was shown to permit HEV replication more efficiently than reported for the 2D system.

The aim of this study was to grow swine HEV in PLC/PRF/5 in 3D culture system to test the sensitivity of this system.

The results obtained by qPCR demonstrated that the virus was able to replicate in this system. The general examination of the data indicated that the 3D system is an efficient system to evaluate HEV. This could be due to the different configuration of the cells in 3D that resemble physiological functionality in vivo therefore allowing better virus attachment and replication.

For the first time, this study has demonstrated the ability of PLC/PRF/5 grown in RCCS to propagate HEV infection, justifying further investigation of the system.

RCCS could represent a powerful tool in the study of HEV pathogenesis.

POSTER: DEVELOPMENT OF A PRIPROET REAL-TIME RT-PCR ASSAY TO DETECT ALL TWENTY-FOUR SEROTYPES OF BLUETONGUE VIRUS

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Key words: BTV, real-time PCR, detection, primer-probe energy transfer, PriProET

Introduction and Objectives
Bluetongue virus (BTV) belongs to the genus Orbivirus, in the family Reoviridae and includes twenty-four distinct virus serotypes according to serum neutralisation tests (Maan et al., 2007). Bluetongue is an infectious, non-contagious, arthropod-borne disease that infects all ruminants, including sheep, cattle, deer, goats and camelids. Since bluetongue is recognised by the OIE as a "notifiable" disease, various conventional and real-time RT-PCR assays have been developed. To date only one article that describes universal detection of all 24 BTV serotypes, using MGB and LNA probes (Toussaint et al., 2007).

The objective of this work was the development of a real-time RT-PCR assay based on the primer-probe energy transfer (PriProET) chemistry that is able to detect all twenty-four serotypes of bluetongue virus (BTV).

Materials and methods
RNA of all 24 BTV serotypes and heterologous virus strains (AHSV, EHDV) were obtained from CISA-INIA (Valdeolmos, Spain) and AFSSA-Alfort (Paris, France). The assay performance was also tested on 45 bovine and ovine whole blood clinical samples from the BTV 8 outbreaks in Sweden and Denmark.

The oligonucleotide primers and probe were designed from the published sequence of the NS3/NS3A conserved region of the viral genomic RNA segment 10 of the BTV-9 genome (GenBank accession no AY438034). Primer and probe sequences were selected using Primer ExpressTM (Applied Biosystems, USA) and Beacon Designer (Biosearch Technologies, USA) software.

Results and Discussion
A real-time RT-PCR assay based on PriProET was developed to detect all twenty-four serotypes of bluetongue virus (BTV). The assay sensitivity was in the range of 10-100 target copies and the specificity tests showed no positive results for heterologous pathogens. The assay was tested on clinical samples and the lowest detection limit for BTV 8 serotype, determined with PCR standards, was 57 genome copies. Assay sensitivity for some other serotypes that currently circulate in Europe was also determined. BTV 2, 4, 9 and 16 were tested on available cell culture samples and the detection limits were 109, 12, 13 and 24 copies, respectively. A distinguishing characteristic of the assay is its tolerance toward mutations in the probe region. Furthermore, melting curve analysis immediately following PCR confirms specific probe hybridization and can reveal mutations in the probe region by showing a difference in the melting point. The significance of such an assay for surveillance of BTV is valuable for laboratories in many places in the world where an outbreak is a threat or is already present and must be monitored. The situation of BTV 8 in Europe is a prime example of the utility of a pan-BTV assay.

Conclusion
In summary, this study produced a competent one-step real-time PCR assay for the detection of all BTV strains. This assay provides an important tool for the early and rapid detection of a wide range of BTV strains, including emerging ones.

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POSTER: EVALUATION OF AUJESZKY DISEASE CONTROL STRATEGY AT REGIONAL LEVEL

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Key words: Aujeszky disease, herd, risk factor, serological test

Introduction

Aujeszky disease (AD) was diagnosed for the first time in Italy in 1940. Still now the infection is endemic in pig population of northern Italy. An AD national control program was implemented in 1997. It was based on annual serological control on reproductive herds, vaccination of breeders and fatteners using marker vaccines and the adoption of strict biosecurity measures. After ten years, the prevalence of AD-positive herds in Piemonte slowly decreased until 36% in 2007. A voluntary regional control plan was applied in 2007 to further reduce AD prevalence. Additional measures were requested such as double serological testing on a larger sample of animals and the identification and testing of sentinel animals. The aim of this study was to compare the results of national and regional program and to evaluate risk factors for AD infection in pig herds.

Material and methods

The regional plan involved a random serological testing of 60 adult breeding stock per herd to evaluate withinherd seroprevalence (estimated prevalence of 5%, confidence limit of 95%). All pig farms located in a 5 km radius zone around each adherent herd were tested to evaluate AD virus circulation. Adherent herds were tested twice a year to gain free-AD status. In order to evaluate biosecurity management efficiency a group of sentinel animal was selected, consisting of 12 breeders serologically tested twice a year.

Blood samples were analysed to detect antibodies against viral glycoprotein E by a competitive Enzyme Linked Immunosorbent Assay (ELISA).

Between 2007-2008, serological results obtained were integrated with data relative to herds involved (gathered by a questionnaire submitted to farmers). Data were analysed to provide descriptive statistics using an univariate approach (t-test and chi squared test) and a multivariate one (logistic regression). Comparison between national and regional program was made by McNemar test. SAS 8.2® software was used for all statistical analysis.

Results

A total of 30 breeding herds and 74 neighbouring herds were tested in 2007, 40 and 193 respectively in 2008. AD seroprevalence in neighbouring herds was 35%. Considering the entire period of time, seroprevalence in adherent herds decreased from 31% in 2007 to 17% in 2008, this difference is statistically $x^2=4$; p significant (<0.04). Seroprevalence decreased from 23.1% to 7.69% in farrow-to-feeder farms whereas it didn't vary in farrow-to-finish farms.

The best-fitting logistic-regression model include gilt-replacement policy, number of pig farms in a 6-km radius. The odds of a given farrow-to-finish herd being seropositive for AD are 8 times higher (OR=8; IC95%: 1.3-50) if the replacement gilts are purchased and 9 times higher (OR=9.3; IC95%: 1.8-47.6) if there are more than 5 pig farms in a 6-km radius.

Discussion

The number of pig farms in a 6-km radius was positively associated with seropositivity of breeding farms, this is consistent with data reported by other authors. The association is reasonable both under airborne virus spread and under virus transmission by indirect contacts (vehicles, personnel, etc.). The control measures of the national plan resulted inadequate to reveal the actual AD sanitary status of pig herds. A larger sample size for serological analysis and a testing strategy in neighbouring herds, as requested by the regional plan, resulted more suitable to highlight the current epidemiological situation of AD in the region.

POSTER: CHASING NOTIFIABLE AVIAN INFLUENZA (NAI) IN DOMESTIC POULTRY: A CASE REPORT OF LOW PATHOGENIC AVIAN INFLUENZA (LPAI) H5N2 VIRUS IN TWO BELGIAN HOLDINGS.

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Key words: serological screening, notifiable avian influenza, H5, case report, diagnostic

At the end of December 2008, two geographically distant holdings were found H5 positive during the annual AI serological screening in Belgium. Following these positive results, cloacal swabs were sampled for virological tests and a low pathogenic avian influenza (LPAI) virus of subtype H5N2 was isolated in the first holding and identified by Real Time PCR in the second holding. The first farm was a mixed holding with mixed ornamental birds and poultry (~ 4000 ducks/geese, ~1000 chickens/turkeys/pheasants, 70 pigeons, 150 swans and other birds (raptors, peacocks, cranes…) and the second one was a free range breeding geese farm (about 1500). There were neither clinical symptoms nor mortality. Control measures were taken as foreseen in Council Directive 2005/94/EC with notification in ADNS and to OIE. A stamping out of ducks, geese, chickens, pheasants and turkeys was performed whereas the other birds were isolated and tested again after 1 month. Fifty animals were sampled during the stamping out: 10 were positive in serology for H5 and 12 were positive in RRT-PCR. The quarantine birds were still negative after one month and relaxed. Partial sequencing of the H5N2 showed a close homology with an H5N2 recently isolated in Italy.

It is noteworthy that, during the previous years, these two holdings were already found H5 positive by HI tests and swabs were collected but no virus could be detected. In addition, some years, sera of the two holdings were found positive only with the first line H5 antigen and, as consequence, the flocks were declared as negative several times. It must be noticed that neither clinical symptoms nor mortality were registered during the whole survey. These results may indicate the circulation and maintenance of a LPAI H5 virus in the bird population for an extended period of time before it was detected. Our results indicate several limitations in the serological screening for early detection of LPAI and are a strong indication for increased virological monitoring in at risk farms.

POSTER: THE BELGIAN MOSS : A MONITORING AND SURVEILLANCE SYSTEM FOR THE EARLY DETECTION AND IDENTIFICATION OF EMERGING ANIMAL DISEASES

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

The analysis of the process that led to the identification of the index case of Bluetongue in Belgium (2006) identified a lack of structured communication between field veterinary practitioners, confronted with an emerging disease, and experts spread in several institutions. There was a need for an accelerated disease identification to restrict animal discomfort and economic losses linked with decreased production. As a response to that, the Belgian Veterinary Authorities promoted the development of a focal point based on the online reporting of atypical syndromes by veterinary field practitioners and experts in different fields of expertise. On the website (www.MoSS.be), the veterinary field practitioners and experts are encouraged to notify three categories of atypical syndromes: i) unknown, emerging syndromes/diseases, ii) diseases with an unusual clinical expression and/or non-responding to the usual treatment and iii) rare diseases. At the moment, the system focuses on production animals but is open to all species. In addition, the website allows for multilingual management and cross-border reporting.
At the website, an enquiry form collects general and specific information about the case and compares them in real time with all previous recorded notifications. A fully definable hierarchical ascending clustering process (HACP) identifies groups of notifications according to a proximity matrix calculated using the information about species, clinical symptoms and spatio-temporal distances.
The discrimination potential of the HACP has been validated in cattle using historic data on model diseases. The chosen diseases were: i) Bovine Spongiform Encephalopathy, ii) Bluetongue and iii) the currently emerging syndrome in Europe: Bovine Neonatal Pancytopoenia in calves. The expected results would clearly identify the clusters of notifications grouped by model disease. Additionally, the system shall be tested in "real life" by a network of sentinel veterinary practitioners before becoming accessible to all Belgian practitioners. Detailed results of the validation process will be presented at the meeting.
Aiming at fast disease identification and control, an alert signal provided by the onset of a new cluster should be followed by efficient communication between veterinary field practitioners and experts to switch from detection to identification of the potentially emerging and re-emerging diseases. Efficient communication will be proposed via a forum where structured information will be shared. The forum should connect all levels of expertise and coordinate the diagnostic approach leading to a fast identification of the causative agent.

POSTER: IN VITRO AND IN VIVO STUDIES OF A PORCINE CIRCOVIRUS (PCV) 2-DERIVED REPLICATIVE PLASMID

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Key words: DNA vaccines, replicative plasmids

DNA vaccination is usually performed with plasmids that do not replicate into eukaryotic cells. We [1] and others [2] found that the use of a plasmid amplifying the level of gene transcription of the proteins of interest enhances the vaccine efficacy. Based on these results, one other way to enhance vaccination efficacy would be to render the vaccinating plasmids replicative into mammals. In fact, a plasmid molecule that enters the nucleus can theorically replicate and finally more of the protein of interest may be produced. Here the Porcine Circovirus 2 (PCV2) replicative elements [3] were inserted into plasmids under different conditions. After having developed and optimized an in vitro technique measuring replication rates of plasmids in eukaryotic cells, the different constructs were evaluated. Replication of the plasmid is observed only if a booster plasmid coding for the PCV2 Rep protein is co-transfected with the replicative plasmid. Replication rates of the plasmids are dependant on the size of the plasmids, but not on the presence of strong eukaryotic promoters. One plasmid was choose for further vaccination efficacy evaluation in pigs. For this purpose, the gene encoding the Pseudorabies Virus (PrV) glycoprotein C was inserted either into the expression cassette of this plasmid or into the one of a plasmid where the replicative elements were inactivated by point mutations. The plasmids were injected intramuscularly twice. No improvement of the protection against a lethal PrV challenge and no differences in the body distribution of both plasmids were observed.

