EPIZONE

Abstracts

6th Annual Meeting EPIZONE

"Viruses on the move"

12th-14th June 2012

The Dome Complex
Brighton, United Kingdom

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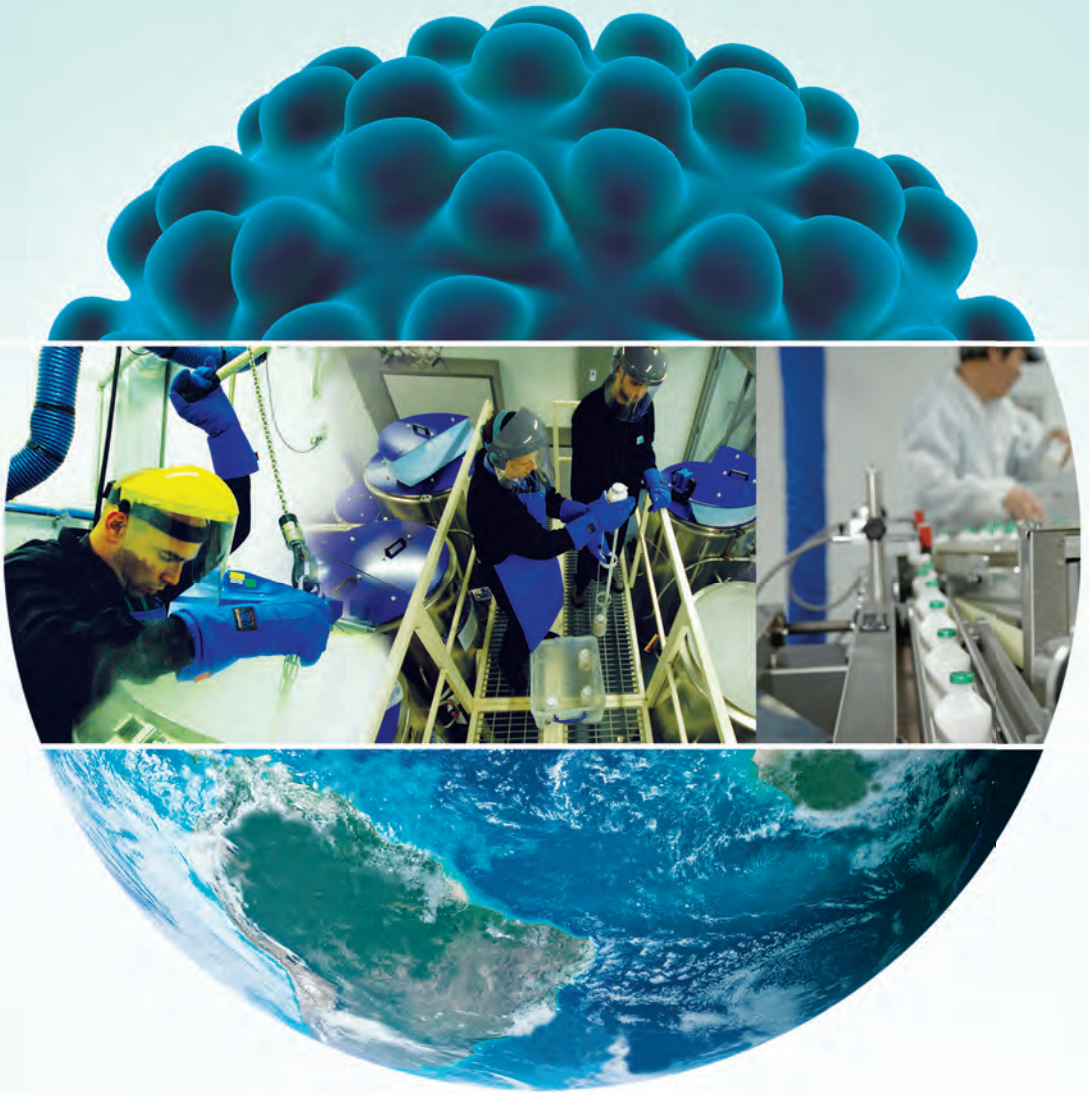

