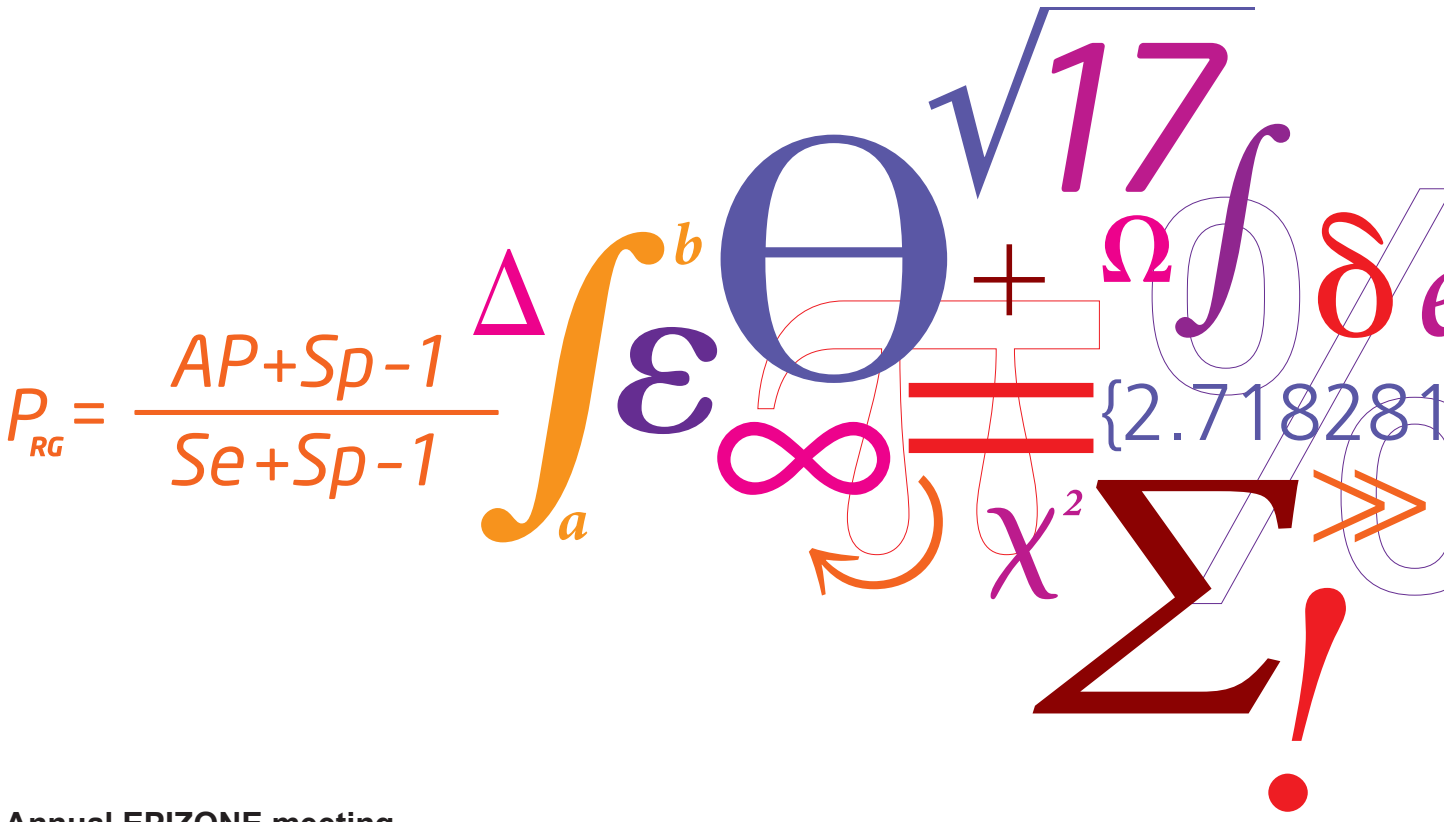
EPIZONE

Abstracts

8th Annual EPIZONE Meeting

"Primed for tomorrow"

23 - 25 September 2014 in Copenhagen, Denmark
Hosted by DTU Vet



8th Annual EPIZONE meeting
September 2014
Copenhagen, Denmark

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- Administration Bureau EPIZONE at Wageningen University
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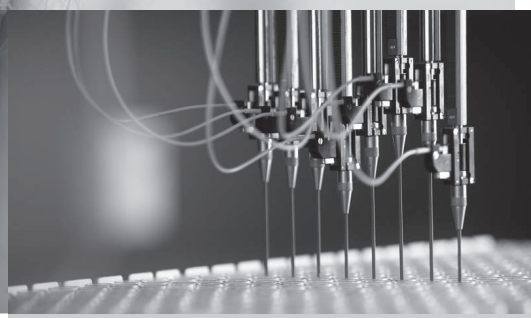

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- Tasks and activities in Danish Pig Research Centre are laid down by a Sector Board that consists of 12 pig farmers.

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Welcome to the 8th Annual meeting

We are very pleased to welcome you to the 8th Annual Meeting of EPIZONE 23-25 September 2014 in Copenhagen, Denmark.

This meeting is for scientists and stakeholders interested in the activities of EPIZONE which is the European Research Group for Epizootic Disease Diagnosis and Control.

Theme: Primed for tomorrow
The meeting is entitled: ”Primed for tomorrow” and will address the latest developments aimed at monitoring and understanding the evolution, emergence, transmission and spread of epizootic viruses.

The focus will remain on the EPIZONE themes aimed at improved disease control through integration and collaboration of research in diagnosis, intervention strategies, risk assessment, surveillance and epidemiology.

A stimulating scientific programme will be provided by invited speakers and selected poster and oral presentations describing recent research on epizootic diseases of cattle, pigs, poultry, sheep, goats, fish and horses.

This meeting builds on previous highly successful EPIZONE meetings and will provide extensive opportunities for networking, scientific exchange and fostering collaborations.

Have a great time
The 8th Annual Meeting is hosted by DTU Vet - the National Veterinary Institute at DTU Technical University of Denmark and together with the Scientific and Organizing Committees we wish you a great time here in Copenhagen, Denmark.

Committees

The 8th Annual EPIZONE Meeting is organised by DTU Vet - the National Veterinary Institute at DTU Technical University of Denmark.

Scientific Committee

Graham Belsham, Professor in Section for Virology, DTU Vet *(Head of Scientific Committee)*
Anette Bøtner, Deputy Head in Section for Diagnostics and Scientific Advice, DTU Vet *(Head of Organizing Committee)*
Anette Boklund, Senior Advisor in Section for Epidemiology, DTU Vet
Thomas Bruun Rasmussen, Senior researcher in Section for Virology, DTU Vet
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Niels Jørgen Olesen, Professor in Section for Virology, DTU Vet
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Dr Martin Beer, FLI, Friedrich-Loeffler-Institut, Germany
Dr Jean-Francois Valarcher, SVA, Statens veterinärmedicinska anstalt, Sweden
Dr José-Manuel Sánchez-Vizcaino, UCM, Universidad Complutense Madrid, Spain
Professor Stuart Reid, RVC, Royal Veterinary College, UK
Dr Frank Koenen, CODA-CERVA, Belgium

Organizing committee, DTU Vet

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Graham Belsham, Professor in Section for Virology *(Head of Scientific Committee)*
Anette Boklund, Senior Advisor in Section for Epidemiology
Thomas Bruun Rasmussen, Senior researcher in Section for Virology
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Maria Gerner-Rasmussen, Executive Secretary
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Programme for the 8th Annual Meeting of EPIZONE

Day 1: Tuesday September 23rd 2014 DTU Vet, Bülowsvej 27		
09:00-17:00	Young EPIZONE	
18:30-19.30	Registration and welcome desks	
19:00-22:00	*Welcome drinks and buffet*	
Day 2: Wednesday September 24th 2014 DGI-Byen, Tietgensgade 65		
08:00-onwards	Registration	
09:00-09:20	**Introduction and welcome** Chair Organizing Committee Anette Bøtner CVO Per Henriksen Epizone Coordinator Wim van der Poel	
Plenary Session Chair: Graham Belsham		
09:20-10:00	**Keynote: Donald P. King** The consensus and beyond: developing new tools to reconstruct transmission pathways of foot-and-mouth disease virus.	
10.00-10.40	**Keynote: Jean-Claude Manuguerra** New coronaviruses - what next?	
10:40-11:10	*Coffee break*	
	Session 1: Antivirals, vaccines Room: Nørrebro Runddel Chairs: Martin Beer & Thomas Bruun Rasmussen	**Session 2: Diagnostics** Room: Skt. Hans Torv Chairs: Willie Loeffen & Helen Crooke
11:10-11:25	**Alexander Postel:** Genetically modified viruses as vaccine candidates – Lessons learned from a chimeric Pestivirus	**Piet A. van Rijn:** A NS3-ELISA accompanying newly developed vaccines for Bluetongue to differentiate infected from vaccinated animals
11:25-11:40	**Mariano Domingo:** The lapinized Chinese CSFV vaccine strain in an endemic situation: Protecting or promoting viral evolution?	**Cécile Beck:** The Luminex Microsphere immunoassay, a technologic innovation for the serological diagnosis of flavivirus infections

11:40-11:55	**Eefke Weesendorp:** Characterization of immune responses following homologous reinfection of pigs with EU subtype 1 and 3 PRRSV strains that differ in virulence	**Denise Meyer:** A new Concept for the Development of a CSF-DIVA ELISA
11:55-12.10	**Femke Feenstra:** Bluetongue virus without NS3/NS3a expression is avirulent and protects against challenge in a sheep model	**Almudena Sánchez-Matamoros:** New Diagnostic Technique for Serological Detection of African Horse Sickness Virus: Luminex Technology
12.10-12.25	**Annebel De Vleeschauwer:** Proof-of-concept for the reduction of Foot-and-Mouth disease by an antiviral drug	**Letizia Ceglie:** Presence of SBV in semen after experimental infection of bulls
12:25-13.30	*Lunch and poster viewing*	
	Session 3: Vaccines Room: Nørrebro Runddel Chair: Günther Keil & Peter M. H. Heegaard	**Session 4: Vector borne diseases** Room: Skt. Hans Torv Chair: Piet van Rijn & Jean-Francois Valarcher
13:30-13:45	**Ulrik Fahnøe:** Uncovering of Classical Swine Fever Virus adaptive response to vaccination by Next Generation Sequencing	**Núria Busquet:** Vector competence of Culex pipiens and Aedes albopictus from Catalonia (Northeastern of Spain) for different arboviruses
13:45-14:00	**Maria Jenckel:** Characterization of atypical BVDV-2c isolates of a German outbreak using Next generation sequencing	**Simon Carpenter:** Studies of Culicoides biting midges at the Pirbright Institute: from Genomics to the Field
14:00-14:15	**Willie Loeffen:** Efficacy of CSF vaccine CP7_E2alf in piglets with maternally derived antibodies	**Stéphan Zientara:** Emergence of Bluetongue virus in Corsica in 2013
14:15-14:30	**Julie Gouzil:** Interaction of Schmallenberg virus (SBV) NSs virulence factor with the cellular Major Vault protein (MVP)	**Sylvie Lecollinet:** Proline residue at NS3 249 is a primary determinant of West nile virus virulence in mammals and birds
14:30-14:45	**Graham J. Belsham:** Targeted modifications of foot-and-mouth disease virus; towards improved vaccine candidates	**Jennifer Bernard:** Effect of salivary gland extract of tick Ornithodoros porcinus on African swine fever virus infection in pigs
14:45-15:00	**Carolin Dräger:** CD46 is also a cellular receptor for Classical swine fever virus	**Derah Saward Arav:** The effect of African swine fever virus and two of its proteins on host MHC class I expression
15:00-15:30	*Coffee and poster viewing*	

	Session 5: Emerging diseases Room: Nørrebro Runddel Chair: Stéphan Szientara & Mariano Domingo	**Session 6: Epidemiology (I)** Room: Skt. Hans Torv Chair: Stuart Reid & Sandra Blome
15:30-15:45	**Sarah White:** Serological Evidence of Equine Influenza Infections among Persons with Horse Exposure in Iowa	**Tariq Halasa:** Impact of clinical surveillance during a foot-and-mouth disease epidemic
15:45-16:00	**Béatrice Grasland:** Preparedness for potential PED emergence in France: Development of diagnostic tools	**Lina Mur:** Combination of simulation and statistical modeling to identify factors contributing to ASF endemicity in Sardinia
16:00-16:15	**Alice Fusaro:** A deep insight into an H7N7 highly pathogenic avian influenza epidemic	**Francesco Feliziani:** ASF in Sardinia: Regional risk categorization to improve surveillance effectiveness
16:15-16:30	**Nathalie Kirschvink:** Passive immunity against Schmallenberg virus in lambs born from naturally infected ewes	**Claire Guinat:** Experimental transmission of African swine fever virus among domestic pigs and wild boars
16:30-16:45	**Marta Martínez-Avilés:** Early detection of transboundary animal diseases with a real-time monitoring system online.	**Sofie Dhollander:** Expert Knowledge Elicitation on African swine fever in the eastern neighbouring countries of the European Union
16:45-17:00	**Hakan IŞIDAN:** Spreading Lumpy Skin Disease to the North	**Aline de Koeijer:** Method for integral risk assessment of vector-borne livestock infections (MINTRISK)
17:00-19:30	*Poster session and drinks*	
19:30-23:00	*Meeting dinner*	

Day 3: Thursday September 25th 2014
DGI-Byen, Tietgensgade 65

08:00-onwards	Registration	
	Plenary session Chair: Anette Bøtner	
09:00-09:40	**Keynote: Nils Toft** Big Data and disease monitoring – The next new thing or old news?	
09:40- 10:20	**Keynote: Norbert Stockhofe-Zurwieden** High Containment Infrastructure Projects to optimize Animal and Public Health Innovations	
10:20-10:50	*Coffee and poster viewing*	
	Session 7: Domestic and Wildlife Room: Nørrebro Runddel Chairs: Wim van der Poel & Louise Lohse	**Session 8: Epidemiology (II)** Room: Skt. Hans Torv Chairs: Yves van der Stede & Tariq Halasa
10:50-11:05	**Manjunathareddy Bayyappa:** Wild animal rabies in India: Possibility of species spill over	**Birgit Schauer:** "Surveillance is a public good" – but how public is it?
11:05-11:20	**Sara Verpoest:** Age and strain dependent differences on the outcome of experimental infections of domestic pigs with Belgian wild boar pseudorabies virus isolates	**Eefke Weesendorp:** Quantification of different classical swine fever virus transmission routes within a single compartment
11:20-11:35	**Thomas Bruun Rasmussen:** Detection of European bat lyssavirus type 2 in Danish Daubenton's bats	**Anette Boklund:** Resource estimations in contingency planning for foot-and-mouth disease
11:35-11:50	**Deborah Kukielka Zunzunegui:** Constant Hepatitis E virus circulation in wild boar, red deer and Iberian pig in Spain: An 8 years longitudinal study	**Sarah White:** A Systematic Review and Meta-Analysis to Measure Sero-Prevalence of Influenza A (H9N2) Virus Infection in Humans
11:50-12.05	**Juliette Hayer:** The metagenomic in silico lab: Design, Analysis and Validation of metagenomic investigations	**Bianca Zecchin:** Identification of the genetic structure of the red fox (Vulpes vulpes) population in association to canine distemper and rabies viruses circulating in Northern Italy
12.05-12.20	**Maja Malmberg:** Kobuvirus in brains of piglets diagnosed with congenital tremor type A-II	**Antoine Poskin:** The impact of Schmallenberg in Belgian cattle and sheep: a case-control study

12:20-13.30	*Lunch and poster viewing*
Special topic session: ASF (keynotes) Chair: Frank Koenen	
13:30-14:10	**Keynote: Lina Mur** Epidemiology of ASF in Eastern Europe and the risk derived from it
14:10-14:50	**Keynote: Sandra Blome** African swine fever in domestic pigs and European wild boar – Experiences from recent animal studies
14:50-15.20	*Coffee break*
Special topic Session: ASF (short presentations) Chairs: Jose Manuel Sanchez-Vizcaino & Linda Dixon	
15:20-15.35	**Willie Loeffen:** Suitability of feces samples in a non-invasive sampling strategy to monitor African swine fever virus in wild boar
15:35-15:50	**Helena Redondo:** Diagnostic tools for rapid detection of ASFV in blood samples: Lateral Flow Assay and Real Time PCR
15:50-16:05	**Claire Barber:** Defining mechanisms used by African swine fever virus to evade the host stress response
16.05-16:20	**Günther Keil:** The African swine fever virus ORF I8L which encodes a putative member of the SH2 domain superfamily is not essential for ASFV replication in vitro
16:20-16.35	**Luisa Arias Neira:** Assessment of the carrier state induced in experimentally infected pigs with African Swine fever (ASF) attenuated and virulent strains.
16:35-16:50	**Alexsey Igolkin:** Comparative analysis of molecular and biological properties of African swine fever virus isolates collected in 2013 from Russian Federation
16:50-17:00	Closing session
End of meeting	

Keynote speakers

Keynote presentation: Donald P. King

The consensus and beyond: developing new tools to reconstruct transmission pathways of foot-and-mouth disease virus

Donald P. King[1], Grace Logan[1,2], Begoña Valdazo-González[1], Graham L. Freimanis[1], Caroline F. Wright[1], David J. King[1], Nick J. Knowles[1], Jemma Wadsworth[1], Katarzyna Bachanek-Bankowska[1], Antonello Di Nardo[1,2], Richard Orton[2], Daniel T. Haydon[2]

[1]The Pirbright Institute, Ash Road, Pirbright, GU24 0NF, UK and [2]Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, G12 8QQ, UK.