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POSTER: DIFFERENTIAL PCR ANALYSIS FOR MONITORING CLASSICAL SWINE FEVER VIRUS CHALLENGE STRAINS DURING EXPERIMENTAL INFECTION OF C-STRAIN VACCINATED PIGS

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Key words: Pestivirus, CSFV, differential PCR

The pestivirus Classical Swine Fever Virus (CSFV), causes a severe hemorrhagic disease of pigs that has considerable economic and welfare impact. The live, attenuated C-strain is a safe and effective vaccine, but its use in the EU is banned, except during emergency situations, owing to an inability to distinguish vaccinated and infected animals by serological testing. To investigate C-strain vaccine performance, differential real-time RT-PCR was used to monitor virus levels in samples from vaccinated animals that were challenged with field virus at 1, 3 or 5 days post-vaccination. Two field strains were used, namely, the genotype 2.1 isolate UK2000/7.1 that caused the most recent CSFV epizootic in the UK and the genetically divergent genotype 3.3 CBR/93 strain that was isolated in Thailand. Vaccine C-strain viraemia was low or undetectable whereas levels of challenge strain declined in each group as the interval between vaccination and challenge increased from 1 to 5 days. Interestingly, vaccine and field virus could occasionally be detected in tonsil at extended periods post-exposure in clinically healthy, non-viraemic animals. In these samples, field strain was rarely present in the absence of vaccine virus. Field strain could be detected in the absence of vaccine strain in those animal groups where protection afforded by the vaccine was incomplete and the field strain transmitted to in-contact animals. A differential PCR approach could be used to inform the use of a live attenuated vaccine to reduce virus dissemination in the event of a CSFV epizootic.

POSTER: INFRARED THERMOGRAPHY -

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Key words: infrared thermography, CSF, fever, diagnostic

In acute outbreaks of classical swine fever (CSFV) the early symptoms can be very unspecific and extremely variable and may be mistaken for many other diseases. The typical hemorrhages of the skin are often missing or can be found only in later stages of the disease which makes a prompt diagnosis difficult. Though, the CSFV infection can be undiscovered for a longer period and a rapid further spread of the virus can occur. Substantial economic losses, particularly in regions with high pig density would be the consequences.

Because increased internal body temperature is an early and reliable indicator for various infectious diseases including CSF, the body temperature measurement is already used for a risk based sampling scheme (Council Directive 2001/89/EC). But manual rectal temperature measurement can be complicated and often leads to substantial stress to the animals which results in an increase of the body temperature. Therefore, non-invasive methods like infrared thermography could maybe help to overcome this problem. After several experimental and field studies with an infrared thermograpy camera it was obvious that the results were influenced to a great extent by physical factors (transmission, emission, reflected infrared radiation and angle of shot), environmental factors and individual factors. Although a correlation could be observed between rectal and skin temperature of all analyzed skin areas, the sensitity of the method especially for single animals in groups is not sufficient and does not guarantee an exact diagnosis of feverish pigs.

POSTER: SURVEILLANCE AND CONTROL OF PARATUBERCULOSIS IN PIEDMONTESE BEEF CATTLE SEASONALLY TAKEN TO MOUNTAIN PASTURES IN PIEDMONT, NORTHWESTERN ITALY

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Key words: Paratuberculosis, ELISA test, beef cattle, transhumance

Paratuberculosis (PTBC) is a chronic enteritis caused by an acid-resistant bacillus: Mycobacterium avium subsp. paratuberculosis (MAP). MAP affects a broad spectrum of animal species including cattle, sheep, goats, rabbits, wild animals (captives or free-living) and primates. The disease is characterized by irreversible wasting, diarrhea and death from cachexia and its incidence seems to be increasing worldwide. In ruminants, MAP is mainly transmitted by the fecal–oral route. Calves generally become infected soon after birth, but only few animals develop overt disease and rarely show clinical signs before they are two year old. Many infected animals become chronic carriers and the incubation period is usually months or years (from 4 months to 15 years). Unless measures are taken to control the microrganism, the prevalence of infection gradually increases and a greater number of animals become clinically ill. The aim of this study was to evaluate the effectiveness of a strategy for the control of MAP in Piedmontese beef cattle subject to transhumance in the Alps. A serological survey was conducted from 2004 to 2008 in 15 herds of Piedmontese beef cattle migrating to higher pastures in summer and showing overt clinical signs of PTBC. Repeated serological test at yearly intervals were used to confirm PTBC in animals with clinical signs, to identify presumptive infected animals as well as to estimate the prevalence of infection in the herd. All adult cattle of 24 months of age or older were tested for the presence of antibodies against MAP using a commercial available absorbed ELISA kit, for a total of 5148 tests over 5 years. Test-positive animals were forbidden to graze, separated from the rest and/or culled. Between 2004-2008, the within-herd prevalence decreased from 87% to 53%, while the PTB individual-animal seroprevalence decreased from 6% to 0.9%. The age of positive animals ranged from 1 year to 13 years and about 27% were younger than 3 years.
Control of paratuberculosis is difficult because of the prolonged course of infection and the predominantly subclinical nature of the disease. Historically, serology was known to be less sensitive than coproculture but the improvement of ELISA technology is now allowing to detect a higher number of infected animals with the advantage that antibody detection is rapid, easy to run on a large number of samples and is not very expensive. In herds showing overt PTBC clinical signs and high seroprevalence ELISA test, together with culling of infected animals, could represent an useful tool in the control of the disease.

POSTER: ASSESSMENT OF SEROLOGICAL METHODS FOR DETECTION OF ANTIBODIES AGAINST KHV (CYHV-3) IN CARP (CYPRINUS CARPIO).

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Key words: serology, KHV, carp, diagnostics

Koi herpes virus disease (KHVD) is an emerging highly contagious disease responsible for mass mortality in common carp and in its koi ornamental variety. The world-wide distribution of the disease is presumably caused by the intensive international trade of koi carp. Because the fish exposed at non-permissive water temperatures or surviving an infection can become persistently infected with the virus, they are considered as potential carriers able to spread the virus during international trade. Detection of the virus in such carrier fish is very difficult using the same methods as those recommended by the OIE for the diagnosis of the disease. For this reason, the development of an efficient, non-lethal test for surveillance of common carp and koi in connection with trade is considered as a priority. Serological methods are highly expected to be a good alternative to assess the health status of carp regarding KHV disease. Different techniques: serum-neutralisation test, antibody ELISA and IFAT have been adapted and apply to sera from carp experimentally infected with KHV at two different temperatures. Those techniques were used to establish antibodies kinetics in individually labeled carp over a period of two years. The sensitivity and specificity of these tests were evaluated. The test validation was carried out through a proficiency test using sera from carp experimentally or naturally infected with KHV.

POSTER: ARTHROPOD-BORNE VIRAL EPIDEMIC THREATS TO UK LIVESTOCK

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Key words: arbovirus, vector, risk, policy

As a consequence of climate change, international transport and other global environmental changes, new diseases are likely to threaten UK livestock during the 21st century. In order to respond to risk as cost-effectively and efficiently as possible with limited resources, the early identification and prioritisation of potential threats is essential for UK policy.

Viruses spread by arthropods are likely to be among the first to be affected by global environmental change due to the high mobility, small size, rapid breeding potential and ectothermic nature of their vectors. This assertion is supported by major outbreaks of bluetongue virus (BTV) in Europe and of West Nile virus (WNV) in the USA during the last ten years, as well as smaller outbreaks of WNV and chikungunya virus (CHIKV) in southern Europe. Until recently, research into arthropods as potential vectors of disease in the UK has been a relatively low priority, and as a result relatively little is known about the distribution and movements of these species and their capacity for transmission. Research into arthropod-borne viruses and their vectors requires unique skills and biosecure facilities which may take many years to develop and are expensive to maintain.

This presentation will describe the initial findings of a research project to identify possible threats to UK livestock and the UK's capacity to respond to those threats. It will present a comprehensive hazard list of arthropod-borne livestock viruses, and an inventory of UK facilities and expertise in the field of arthropod-borne viral diseases of livestock, identifying possible gaps in national capacity. It will then explore some ways of quantifying the relative importance of different viruses based on approaches such as direct financial cost, food security, animal welfare and potential human health impact, illustrated with real examples.

POSTER: TRANSPLACENTAL INFECTION INDUCED BY A WILD-TYPE BLUETONGUE VIRUS SEROTYPE 8 NATURAL INFECTION IN BURGUNDY (FRANCE)

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LDA 58[1]; LSI[2]; AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES ALIMENTS[3]; INSTITUTE FOR ANIMAL HEALTH[4]; MERIAL[5]

Key words: Bluetongue Transplacental Infection

Introduction :
During the 2007 and 2008 bluetongue (BT) epidemics in France, a significant increase in the incidence of abortion, stillbirth and weak calves was reported. While clinical disease, especially fever in pregnant dams can result in the above, it has been reported that BTV-8 is able to cross the placental barrier and infect foetuses in utero.

Material and methods :
A study including more than 850 dam/foetuses or newborn pairs was conducted in France (Burgundy) from November 2008 to April 2009 to investigate the occurrence of transplacental infection caused by wild-type BTV-8.

Dams were sampled at the time of abortion and tested for the presence of BTV antibodies by ELISA (Bluetongue virus Antibodies test kit ® - LSI) and for BTV RNA by real-time RT-PCR (TaqVet BTV All genotypes ® - LSI). At the same time spleen was collected from dead calves or blood from newborn calves and tested for the presence of BTV RNA by RT-PCR. Some newborn calves were also tested for the presence of BTV antibodies .

RNA was extracted from either EDTA blood or spleen samples using the Universal Extraction Robot ® (QIAGEN) and PCR was performed as the manufacturers protocols. Epidemiological data were collected : vaccination, date of beginning of pregnancy, date of abortion, abnormalities. Co-infection, especially with BVD virus was also investigated.

Results:
Increase of incidence of abortions was reported in comparison with previous years (25% to 30 %) mainly in December. More than 98 % of the dams were tested positive for BTV antibodies.

BTV RNA was present in about 12% of the tested dams and in about 15% of the tested foetuses (spleen or blood). The major incidence was reported in December to January.

Abnormalities reported were mainly blindness, abnormal behaviour like "tremor" and "dummy syndrome". Hydranencephaly was also reported.

Some foetuses were tested positive for both BT virus and BVD virus.

Epidemiological data showed that the dams were pregnant for about 4 to 5 months, at a time of higher vector activity , vaccination of the dams occurred too late during pregnancy to prevent transplacental infection.

Quantification data showed that viral RNA in foetuses or newborn calves was much higher than those reported in dams.

Discussion and conclusion :
BTV-8 is capable of crossing the placental barrier to infect the foetus. The results of sampling dam/calf /foetus pairs provides evidence of almost 15% transplacental BTV infection.

The current study concludes that the combined serological and rRT-PCR results in pregnant dams gives no indication of the infection status of the calves, excepted if dams are seronegative and PCR negative.

Further investigations must be conducted to know if the RT-PCR positive foetuses or calves with high viremia could be infectious for the midges.

POSTER: VALIDATION OF COMMERCIAL REAL TIME RT-PCR KITS FOR DETECTION OF WEST NILE VIRUS.

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Key words: Real-Time PCR, West Nile Virus, Taqvet, WNV, Kit

Context

The West Nile virus (WNV), also called West Nile virus is a flavivirus transmitted by mosquitoes. This virus can cause fatal neurological diseases in humans and horses (in about 1% of infections). Birds are the main reservoir hosts of the virus.

The monitoring of this virus by health authorities has helped to highlight an increase in severe forms of the disease, especially a worsening of nerve damage. Indeed, several human epidemics have developed in Europe, North America and around the Mediterranean. Also, several epidemics affecting only the horses have been described in Europe.

The West Nile virus presents a significant variability of its stem: the stem lineage 1 were responsible for the vast majority of recent outbreaks and epidemics, lineage 2 is responsible for the epidemic and equine epizootic in Hungary in 2008.

Therefore, it is necessary to develop molecular tools adapted to efficiently amplify a majority of strains of West Nile virus circulating in Europe, especially various strains on the nucleotide level (lineages 1, 2, 3 ...).

According to epidemiological data provided by the authorities, LSI wished to propose a kit for RT-PCR in real time to improve the detection of the genome of various strains of WNV, in collaboration with the French reference laboratory

Material & Methods

The kit has been developed from the work of the French reference laboratory for virus Nile fever. The detection system was designed to detect a majority of strains of West Nile, particularly the strains involved in human and equine outbreaks occurred in Europe in recent years.

The system was evaluated in silico and experimental strains of lineage 1 and 2.

The specificity of the kit was evaluated in silico and experimentally on a panel of genetically similar strains of the virus from West Nile (flavirus) and strains potentially present in the hosts of the virus (equine and avian strains)

The diagnostic kit of WNV has been developed in duplex to allow the detection of the target and a control of the extraction and amplification of the target due to the presence of a IPC (Internal positive control) endogenously present in the native samples . It is also possible to validate the extraction of acellular matrices with a IPC exogenously added during the extraction of nucleic acids.

The characterization of rtRT-PCR West Nile (determination of the sensitivity, specificity, repeatability and reproducibility of the system) has helped to highlight the robustness of the kit.

RNA were amplified by a duplex rtRT-PCR assay in an ABI Prism 7500 Apparatus (Applied Biosystems) using (i) a set of primers and probe labelled with FAM reporter dye targeting the Pro C gene of WNV, and (ii) a set of primers and probe labelled with VIC reporter dye targeting the BetaActin gene (internal positive control). The detection limit and efficiency of the rtRT-PCR was determined by testing a 10-fold dilution series of a WNV quantified RNA.