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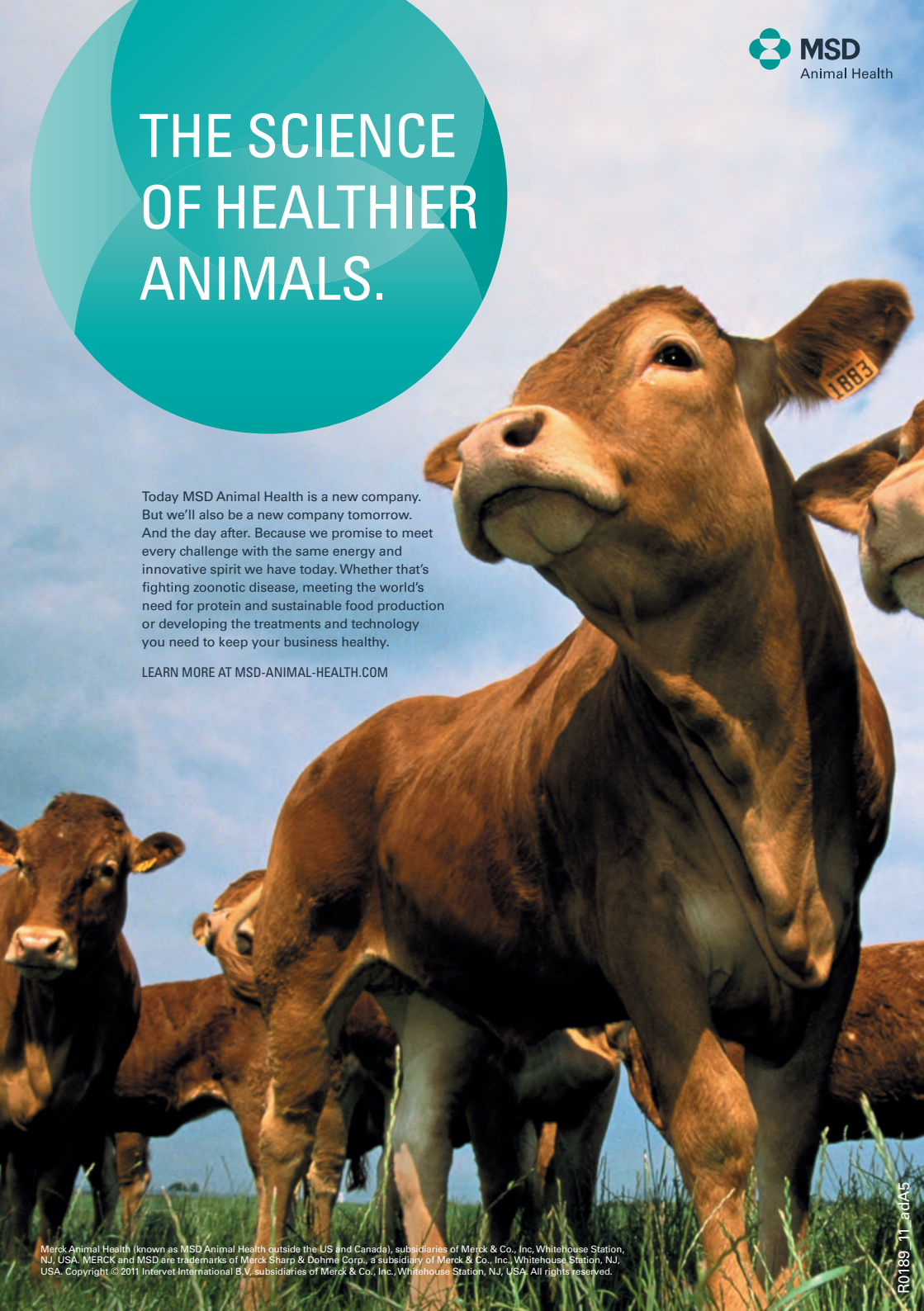
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EPIZONE 6th Annual Meeting "Viruses on the move"

The theme of the 6th Annual Meeting reflects the ever changing virus landscape. This poses continuing and new threats to animal and human health and food security and provides challenges for control of animal diseases. The meeting addresses the latest developments aimed at monitoring and understanding the evolution, emergence, transmission and spread of epizootic viruses. Focus remains on EPIZONE themes aimed at improved disease control through integration and collaboration of research in diagnostics, intervention strategies, risk assessment, surveillance and epidemiology.

Special topics:
"African swine fever and other emerging virus diseases".
African swine fever (ASF) is a devastating, highly fatal disease of pigs which has severe socio-economic consequences in affected countries. The recent spread of ASF to the Trans Caucasus region and Russian Federation emphasises the risks for further global spread and the threat posed to global food security.
"Application of next generation sequencing technologies to virus research".
New developments in sequencing technologies offer enormous potential for virus discovery and diagnosis as well as opportunities for understanding virus, host interactions. This session will review the latest technologies and their applications to virology.