Control of transboundary animal diseases such as foot-and-mouth disease (FMD) is reliant on understanding the critical factors that impact upon the epidemiology of the infectious agent. This presentation reviews the uses of genomic sequences to monitor the spread of FMD virus, and to reconstruct transmission pathways over the course of disease outbreaks. The genome of FMDV (genus Aphthovirus, family Picornaviridae) evolves rapidly as a consequence of mutations that are introduced and inherited during replication. These characteristics allow nucleotide sequence data to be used to reliably reconstruct the relationship between viruses recovered from different locations, and/or at different times. At the broadest scale, analyses of sequences encoding a capsid protein (VP1/1D) are used to categorise field strains into discrete variants (or topotypes) which frequently show geographical clustering based on the historical distribution of FMDV. The pattern of serotypes and variants around the world is not static and sequencing of these viruses allows us to precisely characterise new isolates of FMDV and trace their origin and movements across international boundaries. More recently, complete genome sequences have been used to define the relationship between closely related samples collected during FMD outbreaks in the UK (2001 and 2007) and Bulgaria (2011). Analysis of these complete genome sequences provides data at high resolution allowing transmission events at the farm-to-farm level to be reconstructed. Next-generation sequencing (NGS) platforms are now being used to develop unbiased methods without using PCR to analyse larger numbers of consensus-level sequences within a routine high-throughput diagnostic environment. At the finest scale, the unprecedented step-change increase in the amount of sequence data generated from an individual sample by NGS platforms can also be used to provide snapshots of the evolving viral population structures within different animals and tissues. Using an NGS (Illumina) approach, analysis of polymorphism frequency has revealed that a number of minority variants are transmitted during host-to-host infection events, while the size of the intra-host founder populations appear to be smaller. These data indicate that viral population complexity is influenced by small intra-host bottlenecks and relatively large inter-host bottlenecks. The greater depth of detection, achieved by NGS, demonstrates that this method is a powerful and valuable tool for the dissection of FMDV micro-evolution.

Keynote presentation: Jean-Claude Manuguerra

New coronaviruses - what next?

Jean-Claude MANUGUERRA

Institut PASTEUR, Environment and Infectious risks Research and Expertise Unit, Laboratory for Urgent response to biological threats, Paris, France

Coronaviruses (CoVs) are enveloped viruses that have positive-sense, non-segmented RNA genomes that are 27-32 kb in length. The viral particles are pleomorphic, and their crown aspect - visible under the electron microscope - is due to the presence on the envelope of mace-shaped spikes 20nm high and expressing the high variable S protein. The basic gene organization and replication are well conserved for all CoVs.Among the RNA+ viruses, CoVs clearly distinguish themselves by encoding the most complex and largest genomes. The low replication fidelity of RdRp is thought to be a key determinant of RNA virus quasispecies diversity, adaptation, and virulence. Moreover, recombination events shape the viral population structure, promoting cross-species transmission. The high recombination frequency is likely due to the very large size of the genome paired with a replication complex naturally equipped to associate and reassociate from the template RNA. Recombination combined with high mutation rates allow for the rapid evolution of the CoV genome, which undergoes high positive selective pressure during emergence and host-shift events. The CoV genome is the largest RNA virus genome, and probably possess mechanisms to limit the excessive numbers of deleterious mutations leading to a dramatic loss of fitness while concomitantly maintaining the genetic diversity required for adaptation to new environmental conditions. This allows CoVs to jump from one host species to another and to sucessfully adapt to its new host.

CoVs have a wide spectrum of hosts including a great number of both mammalian and avian species. The pathologies for which they are responsible are essentially respiratory and enteric. Their severity is variable with respect to the host and its immune status. The first animal coronaviruses were described in the 1930s: the IBV or “infectious bronchitis virus” in domestic fowl, the “transmissible gastroenteritis coronavirus” in domestic pigs, and the MHV or “mouse hepatitis virus”. During the 1980s and 90s, the porcine epidemic diarrhoea virus (PEDV) was prevalent throughout Europe and is currently a source of concern in Asia and North America. These viruses, responsible for serious pathologies in these species, were first studied in veterinary science. Human coronaviruses OC43 and 229E were described in the 1960s, but have attracted limited interest because of the mild respiratory symptomatology which they were linked to.

The evolutive and emerging potential of CoVs came into the limelight during the 2003 SARS (Severe Acute Respiratory Syndrome) pandemic. The identification in September 2012 of the MERS-CoV, responsible for a SARS-like syndrome, provides a clear warning that this risk is of current concern.

Keynote presentation: Nils Toft

Big Data and disease monitoring – The next new thing or old news?

Nils Toft

DTU Vet, National Veterinary Institute, Technical University of Denmark

Big data is a relative term which is being used increasingly to describe a situation where the available data exceed an organization's storage or compute capacity for accurate and timely decision making. However, big data is defined less by volume, which seems to be an ever-expanding limit, than by its ever-increasing variety, velocity, variability and complexity. Variety because big data are inherently unstructured (text, video, geodata, etc) and often non-numeric; Velocity because the use of real time imaging, intelligent and electronic identification and other automations potentially permits real time disease monitoring; Variability because data flows can have daily, seasonal or event-triggered peaks; Complexity because it is necessary to understand complex relationships such as hierarchical structures and data links in all data. However, is this really something new or is the above a description of the reality that researchers, authorities and decision makers in the agricultural sector has been facing for years? Following a more in depth definition of big data, the presentation will address this question and provide examples of current, future and past big data-like challenges in disease monitoring.

Keynote presentation: Nobert Stockhofe-Zurwieden

High Containment Infrastructure Projects to optimize Animal and Public Health Innovations

Norbert Stockhofe-Zurwieden[1], Frederic Lantier[2]

[1]Central Veterinary Institute, part of Wageningen University&Research Institutes,
[2]Infectiology and Public Health, INRA, Tours-Nouzilly

In recent years emerging infectious diseases in farm animals like BSE, Bluetongue virus infections, influenza and others have proven the necessity of high containment infectious disease infrastructures for immediate and effective characterization of pathogen isolates and testing of diagnostic and control tools. Within the EU there are a limited number of farm animal high containment facilities, which contrasts with the larger number of research groups working on human transmissible diseases with the limitation of in-vitro approaches or laboratory animal models. In the last decennium it was recognized at the EC level that research infrastructures play a crucial role in the development of science and competitiveness. A roadmap compiled by the European Strategy Forum on Research Infrastructures (ESFRI) identified relevant infrastructures within Europe in very different research areas. In the light of this road map infrastructure projects were initiated in FP7 to stimulate the integration of existing facilities , and the development of new research infrastructures (RI). In NADIR, one of the recent EU-infrastructure projects (2009-2013) sixteen EU animal infectiology research institutes worked together to build up a so-called distributed RI, a network of resources in infectious disease research with the aims to share expertise and know how, to contribute to innovative research, to organize related training and - most important - to make laboratory and large animal highly confined facilities accessible to ERA scientists through transnational access projects. In a period of four years, NADIR funded 45 different studies from users/user groups from 18 different countries. The proposals extended across the whole field of infectiology research: pathogenesis and virulence studies, vaccine or therapeutics development, host response to infection, genetic susceptibility of the host and epidemiology. Most studies were performed in pigs, but also in other farm animal species, fish and even in wild reservoirs such as badgers. High containment facilities for studies under BSL3 are rare and expensive. They require huge maintenance funds to keep up with biosafety and biosecurity, up to date regulations, which include quality, legal aspects and animal welfare. They also necessitate high professional skills from dedicated teams for upkeep, husbandry, maintenance and management of experiments. Therefore, it is essential that research facilities keep the opportunity to build up sufficient expertise to deal with the diversity of demands. From the experience with NADIR and other infrastructure projects it appears feasible and recommendable to share our infrastructures for optimizing their occupation rate and for strengthening innovative research in animal infectiology through a broad access to top level high containment facilities.

Keynote presentation: Lina Mur

Epidemiology of ASF in Eastern Europe and the risk derived from it

Lina Mur[1], Beatriz Martínez-López[1,2], José Manuel Sánchez-Vizcaíno[1]

[1]VISAVET Center, Universidad Complutense de Madrid
[2]Center for Animal Disease Modelling and Surveillance (CADMS), University of California, USA

African swine fever (ASF) is a notifiable disease to the OIE, with important economic and social consequences, derived from its presence. Although it was discovered and it is widely present in African continent, it acquired its recent relevance due to its introduction into Georgia in 2007 and spread to Armenia, Azerbaijan and Russian Federation. Currenly, disease continue spreading without control to northern territories of Russian Federation causing sporadic outbreaks in nearby countries such as Ukraine (2012), Belarus (2013) and to wild boar cases in European Union (EU) countries such as Lithuania, Poland and Latvia in 2014.

In East Europe, ASF is affecting domestic pigs and wild boar. Other factors as the presence of soft ticks have not been elucidated yet, although they could be important for disease maintenance in certain territories.

This situation poses a risk to EU, and aware of that, the risk of ASF introduction into EU by the most common routes was analyzed. Independent risk assessments were developed employing quantitative (legal imports of pigs and products) and semi-quantitative (illegal imports of products, transport associated routes and wild boar) methods. All these assessments were finally integrated in a common framework for allowing the analysis, visualization and interpretation of the results revealing that at least 48% of EU countries presented a relative high level of risk of ASF introduction for one or more pathways. This framework allows also identifying the EU countries at highest risk for each pathway, which could be used to support risk-based surveillance and mitigation strategies.

This work summarizes the evolution and main epidemiological characteristics of ASF in East Europe as well as an overview of the risk of ASF introduction into EU.

Keynote presentation: Sandra Blome

African swine fever in domestic pigs and European wild boar – Experiences from recent animal studies

Sandra Blome, Jana Pietschmann, and Martin Beer

Friedrich-Loeffler-Institut, Institute of Diagnostic Virology, Germany

African swine fever (ASF) is among the most complex viral diseases affecting domestic pigs and European wild boar. Depending on host factors and strain virulence, clinical signs can vary from almost inapparent infection to a hemorrhagic fever like illness. However, most currently circulating strains are highly virulent and lead to an acute-lethal disease course in both domestic pigs and European wild boar.

With these strains, unspecific symptoms predominate in all age classes of domestic pigs and wild boar. Signs include high fever (often >41°C), inappetence, labored breathing, and general depression. Neurological symptoms and hemorrhagic lesions may aggravate the picture in the final stages of infection. Under experimental conditions, almost 100 % of animals died within less than 12 days post infection.

One of the recently performed animal trials dealt with low dose oral infection of domestic pigs and European wild boar with an Armenian ASFV strain. It was demonstrated that very low virus doses are sufficient to infect weaker animals. After amplification in these hosts, and contact to blood or bloody excretions, the virus was transmitted to all remaining animals in a progressive manner, and the individual disease courses were very similar. However, contagiosity was moderate, even within pens, and the disease lasted 36 days on group level. These facts could be most important for disease dynamics in wild boar populations and under backyard settings as well as for the initial introduction into ASFV free regions. Finally, no indications for prolonged or chronic individual courses upon low dose infection in either species could be found.

In a small trial with sub-adult wild boar, high virulence of a recent Sardinian ASFV isolate was confirmed.

Oral presentations

Session 1: Antivirals, vaccines

Genetically modified viruses as vaccine candidates – Lessons learned from a chimeric Pestivirus

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Live attenuated vaccines often mediate robust protection against viral infection and disease. In the field of veterinary medicine live vaccines additionally have the advantage of oral application which is prerequisite for vaccination campaigns including wild animals. Reverse genetic systems allow a targeted genetic manipulation for attenuation and design of new vaccine candidates. In the present study efficacy and genetic stability of a chimeric pestivirus was investigated. To attenuate the virulent classical swine fever virus (CSFV) isolate Alfort-Tuebingen (genotype 2.3), the Jiv-encoding cassette of bovine viral diarrhea virus (BVDV) isolate cp8 was introduced into the viral genome between the Npro and caspid protein encoding region (Gallei et al., J. Virol. 2008). A first animal trial demonstrated that the cytopathic (cp) phenotype of this chimera, designated “Alfort-Jiv”, correlates with attenuation in infected pigs (n=5). In a subsequent challenge study vaccination of pigs induced protection against infection with a highly virulent heterologous CSFV isolate (genotype 1.1) seven days (n=5) and 21 days (n=5) post vaccination. Nevertheless, one of the vaccinated and infected animals from the early-challenge group developed severe symptoms of CSF 12 days post vaccination. Molecular analyses demonstrated absence of the challenge virus and detected multiple revertants of the chimeric pestivirus “Alfort-Jiv”. In the genomes of these revertants eight unique in-frame deletions were identified which resulted in removal of the Jiv-cassette originating from BVDV. The presence of CSFV-homologous BVDV sequences of the Jiv-cassette as well as the integration into a non-essential genomic region of the viral genome paved the way for recombination events resulting in viable and virulent mutants. Molecular characterization of the revertant viruses shed a light on the high frequency of genetic alternations that can occur in genetically engineered chimeric viruses. Furthermore, the presented results demonstrate that genetic design of chimeric viruses as well as careful testing of the genetic stability is crucial with respect to biosecurity and safety of live vaccines.

The lapinized Chinese CSFV vaccine strain in an endemic situation: Protecting or promoting viral evolution?

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Classical swine fever (CSF) is a highly contagious viral disease caused by a small, enveloped single-stranded RNA virus, genus Pestivirus, family Flaviviridae. Nowadays, CSF represents one of the leading worldwide threats to the pig industry, being still endemic in many countries. Despite the lack of DIVA properties, CSF live attenuated vaccines containing the Lapinized Chinese strain are widely used in most countries in which the disease is endemic. However, and despite control programs for over 20 years in many countries, such as China and Cuba, the disease has not been eradicated yet. Moreover, molecular epidemiology studies conducted in these endemic countries have shown that the evolution of CSFV strains since 1993 until today is possibly being driven by positive selection pressure of the live attenuated vaccine. This has lead to the emergence of the current new attenuated viral field variants, which circulate in spite of intensive vaccination programs (Perez et al., 2012; Wei et al., 2014). Considering this precedent, we design a study to asses the clinical, immunological and virological protective capacity of one Lapinized Chinese vaccine actually in use. The kinetics of replication and excretion of this CSFV vaccine was also investigated. Furthermore, the diagnostic interference between the vaccine and challenge virus in clinical samples and tissues has been studied. One group of 7 pigs (6-week-old) was immunized with 100 protective doses (PD) of a C-strain lapinized vaccine (Labiofam vaccine), and a group of 6 pigs remained unvaccinated (control group). All pigs were challenged 16 days later with 105TCID50 CSFV Margarita strain (Genogroup 1.4), and necropsy was done at 13 days post challenge (dpi). During the study, clinical signs were recorded, and serum samples and nasal, rectal and oral swabs were collected at different time post-vaccination, and at 5, 8 and 13 dpi. Pigs vaccinated with 100 Protective doses (potency test according to OIE Manual) showed a strong clinical protection after challenge, compared to unvaccinated controls, and viraemia of challenge virus in vaccinated pigs was avoided ("sterile protection"). However, the challenge virus was detected in nasal and rectal swabs at 8 and 13 dpi, as well as in tonsil, lung, kidney and lymph nodes from most of vaccinated-challenged pigs at necropsy. Infectivity of these tissues and secretions is currently under study. These results show a failure in the virological protection in secretions and in tissues after vaccination with 100 PD. At least experimentally, this vaccine dose allows viral excretion in exposed vaccinated pigs, and this may well generate a selection pressure favouring the observed CSFV evolution in endemic areas.

Characterization of immune responses following homologous reinfection of pigs with EU subtype 1 and 3 PRRSV strains that differ in virulence

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes significant economic losses to the pork industry worldwide. The disease is difficult to control due to a high degree of genetic variation of field strains, with highly virulent strains emerging in South-East Asia and Eastern Europe, and the limited protection from infection after vaccination. Therefore, understanding host immune responses elicited by different PRRSV strains will help to develop more efficacious vaccines. In the current study we characterized the immunological and virological course in pigs after infection and homologous challenge of the highly virulent European subtype 3 strain Lena, and the moderate to low virulent European subtype 1 strain LV. In previous studies, these strains displayed different immunological and inflammatory responses, but it was not known yet how these different responses relate to protection. Eighteen pigs were infected per strain, and 18 non-infected pigs served as control. Post mortem was performed at days 7, 46 and 60 p.i. At day 46, pigs were homologous challenged. After the first inoculation, pigs infected with strain Lena developed fever and clinical symptoms, while this was not observed in pigs infected with strain LV. Virus titres in serum were about 100-fold higher in pigs infected with strain Lena than in pigs infected with strain LV. An inflammatory response was observed in pigs infected with strain Lena with significantly higher levels of IL-12, IL-1β and TNF-α in bronchoalveolar lavage. IFN-γ ELISPOT assay showed comparable responses between Lena and LV when cells were homologous stimulated. However, cells from strain Lena infected pigs heterologous stimulated, showed higher IFN-γ secretion levels than cells from strain LV infected pigs. Neutralizing antibodies were detected earlier in serum of pigs infected with strain Lena than in pigs infected with strain LV. After the challenge, a boost in antibody levels and IFN-γ secreting cells in both groups were observed. Challenge resulted in complete protection, with no viraemia, clinical symptoms or viral RNA in tissues. In conclusion, although there were clear differences in immunological and virological responses to infection, there were no differences observed in protection against homologous challenge.