Discussions & Conclusions

The rtRT-PCR-N (LSI Taqvet® WNV) was specific and highly sensitive. The detection limit was 6 copies per PCR demonstrating the ability of this assay to detect very few amounts of virus. The rtRT-PCR was able to detect the whole lineages of the west nile virus which allow the kit to be a good diagnosis tool for WN virus detection in case of outbreak or in case of epidemiological surveillance.

POSTER: NEWCASTLE DISEASE VIRUS IN MADAGASCAR: IDENTIFICATION OF AN ORIGINAL GENOTYPE

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Key words: Newcastle virus disease, Madagascar, original genotype

Newcastle disease (ND) is a highly contagious and widespread disease which causes severe economic losses in domestic poultry, especially in chickens. The causative agent of the disease is Newcastle disease virus (NDV), also designated avian paramyxovirus serotype 1 (APMV-1). Based on the analysis of nucleotide sequence of the F protein gene, 10 different genotypes (I-X) of NDV have been identified so far. Since Newcastle disease was first described in 1926, three worldwide panzootics of ND have occurred (Alexander, 2003). The first panzootic (1926 to 1960) was caused by viruses belonging to genotypes II - III - IV and the second (1960 to 1973) and third (1970-1980) ones by genotypes V - VI. Moreover, severe outbreaks in Western and Southern Europe (Lomniczi et al., 1998; Herczeg et al., 1999), South Africa (Abolnik et al., 2004) and Taiwan (Yand et al., 1999) in the 1990s were caused by genotype VII. Genotype VII is the currently genotype circulating through Asia, Africa and Europe. In Madagascar, ND was firstly described in 1946 (Rajaonarison, 1991) and since then, outbreaks were regularly reported on the whole island mainly in the rural poultry sector (Koko et al., 2006). While the vaccination rate is estimated to reach 100% in commercial poultry production of Madagascar, probably less than 10% of the free-range poultries are duly vaccinated. ND is considered to induce more than 40% of mortality in such non protected poultries (Maminiaina, 2007). In spite of the importance and endemicity of MN in Madagascar, no data is available about the virus variants involved on clinical cases and/or maintenance of this disease in the island.

In this study different APMV-1 strains (named here MG group) were isolated in 1992, 2008 and 2009 from poultries. The sequence of the cleavage site of the F protein (112 GRRRRR*FV118) showed five basic amino acids at position 112-116, representing a virulent motif never been reported nowadays. Moreover, phylogenetic analysis based on the F and HN gene showed that these isolates are closer to genotype IV but distant enough to constitute a new genotype named genotype XI. Some of these strains were isolated from sick/dead poultries that had been vaccinated against ND (La Sota or local vaccine). The analysis of the F and HN protein sequences of the MG group strains shows original amino acid substitutions. Some of these substitutions occurred in the globular head on which reside the receptor recognition, the F/HN interaction, the neuraminidase activity (NA) and antigenic sites (Iorio et al., 2001; Morrison, 2003). It is tempting to postulate that the modifications observed on F or HN genes from MG strains may play a role in virulence or emergence of escape mutants and finally the apparent lack of vaccine efficacy observed in the field.

In conclusion, in Madagascar the presence of a new APMV-1 genotype, presumably deriving from an ancestor close to genotype IV introduced in the 50's, show a particular evolution of NDV and reinforce the idea that this island is a unique natural ecosystem for micro-organisms. In addition, the possibility that this genotype represents a variant able to escape of immune response induced by current vaccines should not be under-estimated.

POSTER: DETECTION OF ANTI-RVFV ANTIBODIES IN CAMELS (CAMELUS DROMEDARIUS) USING A NOVEL MONOCLONAL ANTIBODY BASED-COMPETITIVE ELISA

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Key words: Rift Valley fever virus, camel sera, competitive ELISA, MAbs

Rift Valley Fever virus (RVFV) is a mosquito-borne virus belonging to the genus Phlebovirus within the Bunyaviridae family that constitutes a latent threat both for animal and human populations in many regions of Africa. RVFV infections have a major impact in certain livestock species, in particular small ruminants and cattle, where high abortion rates and mortality in neonatal and young animals usually accompanies epizootic outbreaks of the disease. In recent years RVF has gained increasing international attention as a result of global warming that could facilitate the spread of RVFV competent mosquito vector species, as recently demonstrated for other vector borne viral diseases. Antibodies against RVFV can be detected in many wildlife species as well as in other ruminants of veterinary or commercial interest. In camels, abortions are associated with RVFV infection and anti-RVFV IgG antibodies are also induced in adult individuals. Therefore, camels may constitute good indicators of the presence of the virus in the arid and semi arid regions of Eastern Africa where RVF epidemics occur periodically in association with unusually high rainfall and other currently unknown factors. Traditionally several methods have been used for determination of the presence of antibodies against RVFV. These include virus neutralization, complement fixation and haemagglutination tests. More recently different forms of enzyme-linked immunoassays have been developed and evaluated for serodiagnosis in wild and domestic ruminants, including those based on inhibition Elisa's. Previously we have described the generation of monoclonal antibodies against the viral nucleoprotein N. The use of these reagents increases specificity as well improving reproducibility between-assays due to the ability to produce effectively unlimited quantities of standardized reagents. In order to validate the usefulness of these reagents for serological detection of antibodies we tested their ability to compete with the binding of camel sera to captured viral antigen. A competitive ELISA based on monoclonal antibodies against the viral nucleoprotein N was been tested for detection of specific camel anti-RVFV antibodies in serological samples (n=206) from Kenya and Somalia regions that were collected during October 2008. Positive (n=37) and negative (n=169) sera were categorized according to the results of a virus neutralization test. A cut-off value in the 20%-30% inhibition range of was determined, resulting in sensitivity and specificity, of 89% and 99%, respectively, for the C-ELISA. The data indicate that the C-ELISA based on anti-N mAbs can be used for seroepidemiological monitoring of RVFV infections in camels. Dairy camels could be a useful indicator species for monitoring of RVFV seroprevalence in semiarid agro-ecosystems s due to their relative longevity and high value to their owners.

POSTER: EVALUATION OF A RECOMBINANT VP7 PROTEIN OF EPIZOOTIC HEMORRHAGIC DISEASE VIRUS (EHDV) AND THE DEVELOPMENT OF COMPETITIVE ELISA FOR THE DETECTION EHDV SPECIFIC ANTIBODIES

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Key words: EHDV, ELISA, recombinant protein, diagnostics, VP7

Epizootic hemorrhagic disease virus (EHDV) is a member of the genus Orbivirus in the family Reoviridae, and is closely related to Bluetongue virus (BTV). EHDV causes disease in cattle and wild cervids, and has been associated with bluetongue-like disease in cattle. The clinical signs of EHDV in cattle are fever, anorexia, erosive lesions of mucosa, hyperemia of the conjunctival mucosae and teats, hemorrhage, dehydration and lameness. The virus has important implication for the international livestock trade and has been isolated from cattle throughout the world (North America, Morocco, Japan, Australia, Israel, Turkey).

Differentiation of BTV and EHDV is necessary because diagnosis of infection caused by these viruses is often confused. Whereas the detection of BTV antibodies by ELISA (enzyme-linked immunosorbent assay) is commonly used in laboratories and allows to determine exposure to BTV infection and/or vaccination, there is no commercial kit available for the serodiagnosis of EHDV.

This paper reports the evaluation of a recombinant structural VP7 protein of EHDV serotype 6 (EHDV VP7) as antigen for the development of a new competitive ELISA (c-ELISA). The c-ELISA is the most sensitive and specific assay to detect specific antibodies, in which a group-specific monoclonal antibody (Mab) to virus is used to compete with antibodies present in the sample tested. A recombinant baculovirus containing the gene coding for VP7 of EHDV was constructed by the French National Reference Laboratory (AFSSA Lerpaz, France) and used to express the EHDV VP7 protein. The VP7 recombinant protein was detected by the group-specific Mab C31 (Institute for Animal Health, UK). These characteristics suggested the potential for these two reagents to be developed into a c-ELISA assay to detect EHDV specific antibodies. Such an assay was evaluated on EHDV positive samples.

First results show that EHDV sera competed with the Mab C31 for binding sites on the recombinant VP7 antigen indicating that it could be a suitable diagnostic reagent for use in c-ELISA. Due to the antigenic similarity of BTV and EHDV, the sensitivity and specificity of the EHDV assay will be evaluated and compared to reactivity obtained with BTV samples. The diagnostic potential of EHDV VP7 recombinant protein and possible cross reaction with BTV samples will be discussed.

POSTER: MOLECULAR CHARACTERIZATION ON AFRICAN SWINE FEVER VIRUSES ASSOCIATED WITH RECENT OUTBREAKS IN WEST AFRICAN COUNTRIES.

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Key words: ASFV, p72, p54, CVR, genotyping

African swine fever (ASF) is one of the most important and serious diseases of domestic pigs caused by DNA arbovirus belonging to Asfaviridae family. Its highly contagious nature and ability to spread over long distances make it one of the most feared diseases causing devastating effects on pig production as has been manifested in Caucasus since its introduction in 2007. The epidemiology of the disease is complex since the virus is maintained in endemic East Africa regions in ancient sylvatic cycle involving ticks (Ornithodorus genus) and unapparent infected warthogs and bushpigs. In contrast, in West Africa, virus cycling occurs in the absence of sylvatic host involvement and the vast majority outbreaks have been linked to the movement of infected pigs or pig products. These different epidemiological transmission patterns seem to be directly related to genetic variability found on East ASF virus isolates distributed in twenty one diverse p72 genotypes in contrast with a high homogeneity in West ASF virus isolates placed in a single p72 genotype I.

Greater expansion of ASF commenced in West Africa in 1996 when Côte d'Ivoire became infected, followed by Benin, Nigeria, and Togo in 1997, Ghana in 1999 and 2002 and Burkina Faso in 2003. With the exception of Côte d'Ivoire, the disease has not been eradicated and numerous outbreaks have occurred since the introductions. Countries such as Nigeria, Burkina Faso, Ghana and Togo still reporting ASF outbreaks in 2008-09. A wide sampling collection comprising 1876 serum and 444 tissues, has been done among 2006-2009 in these areas, including ASF-free country Cote d´Ivoire. The main goal has been to have a complete knowledge about the current epidemiological situation in West African countries.

The results obtained suggest a high incidence of the disease in Ghana, Burkina Faso, Togo and Nigeria. Forty three haemadorbing ASFV isolates were selected on the basis of date collection and geographical localization for genotyping purposes. The standardized genotyping strategy employed involved sequencing of the 3' end of gene encoding the p72 protein and the full length p54-gene to place isolates into mayor genotypes. All the viruses were classified within the domestic-pig cycle associated p72 and p54 genotype I currently circulating in West African regions. To define virus relationships at higher resolution typing was performed by analysis of tetrameric amino acid repeat regions (TRS) within the Central Variable Region (CVR) of the B602L gene. Sequences within the CVR generated from West ASFV isolates exhibited a high variability in number of TRS and different discrete CVR variants were recovered in single West Africa countries. The type of TRS from West African isolates presented a high identity to viruses isolated from outbreaks in Benin in 1997. The data from the CVR analysis are consistent with the hypothesis that ASFV virus exchange between West neighboring countries has occurred on more than one occasion, although an alternative explanation would be that identical mutations have occurred independently in the CVR of two closely related virus lineages. Such information should prove useful to veterinary authorities for better disease control and highlight the requirement for improved regulation and monitoring of domestic pig movement and the pork product trade.

POSTER: PESTE DES PETIT RUMINANTS VIRUSES (PPRV) CAN ESCAPE RNA INTERFERENCE IN VITRO

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Key words: PPRV, siRNA, RNAi, escape mutant

Peste des petits ruminants (PPR) is a highly contagious and infectious disease of domestic and wild small ruminants. It is caused by an enveloped non-segmented negative single-stranded RNA virus (PPRV).The virus is classified in the genus Morbillivirus within the family Paramyxoviridae. The disease is widely spread in Africa, the Middle-East and South-West Asia. In spite of the existence of efficient vaccines against this disease, no effective or specific treatments exist for infected animals. The development of a curative tool could consequently be interesting to help in the control of the disease. A promising approach is the possibility to block the expression of virus genes by RNA interference (RNAi). RNAi is a mechanism of post-transcriptional gene silencing triggered by double-stranded RNA in a sequence-specific manner. However, 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. CIRAD recently identified three synthetic interfering ARN (siNPPRV1, siNPPRV6 and siNPPRV7) able to prevent in vitro at least 90% of PPRV replication. The three siRNAs target conserved areas of the essential gene encoding the viral nucleoprotein. In this study, we investigated the ability of PPRV to escape the inhibition conferred by these siRNAs after several consecutive transfections in vitro. For the three siRNAs, we could recover new virus populations with punctual nucleotide mutations that were able to escape of siRNA inhibition. These single mismatches in the target can be responsible for the loss siRNA effect either by modification of a critical residue for RISC (RNA-Induced Silencing Complex) activity or by changes in the secondary structure of the target mRNA and consequently the decrease of siRNA accessibility. Interestingly, only for the siNPPRV6, this punctual mutation generated an amino acid substitution. The role of the detected mutations in escaping RNA interference will be soon confirmed by using a reporter gene system based on the luciferase gene placed under the original or mutated target sequences. The perspectives of this study are also to quantify by QPCR the mutant versus wild virus populations after each transfection to determine the exact moment of the emergence of the escape mutants. In parallel, the combination of two or three different siRNA will be tested on the capacity of the virus to resist to a multi-target treatment.