Scientific Committee

Linda Dixon, IAH, United Kingdom
Martin Beer, FLI, Germany
Franz Conraths, FLI, Germany
Markus Czub, Canada
Alex Donaldson, BiovetSolutions, United Kingdom
Elisabeth Erlacher, OIE
Christian Griot, IVI, Switserland
Frank Koenen, VAR, Belgium
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Chris Oura, IAH, United Kingdom
Fuat Özyörük, SAP, Turkey
Dirk Pfeiffer, RVC, United Kingdom
Wim van der Poel, CVI, The Netherlands
Marie-Frederique le Potier, ANSES, France

EPIZONE 6th Annual Meeting Programme

Tuesday 12th June 2012, Old Ship Hotel

09:00 - 17:30 14:00 - 17:00	Young Epizone, CF Meeting Small Meetings	Regency and Boardrooms Fecamp Room
18:00 - 20:00	**Welcome drinks and canapés in Paginini Room**	

Wednesday 13th June 2012, Corn Exchange, Brighton Dome

	Pavilion Theatre	**Founders Room**
08:50 -09:00	**Introduction** Wim Van der Poel, Coordinator, EPIZONE John Fazakerley, Director, IAH	
09:00-09.40	**Keynote 1. Sarah Cleaveland**, University of Glasgow, UK Identifying virus reservoirs in complex ecosystems: new tools for an old problem.	
09:40-10:20	**Keynote 2. Stuart Nichol**. CDC, USA Rift Valley fever: hitting a moving target with a One Health approach	
10:20 - 10:50	**Coffee**	
	Session 1. Epidemiology and emerging diseases **Chairs: Wim van der Poel, Alex Donaldson**	**Session 2. Diagnosis** **Chairs: Martin Beer, Christian Griot**
10:50 -11:05	E&ED- 1: EU FP7 project Anticipating the global onset of novel epidemics [ANTIGONE]: Workpackage 3: Interspecies Barriers for Vector-borne Zoonotic Pathogens **Nick Johnson**, AHVLA, UK	D-1: Validation and establishment of an optimized DNA-microarray system for the non targeted detection of viruses **Bjorn Abendroth**, FLI, Germany
11:05 - 11:20	E&ED- 2: Emergence of three mosquito-borne epornitic Flaviviruses (West Nile Virus, Usutu Virus, and Bagaza Virus in Spain: Challenges for diagnosis and control **Miguel A., Jimenez-Clavero**, INIA, Spain	D-2: Exclusion diagnostics for highly contagious diseases – a new tool for early detection **Barbara Thuer**, IVI, Switzerland

11:20 -11:35	E&ED- 3: An emerging flavivirus has a potential to move from duck to mouse in China **Qiyun Zhu**, LVRI, PR China	D-3: A hand-held platform to detect trans-boundary livestock disease viruses using loop-mediated isothermal amplification (LAMP) **Mikidache Madi**, IAH, UK
11:35 - 11:50	E&ED- 4: BTV8 infections in pregnant cows during late gestation **Giovanni Savini**, IZS, Italy	D-4: Epitope mapping for monoclonal antibodies to the structural protein N of Rift Valley Fever Virus using a peptide phage display approach **G Lorenzo**, CISA/INIA, Spain
11:50 -12:05.	E&ED- 5: A model in sheep and cattle to study the effect of vaccination on vertical transmission of BTV-8 **Mirjam Van Der Sluijs**, MSD Animal Health, Netherlands	D-5: Typing of viral haemorrhagic septicaemia virus (VHSV) by monoclonal antibodies **Niels Joergen Oeleson** VET-DTU, Denmark
12:05 -12:20	E&ED- 6: Detection of Bluetongue outbreak in Smolensk region of Russia in 2011 **Agnesa Panferova**, National Institute of Vet. Virol. and Microbiol., Pokrov, Russia	D-6: Rapid detection of goose circovirus by loop-mediated isothermal amplification (LAMP) **Grzegorz Wozniakowski** NVRI, Poland
12:20 -12:35	E&ED- 7: Bluetongue virus serotype 26 (BTV-26) – evidence for direct contact transmission in goats. **Carrie Batten**, IAH, UK	D-7: EPIZONE: Multicenter study for the comparison of nucleic acid extraction methods **Bernd Hoffman**, FLI, Germany
12:35 -14:00	**Lunch and poster viewing**	
14:00 -14:40	**Keynote 3. James Wood**, University of Cambridge UK. within host evolution of mammalian Influenza viruses	
	Session 3 Epidemiology and emerging diseases **Chairs:**	**Session 4 – Next Generation Sequencing** **Chairs: Don King, ?**
14:45 -15:00	E&ED- 8: Italian H1N2 swine influenza viruses: different evolutionary trends compared to European viruses **Ana Moreno**, IZSLER, Italy	NGS-1: What's in a strain?" Random access next generation sequencing of virus isolates as a diagnostic and quality control tool. **Steven Van Borm**, CODA-CERVA, Belgium
15:00 -15:15	E&ED- 9: A virulent East-European Porcine Reproductive and Respiratory Syndrome virus strain induces pathology and a pronounced immune response **Eefke Weesendorp**, CVI, Netherlands	NGS-2: The first detection of Schmallenberg–virus by metagenomic sequencing and intelligent bioinformatics **Matthias Scheuch**, FLI, Germany