Bluetongue virus without NS3/NS3a expression is avirulent and protects against challenge in a sheep model

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The vector borne bluetongue virus (BTV) is the type-species in the genus Orbivirus. BTV causes Bluetongue in ruminants and outbreaks cause huge economic losses and death in sheep in several parts of the world.

The most effective measure to control vector borne diseases is vaccination. However, commercially available BTV vaccines have shortcomings, such as need for yearly boosting with inactivated vaccines or spread of live-attenuated vaccines.

BTV has a ten-segmented double stranded RNA genome and reverse genetics enables de novo generation of genetically modified BTV. Recently, we showed that NS3/NS3a expression, encoded by genome segment 10, is not essential for virus replication in vitro. Since NS3/NS3a functions in virus release, knockout led to disturbed cytopathogenic effect and reduced virus release in vitro. Since such an attenuation could be useful in vaccine development, we examined NS3/NS3a knockout BTV mutants as vaccine candidates in vivo.

BTV vaccine candidates based on laboratory-adapted BTV1, avirulent BTV6 and virulent BTV8 were generated. All vaccine candidates were serotyped with VP2 of BTV8 and were negative for NS3/NS3a expression by internal deletions in the NS3/NS3a open reading frame.

Sheep were vaccinated once with one of these vaccine candidates and were challenged with virulent BTV8 three weeks after vaccination. Inactivated BTV8 without adjuvant was used as replication-deficient control.

The NS3/NS3a knockout mutants were completely avirulent in sheep, including virulent BTV8, indicating that safety is associated with the NS3/NS3a knockout phenotype.

Viremia was not detected, whereas sheep seroconverted for BTV specific antibodies, which appeared replication-dependent. We assume that NS3/NS3a knockout BTV mutants replicate only locally, due to disturbed virus release, which will minimize uptake and spread by the insect vector.

Sheep that were immunized with the BTV6 and BTV8 based vaccine viruses were protected against virulent BTV8, since no clinical signs and fever were observed. Especially the BTV6 based vaccine protected against disease and completely prevented viremia of challenge virus.

The lack of NS3/NS3a expression potentially enables the differentiation of infected from vaccinated animals (DIVA), which is important for monitoring of virus spread in vaccinated livestock. The here presented Disabled Infectious Single Animal (DISA) vaccine for Bluetongue shows safety, efficacy, DIVA characteristics, low production costs, and applicability for other serotypes, which is very promising and will be subject for future studies.

PROOF-OF-CONCEPT FOR THE REDUCTION OF FOOT- AND MOUTH DISEASE BY AN ANTIVIRAL DRUG

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An antiviral control strategy for foot-and-mouth disease (FMD) outbreaks could support and complement current contingency plans in case of an outbreak in Europe and could spare many healthy animals from being pre-emptively culled. To this end highly potent, selective and pan-serotype inhibitors of FMDV replication are being optimized in vitro and significant progress towards the development of such molecules is being made1. Next, the pharmacokinetic properties, safety and efficacy of selected and safe lead antiviral molecules will be assessed in guinea pigs (GP). GP are susceptible to experimental FMDV infection and have been widely used for FMDV vaccine tests, but the evaluation of protection against FMDV infection remains mainly limited to the clinical quantification of the lesions at the inoculation site and at the uninoculated feet. To our knowledge efficacy studies with small molecule inhibitors of FMDV replication in GP have not been reported in the English literature. Therefore, T-1105, an inhibitor of the influenza virus that has also been shown to inhibit FMDV replication in cell culture2, was used to assess the suitability of the GP model for the evaluation of FMDV inhibitors.

First 32 Dunklin Harley GP were used to develop a refined FMDV serotype O infection model that includes clinical and virological parameters of infection. Intradermal inoculation of the GP-adapted FMDV strain O1 Manisa in one hind footpad resulted within 2 to 4 days post inoculation (dpi) in large primary vesicular lesions at the inoculation site, generalization of secondary lesions to the other feet and mouth, a high level of viremia, virus dissemination to internal organs and salivary excretion.

Next, 16 GP were administered with T-1105 (200mg/kg po, twice daily) for 5 consecutive days or left untreated (n=16). Serum concentrations of T-1105 peaked at 136±12.7µM 2 hours after administration (in vitro EC50=22.4µM). One hour after the first administration, all animals were inoculated with O1 Manisa. All untreated animals showed severe generalized infection and vesicular primary and secondary lesions. Mean Cp-values in serum at 2 and 4 dpi were 20.4±3.2 and 29.2±5.9, resp. Disease severity was markedly less pronounced in treated animals. Only 4/16 treated animals showed a small primary vesicle. At 2 dpi 9/16 animals tested RNA positive in serum (mean Cp-value 38.7±6.8) and half of the animals at 4dpi (mean Cp-value 38.8±6.8). Viral RNA was borderline detected in oral swabs and internal organs of half of the treated animals. The other half were negative. Mean viral RNA levels in serum and organs were reduced compared to untreated controls ($p<0.01$).

In conclusion, T-1105 offered substantial clinical and virological protection against O1 Manisa infection in GP. The GP model seems valid for the preliminary evaluation of inhibitors of FMDV replication. Together with the serotype A infection model that was established in severe combined immunodeficient (SCID) mice3 and that demonstrated successful validity of the antiviral effect of the ribonucleoside analogue 2'-C-methylcythidine4, proof-of-concept for the potential of selective inhibitors of viral replication to control FMD outbreaks is provided4.

1 Goris et al., 2012. Proc. Open Session of the Standing Technical Committee of the EuFMD Commission, Jerez, Spain, 156.
2 Furuta et al., 2009. Antiviral Res 82, 95-102.
3 Lefebvre et al., 2010. Transbound Emerg Dis 57(6), 430-3.
4 Lefebvre et al., 2013. Transbound Emerg Dis doi: 10.1111/tbed.12069.

Session 2: Diagnostics

Vaccine development for Bluetongue and African HorseSickness using reverse genetics: similarities, differences and limitations

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Bluetongue (BT) and African HorseSickness (AHS) are notifiable diseases of respectively ruminants and equines, caused by different virus species within the genus Orbivirus of the Reoviridae family. The course of disease is fast and mortality of AHS in domestic horses can be extremely high (>90%). These orbiviruses are transmitted by bites of specific species of Culicoides midges. Spread of BTV8 in Europe by Culicoides species endemic in a moderate climate has initiated re-evaluation of the emergence of orbiviruses in Europe. Evidently, introduction of BTV or AHSV will have a huge economic and socio-emotional impact on the entire community, whereas control of vector-borne diseases is hardly possible without vaccination. Currently, there are temporarily produced inactivated BT vaccines available for certain serotypes, like in Europe for serotype 8 and a few others. For AHS, there is no registered vaccine available outside Africa. Conventionally developed live-attenuated vaccines are considered unsafe due to several reasons; under-attenuation, restored virulence by reassortment or by 'reverse to virulence', and lack of DIVA characteristics.

Reverse genetics for BTV enabled de novo generation of genetically modified BTV resulting in a next generation vaccine candidate for serotype 8, named Disabled Infectious Single Animal (DISA) vaccine 8 (Feenstra et al., 2014). BT DISA vaccine is based on the vaccine-related BTV6/net08 in which immunogenic NS3/NS3a is deleted. This resulted in complete avirulence, local replication and a strongly reduced viremia of vaccine virus, and DIVA characteristics. Exchange of immunodominant VP2 resulted in serotype specific protection (Feenstra et al., 2014). Exploring of this BT DISA platform for other serotypes will be updated.

Accordingly, reverse genetics based on vaccine virus AHSV4LP (Erasmus 1973) was used as a platform for AHS DISA vaccines. We abolished NS3/NS3a expression and exchanged VP2, resulting in a set of AHS DISA vaccines for all nine serotypes. These are not tested in horses yet, but we assume, in line with BT DISA vaccine 8, that these are safe, efficacious, and will enable DIVA based on immunogenic NS3.

So, BT and AHS DISA/DIVA vaccine candidates are both based on a safe vaccine virus and are negative for NS3/NS3a expression. Since only the serotype determining VP2 protein was exchanged, reassortment between DISA vaccines of different serotypes cannot result in new variants. Further, DISA vaccines for different serotypes will be very similar, and thus can be randomly and safely administered or combined in cocktails.

Studies on DIVA characteristics of DISA vaccines are in progress, and a competition ELISA based on NS3 is developed for BTV. Similarities, differences, and limitations of DISA vaccines for Bluetongue and African HorseSickness will be discussed.

The Luminex Microsphere immunoassay, a technologic innovation for the serological diagnosis of flavivirus infections

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Many flaviviruses are emerging or reemerging diseases threatening humans and /or animals, such as horses (West Nile (WNV), Japanese encephalitis (JEV), dengue...). Due to spatial and temporal overlappings in the circulation of flaviviruses and because serology is mostly used for flavivirus diagnosis, serological differentiation is crucial in the adaptation of surveillance and control efforts. However, it is complicated by the lack of specificity of routine serological tests (ELISAs, IFAs) due to antibody cross reactivity.

The envelope (E) glycoprotein of Flavivirus is structured in 3 domains (D) named DI, DII and DIII. DI and DIII contain virus specific epitopes. We wanted to investigate if the Luminex serology based on the binding of DIII antigens from different flaviviruses on color coded beads could help differentiating flavivirus infections in horses.

Synthetic genes encoding the secreted part of E (for WNV only) or E DIII from JEV, Tick-borne encephalitis (TBEV) and WNV were inserted in a drosophila plasmid expression system and the corresponding antigens were obtained.

Different color coded magnetic beads were coupled to E or flavivirus DIIIs through carboxylate amine bounds. Reference or field sera were incubated with the 4 beads and anti-horse secondary antibodies were added. Fluorescence intensity and bead color coding were measured by two lasers that excite the beads at different wavelengths.

Positive reference horses samples for WNV lineage1 (WNV1, It08 strain) or 2 (WNV2, Aus08) or TBEV (Hypr) or JEV (Nakayama strain) were obtained through experimental infections. Blood was sampled at different days (D) post infection (D0, D8, D11, D14, D20, D35 and D58)

Presence of reacting antibodies was detected as early as D8 for WNV1 and 2 with the Luminex technology and D11 with ELISA (ID screen WNV competition kit, ID Vet) while JEV-infected pony generated positive results concurrently (D20) with both technics. Earlier detection of TBEV antibodies was achieved with the non specific ELISA (D20) than with the Luminex (D35).

To evaluate the capacity of the Luminex technology to differentiate flavivirus infections in the field, 102 WNV-positive horse sera from Pakistan and Madagascar were tested. On these sera, 100 were E positive with the Luminex technology and 2 were negative (Se= 98.0 %). Of these 100 E positive sera, 91 were determined as WNV-positive by Luminex and 93 by WNV VNT.

42 field horse sera sampled in Austria and positive for TBEV-antibodies were screened with the Luminex technology. Of the 42 sera positive in TBEV VNT, 41 were found positive with the TBEV Luminex.

To test for the specificity of the Luminex technology, 160 field horse sera from the Camargue, France and Ireland and found negative with ELISA were screened by Luminex. 4 false positive results were generated (Sp=97.5%)

In conclusion, these results demonstrate a good sensitivity and specificity of the Luminex technology and a good correlation with VNT.

The Luminex multiplexing capacity, in association with the use of Flavivirus DIII antigens is really promising, enabling a good serological differentiation of flaviviruses belonging to the same serocomplex (WNV, JEV), as well as to different complexes (TBEV).

A new Concept for the Development of a CSF-DIVA ELISA

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Emergency vaccination with live marker vaccines represents an effective control strategy in a classical swine fever (CSF) outbreak situation. In the European Union the first prototype of a recombinant live marker vaccine (CP7_E2alf) is in the licensing process and successful application will be dependent on a reliable diagnostic assay that allows differentiation of infected from vaccinated animals (DIVA strategy). As induction of a protective immune response relies on virus neutralizing against the E2 protein of the CSF virus (CSFV), the most promising DIVA immunoassay strategy is based on detection of E^{rns}-specific antibodies.

The aim of the present study was to develop an E^{rns}-specific ELISA which may be used as an accompanying discriminatory test for marker vaccines using the E^{rns} immune response as marker. For this purpose the concept of a double antigen ELISA was applied and tested for proof of principle. A serum sample panel comprising CSFV antibody positive sera, CSFV antibody negative sera, BDV and BVDV antibody positive sera and sera taken from animals vaccinated with the marker vaccine candidate CP7_E2alf was tested.

The concept of a double antigen ELISA is shown to be a reliable strategy for development of a serological DIVA assay. Moreover, detection of serum antibodies against E^{rns} from CSFV isolates belonging to different relevant genotypes could be assessed.

Ongoing studies will focus on further improvement of the prototype CSFV E^{rns}-specific ELISA which will be used for final determination of diagnostic sensitivity, diagnostic specificity, repeatability and reproducibility.

New Diagnostic Technique for Serological Detection of African Horse Sickness Virus: Luminex Technology.

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African horse sickness (AHS) is a vector-borne viral disease of equines that causes high socio-economic and sanitary consequences. Nowadays, the European countries have increased their awareness of the possible AHS re-introduction as other vector-borne diseases, such as Bluetongue or Schmallenberg viruses, have recently emerged. Therefore, the diagnostic laboratories require rapid, sensitive and specific techniques for fast disease detection to establish effective control and surveillance programmes, avoiding the AHS spread. The aim of this study was the development of an immunoassay for serological detection of AHS antibodies using the novel Luminex technology. This new immunoassay was compared with commercial enzyme-linked immunosorbent assay (cELISA) and lateral flow assay (LFA) kits by testing a large panel of 300 horse sera samples.

The most conserved protein of AHS, the structural protein 7 (VP7) (kindly provided by INGENASA), was coupled to magnetic carboxylated-fluorescent microbeads (Bio-Plex Pro Magnetic COOH Beads) using the commercial Bio-Plex Amine Coupling Kit. Luminex assay procedure was then performed to assess the VP7 coupling efficiency and to optimize the dilution of the sample and the concentration of reagents. Next, the optimized Luminex assay was evaluated using reference sera of each serotype, sera from experimentally-infected horses, samples from AHSV-free-areas and from infected-areas. The samples were analysed on the Bio-Plex 200 system obtaining the median fluorescence intensity (MFI) of the reporter signal. The results were normalized (T/PC ratio). Finally, the detection performance of cELISA kit and LFA kit were compared with that of the developed Luminex assay.

The cut-off value for this immunoassay was evaluated using 113 ELISA positive samples, reference sera and samples from horses experimentally or naturally-infected (positive result by the cELISA test); and 92 negative controls, samples from non–infected animals and from Spanish and French healthy horses (areas free of AHSV). The cut-off value was determined as a 4.1 T/PC ratio. This technique allowed detection of antibodies against VP7 for all AHSV serotypes, with 100% sensitivity and 100% specificity in horses experimentally or naturally-infected when compared with cELISA results. This assay was also used to analyse the antibody responses in experimentally-infected horses, with antibodies against VP7 detected as early as 7 days post-infection.

The Luminex results were compared to the results from both commercial immunoassays. Luminex assay showed an adequate correlation with ELISA and LFA in experimentally-infected horses as well as in field samples. The agreement between the results of the Luminex assay developed in this study and the cELISA and LFA were 0.74 (95% CI: 0.66-0.83) and 0.90 (95% CI: 0.84-0.96), respectively. However, the Luminex assay and LFA showed higher sensitivity than the ELISA test, hence, these techniques could be used as alternatives for laboratory and clinical diagnostics, respectively. Furthermore, Luminex immunoassay provides a platform for the development of multiplex assays, allowing serological profiling of the antibody response against multiple AHSV antigens in a single test. Therefore, further studies are required to investigate the potential of VP7 Luminex assay together with other proteins for differentiation of infected from vaccinated animals, serotyping, vaccination monitoring or differential diagnosis of look-alike diseases.