POSTER: HIGHLY PATHOGENIC AVIAN INFLUENZA IN MOEYINGYI WETLAND (MYANMAR): PRELIMINARY FINDINGS OF AN EPIDEMIOLOGICAL STUDY.

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Key words: Myanmar, Duck, H5N1, epidemiology

Myanmar was officially infected by the H5N1 Highly Pathogenic Avian Influenza Subtype in March 2007 (first epidemic). Two other epidemics occurred in 2007 and in 2008 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 located in Bago East District: a wild bird sanctuary hosting 125 species. The Moeyingyi lake is located in the centre of the area and has undefined shores connected with a network of irrigation channels. It is difficult to estimate the population (2-4 million birds) of small size indigenous egg producing ducks which are housed in approximately 3000 household farms (300 – 4000 duck flock size range). Farms, either resident or make short transhumances, raise ducks for about two laying cycles. The ducks 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 supplies ducklings and table eggs to most of the Country, thus being a potential route for the spread of the infection to other locations. The objective of this epidemiological study is to understand: (i) whether the area is infected by Avian Influenza Viruses; (ii) the prevalence of the infection; (iii) the incidence of the infection; (iv) the infection determinants. The study area was subdivided into four strata: (1) Moeyingyi lake; (2) West lake side; (3) South lake side; (4) channel district. Forty farms were initially selected by means of a stratified sampling. Randomisation was pursued but, given the incomplete sampling base it is considered unreliable and partially impairs generalisations. The selected farms were investigated by collecting 30 blood samples, 30 cloacal and/or tracheal swabs and by recording risk factors' occurrence. The cross-sectional study took place in September 2009. Only 18 farms resulted negative and were addressed by a longitudinal investigation (October, November, January) until their infection. As the size of the negative cohort decreased new negative flocks were introduced. Despite antibodies against the H5 AIV subtype were found, the virus was never isolated nor PCR for H5 AIV subtype was positive. The farm sero-prevalence of the initial cross-sectional sampling was 55% (22 out of 40). In October the incidence rate was 22% (4 out of 18); in November the incidence rate was 15% (2 out of 13) while in January the incidence rate was 24% (5 out of 29). As of January 2010 only 8 of the 18 initial negative farms remain uninfected. The association of risk factor occurrence with incident cases will be pursued but is not yet possible at this stage. The study is ongoing.

POSTER: ACTIVITIES OF DIAGNOSTICS THEME WORK PACKAGE: PEN-SIDE TESTS (WP 4.4)

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

Work Package 4.4 members are investigating various systems for the detection of pathogens in the field. The members are focusing on direct detection methods, either by pathogen genomic or protein material. 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. In Genomic testing, various strategies are being employed to develop systems that work completely at pen-side or in a portable lab environment; which can be as simple as a small table, basic lab equipment and portable hardware. The most complete systems designed for pen-side use are also the most advanced and work with these involves two commercial collaborators that are developing so-called "blackbox solutions" to pen-side testing. In this case, sophisticated equipment for sample preparation and advanced PCR analysis are provided; the user simply inserts the raw sample and presses a button. On the other side, the most basic approaches involve using isothermal PCR chemistry so that only a simple heating block is needed, extraction can be performed using filter paper or an LFD or not at all (for ex. use whole or diluted blood), samples are detected using intercalating dyes or on an LFD. In between these extremes, a variety of combinations are being studied including portable real-time PCR instruments (thermocyclers and isothermal devices), including the use of lysophilized reagents kits. The goal of our work is to identify the best performing and most practical methods for pen-side testing, including the development and testing of SOPs in the field. The viruses that the group have focused on are: foot and mouth disease virus, swine vesicular disease virus, lyssavirus, African swine fever virus, influenza virus and peste-des-petits-ruminants virus. Details of some experiments can be seen in the poster presented at the Epizone 4th Annual Meeting, June 7-10, 2010, in Saint-Malo, France.

POSTER: EVALUATION OF FOUR COMMERCIAL SERUM ELISA KITS FOR THE DETECTION OF BOVINE PARATUBERCULOSIS: AN INTERLABORATORY AND FIELD TRIAL

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Key words: paratuberculosis; test evaluation; ELISA,

Serum ELISA is worldwide considered an important tool for the control of paratuberculosis in the cattle industry. In Italy, at the moment of this study, four ELISAs were available for purchase: kit A (ID Screen Paratuberculosis Indirect, ID-Vet, Montpellier, France), kit B (Paratubercolosis screening ELISA, Laboratoires Pourquier, Montpellier, France), kit C (Paracheck ELISA, Prionics AG, Switzerland), kit D (Cattle type MAP, Svanova Biotech, Uppsala, Sweden). Despite the fact that they are marketed as herd-level diagnostic tools, they are commonly used as cow-level tests. The aim of this work was therefore to evaluate these commercial ELISA tests. All the tests are based on the detection of antibodies to protoplasmic MAP antigens and include sample preabsorption with a suspension of the environmental mycobacterium M. phlei.

We determined the robustness of the kits in an interlaboratory trial and diagnostic accuracy on field samples. Thirty coded samples composed by eight replicates of one negative sample and two replicates of 11 positive samples were selected and delivered with the kits to 10 laboratories throughout Italy. All the participants tested each sample in duplicates. Decoded results were analysed for reproducibility within and among laboratories and quantitative results were transformed into S/P values to compare analytical results. Kit A gave 100% of the expected results, and Kit B gave failed to detect one sample in one laboratory while kits C and D gave a higher degree of inconsistent results. Accordance within laboratory results was 1.00, 0.99, 0.97 and 0.98 for kit A, B, C and D, in that order. On the other hand concordance among laboratories was 1.00, 0.99, 0.89 and 0.93, respectively.

For the field trial, diagnostic sensitivity and specificity were determined. Archived sera were selected from 122 faecal culture positive animals at different level of excretion and from 134 negative cows belonging to negative close herds. All the kits demonstrated a specificity of 100.0%. Merging inconclusive and positive results, test sensitivity was 69.7% for kit A, 70.5 for kit B, 66.4 for kit C and 64.8 for kit D. Despite these variations, no significant differences were detected among kit sensitivities and between different Map excretion levels with the same kit.

According to these results, two (A and B) of the four ELISA kits evaluated showed good performances and reproducibility within and among laboratories.

POSTER: SURVEILLANCE OF WEST NILE VIRUS INCURSIONS IN THE NETHERLANDS: VALIDATION OF ANTIBODY DETECTING ELISAS IN CHICKENS

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Key words: WNV;ELISA;surveillance;chickens;validation

West Nile virus (WNV) is an arthropod-borne flavivirus endemic in parts of Africa, North- and South America, Europe and East-Asia. During the last decades the disease was of minor importance as only a number of incidental outbreaks were reported from Africa and Europe. In 1999, however, this drastically changed after the virus was introduced in North America, and quickly spread through the US, Canada, Latin America and South America and is since considered endemic in the American continents. This stimulated the discussion on a possible introduction and similar spread of WNV in Europe
In the enzootic cycle of WNV, birds are the virus' reservoir with ornithophilic mosquito species as transmitting vector. The virus has been isolated from over 100 mosquito species in the field. Several mosquito species serve as bridge vector, transmitting the virus from birds to mammals like humans and horses. Mammals are dead end hosts as their virus titres during the viremic phase are too low to infect mosquitoes. Chickens are also considered dead end hosts, but can be subclinically infected and are consequently also used as sentinels in for example the UK, Romania and the USA. In the Netherlands a sampling scheme for avian influenza surveillance is already operational in the poultry industry, and these samples could potentially be used for WNV surveillance in (free range) chickens.
Therefore, sensitivity and specificity (in the Dutch chicken population) of ELISAs had to be assessed. One ELISA kit (blocking ELISA) and one in house indirect ELISA based on commercial reagents, were evaluated. For sensitivity evaluation it was extremely difficult to obtain samples. Finally, some experimental post infection samples were obtained from the UK (n=2) and the USA (n=4), and small volumes of ELISA positive samples from a WNV surveillance program in Romania were received (n=36). Also chickens were vaccinated with either an inactivated WNV vaccine or a live WNV canarypox vector vaccine, and sequential samples were obtained. For specificity evaluation chicken sera representing different housing systems in the Netherlands were used (n=525).
The in house indirect ELISA based on commercial reagents yielded inferior test results with only 1/6 post-infection sera positive, 20/36 (55%) Romanian field sera positive, and 14% of the Dutch field sera false-positive (no significant effect of housing system on test specificity). The commercial blocking ELISA initially demonstrated a poor specificity (92%, CI 89–95%). Therefore, another pre-dilution of sera was chosen (1/16 instead of 1/2). With this pre-dilution all false-positive sera scored negative, whereas 6/6 post-infection sera and 32/36 (89%) of the Romanian field sera scored positive. In a new version of the blocking ELISA (increased purity of antigen, optimised coating concentration, serum pre-dilution 1/2) a subset of 45 "difficult" sera from the specificity panel all scored negative, whereas all post-infection sera and 24/25 (96%) still available Romanian field sera scored positive. Six out of 7 vaccinated chickens seroconverted after two vaccinations. Repeatability was 4.9 S/N%.
For serological monitoring in the Dutch chicken population an improved commercial WNV blocking ELISA seems to be useful, although a full specificity evaluation is still warranted. Since these ELISAs detect flavivirus genus specific antibodies, positive results have to be confirmed by a WNV plaque reduction neutralisation test (PRNT) which is operational at the Institute for Public Health and the Environment, RIVM in Bilthoven.

POSTER: DESCRIPTION AND VALIDATION OF FOUR REAL-TIME RT-PCR ASSAYS FOR THE SEROTYPING OF BTV-1, BTV-6, BTV-8 AND BTV-11

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Key words: Bluetongue virus, genotyping, RT-qPCR, validation

The control of bluetongue virus (BTV) in Central-Western Europe is greatly complicated by the coexistence of several BTV serotypes. Rapid, sensitive and specific assays are therefore needed to correctly identify the currently circulating BTV strains in field samples. In the present study, 4 serotype-specific real-time RT-PCR assays (RT-qPCR) are described for the detection of the BTV-1, BTV-6, BTV-8 and BTV-11 strains. All assays were designed on segment 2 which encodes the main serotype-determining outer capsid protein of BTV. The analytical sensitivity of the BTV-1/S2, BTV-6/S2, BTV-8/S2 and BTV-11/S2 serotype-specific RT-qPCR assays was comparable to the earlier described serogroup-specific panBTV/S5 RT-qPCR assay (1). In silico and in vitro analyses indicated that none of the assays cross-react with viruses which are symptomatically or genetically related to BTV and only detect the intended strains. All assays exhibited a linear range of at least 0,05 to 3,80 log10 TCID50 ml-1 and a PCR efficiency approaching the ideal amplification factor of 2 per PCR cycle. Both intra- and interrun variations were found to be low with a total coefficient of variation of 1-2% for clear positive samples and <10% for very weak positive samples. Finally, the performance of the described assays was compared with commercially available kits for the detection of BTV-1, BTV-6 and BTV-8. All 3 in-house assays gave exactly the same diagnostic result (positive/negative) as the commercial assays and can thus be used interchangeably. Together with the earlier described serogroup-specific panBTV/IC/EC, the serotype-specific RT-qPCR assays form a cheap, flexible and properly validated set of tools to detect and differentiate the BTV strains currently circulating in Central-Western Europe.

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POSTER: VACCINATION WITH SEROTYPE-SPECIFIC VP2-EXPRESSING MYXOMA VIRUS PROTECTS SHEEP AGAINST BTV-8

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Key words: Bluetongue, Myxoma virus, Vaccine, VP2, sheep

Bluetongue virus (BTV) is the aetiological agent of bluetongue (BT), an arthropod-borne disease affecting domestic and wild ruminants. BTV is the prototype of the Orbivirus genus in the Reoviridae family, composed of 24 serotypes. Its double stranded segmented RNA genome encodes 3 non structural proteins, NS1 to NS3, and 7 structural proteins, VP1 to VP7. Since 2006, BTV serotype 8 (BTV-8) and, to a lesser extent serotype 1 (BTV-1), are responsible for major epizootics causing heavy economic losses to the cattle and sheep industry. To date, the best way to control BT is vaccination of sheep in enzootic area. Currently, inactivated and live attenuated vaccines are commercially available. Inactivated vaccines have been more recently developed and are the only vaccines authorised in some European countries. Based on challenge experiments these vaccines have demonstrated good immunogenicity and safety. However, inactivated vaccines require a rigorous quality control, large amounts of antigen and consequently high costs of production. In addition they are directed only against one or two serotypes of BTV and do not allow the distinction between infected and vaccinated animals (DIVA).
With the aim to develop new approach based on DIVA strategy and major immunogenic proteins, we used a host-restricted poxvirus, myxoma virus (MYXV) as a new vaccine vector. MYXV specifically infects Leporidae, and causes a lethal disease called myxomatosis in European rabbits. Recombinant attenuated strains of MYXV were already shown to be efficient for rabbits and also for cats vaccination. Moreover, MYXV has previously been demonstrated to be an immunogenic and safe vaccine vector in ruminants. These strongly suggested that MYXV can be used as a non replicative viral vector in sheep. In this study, we propose to investigate the capacity of recombinant MYXV expressing the single VP2 protein of BTV-8 (MYXV-VP2), to protect sheep against bluetongue in a homologous challenge. We have shown that immunisation of sheep with MYXV-VP2 elicited good clinical and significant virological protections against a challenge inducing severe symptoms and mortality in control animals. This protection was associated with neutralising antibody and cellular responses. Altogether our data prove that MYXV vectorisation is a pertinent strategy for vaccination against bluetongue.

POSTER: REAL-TIME PCR DETECTION OF PESTIVIRUS AND DIFFERENTIATION OF BOVINE VIRAL DIARRHEA VIRUS TYPE-1 AND TYPE-2 STRAINS FOUND IN TURKISH CATTLE

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Key words: Real-time PCR, BVDV, , sequencing, Turkey

Bovine viral diarrh ea (B VD) is an economically important viral disease of cattle. Rap id detection and culling of the persistently infected animals is essential in the control and eradication of BVD. Therefore, this study was contucted to establish a real-time PCR assay to use for rapid detection of bovine viral diarrhea virus (BVDV) and to do sequence analysis for phylogeny. For this blood and stool samples originated from differ ent regions (Aegean, Black Sea and Marmara) of Turkey were firstly analysed by optimised real-time RT -PCR using a SYBR Green assay for the presence of Pestivirus using spesific primers as reported by Baxi and others (2006) Nine out of 15 sera sample and 2 out of 40 stool samples were found to be positive for pestivirus by this assay. Positive samples (11) were then reanalysed by RT-PCR to distinguish strains of BVDV (BVDV-1 or 2) using the primers as reported by Lee and others (2008). All samples were found to be BVDV-1 but not BVDV-2. The region on glycoprotein E2 (Gp53) was then sequenced as described by Couvreur and others (2002). The sequencing data were then analysed for the phylogeny. In conclusion, we suggest the real-time RT -PCR assay established in this study to use in rap id detection of Pestivirus as a first step then the samples can be subjected to RT-PCR to distinguish BVDV strains and finally the products can be sequenced for phylogenetic analysis.

POSTER: RAPID PATHOTYPING ASSAY FOR NEWCASTLE DISEASE VIRUS

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STATENS VETERINARMEDICINSKA ANSTALT[1]

Key words: Proximity ligation, Newcastle,detection

A Novel proximity ligation assay (PLA) using specific monoclonal antibodies was developed for the detection of Newcastle disease. The technique is based on detection of proteins on virus particles via nucleic acid amplification. In PLA, affinity probes that bind target proteins are equipped with DNA strands that can be joined by ligation when 2 or more such reagents are brought into proximity by binding to the same target molecule. The DNA ligation products are subsequently detected by DNA amplification. The reaction is performed in three steps. Different NDV strains of different virulence (lentogens, mesogens and velogens) have been tested. The sensitivity of the assay has been compared with other methods. Our results showed that PLA assays are high specific and more sensitive than other detection methods.

POSTER: EXPERIMENTAL HETEROLOGOUS AND HOMOLOGOUS INFECTIONS WITH AN H1N1 AND H3N8 LPAI VIRUSES IN MALLARD DUCKS

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VETERINARY AND AGROCHEMICAL RESEARCH CENTRE[1]

Key words: avian influenza; infection; serology; excretion; wild birds

Mallards ducks (Anas platyrhynchos) are the main reservoir of low pathogenic avian influenza (LPAI) viruses. During the influenza A virus surveillance in Belgium in 2007-2008, two LPAI viruses were consecutively detected in mallard ducks: a H3N8 followed by H1N1 infection was detected at 96 days intervals in the natural conditions. The present study aimed at evaluating the humoral response and the viral shedding after homologous and heterologous infection 21 days after the first infection using these two different viruses (H1N1 and H3N8) of duck origin in mallard ducklings held in isolators. The humoral response and the virus excretion were evaluated by conventional serological tests, such as HI test and NP competitive ELISA, and RRT-PCR. Regarding the serological results, the HI test could detect only a minimal antibody response after homologous infection using H1N1 virus and the HI antibody response after H3N8 infection was almost absent; whereas NP competitive ELISA could efficiently detect the humoral response induced by both viruses. As for virus excretion, H3N8 virus had a peak of excretion 4 days earlier than H1N1 viruses after prime infection; whereas the virus shedding was limited to short period after the second infection in all groups. The present study reiterates that serological results in wild birds should be carefully evaluated, because of the low HI response induced by LPAI viruses. In addition, our results suggest that a prime infection did not impede a subsequent homologous or heterologous influenza infection after 21 days; however it limited the virus shedding.

POSTER: FIRST REPORT OF BOVINE NOROVIRUS IN TURKISH CALVES

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Key words: bovine, norovirus, real-time, PCR, calves, Turkey

Importance of bovine norovirus has increased in recent yeras as an aetiologic agent of diarrhae in calves and has been reported mainly in the USA, Europe, and South Korea. However, there is no report on the existence of norovirus in calves in Turkey at present. Therefore, this study was aimed to investigate the presence of norovirus in Turkish calves by real-time RT-PCR using primers specific for bovine norovirus. It was also aimed to do phylogenetic analysis to determine the strain circulating in Turkish calves. For this, fecal samples from calves aged between 1-60 days old were collected and analysed by real-time RT-PCR (SYBR Green). Bovine norovirus was detected in 6 fecal samples. PCR was performed in positive samples using the primers for capsid protein (Nucleotide position 5468-5488) to do sequencing and phylogenetic analyses (Wolf et al., 2007). Results have shown that norovirus found in Turkish calves were belong to bovine newbury agent 2 (GIII-2).

POSTER: APPLICATION OF A SYSTEMATIC PROCEDURE FOR THE PRIORITISATION OF DISEASES AND DESIGN OF SURVEYS IN THE SURVEILLANCE OF EXOTIC DISEASES

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STATENS VETERINARMEDICINSKA ANSTALT[1]

Key words: surveillance criteria, survey design, exotic disease, Sweden

Since the early 90's, the Swedish National Veterinary Institute has been responsible for the design and implementation of surveys with the purpose to demonstrate freedom from important exotic diseases in animals in the country. The choice of diseases to include in the investigations and of survey design has been based on expert opinions on current threats and investigations of optimal sample sizes and selection of samples. To some extent, the choice has also been based on tradition. Lately, several different approaches for the quantified comparison and prioritisation of important contagious diseases, from slightly different perspectives, have been presented. The aim of this study was to develop a standard procedure to be used in the planning of surveys performed with the purpose to demonstrate freedom from exotic diseases in Sweden.

A systematic procedure that was considered to capture the main steps of designing surveys with the purpose to document freedom from disease was set up. The procedure includes, for each disease, an inventory of the surveillance background as well as a description of the target population and its risk profile. It further comprises an investigation and scoring of a number of criteria related to disease surveillance. The scores are summarised by four groups of criteria: 1. International aspects, 2. Epidemiology of disease, 3. Zoonotic aspects and 4. Economy and consequences. Based on this, the general and specific goals of the surveillance activity are set, and design prevalences as well as options as regards sample sizes and expected sensitivities on the level of individual, herd and survey are discussed. Coherence with other on-going surveillance activities in the population is also considered and the efficiency of suggested surveillance alternatives is estimated.

As part of the study, scoring of the defined criteria was applied to a number of exotic contagious diseases. The results showed that avian influenza, brucellosis, Newcastle disease, bovine spongiform encephalitis and paratuberculosis had a high total score, i.e. this supports that surveillance of these diseases is prioritised. Examples of diseases with generally lower scores were Mycoplasma synoviae infection and Infectious laryngotracheitis in poultry and transmissible grastroenteritis in pigs.

The full procedure was implemented on classical swine fever and African swine fever. The inventory revealed that the number of tested samples within the annual serological surveys could be reduced. In order to keep sensitivity of the surveillance activities continuously high, samples need to be selected and tested throughout the year. A need to further investigate means to include so-called hobby herds in the surveillance activities was also identified.

The suggested systematic procedure is recommended for yearly revisions of disease surveillance activities in Sweden.

POSTER: SEQUENCE ANALYSES AND PHYLOGENETIC COMPARISONS OF THE GENOME SEGMENTS OF DIFFERENT ISOLATES AND SEROTYPES OF AFRICAN HORSE SICKNESS VIRUS

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Key words: AHSV, molecular epidemiology, phylogeny, VP2, VP5

Although African horse sickness virus (AHSV) is currently restricted to sub-Saharan Africa, the virus has previously caused significant fatalities and disease outbreaks in horses beyond this 'endemic zone', and was able to persist in affected areas for a number of years. This indicates that environmental conditions and insect vectors that are suitable for successful transmission of AHSV exist in a geographic area that is significantly greater than the current distribution of the disease.

Historically the distribution of Bluetongue virus (BTV) was restricted to approximately 35oS and 40oN, matching the distribution of known BTV vector species (certain species of biting midge - Culicoides sp). However, since 1998 there have been multiple introductions of BTV into Europe, from the east via Turkey, from west and North Africa, and via an unknown route from Sub-Saharan Africa into Northern Europe. These events (which have been linked to climate change) suggest that there may also be an increased risk posed by other insect transmitted viruses in the region. Indeed, the rapid emergence of different BTV types (post 1998), and the massive economic damage that has been caused to livestock industries by BTV-8 (since 2006), suggest that there may also be an increased risk of AHSV outbreaks in Europe, with the potential to cause both significant fatalities in horses and major economic losses.

In order to better prepare for possible introductions of AHSV, the origin, movement and genetic variations of different virus strains should be well understood and monitored. A database containing sequences for isolates of the virus from different and well documented origins could provide a basis for molecular epidemiology studies and would represent a potentially important component of surveillance and control strategies. Such a database has recently been created for bluetongue virus and has been used to examine/determine the origins of different European field strains, identify reassortant field/vaccine strains, and has provided a basis for development of novel RT-PCR based diagnostic-assays. Full-length sequence data were generated for the entire genomes of the nine reference strains of AHSV, as well as other available field and vaccine strains. These sequence data have helped to determine relationships between the different AHSV serotypes, as well as between the different strains and lineages within each type. These studies have also helped determine the level of variation that exists within each of the different AHSV genome segments and if the restricted distribution of AHSV to a single continent (unlike BTV) has had any significant impact on its variability and evolution. A phylogenetic comparison of the two serotype determining outer capsid proteins - VP2 and VP-5 - will be presented.

POSTER: UBIQUITIN FUSED TO RVFV NUCLEOPROTEIN CDNA SIGNIFICANTLY INCREASES IMMUNE PROTECTION IN IFNAR -/- KNOCKOUT MICE

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Key words: DNA vaccine, bunyavirus, self-adjuvant

Rift Valley Fever is a vector-borne disease that primarily affects ruminants in Sub-Saharan and Eastern Africa. In these areas, sporadic outbreaks can cause significant economic damage to livestock, especially that of sheep. One strategy to make vaccine production more economically feasible involves the use of self-adjuvants in DNA vaccines. DNA vaccines are a promising vaccine candidates in combating Rift Valley Fever Virus (RVFV), since they are low cost (making them affordable for widespread use by African farmers), and their ability to be stored under non-refrigerated conditions. Self-adjuvants, by definition, are molecules produced by a host organism with the purpose of producing and enhancing an immune response. Our hypothesis is that when self-adjuvants are incorporated with an RVFV target that has previously been shown to exhibit partial protection, the host immune response can be significantly augmented to the point of providing an increased level of protection against RVFV infection. Over the last year, we have constructed and cloned several plasmid vaccine constructs, targeting the nucleoprotein of RVFV. An expression plasmid encoding RVFV nucleoprotein was constructed alone, as well as fused to previously classified self-adjuvants such as Ubiquitin, LIMPII, CD154 and C3d-trimer. While slightly increased immune responses were observed using C3d-trimer, LIMPII and CD154 fusions, Ubiquitin fusions significantly augmented antibody titers prior to challenge. This increase in Ab titers also correlated with increased protection against RVFV challenge in knockout mice, indicating a novel vaccine candidate for the prevention of Rift Valley Fever.

POSTER: BLUETONGUE VACCINATION IN SHEEP: COLOSTRAL ANTIBODY PROTECTION AND INTERFERENCE.