Presence of SBV in semen after experimental infection of bulls

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Background:
In 2011, in Germany, a novel orthobunyavirus of the Simbu serogroup, the Schmallenberg virus (SBV), was identified using a metagenomic approach. SBV spread rapidly throughout the whole of Europe, causing mild transient disease in adults, while main losses are associated with infections during pregnancy leading to abortions and severe malformations in newborn calves and lambs. The virus is transmitted by biting midges from the *Culicoides spp*. Recently, the virus was also reported to be present in bull semen and thus a venereal transmission could be also possible. This could implicate trade restrictions on bovine semen, subsequently having economic consequence for breeders. For a better understanding on the duration of excretion and the infectivity of SBV in bull semen an experimental infection with two different SBV strains was performed.
Methods:
Three SBV-ab negative bulls, kept in a confined culicoides-free facility, were included in the study: one was inoculated subcutaneously with one culture-grown SBV strain (Italian origin), 2 bulls with one SBV viraemic calf serum (provided by FLI Riems). Throughout the experiment, animals were daily recorded for clinical signs and rectal temperatures. Furthermore serum and semen samples were collected regularly during the study. The bulls were euthanized 22 or 25 days post infection (p.i.) and lymph nodes, spleen, testicles as well as accessory sex glands were collected after slaughtering. Sera were analysed for antibodies by ELISA (ID Screen® Schmallenberg virus Competition Multi-species, IDVet, France) and virus neutralisation test (VNT). The sera, the fresh and extended semen as well as the post-mortem samples were analysed for SBV by an S3-segment-specific real-time RT-PCR (RT-qPCR). PCR positive extended semen samples will be used to inseminate artificially sero-negative cows and the subcutaneous inoculation of susceptible ruminants.
Results:
Throughout the experiment, all black-light traps used for screening the animal facility for vector-freedom were negative for *Culicoides spp*. None of the animals showed any clinical signs, temperature rose above 39°C only in one animal for one day. All bulls showed sero-conversion after 9 days p.i. when the virus was no more detectable in serum. Viremia was detected in the sera of all 3 bulls, independently whether virus strain was used for inoculation. The viremia length was consistent with the published experimental findings. Cq-values recorded in serum of the bull inoculated with the culture-grown virus were higher, compared to bulls inoculated with the infectious serum. RNA in fresh semen was detectable in two bulls infected with bovine serum. One bull was positive in the collected semen only at 6 dpi with a Cq value of 36. In contrast the semen of the second one was consistently SBV positive with lower Cq values starting from day 13 p.i. Extended semen batches are currently under analysis. The RT-qPCR of the post-mortem samples confirmed the persistence of SBV genome in spleen and lymph nodes, whereas accessory sex glands and testicles were PCR negative.

Session 3: Vaccines

Uncovering of Classical Swine Fever Virus adaptive response to vaccination by Next Generation Sequencing

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Next Generation Sequencing (NGS) has rapidly become the preferred technology in nucleotide sequencing, and can be applied to unravel molecular adaptation of RNA viruses such as Classical Swine Fever Virus (CSFV). However, the detection of low frequency variants within viral populations by NGS is affected by errors introduced during sample preparation and sequencing, and so far no definitive solution to this problem has been presented. Here we present NGS data from an immunisation/challenge experiment obtained by two different NGS-platforms (Ion PGM and Roche FLX). The pigs were immunised with the DIVA vaccine candidate, vR26_E2gif, and subsequently challenged with the highly virulent CSFV strain “Koslov”. NGS data of Koslov RNA derived from serum of vaccinated pigs and mock-vaccinated controls was obtained and analysed. Before low frequency analysis, we performed a rigorous error correction. Several tools were benchmarked and the RC454 developed by the Broad institute outperformed the others by removing most indels from detection and strengthened the variation detection limit. Subsequently, variation analysis revealed significant differences between the CSFV sequence data from the vaccinated and the mock-vaccinated groups. The viral sequences obtained from the mock-vaccinated pigs had a similar single-nucleotide polymorphism (SNP) distribution as the challenge virus, which was not the case for the sequence data from the vaccinated group where a complete change of the SNP distribution was observed. Additionally, new detectable non-synonymous SNPs were found in the vaccinated pigs indicating selection pressure onto the challenge virus, which was not observed in the mock-vaccinated group.

Characterization of atypical BVDV-2c isolates of a German outbreak using Next generation sequencing

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In February 2013, a BVDV outbreak caused very severe acute clinical symptoms in calves, heifers, and dairy cattle in several farms in North Rhine Westphalia and Lower Saxony, Germany with a mortality of up to 80%. First investigations including phylogenetic analysis of the 5'-UTR identified Bovine viral diarrhea virus of genotype 2c. For further characterization Next generation sequencing was used for full-genome determination. In depth data analysis of the virus-specific sequence reads revealed the coexistence of three distinct genome variants within a number of isolates from the recent outbreak series. In all cases only the minority (approximately 5%) reflected the standard structure of a BVDV-2 genome while the vast majority (ca. 95%) of the genomes harbored a duplication of a 222-nucleotide stretch within the p7-NS2-encoding region. Additionally, unusual mutations were found within the highly conserved p7 protein and close to the p7-NS2 cleavage site in both the elongated and the standard genomes. Using a reverse genetics system of a BVDV-2a strain harboring a similar duplication, it could be shown that during viral replication the duplication can be deleted giving rise to genomes with standard structure and hence a compulsory mixture of both variants. RNA secondary structure prediction provided a possible route for the elimination of the duplication. Furthermore, the existence of one of three possible standard genomes could be ruled out by analysis of p7-NS2 specific reads. In conclusion, our results suggest that the variant with duplication plays the major role in the highly virulent phenotype.

Efficacy of CSF vaccine CP7_E2alf in piglets with maternally derived antibodies

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There is a need for live DIVA (Differentiating Infected from Vaccinated Animals) vaccines against Classical Swine Fever (CSF). Live vaccines tend to be more efficacious than inactivated or subunit vaccines, and can also be used for oral application, which may be important for vaccinating wild boar. The aim of this study was to investigate whether vaccination with the chimeric pestivirus vaccine CP7_E2alf is efficacious to protect young piglets born from vaccinated sows, thus with maternally derived antibodies (MDAs). Groups of 10 piglets each, with or without MDAs, were vaccinated either intramuscularly (IM), at an age of 3 or 6 weeks, or orally, at an age of 6 weeks. Five piglets of each group were challenged with CSFV strain Koslov and protection against clinical disease, virus shedding and transmission were studied. Vaccination with CP7_E2alf, both in the presence of MDA's and in piglets without MDA's, protected against severe clinical signs, but virus shedding from most inoculated piglets and transmission to contact pigs was observed. However, virus transmission in the vaccinated piglets was significantly reduced as compared to non-vaccinated piglets, although the reproduction ratio's calculated from the results in the vaccinated pigs from our study were not yet significantly below 1. The efficacy of vaccination with CP7_E2alf in the presence of MDAs seemed to be slightly less as compared to vaccination in the absence of MDAs.
On a population level, the results suggest that the CP7_E2alf vaccine is an effective tool in the control and eradication of CSF and, moreover, can be applied for both IM and oral use for young age groups, with MDAs having a limited effect on the efficacy.

Interaction of Schmallenberg virus (SBV) NSs virulence factor with the cellular Major Vault protein (MVP)

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In 2011, an emerging arbovirus named Schmallenberg virus (SBV) and belonging to the *Bunyaviridae* family was identified in German cattle herds, and has rapidly spread through Europe. SBV infects ruminants and although adult animals' infection is benign, pregnant females' infection can lead to stillbirth and foetal abnormalities due to SBV ability to cross placental barrier.
Among Bunyaviruses encoded genes, the non-structural protein NSs is a virulence factor involved in transcription and interferon inhibition. It has been shown that a NSs-deleted recombinant virus was unable to grow efficiently and to disrupt interferon synthesis in vitro and in vivo, referred to the wild type strain. To study NSs roles in SBV pathogenesis, we have searched for its cellular partners using a yeast two-hybrid screening. We have identified the Major Vault Protein (MVP) as a NSs putative cellular partner. MVP is the main component of a large ribonucleoproteic complex and is considered as a cytoplasmic hub for regulation of several signalling pathways controlling cell proliferation and immunity. To study this interaction in mammalian cells, we have developed gene constructs whose expression is driven by a CMV promoter, but unfortunately NSs protein expression remained undetectable using this approach. This is likely due to the potential inhibitory function of NSs on RNA polymerase II based transcription. To investigate this hypothesis, we measured the effect of NSs on CMV promoter activity in a luciferase reporter assay. As expected, co-expression of NSs triggered a strong decrease of CMV promoter activity. To go further, we are currently trying to identify which domains of NSs are involved in this function by designing several deletion mutants. These mutants will also be used in a yeast-two hybrid assay to map the minimal domains involved in MVP interaction. This will help to generate SBV recombinant viruses lacking MVP interacting domain in order to measure the consequences of the NSs-MVP binding at the virus level. Interestingly, silencing of MVP expression in mammalian cells using RNA interference lead to a drastic decrease of SBV viral titers, antigens and mRNA expression, suggesting a promoting role of MVP on SBV replication. Moreover, we handle a reverse genetics system for SBV in our laboratory (Collaboration with Massimo Palmarini, University of Glasgow). This tool will be useful to assess the function of NSs-MVP interaction on viral pathogenesis, especially in RNA interference experiments and also *in vivo* in a natural host model (ovine). Finally, this work could contribute to a better understanding of SBV pathogenesis molecular mechanisms.

Targeted modifications of foot-and-mouth disease virus; towards improved vaccine candidates

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Foot-and-mouth disease virus (FMDV) is responsible for one of the most economically important diseases of farm animals (estimated annual costs are about US$10 billion globally). The virus is the prototypic *Aphthovirus* within the family *Picornaviridae* and has a positive sense RNA genome (ca. 8.3kb) encoding a single large polyprotein that is processed to generate about 15 mature proteins plus precursors. The virus particle comprises 60 copies of 4 separate capsid proteins (VP1-VP4) plus a single copy of the genome. By modifying full length cDNAs, producing RNA transcripts in vitro and then introducing these into susceptible cells, it is possible to rescue specifically altered FMDVs. We have used this approach to generate modified viruses that have particular properties; these studies can assist in the development of improved and safer vaccines to protect against FMDV. For example, we have made changes to the leader (L) protein coding sequence. The L protein is the first component of the viral polyprotein and is produced in two forms (termed Lab and Lb) as the result of use of alternative initiation codons, 84 nt apart. Both forms have protease activity (which separates the L protein from the capsid precursor) and induce the shut-off of host cell protein synthesis. When the shorter form, Lb, is precisely deleted then FMD viruses that grow well in cell culture are produced (Belsham, 2013). However such viruses are attenuated within cattle. In contrast, when the entire Lab coding sequence is deleted then no viable viruses are generated. In an alternative approach, we have modified a processing site within the viral polyprotein so that incomplete processing occurs. It has been shown that a single amino acid substitution that blocks cleavage of the VP1/2A junction within the capsid precursor results in the production of modified “self-tagged” virus particles that contain the VP1-2A precursor (Gullberg et al., 2013). This approach works for two of the most common FMDV serotypes (O and A). This system offers the possibility of a single method for purifying virus particles from different serotypes using reagents targeted to the conserved 2A peptide.

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CD46 is also a cellular receptor for Classical swine fever virus

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Classical swine fever virus (CSF) is the causative agent of a severe multi-systemic disease of pigs with tremendous socio-economic impact. While several aspects of virus-host-interaction are known, early steps of infection, i.e. receptor binding, remain still unclear. For the closely related Bovine viral diarrhea virus (BVDV), a cellular receptor is known: bovine complement regulatory protein CD46. Given the close relation of both viruses, porcine CD46 is the most probable receptor for CSFV. Apart from the use of a specific cellular receptor, cell culture adapted CSFV strains were shown to use heparan sulfates as an alternative receptor.

In the presented study, the interaction of a field-type 2nd and a cell culture adapted 26th or 30th passage of CSFV “Roesrath” (recent CSFV isolate from Germany) with the host cell was assessed through the use of monoclonal antibodies directed against porcine CD46 and heparan sulfate blocking compound DSTP27 (kindly provided by M. Schmidtke, Jena). Blocking assays were conducted with the mentioned blocking reagents either alone or in combination. To use both an established cell culture system and natural target cells of CSFV, assays were conducted on permanent porcine kidney cells and on macrophages derived from primary blood monocytes. The influence of receptor blocking was assessed (semi-) quantitatively through the use of virus titration and RT-qPCR of cell culture supernatants.

The treatment of cells with monoclonal antibodies against porcine CD46 led to a reduction of viral growth in both permanent and primary cells. The effect was most pronounced with the field-type CSFV passage. The blocking could be enhanced by addition of DSTP27, especially in cell culture adapted passages of CSFV. However, the combined use of both blocking compounds only led to a significant reduction of viral growth but was not able to abolish infection completely.

The results obtained in this study showed that porcine CD46 plays an important role in the initial steps of CSFV infection and could be one receptor of CSFV. Yet, an additional receptor seems to play a role in viral entry as a complete block of infection could not be achieved. Future studies will target this aspect.

Session 4: Vector borne diseases

Vector competence of Culex pipiens and Aedes albopictus from Catalonia (Northeastern of Spain) for different arboviruses.

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Mosquitoes vary in their competence or ability to transmit arthropod-borne viruses (arboviruses). Many arboviruses cause disease in humans and animals. Identifying the environmental and genetic causes of variation in mosquito competence for arboviruses is one of the great challenges in public health. Therefore bio-ecological characteristics of mosquito populations in combination with some environmental factors can determinate the risk of the emergence of these viral disease in a disease free zone. *Culex pipiens* and *Aedes albopictus* species have been involved as vectors of different vector borne diseases. Different species of *Culex* genus are the main vectors of West Nile virus (WNV), which maintains the virus in enzootic cycles involving primarily avian hosts, and *Ae.albopictus* has been highly involved in Chikungunya virus (CHIKV) transmission. CHIKV causes a major public health problem. In 2004, CHIKV began an unprecedented global expansion and has been responsible for epidemics in Africa, Asia, islands in the Indian Ocean region, and surprisingly, in temperate regions, such as Europe. On the other hand, WNV causes epizootic spread to mammals, including horses and humans. The resurgence of WNV in North America and Europe in recent years has raised the concerns of local authorities and highlighted that mosquito-borne disease is not restricted to tropical regions of the world. All these facts lead to question about the potential risk of introducing these arboviruses in Catalonia from neighboring countries where WNV and CHIKV circulates. In the present study, vector competence of different populations of *Cx. pipiens* and *Ae. albopictus* species from Catalonia was evaluated towards different strains of WNV. It was also evaluated for *Ae. albopictus* towards different strains of CHIKV. All these assays were performed at different environmental conditions in Biosecurity level 3 facilities. Nine-twelve days after imbibing an infective blood meal simulating host viremia concentrations mosquitoes were dissecionated to evaluate the viral load in bodies and paws. High-disseminated infection rates were found in *Ae. albopictus* infected with both CHIKV strains (wt and E1- A226V). *Ae. albopictus* could also be infected with WNV and disseminate it. Moreover, *Cx. pipiens* was able to sustain an amplification of WNV. The results obtained from these vector competence assays will be useful to assess risk of mosquito-borne disease and provide novel strategies to mitigate mosquito-borne arbovirus transmission in Europe.

Studies of Culicoides biting midges at the Pirbright Institute: from Genomics to the Field

Simon Carpenter

The Pirbright Institute, United Kingdom

In this talk I will give an overview of current research on *Culicoides* biting midges carried out at The Pirbright Institute (TPI). This research ranges from the first de *novo* genome build for the genus (using the colony line of *C. sonorensis* that has been maintained at TPI for over forty years) to fundamental field-based studies in the UK, continental Europe and India. By placing these studies in context with global research on the genus I hope to illustrate some of the specific challenges that *Culicoides* provide for manipulative research and additionally to suggest areas that may be of importance in the future epidemiology of emerging arboviruses in Europe. I will also provide a critical review of specific resources currently available to *Culicoides* workers in the field and laboratory and suggest methods that require development for studies of European species. Finally, I will examine the recent development of genetic and microbe-based control methodologies and discuss the potential for their implementation in *Culicoides* populations by comparing and contrasting the biology of the genus with mosquito vectors of arbovirus. These emerging methods will also be contrasted with vaccination as a means of control of *Culicoides*-borne arboviruses in Europe.

Emergence of Bluetongue virus in Corsica in 2013

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Since 2000, French Corsica Island has been exposed to the emergence of three different BTV serotypes: serotype 2 in 2000 and 2001, serotype 4 in 2003 and serotype 16 in 2004. Between 2005 and August 2013, no outbreak has been reported in the French island. At the beginning of September 2013, sheep located in the south of the island showed clinical signs suggestive of BTV infection. Laboratory analyses identified the virus as BTV serotype 1. The virus has spread rapidly over the Island. The phylogenetic studies showed that the sequences of this strain are closely related to the BTV-1 strain that was circulating in the Mediterranean basin and in Sardinia in 2012. By testing biological samples as part of the corresponding monitoring program, a novel BTV strain was isolated, displaying nucleotide sequences evocative of a novel serotype, was identified in several goat samples. After a short description of the past outbreaks in Corsica, this paper provides an overview of the 2013-2014 outbreaks, describes the measures that have been put in place and reports the conditions of detection of the new BT virus isolated in this Island.

Proline residue at NS3 249 is a primary determinant of West nile virus virulence in mammals and birds

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Introduction: West Nile virus (WNV) is a neurotropic flavivirus mainly transmitted through mosquito bites and whose reservoir hosts are wild birds. Equids and humans are incidental dead end hosts and can develop severe neurological symptoms in 1-10% cases. Despite enhanced reporting of WNV outbreaks in Europe during the past 5 years, much remains to be explored about the virulence level and determinants of WNV strains circulating in Europe. WNV epidemics in Europe involve strains that belong to the South Europe/Kenyan and the Israeli/American clusters of lineage 1a, or to lineage 2. Of note, expanding lineage 2 virus has gained a proline residue at position 249 in the non structural 3 (NS3) protein and has caused the worst epidemics ever experienced in central and Southern Europe (Greece, Serbia, …). Interestingly, Brault et al. (2007) identified NS3 249P as being crucial for the virulence of WNV in American crows. The mutation is located in the helicase portion of NS3 and could affect the replicative efficacy of WNV.

Objectives: We aimed at deciphering the role of the NS3 249P mutation in WNV virulence for birds and mammals.

Materials and methods: An infectious clone, based on WNV lineage 1 IS-98-ST1 strain, a highly neuroinvasive strain, harbouring NS3 249P, was previously constructed (Bahuon et al., 2012) and a NS3 P249T mutant was generated by directed mutagenesis. The properties of recombinant viral particles *in vitro* and *in vivo*, in mammalian and bird models (Dridi et al, 2013) were assessed.

Results: In Vero cells, virus with a NS3 249T protein proved to replicate at a slower rate than the parental NS3 249P virus. When injected intraperitoneally in female Balb c/J mice, NS3 249P virus was found to be highly virulent (Lethal Dose 50 (LD50) < 1 pfu), while only 1 out of 20 animals infected with the NS3 249T virus succumbed, regardless of the initial infecting dose (0,1-102pfu). Mice infected with NS3 249T virus experienced milder clinical and virological outcomes, with delayed and non fatal weight loss as well as decreased viremia 4 days pi (5x102 vs 7.5x103 viral copies/mL blood in groups inoculated with 10pfu). However, virulence of NS3 249T and 249P viruses were comparable upon intracerebral inoculation of Balb c/J mice, indicating that NS3 249T virus was impaired in early replication events at the periphery. Birds, eg. one-day old chicks and young corvids (*Corvus corone*), also indicated that NS3 249T virus was attenuated for model and susceptible European birds. In particular, in young crows, 16.7% (1/6) animals died after subcutaneous infection with NS3 249T virus whereas a 100% lethality (7/7) was observed with parental NS3 249P virus.

Conclusions: The presence of a Proline residue at position 249 in NS3 appears as a primary determinant for WNV virulence in wild birds, as well as in mammals and could be a genetic factor accounting for enhanced reporting of WNV neuro-invasive diseases in humans infected by lineage 2 viruses in Europe.

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Effect of salivary gland extract of tick Ornithodoros porcinus on African swine fever virus infection in pigs

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African swine fever (ASF) is a lethal, hemorrhagic disease of domestic pigs for which animal slaughtering and area zoning for restricting risky movements are the only control methods available. ASF is historically endemic in eastern and southern Africa and has been introduced a few years ago into Caucasus to recently spread to Eastern Europe.
Several studies have been conducted on the role of Ornithodoros ticks in ASFV vector transmission to pigs during blood feeding. However, no study has investigated this peculiar interaction during tick bite, especially the role of tick saliva on the success of pig contamination. The pharmacological arsenal of tick saliva is known, mainly in hard ticks and their related pathogens, to modulate inflammatory and immune mechanisms deployed by vertebrate hosts during blood feeding. This phenomenon is suspected to exist between ASFV and soft tick.

A first one-month study was initiated on the impact of salivary gland extract of Ornithodoros porcinus domesticus on the virus infection and the local recruitment of myeloid cells in pigs. We compared the effect of tick bite with intradermal injection of ASFV and intradermal injection of virus added to salivary gland extract on clinical signs, immunological modifications and virus detection in pigs. Cell recruitment at injection site or biting site was also analyzed by immunohistological antibody labelling of cryosections, 1h and 48h post inoculation. Preliminary results indicate a significative lag in virological detection on the injection side parotid lymphnode in group of pig with salivary gland extract.

This study should show that gland salivary extract of tick tends to modulate innate immune response of pigs, as it has been shown for some other tick-borne diseases. The analyses of cell recruitment, notably of macrophage cells, are in progress and could indicate what is it incidence on the infection.

The effect of African swine fever virus and two of its proteins on host MHC class I expression

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Various DNA viruses, including pox- and herpesviruses, express proteins which downregulate host MHC class I antigen expression as an immune evasion strategy. To date, studies into whether this is also true of ASFV have not provided a clear or consistent answer.

In the current study, we have used flow cytometry to show that modulation of surface MHC class I expression varies between ASFV isolates and also with the presence or absence of two ASFV proteins, EP153R and CD2v.

The cytoplasmic tail of CD2v has previously been shown to bind mABp1, an actin binding adaptor protein which has been implicated in vesicular transport and modulation of signalling pathways. Higher expression of surface MHC class I was observed in macrophages infected virulent ASFV isolates Benin 97/1 and Malawi Lil 20-1than in cells infected with their ΔCD2v deletion mutants. Through biding to mABp1, CD2v may indirectly affect transport and cell surface expression of MHC class I molecules.

The C-type lectin domain of EP153R shows similarities to that of Clec2B, the ligand of NK cell activating receptor NKp80, and is predicted to be involved in immune evasion. Previous studies have suggested that EP153R may be directly involved in the modulation of MHC class I expression, since cells transfected with EP153R from tissue culture adapted isolate ASFV BA71V showed reduced MHC class I up-regulation in response to stimulation with PMA/ion. Our own investigations have confirmed a reduction in up-regulation of surface MHC class I in porcine kidney (Max) cells stimulated with both PMA/ion and IFN-γ when transfected with EP153R from virulent ASFV isolates Georgia 2007 and Benin 97/1.

Investigations into the localisation and processing of EP153R found that it shows different patterns of expression in ASFV-infected and uninfected Vero and Max cells. In uninfected cells, the expressed protein has a molecular weight of ~37 kDa glycosylated and ~18 kDa unmodified, with an additional modified form of ~76 kDa expressed in Max cells, and localises with markers for the ER with no surface expression detected. In ASFV-infected cells, the expressed protein is 2-3 kDa smaller and is detected throughout the cytoplasm and at the cell surface. This suggests that EP153R is processed by a virus-encoded/induced enzyme and that localisation and processing of the protein may be important for its function(s) during virus infection. EP153R contains no predicted sites of processing or cleavage, but has putative di-lysine ER retention motifs at the N-terminus. Constructs expressing EP153R with mutated di-lysine motifs are being used to determine the role of these motifs in the ER localisation of EP153R in uninfected cells. Additionally, pulse-chase radiolabelling is being used to track translation and processing of EP153R in ASFV-infected and uninfected cells.

Furthering our understanding of the expression, processing and function of ASFV proteins implicated in the modulation of host MHC class I expression will play an essential role in elucidating the reason behind the disparity seen in MHC class I responses to ASFV infection.

Session 5: Emerging diseases

Serological Evidence of Equine Influenza Infections among Persons with Horse Exposure in Iowa

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Background
According to the World Organisation for Animal Health (OIE), equine influenza virus (EIV) is considered enzootic in North America and each year US horses experience sporadic infections. Previous experimental studies have documented EIV infection in humans, but few experiments have investigated natural human infections.
Objectives
In this cross-sectional study we sought to examine evidence of previous EIV infection among persons with equine exposure, both occupational and recreational, and to determine factors which may place those persons at higher risk of infection.
Methods
In 2005, we collected sera from 94 horse-exposed adult Iowans and thirty-four non-exposed controls, and over time evaluated their sera for antibodies against two h3N8 EIV, 1 H7N7 EIV, and two human H3N2 viruses. We used microneutralization (MN), neuraminidase inhibition (NI), enzyme-linked lectin (ELLA) and hemagglutination inhibition (HI) serological assays.
Results
Regarding the MN assays against the 3 EIVs, there was only evidence of previous infection (MN titer ≥1:80) among 11 (11.7%, maximum titer 1:320) horse-exposed and 2 (5.9%, maximun titer 1:160) control subjects against the A/equine/Ohio/2003(H3N8) strain. The NI assay seemed to validate the MN results with 18% of horse-exposed showing reactivity against A/equine/Ohio/2003(H3N8) (controls not run). The ELLA assay seemed to validate the MN results with 8 (8.5%) of horse-exposed subjects (most were MN and/or NI assay positive) having elevated titers compared to 1 (2.9%) of controls against a recombinant N8 neuriminadase derived from A/equine/Pennsylvania/1/2007 (H3N8) virus. Both exposed and control populations had similar percentages of elevated antibody (HI ≥1:40) against 2 recently circulating human H3N2 viruses. Bivariate modeling for MN titers ≥1:40 against A/Equine/Ohio/03 (H3N8) revealed increased odds of infection among veterinarians with horse exposure (OR= 5.8, 95%CI=1.1-31.8) and horse breeders (OR=3.1;95% CI=1.0-9.4). Upon multivariate analysis, only being a veterinarian with horse exposure (OR=21.9; 95% CI=2.4-200.9, a history of smoking (OR=6.2;95%CI=1.1-34.9), and receipt of seasonal influenza vaccine 2003/4 (OR=8.3;95%CI=1.5-44.9) were important predictors for evidence of infection.
Conclusions
These data provide evidence that persons occupationally exposed to horses may occasionally be subclinically infected with EIV.

Preparedness for potential PED emergence in France: Development of diagnostic tools

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Since april 2013, a severe epizooty of porcine epidemic diarrhea (PED) has been striking USA and Canada that were previously free from this disease. More than 6400 herds have been affected with up to 90-95% mortality on suckling piglets. In Europe, PED, characterized by watery diarrhea and vomiting, was first described in England in 1971 then throughout Europe till the end of the 1990's. In 2006 a limited PED outbreak (63 herds) has been described in Italy. The epidemic character of the PED outbreak in Italy suggested that the current immunity of the pig population against PEDV is low, at least in Italy and probably throughout Europe.

The etiologic agent of PED is an *Alphacoronavirus*, the PED virus (PEDV) that differs from two others porcine *Alphacoronavirus*, the transmissible gastro-enteritis virus and the porcine respiratory coronavirus. PEDV is not zoonotic. The PEDV isolates identified in USA in 2013 present 99.5% of nucleotide identity with Chinese strain isolated in 2012.

Regarding the current situation in North America, with a very fast and dramatic spread of the virus, European countries have to be prepared to prevent the new PEDV variant from entering in the union area and eventually to face an outbreak. Here is presented the logistic carried out at Anses to detect and face up a potential PED outbreak in France.

The first step has been to develop a quantitative RT-PCR specific of PEDV to promptly detect and quantify the viral genome. The test targets the amplification of a specific region of the N gene of PEDV according to Kim et al. (2007) with few modifications of the primer sequences. In a second step we have implemented a pipe line for the treatment of NGS data for a rapid sequencing, identification and assembly of any pathogen genome. The pipe line has been tested on historical PEDV (C777 strain) and has allowed the full sequencing of the PEDV genome. Finally, the last step has been to produce the S protein of PEDV in recombinant baculovirus and develop an ELISA test to detect PEDV antibodies in order to perform seroprevalence study in France.

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A deep insight into an H7N7 highly pathogenic avian influenza epidemic

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Avian influenza (AI) viruses of the H7 subtype have the potential to evolve into highly pathogenic (HP) forms from low pathogenic (LP) precursors following introduction into domestic poultry. The emergence of HPAI viruses results from insertion or substitutions of basic amino acids at the hemagglutinin (HA) cleavage site. Between 14 August and 5 September 2013 Italy experienced an avian influenza epidemic caused by a HPAI virus of the H7N7 subtype, which infected five industrial poultry holdings and one backyard flock. The epidemiological investigation indicated that the contact between free-range hens and wild waterfowl may have favoured the introduction of a LPAI virus in the first affected area, which rapidly mutated into a HP form within the holding.

To better characterize the source and spatial spread of this viral strain, we sequenced the complete genome of 15 clinical samples collected from all the six affected farms using the Illumina Miseq platform (deep sequencing analysis, 14 samples) or Sanger sequencing (1 sample).

The maximum likelihood phylogenetic trees obtained for the two surface glycoproteins suggest that the Italian H7N7 samples cluster with LP H7 viruses collected in poultry and wild birds in Europe between 2009 and 2013. Differently, the remaining gene segments were acquired through multiple reassortment events from different AI subtypes.

Our deep sequencing analysis of eight samples collected from the index case revealed the co-circulation of two main viral populations displaying a cleavage site of the HA gene of different length (PKRKRRG and PKRRERRG) and several minority variants showing cleavage sites of intermediate lengths. In contrast, all samples collected during the following outbreaks showed a single cleavage site (PKRRERRG), with only few reads displaying deletions in this position. In addition, analysis of the whole genome allowed the identification of twelve nucleotide and six amino acid substitutions, which may have been acquired during the circulation of the viral strain in the first holding and subsequently transmitted to all the other affected farms.

Our Bayesian phylogeographic analysis of the eight concatenated gene segments allowed us to trace the spread of the virus among the farms. This has been confirmed also through the analysis of the transmission dynamics of the minority variants.

Although we did not identify the LPAI progenitor virus among the analysed samples, the great variability observed at the cleavage site of the HA gene in the samples collected at the index case suggests that evolution of the virus pathogenicity had occurred within the first infected flock.

This finding highlights the importance of implementing prompt and effective eradication measures in case of an H7 epidemic to prevent the emergence and spread of viruses with unpredictable pathogenic properties.

Passive immunity against Schmallenberg virus in lambs born from naturally infected ewes

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Schmallenberg virus (SBV) emerged in early September 2011 in the Ovine Research Center (ORC) of the University of Namur (Belgium) leading to rapid infection of the entire sheep flock (n=400) and observation of clinical evidences of SBV transplacental infection. Deformed newborn lambs and late aborted foetuses were detected during the lambing periods of January and March 2012. In 2012, SBV re-emergence was detected within the same sheep flock among 50 ewe lambs between mid-July and mid-October. If first information about duration of passive immunity against SBV in calves has been published, no study focuses on decay of colostral antibodies against SBV in lambs. Therefore, this point is of major interest because maternal antibodies acquired by colostrum ingestion and absorption could interfere with vaccine efficacy. The objectives of this investigation were (1) to evaluate decay of colostral antibodies in lambs born from naturally infected ewes and (2) to estimate impact of SBV re-emergence on colostral anti-SBV antibody transfer.

A total of 64 lambs and their dams (n= 34) were used for this study. Group A lambs (n=35) were born in January (n=12), March (n=12) and May 2012 (n=11). Group B lambs (n=29) were born in January (n=10), March (n=10) and June 2013 (n=9). Serum was collected from lambs at birth (t0), 48 hours after first colostrum ingestion (t48) and then bimonthly until 150 days after birth. Colostrum and serum were collected from dams at lambing. Virus neutralization tests (VNT) were performed on serum and colostrum samples at serial dilutions. Results of VNT were expressed as the log2 transformed dilution that neutralizes 50% of the challenge virus (ED50) and were considered positive if log2 ED50 was > 3.49.

Median (min – max) log2 ED50 anti-SBV antibody titers of serum samples collected at t48 equaled 9.47 (6.98 - 10.97) for group A lambs and 10.46 (8.47 – 12.46) for group B lambs and differed significantly ($p<0.05$), whereas no effect of lambing period existed ($p>0.05$). The median (min – max) length of time between birth and the first detection of VNT negative result was significantly lower for lambs born in 2012 (81 days; 69 – 132) than for lambs born in 2013 (122 days; 74 – 144). Although no year-related differences were evidenced in ewes serum, log2 ED50 titers in colostrum were significantly higher in 2013 than in 2012 (11.46 [9.47-12.46] vs 12.46 [10.96-12.46]) ($p<0.05$).
This study provides precise information about decay of colostral antibody titers in sheep and certainly could help to define the age at which lambs could be vaccinated against SBV. Moreover, this study shows that SBV re-emergence significantly affected passive immunity.

Early detection of transboundary animal diseases with a real-time monitoring system online

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Rapidly detecting notifiable diseases at high risk of introduction into a free country is crucial to avoid the negative consequences derived from its spread. Testing efforts for emerging diseases in Europe increased considerably after a delayed detection by passive surveillance of epizootics like classical swine fever (2003) resulted in huge economic losses. Oppositely, the cost in wild bird sampling for avian influenza in 2006-2007 was not proportional to the risk of infection in many European countries. Risk assessments have helped identifying the locations and frequency at which farms should be sampled based on an expected higher exposure to the infection. Today, after almost 10 years without significant epizootics in Europe, there is a tendency to decrease active sampling and testing efforts, justified by the fact that many of the animals sampled will yield negative results incurring in an unnecessary cost. To avoid reversing to the situation a decade ago, new methods need to be developed to keep a high sensitivity of infection detection in the field without the expenses and logistic efforts incurred by active sampling.

Biosensors have been predominantly used to manage livestock production, but can also be very useful to monitor health. Under the EU project Rapidia Field, we carried out an experiment to present a novel system based on the remote monitoring of animal behavioural and physiological parameters through three type of sensors that can alert the farmer if an infection is present in real time. Behaviour is measured by a water consumption sensor located in the water pump, and by an accelerometer that measures motion and is placed in the animal's ear in the form of an eartag device. Fever is measured with a subcutaneous sensor inserted over the cleido-occipital muscle behind the ear. Transmission of data sensors is ensured by radiofrequency identification (RFID) systems (temperature and accelerometer) and by cable (water consumption) to a base unit wirelessly connected to a core server where the data is stored.