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Key words: Bluetongue; Vaccination; Colostral antibodies; Interference

Bluetongue (BT) is a non-contagious culicoides-borne viral disease affecting cattle, sheep and goats, as well as camelids and wild ruminants such as deer. From 2006 to 2008 Bluetongue virus serotype 8 (BTV-8) has caused major economic losses in many countries across northern and western Europe. In 2008 the European Union agreed to support a mass-vaccination programme and many pharmaceutical companies developed inactivated vaccines against BTV-8 which were brought onto the market throughout 2008. These vaccines have been used extensively and effectively across countries affected by BT in Northern and Western Europe and outbreaks of BTV-8 reduced dramatically in 2008 and 2009.

The BTV-8 inactivated vaccines used across Europe were produced very rapidly and were licenced for emergency use without any associated efficacy guaranties. Although many millions of animals in Europe have now been vaccinated across Europe, there remain some unanswered questions about the levels of seroconversion and neutralising antibodies in vaccinated animals, the length of the protective period, the safety of the vaccine in pregnant animals and both the extent and length of colostral antibody protection in lambs and calves born from vaccinated dams. This study aimed to address two key questions for sheep farmers. Firstly, what is the extent and length of colostral antibody protection in lambs born from vaccinated dams. If the duration of colostral antibody protection is shorter than the time taken for the lamb to reach slaughter weight it will be necessary to vaccinate the lambs as they will be alive during a high risk period when both culicoides vector midges and BTV is likely to be circulating (May - October). Secondly, what is the optimal time at which lambs born from vaccinated dams should be vaccinated thus avoiding interference of the immune response to the vaccine by colostral antibodies.

This study investigates the extent and length of colostral antibody protection, as well as the degree of colostral antibody induced interference of the immune response to BTV-8, in sheep. Significantly lower titres of neutralising antibodies were transferred in colostrum to lambs born from sheep vaccinated once as opposed those vaccinated twice (single vaccine in the first year and a booster vaccine in the second year). On BTV-8 challenge, lambs born from sheep vaccinated on two occasions, with the second booster vaccine given approximately 1 month prior to lambing, were protected from clinical disease for up to 14 weeks. BTV-8 was isolated from 5 of the 22 challenged lambs, although only one of these lambs showed a transient rise in body temperature with no other clinical signs. Lambs born from ewes given a second booster vaccine 1 month prior to lambing, are likely to be protected from clinical disease for at least 14 weeks, whereas lambs born from ewes vaccinated once are likely to be protected for a shorter time. Colostral antibodies present in the 13–14-week-old lambs appeared to interfere with the humoral response to challenge virus. These results suggest that colostral antibodies may interfere with vaccination in lambs up to at least 14 weeks of age.

POSTER: RABIES LYSSAVIRUS DETECTION AND GENOTYPING USING PYROSEQUENCING

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Key words: lyssavirus pyrosequencing identification

Introduction

Rabies is an acute encephalomyelitis caused by a non-segmented negative strand RNA virus belonging to the genus Lyssavirus transmitted to humans by rabid animals. The genus Lyssavirus include seven genotypes among which the genotype 1 (Gt1) corresponds to the classical rabies virus (RABV), largely distributed and mainly responsible of human deaths. Additional six genotypes are officially recognised as Gt2 to Gt7, and lyssaviruses waiting for official classification have been also recently detected in different bat ecosystems, through the application of molecular diagnostic methods.

To date, molecular methods have been adopted as confirmatory assays, or as first choice method, in case of intra-vitam diagnosis in humans. However, molecular techniques currently developed for rabies diagnosis needs further sequencing of the PCR products for lyssavirus characterization and typing. The requirement for further genomic sequencing increases the turn-around-time and final cost of the complete analysis in order to clearly define the genotype involved in the infection.

Pyrosequencing is one of the most promising molecular techniques for pathogens genotyping and barcodes identification. In the present study, we describe the development of a One-Step RT-PCR followed by pyrosequencing analysis for rapid lyssavirus genotyping. To date, this is the first application of pyrosequencing to lyssavirus typing.

Material & Methods

Viral RNA was extracted from clinical samples and supernatant of cell cultures. A One-Step RT-PCR was developed targeting the gene encoding for the nucleoprotein. A Lyssavirus specific primer set was designed using complete nucleotide sequences for the nucleoprotein gene publicly available for GTs 1 to 7. Sequences from 5 different unclassified lyssaviruses were also aligned. Sensitivity and specificity of the method were tested.

Pyrosequencing reactions were performed, and a stretch of 30-50 nucleotide reads was obtained using the Pyromark ID platform. Conventional sequencing (Sanger) method was also applied to validate pyrosequencing results.

Results

The One-Step RT-PCR developed was able to detect RNA for all tested lyssaviruses. No amplification was observed with other pathogens tested. The limit of detection of the method was determined as 103 to 101 gene copies/µl of target RNA, depending on the genotypes. Further pyrosequencing analysis allowed the identification and classification of all the different lyssavirus genotypes tested. The comparative Sanger method results confirmed and validated the identification obtained by pyrosequencing.

Discussions & Conclusions

We have developed a One-Step RT-PCR assay for the simultaneous detection of all known lyssaviruses, followed by pyrosequencing for rapid genotyping of the virus. The method demonstrated high sensitivity and specificity. Although this method can not totally replace gold standard for rabies diagnosis, the method developed offers several advantages over standard and molecular methods.

POSTER: SEROPREVALENCE OF A NOVEL CANINE NOROVIRUS IN PET DOGS

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Key words: canine norovirus, seroprevalence, pet dogs

Enteric caliciviruses are a common cause of acute gastroenteritis in both humans and animals. Within the genus Norovirus, zoonotic transmission has been suggested because of the genetic relatedness between human and some animal strains. Recently, we identified a novel canine norovirus (NoV) in Portugal that was genetically unrelated to any other animal or human norovirus. To determine the seroprevalence of this new virus, 309 serum samples from pet dogs (age range 3 months to 18 years-old) were tested for the presence of canine NoV antibodies. Blood samples were collected between September 2009 and January 2010 in veterinary clinics across Portugal. The VP1 gene of the canine NoV strain was cloned into baculovirus and virus-like particles (VLP) were purified by CsCl gradient ultracentrifugation. Using a VLP-based ELISA canine NoV – specific IgG antibodies were detected in 226 (73 %) of the 309 serum samples tested. This high seroprevalence demonstrates that infection with this novel canine NoV strain is widespread among dogs in Portugal which, given their intimate contact with humans, may represent a potential zoonotic risk.

POSTER: INTERACTIONS BEWEEN ADENOVIRAL VECTORS AND DC SUB-SETS

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Key words: vectored vaccines, dendritic cells

Given the importance of dendritic cells (DC) in the initiation of immune responses (IR), it is probable that antigen transfer to DC represents a limiting factor in vaccine efficacy. Indeed, when different model antigens are targeted toward endocytotic receptors present at the surface of murine DC, antigen-specific IR are considerably enhanced. Nonetheless, different sub-sets of DC play distinct roles in induction of IR. In particular, murine CD8alpha+ and CD8alpha- DC appear to be specialised in antigen presentation by class I and class II pathways, respectively. This division of labour would explain why antigen targeting toward endocytotic receptors expressed by CD8alpha+ or CD8alpha- DC selectively activates CD8+ or CD4+ T lymphocytes, respectively. These considerations are largely unexplored for vectored vaccines; that is, for vaccines that deliver a gene that encodes an antigen rather than the antigen itself. Paradoxically, vectored vaccines derived from human adenoviruses (Ad) of species C elicit robust IR, despite inefficient transduction of DC. It is presently unclear whether Ad transduce different functional sub-sets of DC in a differential manner, and especially whether the IR so elicited can be modified in a quantitative or even qualitative manner by retargeting Ad to these different sub-sets. This information is needed to optimise the tropism of Ad-based vaccines so as to elicit the immune effector functions that are most appropriate for protection against the pathogen in question.

This study aims 1) to gain a better understanding of the role of different functional sub-sets of DC in the induction of IR by Ad-based vaccines and 2) to evaluate the impact of retargeting such vaccines toward these different sub-sets on the amplitude and quality of IR.

To this end, splenic DC (CD11c+ cells) have been isolated from C57Bl6 mice. Successive steps involved in gene transfer by Ad to DC subsets have then been examined by flow cytometry. In particular, attachment of Ad to CD8alpha+ or CD8alpha- sub-sets was evaluated by using a monoclonal antibody (MAb) directed against a viral capsid protein, while transduction was assessed by using an Ad expressing Green fluorescent protein. Antigen presentation by the class I pathway was assessed by using an Ad expressing the model antigen ovalbumin and a MAb directed against the complex composed of an immunodominant Ova peptide and the H-2 Kb molecule, while T-cell activation was evaluated by using Ova-specific CD4+ and CD8- cell lines.

Second, the impact of retargeting Ad toward CD8alpha+ and CD8alpha- DC was adressed by using biotinylated Ad. This modification allows coupling of Ad to a large diversity of biotinylated ligands, by virtue of an avidin bridge, and hence evaluation of the utility of retargeting Ad toward different cell types. Using MAb that recognize endocytotic receptors selectively expressed at the surface of CD8alpha+ or CD8alpha- DC, the impact of retargeting Ad toward these sub-sets will be examined at each step of induction of IR by Ad; that is, attachment, transduction, antigen presentation and activation of antigen-specific T cells.

This study explicitly addresses the role of Ad tropism, and in particular for differents sub-sets of DC, in induction of IR. Once a proof of principle is obtained ex vivo for certain ligands, the impact of retargeting Ad toward the DC sub-sets will be explored in vivo. Since division of labour among DC sub-sets is emerging as a shared feature among diverse species, including mice, humans and ruminants, the strategy may be of general interest in vaccine development.

POSTER: ANTIGENIC DOMAINS ON THE ERNS PROTEIN OF PESTIVIRUSES

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Key words: Classical swine fever virus, structural glycoprotein Erns , epitope mapping

Classical swine fever (CSF) is a highly contagious disease of domestic pigs and wild boar and is caused by the Classical swine fever virus (CSFV). Together with the Bovine viral diarrhea virus (BVDV) and the Border disease virus (BDV) it forms the genus Pestivirus within the family Flaviviridae. Besides the E2 protein, the viral glycoprotein Erns is one of the main targets for the immune response and can be used for the development of a DIVA (differentiation of infected and vaccinated animals) test. For this, the knowledge of the epitopes involved is of great importance. To supplement the existing knowledge concerning the antigenic domains and epitopes of the CSFV- and BVDV Erns protein, chimeric and truncated constructs were generated and the reactivity of 13 monoclonal antibodies (mabs) was analyzed. The cross reactivity of the mabs was determined to obtain information concerning the degree of conservation of epitopes on the Erns proteins of Pestiviruses. The amino acid sequence identity between the Erns protein of CSFV strain Alfort/187 and BVDV strain NADL is 73%. Structural elements like cysteins involved in intramolecular disulfid bridges and potential glycosylation sites are conserved in both proteins and in the chimeras. All mabs tested reacted with at least one of the chimeras. The mabs allowed the identification of one antigenic domain on the CSFV Erns protein (aa 55-110) and one on the BVDV Erns protein (aa 111-167). Furthermore most mabs were CSFV or BVDV specific, respectively.

POSTER: FOOT-AND-MOUTH DISEASE (FMD) IN CENTRAL ASIA: FINDINGS AND ISSUES

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Key words: FMD; Central Asia; Progressive Control Pathway

Foot-and-Mouth disease (FMD) is known to be present at endemic level in Afghanistan and Pakistan. Both countries are beneficiary of the FAO regional project GTFS/INT/907/ITA ("Controlling Trans-boundary animal diseases in Central Asian countries") which in addition to those two countries include: Tajikistan, Turkmenistan and Uzbekistan. The project is currently supporting the implementation of field activities coherent with the FMD Progressive Control Pathway (PCP) proposed by FAO and supposed to assist countries to progressively reduce the load of FMD virus. All countries had previously self-assessed themselves (in 2008) as being in stage 0 whereas the requirements for moving to stage 1 are that a monitoring system (at least in large ruminants) should be put in place enabling: (i) to assess the level of exposure to FMD virus in different productive sectors; (ii) to identify and characterize prevalent FMD strains circulating in any given country; (iii) to identify sectors that should receive priorities for control purposes. In this regard the results of the activities carried out in Afghanistan, Pakistan and Tajikistan in 2008 and 2009 are presented in this paper referring to: (i) 11,047 serum samples for the detection of non-structural proteins (NSP) antibodies using an ELISA assay; (ii) 1,510 oral swabs collected from clinically healthy animals in different productive systems (mainly live animal markets and dairy colonies) and tested with a diagnostic rRT-PCR assay; (iii) 144 individual samples from clinically affected animals in 49 different outbreaks (35 in Afghanistan and 14 in Pakistan) occurred between December 2008 and August 2009. Samples were tested with a rRT-PCR and were further sequenced (VP1 coding region) to determine the serotype and sub-type of the virus.

Virus circulation as determined through the NSP ELISA has provided evidence of a high level of exposure to FMD virus. In all productive systems sampled (mainly villages at household level) evidence of NSP antibodies was found with an overall average adjusted prevalence of more than 50%. Collection of oral swabs from non-clinically affected animals in live animal markets and dairy colonies, under the current epidemiological situation, has proved to be an efficient component of the monitoring system enabling to generate reliable information on the current circulating serotypes, lineages and sub-lineages. In 75 out of the 1,510 oral swabs collected FMD genome virus was identified. Further characterization allowed to detect sero-type A/Irn05BAR-08; serotype A/Irn05AFG-07; serotype O PanAsia 2 and a variant tentatively designated as PanAsia 3. In clinical outbreaks the same serotypes as above were identified with the addition of Asia 1 group I (only in Afghanistan) and group II (only in Pakistan). Of note that sero-type A/Irn05BAR-08 was almost exclusively detected on samples from non-clinically affected animals whereas A/Irn05AFG-07 was detected exclusively in clinically affected animals. The results obtained, besides allowing the countries to move from stage 0 to 1, are the basis for the design of targeted control measures in high risk productive sectors.

POSTER: GENETIC HETEROGENEITY OF SMALL RUMINANT LENTIVIRUSES IN ITALY: EVIDENCE FOR THE PRESENCE OF A NOVEL GENOGROUP

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Key words: small ruminant lentiviruses phylogenetic analysis

Small ruminant lentiviruses (SRLVs) are distributed worldwide and cause slow progressive multi-systemic diseases. The nucleotide and amino acid sequences of these viruses are related to the antigenicity and virulence and may affect their persistence and escape from the immune system. The genetic analysis may further help to understand the epidemiology and the phylogenetic relationships of these viruses. It may also be the key to increase the sensitivity of diagnostic tests. The SRLVs are classified into four genetic groups, A-D, based on differences in gag and pol sequences. Group A corresponds to the Maedi Visna Virus (MVV) prototype and can be also divided into at least nine subtypes (A1-A9). Group B refers to the genetically less complex Caprine Arthritis Encephalitis Virus (CAEV) prototype and can be further divided into at least two subtypes (B1-B2). In addition two equidistantly related groups (C and D) are represented by few isolates and have been recognized only by pol sequence. Furthermore, an highly divergent genotype E has been recently characterized in northern Italy and in Sardinia and comprises two distinct subtypes (E1-E2). Interspecies transmission may occur in the field as well as mixed infections in goat and sheep.

The present study has the aim to analyse 29 Italian SRLV isolates, mainly collected during the period 1998-2010 from five different regions. Our genetic study was based on the gag and pol sequences. DNA was extracted from individual buffy-coat, milk and tissue samples and used to amplify a partial region of gag and pol genes. The DNA was analyzed by a nested PCR designed to amplify a 0,8 Kb fragment. The result of the nested amplification was sequenced directly using an ABI PRISM 3130 genetic analyzer (Applied Biosystem).

The phylogenetic analysis revealed that the isolates belonged to the subtype A9 (n=1, sheep), B1 (n=4, goat), B2 (n=3, sheep) and E2 (n=3, goat) and interestingly, to a new putative subtype A10 (n=13) (sheep and goat). Furthermore, five viruses isolated from both sheep and goat, significantly differed from all the other SRLVs previously described and form a separate cluster.

In conclusion, these results showed a high SRLV genetic heterogeneity in Italy revealing the presence of a new putative A10 subtype, which has been never shown before. More interestingly, the sequence analysis also provided evidence for the first time of SRLV isolates which could represent members of a novel genogroup. This variability is probably the result of the absence of any SRLV systematic control measures. Indeed, in Italy there is no SRLV national control program and the management practices, such as trade and movement of animals, expose animals to a high risk of introduction of SRLV infection as well as of a new genetic variants. Further knowledge on nucleotide and amino acid sequences of SRLV from different geographic origin may help to improve the sensitivity and specificity of molecular diagnostic and serological techniques.

POSTER: MOLECULAR EPIDEMIOLOGY OF THE HA GENE OF H9N2 VIRUSES IN THE MIDDLE EAST, 2004-2009

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Key words: Avian influenza virus; H9N2; Middle East; Phylgeny

Since the mid-1990's H9N2 influenza viruses have become prevalent in many European, Asian and Middle Eastern countries and have been sporadically transmitted to pigs and humans. Previous phylogenetic investigations revealed that at least three lineages have become established in domestic poultry in Eurasia (Guan et al., 1999). To date few information are available on the genetic properties of the H9N2 viruses circulating in the Middle East. The present study was conducted to elucidate the molecular characteristics and the phylogenetic relationships of 21 H9N2 viruses identified in 4 distinct Middle Eastern Countries, namely Jordan, Iraq, Saudi Arabia and the United Arab Emirates (UAE) between 2004 and 2009. The HA gene segment of the 21 viruses was completely sequenced and the sequences were phylogenetically analysed using Bayesian methods implemented with the program MrBayes v3.1.2. Topology of the phylogenetic tree showed that all the HA sequences clustered in the G1-like lineage previously described by Xu et al., 2007. This lineage comprises human H9N2 isolates from Hong Kong and several H9N2 viruses from Asia and Middle Eastern countries. Within G1-like lineage, 2 main sublineages, named G1-A and G1-B, and several distinct monophyletic clusters were recognized. Viruses from Jordan, Iraq and one virus from Saudi Arabia, isolated between 2004 and 2007, belonged to sublineage G1-A and clustered with viruses isolated in Israel from 2000 to 2007. Viruses from Dubai, Kurdistan-Iraq and 8 samples from Saudi Arabia isolated between 2005 and 2008 fell within the G1-B sublineage and clustered with viruses isolated in Israel and Pakistan from 2005 to 2009. Interestingly, the sequences of the H9N2 viruses isolated in the UAE in 2008 clustered separately from the respective gene sequences of the UAE viruses identified in 2000-2003, belonging to the G1-A sublineage. Interestingly, in some countries (Jordan and Iraq) only one sublineage is present (2004-2007) while in Saudi Arabia and UAE the co-circulation of two sublineages exists (2000-2008)

Analysis of deduced amino acid sequences of the HA glycoprotein revealed that 6 viruses from Saudi Arabia, two viruses from Dubai and all the viruses isolated in Kurdistan-Iraq contained the substitution Q226L (H3 numbering) at the receptor binding site. This mutation is associated with a preferential receptor binding specificity for sialic acid α2,6-linked galactose and displayed human virus-like cell tropisms. Molecular analysis allowed us also to identify a Saudi Arabian strain, exhibiting 3 out of 4 amino acid residues at the receptor binding site previously associated with respiratory transmission in ferrets (Sorrel et al., 2009)

The genetic variability identified between the H9N2 viruses used in the present study combined with the identification of specific substitutions which are believed to modify the efficiency of the replication in non avian species, highlight the need to constantly monitor the evolution of this potential pandemic strain in the Middle East.

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POSTER: QUALITATIVE ASSESSMENT OF THE RISK OF RELEASE OF H5N1 HPAI THROUGH THE TRADE OF POULTRY AND POULTRY PRODUCTS

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Key words: Risk assessment, release, pathways, avian influenza, H5N1 HPAI

1. Introduction and Objectives

A qualitative release assessment has been carried out to assess the risk of releasing H5N1 highly pathogenic avian influenza (HPAI) virus into Great Britain (GB), Italy (IT) and the Netherlands (NL). In total, four different release pathways have been considered, namely: legal trade of captive birds, live poultry, poultry products and the movement of wild birds. The release assessments for live poultry and their products are presented here.

2. Material and Methods

Using the OIE guidelines, release assessment describes the "biological pathway(s) necessary for an importation activity to 'release' (that is, introduce) pathogenic agents into a particular environment, and estimating the probability of that complete process occurring" (OIE, 2009). The release pathways for introduction of H5N1 HPAI were constructed and validated by experts in the field. For each step of the release risk pathway data were collected from the published and unpublished literature and, where necessary, expert opinion was sought. In particular, data on recent reported outbreaks of H5N1 HPAI in birds in exporting countries, clinical signs and incubation periods of H5N1 HPAI in different poultry species, survival characteristics of the virus in meat, trade routes of poultry and their products into GB, IT and NL and the legislation surrounding these movements; quarantine, vaccination etc, were collected. Therefore, using the data collected, for each step in the pathway a qualitative risk value was assigned.

3. Results

Combining the qualitative risks at each step of the pathway determines the overall risk of release of H5N1 HPAI into GB, IT and NL per import (bird or kilogram of meat) and annual risk from an exporting region. The results focus on important differences, if any, between the types of imports (e.g. poultry species) and the three Member States in question.

4. Discussion and Conclusions

The release pathways investigated here represent a summary of the risk of H5N1 HPAI entering GB, IT and NL due to the worldwide trade in poultry and poultry products and internal EU movement of these commodities. The results identify regions from which imports are associated with higher and lower risks of H5N1 HPAI release, hence providing a method through which risks can be assessed and managed. The modular integration of trade data is potentially important since trade routes can change over time, making it possible for the risk assessment to be used to predict the risks associated with initiating or increasing trade in poultry and their products from different global regions.

5. Acknowledgements

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POSTER: IS THERE A CORRELATION BETWEEN NEUTRALIZING ANTIBODIES TITERS

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Key words: Bluetongue, neutralizing antibodies, viremia, vaccine

Introduction

Bluetongue virus (BTV) has caused a major outbreak of disease in cattle and sheep in several countries. Classical inactivated vaccines directed to a specific serotype generate protective immunity and may help control current epidemic situations. Vaccination appeared to be the most effective way to eradicate the disease.

After much debate about the advantages and disadvantages of the use of live attenuated and inactivated vaccines against BTV , countries in Northern and Western Europe decided to use inactivated as opposed to live attenuated vaccines in their BTV control plans. In 2008 the European Union instigated a mass-vaccination programme in affected countries using whole virus inactivated vaccines.

Although many millions of animals in Europe have now been vaccinated, there remain some unanswered questions about the immunological mechanisms resulting in vaccine efficacy.

Are neutralizing antibodies a critical component in the protection or recovery from viral infections?

The aim of this study was to analyze if there is a correlation between the neutralizing antibody titer and viremia preventions in vaccinated animals with Zulvac® vaccines and challenged. For this purpose, the neutralizing antibodies titers before challenge were evaluated in animals (sheep and cattle) vaccinated with different Zulvac® vaccines (Zulvac®4 Ovis, Zulvac® 1 Ovis, Zulvac® 1 Bovis, Zulvac® 8 Ovis, Zulvac® 8 Bovis, Zulvac® 1+8 Ovis, Zulvac® 1+8 Bovis and Zulvac® 1+8 Ovis ONE).

Materials and Methods

Nine hundred and seven animals (sheep and calves) were vaccinated and revaccinated 3 weeks later by intramuscular (sheep) and subcutaneous (cattle) route with different Zulvac® vaccines. Three hundred forty-six animals were used as unvaccinated controls.

All animals were seronegative to BTV by ELISA and by a real time reverse transcription-polymerase chain reaction (rRT-PCR) before the start of the study.

At the moment of challenge the titers of neutralizing antibodies were tested by seroneutralization technique.

Conclusion

From the analysis of all experiments conducted, it can be concluded that:

1. The presence of neutralizing antibodies is indicative of prevention of viremia.
2. In function of the titer of neutralising antibodies, prevention of viremia can be predicted.
3. A percentage of animals without neutralizing antibodies can also be protected.

POSTER: EFFICACY OF ZULVAC® 1+8 OVIS, INACTIVATED AND ADJUVANTED VACCINE, AGAINST BLUETONGUE VIRUS SEROTYPE 1 AND SEROTYPE 8

PARADELL, H[1]; GARCIA, L[1]; MOURIÑO, M[1]; VILA, A[1]; URNIZA, A[1]; ALBERCA, B[1]; TARRATS, M[1]; JUANOLA, S[1]; GÓMEZ-TEJEDOR, C[2]; AGÜERO, M[2]; SAN MIGUEL, E[2]; RUANO, M J[2]; PLANA-DURÁN, J[1]

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Key words: Bluetongue, efficacy, vaccine, serotype 1, serotype 8

Introduction
Bluetongue (BT) is an economically significant disease for ruminant animals caused by a virus belonging to the family Reoviridae, genus Orbivirus and transmitted by Culicoides spp. biting midges. Sheep are generally the worst affected. The aim of this study is to verify if Zulvac® 1+8 Ovis vaccine is able to prevent the viremia in vaccinated and challenged animals, stressing the role of the vaccines as an aid for the epidemiological control of the bluetongue disease in sheep.

Materials and Methods
Ninety-nine 1.5-month-old lambs were allocated into three treatment groups as follows: Group 1: 33 lambs vaccinated with Zulvac® 1+8 Ovis (vaccine A); Group 2: 33 lambs vaccinated with Zulvac® 1+8 Ovis (vaccine B); Group 3: 33 control lambs, unvaccinated. Groups 1 and 2 were vaccinated and revaccinated 3 weeks later by subcutaneous route (s.c.). Twenty-one days after revaccination, the lambs (vaccinated and unvaccinated) were challenged. Half of the animals of each group were challenged with BT virus serotype 1 and the other half with BT virus serotype 8. Animals were monitored for clinical signs and blood samples were taken post infection during several days in order to detect presence of the viral genome by a real time reverse transcription-polymerase chain reaction (rRT-PCR) .