The first results of an assay with biosensors in pigs experimentally infected with an attenuated strain of African swine fever virus show that the system works well and that viraemia is related with the increase in temperature, decrease in water consumption, and decrease in motion once infected. Baseline data need to be adjusted to be able to set a cut-off that will differentiate healthy from infected animals by the combination of all three parameters. The cut-off would set the alarm that allow an early suspicion of disease.

Spreading Lumpy Skin Disease to the North

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An outbreak of lumpy skin disease (LSD) occurred in Sivas city located in central portion of Turkey at an unexpected time for the disease occurrence when the middle of winter season in 2014. We have sampled blood and skin lesions from clinically infected and subclinical animals. Viral nucleic acid extracted, amplified and sequenced directly lumpy skin disease virus RNA polymerase 30 kD subunit (RPO30) and G-protein-coupled chemokine receptor (GPCR) gene for the molecular characterization. Genetic analysis has been conducted using sequence results. This is the first scientific report of lumpy skin disease in Turkey. We suggest that the disease is an emerging disease in Turkey and has very high potential to be spread out into Europe and Asia.

Session 6: Epidemiology (I)

Impact of clinical surveillance during a foot-and-mouth disease epidemic

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The objectives of this study were to assess, whether the current surveillance capacity is sufficient to fulfill EU and Danish regulations to control a hypothetical foot-and-mouth disease (FMD) epidemic in Denmark, and whether enlarging the protection and/or surveillance zones could reduce epidemic duration, number of infected herds and the economic losses from an epidemic.

The stochastic spatial simulation model DTU-DADS was enhanced to include simulation of surveillance of herds within the protection and surveillance zones and the model was used to model spread of FMD between herds. A queuing system was included in the model, and based on a daily surveillance capacity of 450 herds per day, it was decided whether herds appointed for surveillance would be surveyed on the current day or added to the queue. The model was run with a basic scenario representing the EU and Danish regulations, which includes a 3 km protection and 10 km surveillance zone around detected herds. In alternative scenarios, the protection zone was enlarged to 5 km, the surveillance zone was enlarged to 15 or 20 km, or a combined enlargement of the protection and surveillance zones was modelled. Sensitivity analysis included changing 1) surveillance capacity to 200, 350 or 600 herds per day, 2) frequency of repeated visits for herds in overlapping surveillance zones from every 14 days to every 7, 21 and 30 days, and 3) the size of the zones combined with a surveillance capacity increased to 600 herds per day.

The results showed that the default surveillance capacity is sufficient to survey herds within one week of the zones establishment, as the regulations demand. Extra resources for surveillance did not reduce the costs of the epidemics, but fewer resources could result in larger epidemics and costs. Furthermore, enlarging the surveillance zone may result in shorter epidemic duration, and lower number of affected herds, while enlargements of the protection zone resulted in lower economic losses when epidemics were large. Given the assumptions, enlarging the surveillance zone did not reduce the economic losses.

Combination of simulation and statistical modeling to identify factors contributing to ASF endemicity in Sardinia

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African swine fever (ASF) is one of the major threats of swine industry due to the important implications derived from its presence in affected territories. It is currently present in many Sub-Saharan countries, some East European countries, and Sardinia, that remains endemic since the introduction of the virus in 1978. Control programs have been implemented in Sardinia since 1982 with no favorable results. The present work aims to use an ASF-adapted spatial and stochastic model (Be-FAST) in combination with a multilevel logistic regression model to evaluate dynamics of the ASFV outbreaks in Sardinia as well as to identify the most important factors contributing to the endemicity into the island. Data on pig demographics, pig movements, surveillance and control measures as well as ASF cases in domestic and wild boar during 2012 and 2013 were used for such purpose. Illegal pigs documented by Veterinary Authorities in Sardinia were also included.

The results of the Be-FAST model reflect that, given the demographics, contact patterns and control measures implemented, ASF outbreaks in Sardinia should have small size (2.16 farms affected per outbreak) and an average duration of 18 days, being the most common route of transmission the local spread, which agrees with the ASF history in the Island. Areas at high risk for having ASF epidemics in the last years were well captured by the model, but not so the hypoendemic/endemic infections. Factors included in the multilevel logistic regression model, which were the prediction of the Be-FAST model, the wild boar presence/absence and the illegal presence of pigs were all significant predictors of ASF outbreak occurrence in Sardinia. However, model reflects that whereas in some areas the domestic cycle is explaining the ASF spread, in other regions, illegal pigs (OR=1.24) and potential presence of wild boars (OR=1.2) play a more important role. These results suggest that the strict application of biosecurity and control measures in ASF outbreaks is not enough for controlling the disease and more attentions should be paid, mostly to illegal raising and trade of pigs to be successful in the eradication process.

ASF in Sardinia: Regional risk categorization to improve surveillance effectiveness

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African Swine Fever (ASF) is a devastating infectious disease of domestic and wild pigs and is considered a relevant threat to the swine industry. In the past, ASF was confined to certain African countries. ASF was first detected in Sardinia in 1978, but initially, its presence was not considered a threat to the surrounding European countries; during this long period, the ASF virus did not escape Italy.

However, the infection has since spread to Eastern Europe, even involving EU countries (Lithuania and Poland), and outbreaks have resurfaced in Sardinia. As a result, the alert status was changed, and the European Commission was urgently requested to intervene.

In Sardinia, beginning in 2011, pork exports were banned and an extremely rigorous response was imposed, primarily comprising: efficient registration of swineherds, increased biosecurity at swine farms, and comprehensive (clinical, serological, and virological) surveillance of the swine population including wild boars. Furthermore, the European Commission has proposed taking strict action to eliminate the illegal breeding of feral pigs, which are considered the ASF reservoir, particularly in east-central Sardinia.

Despite adopting this onerous eradication plan, the ASF incidence has not significantly decreased, and questions regarding the long-term sustainability of these measures have arisen. In this context, considering the history as well as the recent epidemiologic dynamic of ASF infection, a risk categorization was proposed for the Sardinian territory. The objective was to indicate the areas at higher risk of viral circulation.

The Sardinian municipalities formed the territorial basis of the present study, and four distinct risk levels were defined describing the viral persistence or circulation potential. The analysis was based on several variables allocated into three main categories: 1) outbreaks reported in the domestic swine or wild boar population, 2) evidence of illegal feral swine breeding, and 3) serologic and virologic laboratory findings.

In this initial evaluation, 70% of the Sardinian municipalities were allocated into the lowest risk category, whereas only 10% fell into the high-risk category. This assessment indicates that most of the intervention can be targeted to select areas, creating a more effective eradication program. In the remaining island territories, a gradually less intensive surveillance should be employed to prevent ASF circulation. In conclusion, ASF eradication is ongoing, but a comprehensive effort is currently underway in Sardinia to reach this important veterinary health goal.

Experimental transmission of African swine fever virus among domestic pigs and wild boars

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African swine fever (ASF) is a highly virulent swine disease without effective vaccine or treatment available so far. ASF has been reported since the 1920s in Africa. In 2007, the African swine fever virus (ASFV) was introduced into Georgia from where it has spread throughout Eastern Europe since then. ASF is of great economic importance for the pig sector in affected countries due to the high mortality rates, the cost of stamping out measures and the impact on the ability to trade. There is a high risk that ASFV will spread to more European countries, in particular into the European Union. An improved understanding of quantitative ASFV transmission dynamics among domestic pigs (DP) and wild boar (WB) will allow the development of more effective control and prevention strategies. Here, we used a stochastic Susceptible-Exposed-Infectious-Removed (SEIR) model fitted to experimental data for estimation of the basic reproduction number, R0, of the currently circulating ASFV strain. The average number of new infected cases caused by one infectious individual during its infectious period in a susceptible population (R0) is a key determinant of the transmission potential and therefore very useful for determining the likely impact of control strategies. Six transmission studies were performed in a controlled environment, in which susceptible DP and WB were exposed to experimentally infected individuals of the same species. We investigated five scenarios: direct contact transmission within groups of (1) DP, (2) WB, (3) DP and WB, and indirect contact transmission between groups of (4) DP and WB, (5) DP and DP. Virus titres in blood samples regularly collected from the animals were used to reconstruct the transmission process, i.e. to determine the time of infection and onset of infectiousness for each individual. R0 was estimated using a maximum likelihood approach. Results show that in the absence of specific control measures, ASFV is moderately transmissible within DP and WB population. Assuming a latent period of 3 days and an infectious period of 3-6 days, R0 was estimated to be 2.2 (CI 95%: 1.0-3.9), 3.9 (CI 95%: 0.4-7.9) and 3.5 (CI 95%: 1.1-6.2) for scenarios (1), (2) and (3) respectively, meaning that infectious WB and DP are likely to infect on average 2-3 animals via direct contact within-pen. Lower R0 values were observed for scenarios (4) and (5), i.e. 0.41 (CI 95%: 0.0-0.9) and 1.2 (CI 95%: 0.5-2.0), emphasizing the impact of separating infectious and susceptible animals into different pens to reduce the risk of ASFV infection. Simulations of ASF outbreaks within larger populations helped to predict the extent of the disease spread and defined protection level required of future vaccines at around 75%. Such results gives strong support that high biosecurity measures, quarantine of suspected animals and future vaccination could be effective in reducing the risk of further ASF dissemination.

Expert Knowledge Elicitation on African swine fever in the eastern neighbouring countries of the European Union

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Following a request from the European Commission, EFSA's Panel on Animal Health and Welfare assessed the risk for endemicity of ASF in the eastern neighbouring countries of the EU. The assessment was based on a literature review, which was updated until 31/01/2014 and expert knowledge elicitation (EKE). A qualitative risk assessment model was used, which was already developed for a previous risk assessment on ASF of EFSA, in 2010. The approach that was followed for the EKE was a nominal group technique. The process of the elicitation consisted out of two anonymous, web-based, elicitation rounds, and a third round, where the experts met and discussed the outcomes of the EKE. During the third elicitation round, the statistical measure "Entropy" was used to express the uncertainty of the answers and a cut off-value of the maximum entropy was agreed upon. Only for those parameters with an entropy above the cut-off value, the experts provided their judgements again individually and anonymously after the groups discussion. The median of the distribution of the answers was taken as the group response, and used in the risk assessment model. Feed-back on the updated outcomes of the model was provided to the group of experts, and conclusions were formulated and agreed upon. The most important conclusions were that the risk that ASF is endemic in Georgia, Armenia and the Russian Federation has increased from moderate to high, particularly due to challenges in outbreak control in the backyard production sector. The risk that ASFV will spread further into unaffected areas from these countries, mainly through movement of contaminated pork, infected pigs or contaminated vehicles, has remained high. In Ukraine and Belarus, the risk for ASF endemicity was considered moderate. Although only few outbreaks have been reported, which have been stamped out, only limited activities are ongoing to facilitate early detection of secondary spread. Further, there is a continuous risk of ASFV re-introduction from the Russian Federation, due to transboundary movements of people, pork or infected wild boar. The number of backyard farms is greatest in the west of Ukraine and westwards spread of ASFV could result in an infected area near the EU border, difficult to control. In Georgia, Armenia and the Russian Federation, the risk for endemicity of ASF in the wild boar population is considered moderate, mainly due to spill-over from the domestic pig population, whereas in Ukraine and Belarus this was considered to be low. In those areas in the Russian Federation, where wild boar density is high, this risk may be higher. Intensive hunting pressure in affected wild boar populations may increase the risk for spread, possibly with severe implications across international borders. The EKE approach proved to be valuable for risk assessments in a data-scarce environment, which need to be carried out in a short time-frame. It facilitated to reach a consensus between experts with different expertise and political backgrounds, whilst, at the same time, it dealt appropriately and transparently with uncertainty related to the risk estimates.

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Method for integral risk assessment of vector-borne livestock infections (MINTRISK).

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Increasing occurrence of vector-borne infections in livestock bring about the need for tools to evaluate and compare the risk of exotic vector-borne infections. Recent requests both from the Dutch government and from the European Commision1 to assess and compare a series of vector-borne infections with respect to their risk, prove the need for such tools.
Tools that can integrate different risk aspects, while distinguishing uncertainty, probability and impact, are important in dealing with such questions. Although several methods for risk comparison exist, the specific needs of vector-borne infections are often lacking and the way these methods deal with uncertainty, probability and impact restrict their value.
Therefore we have developed MINTRISK, an integral calculation method, using a structure provided by the Framework to assess Emerging VEctor-borne disease Risks for livestock (FEVER), which was presented at the 2013 EPIZONE meeting. A questionnaire, also taken from FEVER, is used to summarize knowledge and information about a vector-borne infection for risk assessment. An underlying quantitative model was developed to combine the (qualitative) answers to the questions, while taking account of assessed uncertainty using Monte Carlo simulation. The risk is evaluated separately for each of the six risk elements distinguished in FEVER: 1) Probability of entry, 2) Probability of transmission, 3) Probability of establishment, 4) Likelihood of persistence, 5) Extent of spread and 6) Impact of the disease. The results are subsequently summarized into an overall risk that allows for comparison and prioritization. For the overall risk, the impact can be assessed as economic impact only, but may also include other aspects such as animal welfare and impact on the environment.
Three main aspects of risk: probabilities, impact and uncertainties are usually interlinked in risk assessments, making effective risk communication difficult. By separating probabilities or rates as opposed to numbers or amounts, a good overview of the different risk aspects is created, while for each of these aspects the uncertainty is evaluated separately. Thus, the tool facilitates communication regarding important risk aspects, helps answering more specific questions by separating risk in six sections, and can help policy makers to prioritize based on overall risk. Several infections were evaluated with this new tool, to test and prove its methodological and scientific validity for policy support.

[1]Request of the European Commission for a Scientific Opinion on entry routes into the EU of vector-borne diseases EFSA-Q-2014-00187;

Session 7: Domestic and Wildlife

Wild animal rabies in India: Possibility of species spill over

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Rabies is endemic in most parts of India, with the exception of Andaman and Nicobar and Lakshdweep islands and to some extent in Nagaland. In India, dogs play an important role in rabies transmission and maintenance, however very less is known about the role of wild animals in rabies transmission and maintenance. Phylogenetic analysis supports the evolution of lyssaviruses in bat vectors with occasional but regular spill over and host switching to carnivore vectors to extend the virus host range.The present study is aimed at sequencing and phyloegnetic analysis of wild animal rabies virus isolates by targeting the nuceloprotein gene.

We collected 12 rabies suspected brain samples from different species of wild animals in India. The confirmatory diagnosis was made by direct fluorescent antibody test (dFAT) and nuceloprotien gene specific real time polymerase chain reaction (RT-PCR). The partial nucleoprotein gene was amplified and sequenced. The phylogenetic analysis was carried out with published N gene sequences from wild, domestic and human origin by DNAstar software.

Eight out of twelve samples were found positive by dFAT and RT-PCR after run with specific controls. The partial N gene (803bp) sequence analysis revealed the all rabies isolates were belonged to classical rabies virus of genotype 1 of rhabdovirus. These isolates were more closely related to other animal rabies virus isolates, indicating the spillover of species. Even though this event is rare, occasionally such an event can initiate a new virus-host relationship in which sustained propagation and independent transmission of the virus within the new host species occurs such that the new host species becomes a rabies reservoir. The full length nucleotide sequence analysis is also underway along with more number of samples to know precise virus type and the transmission of rabies virus from the reservoir host to the other host.

In conclusion the rabies virus isolates circulating in wild and domestic animals in India are more closely related genotypically and suggestive of species spill over.

Age and strain dependent differences on the outcome of experimental infections of domestic pigs with Belgian wild boar pseudorabies virus isolates

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Aujeszky's disease is an economically important disease in domestic swine caused by the porcine alphaherpesvirus, pseudorabies virus (PRV). As a result of large scale vaccination programs, the disease has successfully been eradicated in domestic pigs in a large part of Europe, including Belgium. Serological studies however show that the virus is still present in the wild boar population. A limited number of infection studies suggest an attenuated nature of these wild boar strains. Care should however be taken when extrapolating these results since phylogenetic studies identified the existence of important genetic diversity among European wild boar strains.

To get more insight into the virulence of Belgian wild boar PRV strains, an in vivo infection study using two genetically distinct wild boar isolates and the virulent NIA3 reference strain was performed. Six female domestic pigs of 15 weeks old were intranasally inoculated with 105 TCID50 of the NIA3 strain or 106 TCID50 of the wild boar isolates BEL24043 or BEL20075. Furthermore, six naive contact animals were added to the boxes with pigs inoculated with the wild boar isolates at 24hpi to assess possible virus transmission. Since clinical disease induced by PRV is dependent on the age of the pigs, also six animals of two weeks old were inoculated with the NIA3 strain or the wild boar isolate BEL24043. On regular time points temperature and clinical symptoms were determined and serum, nasal and vaginal swabs were collected. Animals were euthanized and tissues collected at the end of the experiment at 28 dpi or at intermediate time points when mandatory on ethical grounds.

A clear difference between clinical symptoms induced by the NIA3 strain and the wild boar isolates BEL24043 and BEL20075 was observed. In 15 weeks old pigs, infection with the NIA3 strain led to severe respiratory and neurological symptoms. In contrast, no clinical symptoms were observed when pigs of the same age were inoculated with the wild boar isolates BEL24043 or BEL20075. In two weeks old piglets, the symptoms induced by the NIA3 strain were even more pronounced, leading to euthanasia of all piglets at 3 and 4 dpi. Also the wild boar isolate BEL24043 was able to induce respiratory and neurological disease in these two weeks old piglets, although symptoms were less severe than after NIA3 infection and only one piglet had to be euthanized before the foreseen date.

Viremia was detected via qPCR in 15 weeks old pigs after infection with the NIA3 strain, but could not be found upon inoculation with the wild boar isolates BEL24043 and BEL20075. In the two weeks old piglets viremia was observed after infection with both the NIA3 strain and the wild boar isolate BEL24043. Furthermore, inoculation of 15 weeks old pigs with the NIA3 strain and the wild boar isolate BEL24043, but not with the wild boar isolate BEL20075, resulted in seroconversion. The wild boar isolate BEL24043 was also able to induce seroconversion in two weeks old piglets.

PCR analysis of swabs showed a prolonged nasal and vaginal virus excretion after inoculation of 15 week old pigs with the wild boar isolate BEL24043. Importantly, efficient transmission of the virus to contact animals was evidenced by nasal and vaginal excretion, seroconversion and the presence of virus in the tonsils of these contact animals. On the other hand, no clear evidence of transmission of the isolate BEL20075 to contact animals was found, since only occasionally positive nasal or vaginal swabs were found.

In conclusion, our study identified differences between Belgian wild boar isolates in their capacity to infect and spread between domestic pigs. The difference in induced clinical symptoms in two and 15 week old piglets showed that the known age dependency of pigs on the outcome of infection with PRV isolates from domestic pigs is also valid for wild boar strains. Although both wild boar strains were attenuated in 15 week old pigs, one was capable to induce seroconversion and was efficiently transmitted to contact animals. Despite the fact that reports of PRV transmission from wild boars to the domestic population are rare, this indicates that a reintroduction could have serious economic consequences.

Detection of European bat lyssavirus type 2 in Danish Daubenton's bats using a molecular diagnostic strategy

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European bat lyssavirus (EBLV) is considered to be endemic in the Danish bat populations, but limited information exists about the types of EBLV strains currently in circulation. EBLV type 1 (EBLV-1) is seen as the predominant type in the Serotine bats (Eptesicus serotinus) with the latest case identified in 2009. EBLV type 2 (EBLV-2) has not been reported in Denmark but a survey in 1986 revealed two EBLV positive Myotis bats; a Daubenton's bat (Myotis daubentonii) and a pond bat (Myotis dasycneme)(Grauballe et al. 1987). Typing of these EBLV isolates was not performed but as Myotis bats are known to be associated with EBLV-2 this result suggested that type 2 EBLV was also present within the country at that time. In the present study, mouth swabs (in total 136) from Danish bats, representing 10 different bat species, were collected during the autumn of 2013. They were screened for the presence of EBLV RNA using a new molecular diagnostic strategy based on pan-lyssavirus RT-qPCRs (Fischer et al. 2014). In two samples, obtained from Daubenton's bats, EBLV RNA was detected by the pan-lyssavirus RT-qPCR targeting the highly conserved L-gene. Subsequent analysis with EBLV specific RT-qPCRs showed the presence of EBLV-2 in the bats. Furthermore, nucleotide sequencing of the RT-PCR products obtained from the pan-lyssavirus assay, revealed a distinct sequence with 98% similarity to published EBLV-2 strains from neighbouring countries. Our results show that EBLV-2, in addition to EBLV-1, is circulating in the Danish bat populations.

CONSTANT HEPATITIS E VIRUS CIRCULATION IN WILD BOAR, RED DEER AND IBERIAN PIG IN SPAIN: an 8 years longitudinal study.

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Hepatitis E is a viral zoonosis affecting multiple wildlife and domestic hosts. The complete dynamics of infection in wild life are still unknown but the previous fact facilitates the maintenance and circulation of the virus, posing a risk to human health in the case of meat consumption from susceptible animals, as shown by past transmission events between humans, deer and wild boar. In Spain, it has been shown how domestic Iberian pigs, cattle and wildlife (i.e. wild boar and red deer) clearly interact in hunting farms, where there is an increased intensive management of wildlife populations for hunting purposes, generating a complex epidemiological situation in terms of interspecies pathogen transmission. Therefore, in this study we aimed to (i) evaluate the circulation of the virus in geographically close domestic (Iberian pigs) and wild animals (wild boar and deer) living in hunting areas from Central Spain over 8 years (2003-2010) and (ii) to determine if HEV could be used as a marker of domestic-wildlife contact. For this purposes, a longitudinal analysis of Iberian pig, wild boar and red deer samples (n=287) through virological and serological tests shed light to the circulation events of HEV. Regarding HEV RNA detection by real time RT-PCR, 10, 12% samples (95% CI: 5, 44-14, 8) from wild boar and 16, 05% samples (95% CI: 8,06-24,04) from red deer resulted positive. As for the Iberian pigs, none of the 48 samples were positive to HEV RNA detection. In the serological analysis 43,75% (95% CI: 29,75-57,75) from Iberian pig, 57,40% (95%CI: 48,10-66,70) from wild boar and 12,85% (95% CI: 5,01-20,69) samples from red deer presented anti-HEV antibodies. Positive samples were distributed along all study years (2003-2010).

These results depict the urgent need to improve the inspection and surveillance of these species and their products. In the case of HEV, it is clear that the stable and constant presence of the virus in wildlife and their contact with Iberian pigs pose a risk for human health as they are all destined to human consumption.

The metagenomic in silico lab: Design, Analysis and Validation of metagenomic investigations

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Background

The advent of sequencing based methods for unbiased detection of nucleic acids in environmental samples, metagenomics, now provides investigators with a powerful tool for monitoring emerging infectious disease of both enzootic and zoonotic potential. The recent advances in DNA sequencing technology have opened the field further, allowing rapid, cheap, and accurate detection of pathogens. While it can be an effective method for pathogen detection and discovery, these studies results are only as accurate as the quality of the bioinformatic data processing and analysis allows. In addition to bioinformatics, any successful project requires proper experimental design as well. This study describes a fully automated pipeline system to perform the *in silico* analysis of a viral metagenomics project, as well as support with the experimental design.

Methods

Bioinformatic classification and annotation of metagenomic data is commonly divided into four step: i) quality control and filtration of the raw sequences, ii) de-novo assembly of the data into contiguous sequences (contigs), iii) taxonomic classification of the contigs, and iv) gene prediction and functional annotation. While there are several tools available to perform these analyses, we found none of them well adapted to viral metagenomics data. Many taxonomic classification methods are based on bacterial marker genes, making adaptation to viral sequences impractical. Classification with BLAST followed by Lowest Common Ancestor (LCA) is also common, but limited due to its reliance on homology to published sequences.

Estimation of sequencing depth, often called target coverage, is a common design problem in sequencing experiments. While sequencing depth estimation for single organism experiments is straightforward, coverage estimation for metagenomic sequencing is far more complex, being one of the most difficult parts of metagenomic experimental design, as it involves advanced probability theory. We have written high-precision mathematic modules to easily help with this otherwise daunting step.

Results and Conclusions

A modular *in silico* environment was created for evaluation of experimental design, assembly and classification. We developed a tool for simulation of metagenomic datasets based on estimated experimental design and error profiles from almost all currently available sequencing platforms. This tool, MetaMaker, was employed within the environment for simulation of plausible datasets. Several taxonomic classification methods were evaluated using these simulated viral datasets, as well as on previously produced datasets, and only a handful was suitable for viral classification.

Our conclusions are that viral metagenomics must rely on a combination of methods for classification, both homology based and unsupervised methods. Homology based methods are highly reliant on curated databases and as such the need for whole genome sequencing of microorganisms will rise in the future. Metagenomic experiments must estimate coverage based on genome size, genome abundance and stochastic genome pool. The *in silico* environment presented here provides scientists with tools for Designing, Simulating, Validating and Analysing metagenomic experiments. The tool will be of value for estimating the feasibility of using metagenomics to solve biological questions.

Kobuvirus in brains of piglets diagnosed with congenital tremor type A-II

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Congenital Tremor (CT) is a sporadic disease seen in neonatal pigs. It causes symptoms such as muscle tremor, shaking of the body and incoordination. These symptoms can disable the piglet from finding a teat, potentially leading to its death. CT has been linked with inheritance, viruses and toxins. It is classified as type A (displaying histopathological lesions in the central nervous system) and B (with no evident microscopic findings). The latter one is considered of unknown cause, while type A has been subdivided in sub-types I to V. The most common form worldwide is considered to be subtype A-II, which is transmissible and likely to be caused by an unknown virus. A number of efforts have been done to clarify the etiology of CT A-II, but so far, no definitive conclusions have been drawn. The aim of this study was to identify possible viral causes for CT A-II using a high-throughput sequencing (HTS) approach.

A total of 13 piglets showing clinical signs of CT were submitted to the *Servei de Diagnòstic de Patologia Veterinària at the Universitat Autònoma de Barcelona* (Spain). Causes of CT subtypes A-I, A-III, A-IV and A-V were ruled out by means of clinical history and epidemiological findings. In consequence, CT subtype A-II was established. Brain samples from these 13 pigs were transferred to *Swedish University of Agricultural Sciences* and prepared for HTS. In brief, sample preparation was performed as earlier described1 with the exception that homogenization was performed using Omni Tissue Homogenizer (OMNI International) and freeze thawing. Libraries were prepared by standard Nextera XT protocol and sequencing was performed on MiSeq (Illumina). Sequence data was processed with a previously described method1 and a new methodology involving data cleaning performed with PRINSEQ and a taxonomic classification of the sequences using Kraken2. Computing resources at UPPNEX and SLU Global Bioinformatics Centre, Uppsala, Sweden, were used.

The MiSeq generated 16.4 GB of data, varying between 0.9-1.6 GB per sample. On average 36% of the sequences, i.e. 33.9 million, were of good quality and used for classification. One of the most notable findings was that porcine kobuvirus, a positive-sense ssRNA virus of the picornaviridae family, was identified in 5 out of 13 samples (38%).

Porcine kobuvirus (Aichivirus C), was first identified in 2007 in stool samples from healthy piglets in Hungary3. It has thereafter been found in many other countries, in serum samples and been inconclusively associated with piglet diarrhea. Close relatives to Kobuviruses within the Picornaviridae family are known to cause neurological symptoms. However, to date no Kobuvirus have been identified in the brain. The relatively high prevalence of Kobuvirus identified in CT A-II pigs could be a step closer to clarifying the etiology of this devastating disease. Work to validate the findings in larger sample sets including healthy animals and by alternative methods (PCR and/or RT PCR) is ongoing.

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Session 8: Epidemiology (II)

“Surveillance is a public good” – but how public is it?

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Animal health surveillance plays a key role in early detecting the introduction of diseases, facilitating their eradication and demonstrating disease freedom. Therefore, it is recognised as a global public good to support improved animal and global public health. In recent years, country-specific risks such as the risk of introduction from other countries (i.e. trading partners or geographical neighbours) are increasingly assessed in order to develop risk-based approaches and thus improve the efficiency of surveillance. However, risk assessments rely on the availability of information.

For co-financed surveillance systems, detailed information is provided on surveillance design (2004/450/EC), whilst for other hazards only the extent of surveillance (2003/886/EEC) or any detected cases have to be reported (notifiable and reportable diseases). However, knowledge on both the design and extent of surveillance would be desirable in order to assess important epidemiological measures such as population coverage and surveillance sensitivity. Hence, the aim of this work was to describe the degree of publicly available information on surveillance designs in EU countries.

A review of surveillance systems was carried out as part of the RISKSUR project to describe basic epidemiological characteristics of surveillance systems of eight EU countries (DE, DK, ES, FR, GB, IT, NL and SE) and Switzerland in 2011. Surveillance experts from seven RISKSUR-partner countries collected the data, including 40 variables relating to the target population, sampling methods, data generation and data translation process as well as the number of herds and animals sampled. A questionnaire was sent to the competent persons involved in data collection with the aim to determine the number of staff collecting the data, the availability and use of sources (reports, in-country contacts), the existence of documentation and the completeness of public and private surveillance data.

Results showed that centralized systems, which document design details of surveillance activities, do not exist in most countries. It is particularly difficult to capture regional variation in decentralized countries and private surveillance efforts. Furthermore, there is a lack of consistency in information being reported.

Lacking documentation of surveillance efforts and the lack of standardisation of terms presents a major hindrance to evaluate the quality of surveillance systems within a country and between countries. It is recommended to define a minimum set of variables describing the design of surveillance systems and encourage decision makers to document their efforts in public domain and/or surveillance databases. This would facilitate a better comparison of surveillance activities and indicate opportunities to coordinate efforts (e.g. public and private surveillance). It is concluded that the current documentation of animal health surveillance for notifiable diseases does not fulfil the criteria of a global public good (non-rivalrous, non-excludable, and available worldwide).

Quantification of different classical swine fever virus transmission routes within a single compartment

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During outbreaks of classical swine fever (CSF), CSF virus (CSFV) can be transmitted via different routes. Understanding these transmission routes is crucial in preventing the unlimited spread of the virus in a naïve population, and the subsequent eradication of the virus from that population. The objectives of the present study were to quantify virus transmission within a compartment, differentiating between transmission within a pen, transmission between pens via contact through (open) pen partitions, and transmission via the air. Furthermore, the possible contribution of each of these routes to infection of individual pigs was quantified. A CSFV outbreak was mimicked in a compartment housing 24 pigs in six different pens. Two pigs in one pen were inoculated with the moderately virulent Paderborn strain, and virus transmission to other pigs was followed in time. Virus transmission rates for transmission via the air (β of 0.33 (0.14 – 0.64) per day) and transmission between adjacent pens (β of 0.30 (0 – 0.88) per day) were comparable, but significantly lower than for virus transmission within a pen (β of 6.1 (0.86 – 18) per day). The route via the air created new focal points of infection, from which virus transmission continued through other routes. This shows that, at least within a compartment, transmission via the air is expected to play a relevant role in the fast spread of the virus after an initial slow start. This will have consequences for efficacy of intervention measures, including vaccination during an outbreak.

Resource estimations in contingency planning for foot-and-mouth disease

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Preparedness planning for a veterinary crisis is important to be fast and effective in the eradication of disease. For countries with a large export of animals and animal products, each extra day in an epidemic will cost millions of euros due to the closure of export markets. This is important for the Danish swine industry, which had an export of €4.4 billion in 2012.
The purposes of this project were to: 1) estimate the resources needed during an outbreak of foot and mouth disease (FMD) in Denmark, 2) identify areas, which can delay the control of the disease, and 3) develop an iterative tool, which can easily be updated, when knowledge is gained from other veterinary crises or during an outbreak of FMD.
A stochastic simulation model was developed in InterSpread Plus to simulate spread of FMD in Denmark. The personnel and resource needs was estimated using results from this model.
It was estimated that the need for personnel would peak on day 7 with a requirement of approximately 170 veterinarians, 70 technicians and 45 administrative staff. However, the need for personnel in the Danish Emergency Management Agency (responsible for the hygiene barrier and initial cleaning and disinfection of the farm) would peak already on day 4 with a requirement for almost 500 persons, mostly recruits.
On average, 53000 animals were culled during the simulated epidemics, leading to a daily need for rendering capacity of up to 210 tons for swine and 379 tons for ruminants.
Based on results from a stochastic simulation model, it was possible to create a simple model in excel to estimate the requirements for personnel and materiel during an FMD outbreak in Denmark. The model can easily be adjusted, when new information on resources appears from management of other crisis or from new model runs.

A Systematic Review and Meta-Analysis to Measure Sero-Prevalence of Influenza A (H9N2) Virus Infection in Humans

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Introduction

Influenza A H9N2 subtype is prevalent among domestic poultry populations in many countries. The H9N2 virus occasionally causes human infections, most of which are mild or asymptomatic. Recent studies suggest that the virus has acquired genetic material from other pandemic subtypes including H5N1 and 2009 pandemic H1N1 viruses. Should H9N2 strains become more virulent and more efficient in human-to-human transmission, H9N2 strains have potential to cause pandemics. The objective of this study was to assess the overall burden of H9N2 infections among the poultry-exposed human populations worldwide using a meta-analysis of the published serological data.

Methods

Considering the period January 1997 to December 2013, we performed a systematic search using the PubMed, AGRICOLA, and Cab abstracts databases for the following terms, all in the "explode" function: "influenza H9N2" AND "serological surveys" OR "seroprevalence" OR "sero-prevalence" OR "seroepidemiology" OR "sero-epidemiology". Related studies in all languages were all considered. Articles were selected if they were population-based (surveillance reports, cross-sectional or prospective) and contained laboratory serological data. Herein we report and compare positive hemagglutination inhibition (HI ≥1:160 titer), positive microneutralization (MN ≥1:80 titer) assays, and seroconversion (4-fold rise in HI or MN titer over time) as a standardized case definition for seropositivity with the reports from the studies. We calculated non-adjusted seroprevalance through a random effect meta-analysis model. We calculated heterogeneity by Pearson's chi-squared test, which was determined using I2 index statistics to estimated the proportion of total variation.