Results
None of the animals vaccinated with Zulvac® 1+8 Ovis (vaccine A and vaccine B) presented any systemic reactions. One hundred per cent of the vaccinated animals presented a significantly reduction of hyperthermia after challenge with BT virus serotype 1 or BT virus serotype 8, whereas the control group showed a rise of body temperature (>40 ºC).
One hundred per cent of the vaccinated animals elicited neutralizing antibodies before challenge while unvaccinated animals neutralizing antibodies can not be detected.
In one hundred per cent of the vaccinated animals, viral genome was not detected. In contrast, it was detected in all unvaccinated animals.

Conclusion
Zulvac® 1+8 Ovis, inactivated and adjuvanted vaccine, inoculated in 1.5-month-old lambs is able to induce an active immunity capable to prevent the viremia, blocking the infection cycle caused by the mosquito.

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POSTER: DURATION OF IMMUNITY OF ONE YEAR FOR ZULVAC® 1 BOVIS AND ZULVAC® 1 OVIS, INACTIVATED AND ADJUVANTED VACCINE, AGAINST BLUETONGUE VIRUS SEROTYPE 1

PARADELL, H[1]; GARCIA, L[1]; MOURIÑO, M[1]; VILA, A[1]; URNIZA, A[1]; ALBERCA, B[1]; TARRATS, M[1]; JUANOLA, S[1]; GÓMEZ-TEJEDOR, C[2]; AGÜERO, M[2]; SAN MIGUEL, E[2]; RUANO, M J[2]; PLANA-DURÁN, J[1]

FORT DODGE VETERINARIA, S.A.[1]; LABORATORIO CENTRAL DE VETERINARIA[2]

Key words: Bluetongue, Duration of Immunity, Serotype 1, Cattle

Introduction
Bluetongue (BT) is an economically significant disease for ruminant animals caused by a virus belonging to the family Reoviridae, genus Orbivirus and transmitted by Culicoides spp. biting midges. Vaccination is regarded as one of the most effective ways of controlling and eventually eradicating bluetongue disease in affected areas.
The aim of this study is to verify the duration of immunity of one year for Zulvac® 1 Bovis and Zulvac® 1 Ovis in calves and sheep.

Materials and Methods
Animals were vaccinated at 2.5 to 4-month-old (calves) and 1.5 month-old (lambs) and revaccinated 3 weeks later by intramuscular and subcutaneous route respectively. Eight calves and twenty lambs were vaccinated; eight calves and ten lambs were used as unvaccinated controls. One year after revaccination the animals (vaccinated and unvaccinated) were challenged. Animals were monitored for clinical signs and blood samples were taken during several days post infection to detect the presence of the viral genome by a real time reverse transcription-polymerase chain reaction (rRT-PCR) .

Results
None of the calves and lambs manifested any systemic reactions after vaccination.
After BT virus serotype 1 infection, unvaccinated animals presented a higher rectal temperature than the vaccinated and infected animals.
In one hundred per cent of the vaccinated animals, viral genome was not detected. In contrast, it was detected in all unvaccinated animals.

Conclusion
Zulvac® 1 Bovis and Zulvac® 1 Ovis, inactivated and adjuvanted vaccines, are able to prevent viremia in animals challenged one year after revaccination.

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(1) Toussaint JF, Sailleau C, Breard E, Zientara S, De Clercq K J. Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments. Virol Methods 2007; 140(1-2):115-123.

POSTER: DURATION OF IMMUNITY OF ONE YEAR FOR ZULVAC® 8 OVIS, INACTIVATED AND ADJUVANTED VACCINE, AGAINST BLUETONGUE VIRUS SEROTYPE 8

PARADELL, H[1]; GARCIA, L[1]; MOURIÑO, M[1]; VILA, A [1]; URNIZA, A[1]; ALBERCA, B[1]; TARRATS, M[1]; JUANOLA, S[1]; GÓMEZ-TEJEDOR, C[2]; AGÜERO, M[2]; SAN MIGUEL, E[2]; RUANO, M J[2]; PLANA-DURÁN, J[1]

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Key words: Bluetongue, Duration of Immunity, Serotype 8, Sheep

Introduction
Bluetongue (BT) is a devastating disease of wild and domestic ruminants caused by a virus belonging to the family Reoviridae, genus Orbivirus. The virus is transmitted to ruminants by small blood-feeding midges of the genus Culicoides. Considering the way of transmission, it is very useful to have efficacious vaccines able to prevent viremia and block the infectious cycle caused by the mosquito. The objective of this study is to verify the duration of immunity of one year for Zulvac® 8 Ovis in sheep.

Materials and Methods
Twenty-four 1.5 month-old lambs were allocated into two treatment groups, as follows: Group 1: twelve lambs vaccinated with Zulvac® 8 Ovis; Group 2: twelve lambs unvaccinated. Lambs were vaccinated and revaccinated 3 weeks later by subcutaneous route. One year after revaccination the animals (vaccinated and unvaccinated) were challenged. Animals were monitored for clinical signs and blood samples were taken during several days post infection to detect the presence of the viral genome by a real time reverse transcription-polymerase chain reaction (rRT-PCR) .

Results
None of the lambs presented any systemic reactions after vaccination.
After BT virus serotype 8 infection, unvaccinated animals presented a higher rectal temperature than the vaccinated and infected animals.
In one hundred per cent of the vaccinated animals, viral genome was not detected. In contrast, it was detected in all unvaccinated animals.

Conclusion
Zulvac® 8 Ovis, inactivated and adjuvanted vaccine, is able to prevent viremia in animals challenged one year after revaccination.

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POSTER: MORBILLIVIRUS THREATS: FROM RINDERPEST TO PPR

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Key words: rinderpest, Peste des Petit Ruminants, OIE, GREP

Morbillivirus threats: from rinderpest to PPR
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Rinderpest is principally a disease of ruminants that has been known for millennia and at times has been responsible for changing the course of history. Wherever it occurred, it was the most dreaded animal disease, killing livestock, wildlife, destroying rural and pastoral livelihoods, and was detrimental to food security in numerous regions. Since the late 1940s, FAO has convened a series of meetings that included affected countries and institutions. Guided by these meetings, the fight against rinderpest has been conducted through a serial of internationally coordinated projects and programmes (Africa, Middle East and Asia) implemented over the last seven decades. In 1994, rinderpest control worldwide became a truly global effort with the launch by FAO of the Global Rinderpest Eradication Programme (GREP), which provided the framework for progressive time- bound eradication of the disease. A GREP Secretariat was established which promoted a strategy to contain rinderpest within several infected ecosystems and the elimination of infection reservoirs through epidemiologically and intelligence-based control actions and monitoring and surveillance activities supported by a global laboratory network. Despite mounting and accumulated evidence that the disease had been clinically eradicated, assistance was provided to countries to implement surveillance required for the pathway towards official international declaration of freedom from rinderpest disease and subsequently from the virus, by the OIE. The last reported rinderpest outbreak was in Kenya in 2001. As of early 2010, the FAO is confident that the disease is eliminated from Europe, Asia, the Middle East and Africa.

Peste des petits ruminant (PPR) is a highly contagious and infectious viral disease of both domestic (goats and sheep) and wild ruminants. It is clinically and pathologically similar to rinderpest and is one of the most economically important viral diseases of small ruminants.
The current epidemiological distribution of the disease is similar to that of rinderpest 30 years ago. PPR infection affected areas extend north to Egypt and south to Tanzania, in the east, and Mauritania to DR Congo, in the west. The disease has not been recognized in most of North and southern Africa (Except Egypt and Morocco). The disease has been described in countries in the Middle East, including Iran and Turkey. PPR infection has been recognized in many Asian countries bordering China, including Mongolia, India, Nepal, Bangladesh, Pakistan, Afghanistan and other central Asian countries (Tajikistan, Turkmenistan and Uzbekistan).
Being so closely related to rinderpest virus, intervention strategies to eradicate PPR should show early promise and early successes. As with rinderpest, PPR is a killer disease and thus would certainly benefit livestock owners and gain their quick agreement and participation. With only one strain in circulation and the fact that the vaccine also gives long lasting protection, an international campaign to eradicate this disease is certainly possible.
The rinderpest virus will be the second globally eradicated disease pathogen, following smallpox virus eradication in 1977 and its certified global freedom in 1980. The pathway from the launch of GREP to the achievement of successful eradication of rinderpest provides valuable lessons for future efforts to control and elimination of other transboundary animal diseases.

POSTER: THE FIRST SWEDISH H1N2 SWINE INFLUENZA VIRUS ISOLATE REPRESENTS AN UNCOMMON REASSORTANT

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Key words: swine influenza

The European swine influenza viruses (SIVs) show considerable diversity comprising different types of H1N1, H3N2, and H1N2 strains. The intensifying full genome sequencing efforts reveal further reassortants within these subtypes. Here we report the identification of an uncommon reassortant variant of H1N2 subtype influenza virus isolated from a pig in a multisite herd where H1N2 swine influenza was diagnosed for the first time in Sweden during the winter of 2008-2009. The majority of the European H1N2 swine influenza viruses described so far possess haemagglutinin (HA) of the human-like H1N2 SIV viruses and the neuraminidase (NA) of either the European H1N2 or H3N2 SIV-like viruses. The Swedish isolate has an avian-like SIV HA and a H3N2 SIV-like NA, which is phylogenetically more closely related to H3N2 SIV NAs from isolates collected in the early '80s than to the NA of H3N2 origin of the H1N2 viruses isolated during the last decade, as depicted by some German strains, indicative of independent acquisition of the NA genes for these two types of reassortants. The internal genes proved to be entirely of avian-like SIV H1N1 origin. The prevalence of this SIV variant in pig populations needs to be determined, as well as the suitability of the routinely used laboratory reagents to analyze this strain. The description of this H1N2 SIV adds further information to influenza epidemiology and supports the necessity of surveillance for influenza viruses in pigs.

POSTER: ENVIRONMENTAL AND CLIMATIC FACTORS INFLUENCING SPATIO-TEMPORAL ACTIVITY OF POSSIBLE BLUETONGUE VECTOR SPECIES OF CULICOIDES IN BELGIUM

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Key words: biting midge, bluetongue virus, flight activity, temperature, ecology

Since the introduction of bluetongue virus serotype 8 (BTV-8) in Belgium in 2006, a vector monitoring programme was set-up in 2007 to study the population dynamics of the BTV vector, Culicoides. From April 2007 till April 2008 about 150000 midges comprising 41 species were caught with OVI light traps at 20 dairy farms spread over the different provinces of Belgium. This study examined the influence of environmental (land cover, vegetation and soil type) and climatic (temperature, wind and rain) factors on apparent flight activity of each of the 8 most frequently captured species, including possible BTV vectors (C. obsoletus s.l., C. dewulfi, C. chiopterus, C. punctatus, C. pulicaris, C. nubeculosus, C. festivipennis, C. achrayi). Non parametric classification and regression trees (CART) were used to identify the possibly complex relationship between the spatio-temporal apparent activity of Culicoides species to the trap and the aforementioned factors. Furthermore parametric poisson and negative binomial regression analyses were used to confirm the influence of each variable on Culicoides apparent activity statistically. Overall, temperature emerged as a predominant factor influencing flight activity. Other important ecological parameters and their complex interplay will be presented and discussed for the 8 Culicoides species.

EPIZONE

List of participants
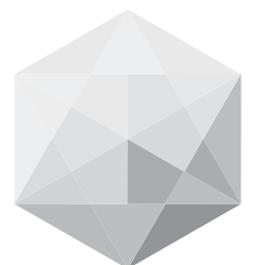
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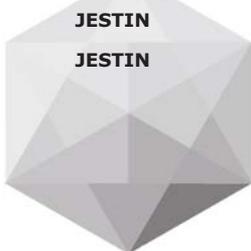
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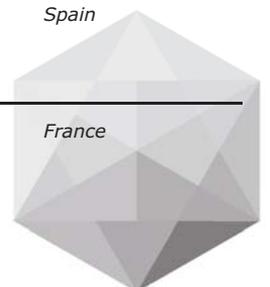
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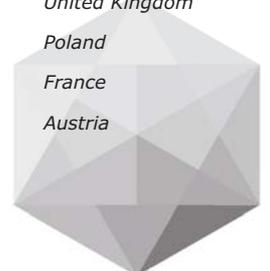
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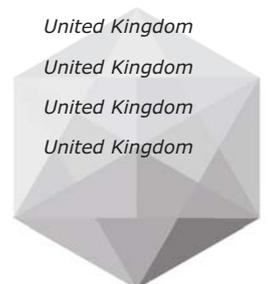
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EPIZONE is an EU funded Network of Excellence for Epizootic Disease Diagnosis and Control to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks of Foot-and-mouth disease, Classical swine fever, Avian influenza, and other relevant epizootic diseases like Bluetongue and African swine fever, through increased excellence by collaboration.