Results

We identified 22 studies for the final review and meta-analysis. Most of these reports were from Asia (86%) and cross-sectional surveys in nature (82%). The majority of the study participants were ≥18 years of age and almost all of them had a history of poultry or wild bird exposure. Meta-analysis demonstrated that the overall HI seroprevalence calculated using the antibody cut-off reported by the studies was 9.6% [95% confidence interval (CI): 08 – 12%, I2 = 98.5%], which was higher compared to the standardized case definition (≥1:160) for seropositivity (seroprevalence: 6.6%, 95% CI: 4 – 9%, I2 = 98.6%). For the MN meta-analysis, the overall reported seroprevalence was 3% [95% CI: 2 – 5%, I2 = 87.8%], which was higher compared to the standardized case definition (≥1:80) for seropositivity (seroprevalence: 0.2%, 95% CI: 0.0 – 0.5%, I2=29.4%).

Conclusion

Findings from the meta-analysis suggest that few poultry-exposed humans are infected with H9N2. However, should H9N2 viruses further adapt to human hosts and develop more virulent characteristics, they may spread quite rapidly among man causing significant morbidity and mortality. Efforts should be increased to conduct more aggressive surveillance for H9N2 strains such that genetic changes might be identified in time to provide pre-pandemic warnings.

Identification of the genetic structure of the red fox (Vulpes vulpes) population in association to canine distemper and rabies viruses circulating in Northern Italy

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Since 2006, the wild red fox (Vulpes vulpes) population in the north-east of Italy has been affected by a Canine Distemper Virus (CDV) epidemic. The incidence in the population appeared significant and the geographic spread of the disease was broadly south-westerly oriented.
In addition, at the end of 2008 fox rabies re-emerged in the same area, where it circulated until the beginning of 2011. The first cases were detected in proximity of the national border with Slovenia and the phylogenetic analysis supported the genetic correlation with the Rabies virus circulating in Slovenia and Bosnia Herzegovina. More than 280 cases of Rabies in foxes were confirmed and since the very first detection, the disease has shown a westerly spread.
In this study we described the genetic and spatial characterization of the red fox population affected by these infections through a microsatellite analysis on 379 samples, collected between 2006 and 2011 in the north-east of Italy (Friuli Venezia Giulia, Veneto and Trentino Alto-Adige). The panel of genetic markers was evaluated from the bibliography and 21 microsatellites were selected for the genotyping of the red fox populations. We investigated the population structure with a Bayesian clustering analysis implemented in Structure 2.3.3 and we spatially characterized the sub-populations by making use of Geneland 4.0.2.
Genetic data were used to investigate the association between the genetic structure of the identified subpopulation and the susceptibility to the diseases. Furthermore we performed a case control study in order to assess whether rabies and distemper infections differed among genotypes.
We identified 4 distinct sub-populations and we hypothesized a spatial segregation influenced by environmental (rivers) and artificial (highways) barriers. As a result of the case control study, we had indications that distinct sub-populations could differ in the susceptibility to canine distemper and rabies viruses. In fact, one sub-population appeared more susceptible to rabies and less to CDV, while it was the opposite for another sub-population, which was more susceptible to CDV.
In order to identify predictable trajectories and high risk situations, the following issues should be taken into account and evaluated: the study of the ecological features of the red fox population, the microsatellite analyses for the assessment of the genetic structure of the red fox population and the phylogenetic studies on the viruses affecting this population. This could help to better understand the disease dynamics and to improve control strategies and surveillance.

The impact of Schmallenberg in Belgian cattle and sheep: a case-control study

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Schmallenberg virus (SBV) emerged during summer 2011. SBV was shown to be responsible for an acute unspecific syndrome in cattle and reproductive diseases in domestic ruminants. To date, the real impact of SBV in Belgium and the risk factors associated with this disease remain unknown. Therefore a case-control study, via a telephone questionnaire, was conducted for cattle and sheep farms. Questions related to reproduction parameters and clinical signs observed in newborn and adult animals were designed. Several management related parameters including treatment with insecticides, surrounding area and vaccination protocols were also investigated. Comparisons between the case and control farms were carried out to identify potential risk factors.

In cattle, 54 case (C+) and 29 control (C-) farms were selected whereas in sheep 50 farms were withdrawn per group (C+ versus C-).

In cattle, the rate of malformed calves born in the C+ herds was elevated during the SBV epidemic and represented 3.37% of gestations, with 3.25% attributable to the SBV infection. In addition 23% of the C+ cattle farmers stated a decrease in fertility (more inseminations per succeeded gestation were needed). According to the results in this case-control study, abortions and still-births were not specific signs of SBV infection in cattle whereas the observed malformations were typical signs observed during the epidemic of SBV.

In sheep, the impact of SBV on reproductive parameters was perceived to be higher compared to cattle. This was reflected in the C+ herds by a high proportion of lambs born aborted (3.26%), still-born (10.49%) and malformed (8.26%). Of these proportions 2.69% of aborted, 8.46% of still-born and 8.16% of malformed lambs, respectively, were attributed to the SBV infection. In sheep, as it was observed in cattle, the birth of a malformed animal was found to be the most relevant sign for the farmer to suspect SBV in the flock.

Twenty-six percent of C+ cattle farmers and even 10% of C+ sheep farmers observed clinical signs in adult animals (temperature and/or degradation of general state and/or diarrhea and/or nervous symptoms). The clinical impact on adult animals was also shown by the fact that 2% of C+ cattle and 24% C+ sheep farmers stated to have observed the death of at least one dam after it gave birth to an SBV confirmed calf/lamb.

No differences could be found between C+ and C- farms in sheep and cattle with relation to management system, treatment with insecticides, surrounding area and vaccination protocols.

In conclusion, the results of this study suggest that the impact of SBV was particularly high in Belgian cattle and sheep farms with relation to reproductive disorders encountered during the epidemic. For the farmers the birth of a malformed animal was the key event to suspect the presence of SBV in the farm. This study also pinpoints the importance of arboviroses in Belgium.

Special topic Session: ASF

Suitability of feces samples in a non-invasive sampling strategy to monitor African swine fever virus in wild boar

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A challenging aspect of ASFV control in wild boar populations is the design and implementation of effective surveillance and monitoring programs, both for early warning, and to determine the on-going epidemiological situation in an infected population. Testing blood samples requires invasive sampling strategies like hunting or capture of wild boar. Besides a bias towards healthy animals, such strategies are also linked to further spread of the virus. Non-invasive sampling strategies would increase the reliability of surveillance of ASFV in wild boar populations, without the negative side effects. This study evaluates the potential of feces samples as a basis for non-invasive sampling strategies for ASFV in wild boar. Three factors are crucial in the use of feces samples: 1) excretion dynamics of the virus in feces, 2) stability of viral DNA in feces (for PCR-diagnostics), and 3) access to feces samples in the field. In the acute phase (0-21 days after infection) viral DNA can be detected in 50-80% of the time in which virus can be detected in blood. This percentage is going down to below 10% for the subacute/chronic phase. ASFV DNA is quite stable in feces. Half-lives range from more than 2 years at temperature up to 12°C, to roughly 15 days at temperatures of 30°C. From literature, several studies have shown that collecting fecal pellets from wild boars is possible with relatively low effort compared to for instance hunting. These findings suggest that sampling of feces could be the basis for an improved and non-invasive sampling strategy to monitor ASFV in wild boar.

Diagnostic tools for rapid detection of ASFV in blood samples: Lateral Flow Assay and Real Time PCR.

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African Swine Fever (ASF) is a highly contagious disease of swine that produces great economic losses in the affected countries. Domestic pigs and European wild boars are susceptible, showing a wide range of clinical forms. Epidemiological studies have demonstrated that the entrance of ASF virus (ASFV) in ASF-free areas is primarily related to feeding pigs with contaminated garbage from international airports and seaports. This fact, together with the extensive commercial trade, puts ASFV-free countries at constant risk of having the disease introduced in their territory. In the absence of vaccine, control and eradication strategies are mainly based on rapid laboratory diagnosis of ASFV positive and carrier animals and on the enforcement of strict sanitary measures.

To this end, INGENASA in collaboration with the European Union Reference Laboratory (EURL) for ASFV (INIA-CISA), have been working in the development and standardization of rapid, easy and reliable diagnostic tools based on Lateral Flow Assay and Real Time PCR (INgene® q PPA) for accurate detection of ASFV in serum, blood and tissue samples.

For real-time PCR, a primer set and a UPL-probe (Universal Probe Library/Roche) specific for ASFV detection were selected (Fernández-Pinero et al., 2013). An exogenous internal control using a primer set and a VIC-labeled hydrolysis Taq-Man-MGB probe was designed for porcine β-actin gene detection. Optimal reaction conditions were established to get a duplex real-time PCR test allowing a correct differential detection of ASFV and the internal control. On the other hand, a Lateral Flow Assay (LFA) for antigen detection based on the use of MAbs against ASFV VP72 protein has been developed. Initially, a spike in test was performed by adding VP72 recombinant protein into blood. To test the applicability of these two assays to detect ASFV, a panel of experimental porcine sera and blood samples were used.

Results of INgene® q PPA test were compared with those using the ASFV UPL-Real Time PCR previously described by Fernández-Pinero et al, 2013 in a validation study performed at the EURL for ASFV (INIA-CISA). Concerning LFA, results were compared with the ones obtained by a commercially available ELISA Sandwich (INGEZIM® PPA DAS), qPCR or hemadsorbing titre.

From the validation study of INgene® q PPA assay, it can be concluded that there is an excellent agreement with the reference UPL-PCR method showing 98% sensitivity and 96,8% specificity and being able to detect all isolates checked (n=21). Moreover, the assay was able to detect the ASFV during chronic, subacute and acute ASF infections with similar sensitivity to those showed by the reference UPL-PCR method.

Regarding the LFA for ASFV detection, the test showed the same sensitivity as the INGEZIM® PPA DAS when recombinant protein VP72 or inactivated culture virus were used. When the assay was performed with blood samples from experimentally infected animals, viral loads between 102-103 HAU could be detected. These results suggest that the developed assays can be useful tools for rapid and reliable detection of ASFV, not only at laboratory level (INgene® q PPA) but also at field level (LFA-ASFV), providing a useful tool in situations where laboratory support and skilled personnel are limited.

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Defining mechanisms used by African swine fever virus to evade the host stress response

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The ER responds to the accumulation of unfolded or misfolded proteins by activating the unfolded protein response in an attempt to restore homeostasis by reducing protein influx, increasing the capacity of the ER and increasing clearance of proteins through autophagy. One way homeostasis is achieved is through the activation of PERK leading to phosphorylation of eIF2α and therefore the inhibition of global protein synthesis, which also restricts viral replication. Whilst most mRNA translation is inhibited during ER stress a small subset are still synthesised. One such mRNA is that for the transcription factor ATF4, a downstream target of which is CHOP; a pro-apoptotic transcription factor responsible for most ER stress related apoptosis.

DP71L is one of several virulence factors encoded by the large double stranded DNA virus, African swine fever virus (ASFV). DP71L shares sequence similarity to both cellular GADD34 (growth arrest and DNA damage-inducible protein 34) and the neurovirulence factor ICP34.5 of Herpesvirus. It has previously been shown that, like ICP34.5 and GADD34, DP71L recruits PP1 to dephosphorylate phosphorylated eIF2α, restoring global protein synthesis and inhibiting CHOP activation via the ATF4 pathway.

We have established the key residues which are required for this function by assessing DP71L mutants for their ability to inhibit induction of CHOP. We propose that the previously described PP1 binding motif and a putative eIF2α binding domain are critical for function.

It has previously been demonstrated that deleting DP71L from ASFV does not result in an increase in either eIF2α phosphorylation or induction of CHOP; therefore there must be another gene, or genes, which perform the same or similar function. A library of plasmids each expressing a single HA-tagged ASFV gene has been evaluated for their ability to inhibit CHOP activation post treatment with the ER stress inducer, tunicamycin. Three candidate genes have been identified and these are A179L, K145R and D129L.

The current study aims to elucidate how these genes of interest function in their ability to inhibit CHOP post treatment with tunicamycin.

The African swine fever virus ORF I8L which encodes a putative member of the SH2 domain superfamily is not essential for ASFV replication in vitro.

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African swine fever virus (ASFV) causes an acute haemorrhagic fever. Due to high mortality in domestic pigs and wild boar, the disease can have serious economical and social consequences. The lack of a vaccine limits options for disease control in both ani-mal husbandry and wildlife. ASFV has the capacity to expresses more than 160 proteins of which most are not yet characterized functionally. To better understand the biology of this complex virus, we started to delete genes encoding proteins with unknown function. One of them is the SH2 domain superfamily member pI8L. The SH2 (Src Homology 2) domain is a structurally conserved protein domain within the Src oncoprotein and several other proteins involved in signalling pathways. For deletion of the IL8 ORF, a p72 promoter regulated expression cassette for the red fluorescing protein (RFP) was flanked with LoxP sequences and about 2.5 kbp upstream and downstream from the I8L ORF were added to provide sequences for homologous recombination. This plasmid was transfected into WSL cells. Cultures were superinfected with the TK-GFP+ ASFV recombinant NHVdTK/GFP and monitored for appearance of both green and red autofluorescing cell foci. Viruses from double-autofluorescing cells were plaque purified to homogeneity and one isolate, named NHVdTKdI8L/RFP, was selected for further characterization. The property of this recombinant to grow on normal WSL shows that pI8L is not essential for replication of ASFV in cell culture. To remove the RFP expression cassette from the NHVdTKdI8L/RFP genome to avoid unwanted site effects by the RFP expression, the virus was propagated on WSL-Cre cells that constitutively express Cre recombinase (see presentation by Fuchs et al.). Virus progeny was titrated on WSL cells and viruses from green-only foci were selected to yield NHVdTKdI8L/Cre8-2. An I8L-revertant virus is currently generated to analyse the role of pI8L in cultured cells. Respective data will be presented.

Assessment of the carrier state induced in experimentally infected pigs with African Swine fever (ASF) attenuated and virulent strains.

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In endemic areas, moderately virulent ASF virus isolates and low virulent isolates have been described and recovered pigs may remain persistently infected for long periods of time (Wilkinson 1984; Oura et al.2005; Costard et al, 2009, EFSA, 2010). Since ASF introduction of the virus into the Republic of Georgia in 2007 and in Russia Federation ASF has become a large-scale epidemic involving the domestic pig and wild boar population with tendency to form a endemic zone in the central and southern parts of the Russia Federation. This situation together with the recent incursions of the disease into the European Union (Lithuania and Poland, OIE 2014) poses the need to establish the role of recovered pigs or pigs that do not develop clinical signs in the maintenance of the disease.

To this end we present the results of two experimental infections, using (i) a low-virulent, NHV/P68, genotype I and (ii) a virulent Lithuanian LT14/1490 ASFV genotype II isolate (2014). Both viruses were inoculated into four and eight domestic pigs via intramuscular in separated boxes, and two (i) or 10 (ii) pigs were in contact with the inoculated pigs (i) at 65 dpi in case of the low virulence virus isolate or (ii) at the time of infection.

(i) NHV inoculated animals developed ASF moderate to low chronic type lesions described in the Iberian Peninsula (Leitao et al, 2001). In contrast with the high specific antibody response detected from 8-11 days post infection (dpi) and maintained until the end of the experiment, only weak intermittent and variable viremia peaks could be detected in infected pigs and contact pigs. ASF virus was easily detected in target organs by PCR and further isolation in the animals slaughtered after 1 and 2 months post infection. At month three, the virus could be recovered only from lung and mediastinum lymph node. No virus could be detected in organs at 4, 5 month post infection. Transmission of the virus to the two healthy contact pigs (which were introduced after two months of the NHV initial inoculation) occurred, demonstrating that infected animals recovered from infection were able to transmit the virus after two months of being infected. Contact pigs showed a moderate to low unspecific clinical signs, that could be easily unnoticed in the field, although detected by the antibody detection test in the laboratory. Virus persistence was demonstrated in tissue samples.

(ii) In vivo experiments with the Lithuanian LT14/1490 virus isolate induced an acute ASF infection in domestic pigs which resulted in 94, 5% mortality (17/18). The survivor animal showed a weak and intermittent viremia during the course of the experiment without significant clinical manifestation of the disease. Necropsy findings showed lesions compatible with ASFV infection, and virus was detected in some tissues.

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Comparative analysis of molecular and biological properties of African swine fever virus isolates collected in 2013 from Russian Federation

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To investigate the pathogenesis of African swine fever virus, domestic pigs were challenged with different doses (from 50 to 5000 haemadsorbing doses (HAD50)) of several Russian ASFV isolates of 2013. Animals were inoculated using intranasal or intramuscular routes. A subsequent contact challenge experiment was performed in which IN and IM-inoculated pigs were allowed to have a direct contact with healthy pigs. All the tested isolates caused death in experimentally challenged animals, however, some features differed among the isolates and inoculation doses employed, such as: pyrexia, duration of the clinical phase of infection and period of disease progression, differences in clinical symptoms. There were also observed noticeable dissimilarity between isolates from domestic pigs and wild boars. For each isolate pigs inoculated by higher doses of ASFV had the shortest mean survival period.

The study of culture-biological properties of ASF virus isolates collected in the territory of the Russian Federation in 2013 made it possible to discover the variation of pathogenicity towards the reduction of virulence and increase of the disease duration. Pyrosequencing of genome of Kashino 04/13 isolate revealed nucleotide changes in 3 genes that could be potentially responsible of the modification in the virulence of this ASFV isolate.

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Notes

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