15:15 -15:30	E&ED- 10: Survey of several infectious diseases of zoonotic and epizootic significance in Austrian wildlife **Adolf Steinrigl**, Austrian Agency for Health and Food Safety, Modeling, Austria	NGS-3: Deep sequencing of African swine fever virus (ASFV) from direct field samples in multiple outbreaks in Uganda: implications for the spread and control of ASF **Charles Masembe**, Makerere University, Uganda
15:30 -15:45	E&ED- 11:EPIZONE: Use of veterinary databases within the EPIZONE Network: the metadata core group **Nicola Ferre**, IZS Venezie, Italy	NGS-4: Use of full genome sequences to determine transmission pathways of Foot-and-Mouth Disease Virus during animal-to-animal experimental infection **Begona Valdazo-Gonzalez,** IAH, UK
15:45 -16:00	E&ED- 12: Peste des petits ruminants in European sheep and goats experimentally infected with a highly virulent Kurdish strain **Kerstin Wernicke**, FLI, Germany	NGS-5: Intra and inter-host transmission of FMDV studied at the ultra-deep level through space and time **Caroline Wright**, IAH, UK
16:00 - 17:45	**Poster Session**	
18:00 -23:30	**Pier Fairground 18:00 - 19:30** **Evening Dinner at the Pier 19:30 - 23:30**	

Thursday 14th June 2012, Corn Exchange, Brighton Dome

Time	**Pavilion Theatre**	**Founders Room**
09:00 - 09:40	**Keynote 4. Oliver Pybus,** Oxford University, UK, "Viral evolution: endless forms most probable"	
09:40 -10:20	**Keynote 5. Christian Drosten,** University Bonn, Germany**,** "Bats as Virus Reservoirs"	
10:20 - 10:50	**Coffee**	
	Session 5. African swine fever **Chairs: Dirk Pfeiffer, Marie-Frederique le Potier**	**Session 6. Intervention strategies** **Chairs: Frank Koenen, Alejandro Brun**
10:50 - 11:05	ASF-1: Molecular characterisation of African swine fever outbreak viruses in Africa can assist in ASF control **Juanita Van Heerden,** ARC, OVI, S. Africa	IS-1: Rescued 'serotyped' bluetongue viruses based on vaccine-related BTV6\net08 confer full protection in sheep against virulent BTV8\net06 **Piet Van Rijn**, CVI, Netherlands

11:05 - 11:20	ASF-2: Analysis of low input pig farming in western Kenya in relation to prevalence of infection by an African swine fever virus genotype that does not induce apparent clinical symptoms or a detectable serological response. **Richard Bishop**, ILRI, Nairobi, Kenya	IS-2: Bluetongue virus induces type I IFN in primary plasmacytoid dendritic cells via a MyD88 dependent TLR7/8 independent signalling pathway **Suzana Ruscana**, INRA, Jouey en Josas, France
11:20 - 11:35	ASF-3: Spatio-temporal dynamics of African Swine Fever in Gulu district, northern Uganda **Karl Stahl,** Agricultural University of Sweden, Uppsala, Sweden	IS-3: Sensing and control of Bluetongue virus infection in epithelial cells via RIG-I and MDA5 helicases **Virginie Doceul**, Anses-INRA-ENVA, Maisons Alfort, France
11:35 - 11:50	ASF-4: Incidence of African Swine Fever infection in Sardinia during the 2011: a new epidemic or an old epiphenomenon? **Francesco Feliziani**, IZSUM, Italy	IS-4: Potential application of FMDV non-coding RNAs as immune adjuvants on FMDV vaccination **Belem Borrego** CISA/INIA, Spain
11:50 - 12:05	ASF-5: Detection of specific antibodies in the organs of dead domestic pigs and wild boars after an experimental acute ASF infection and at the infection outbreaks in Russia in 2008 to 2011 **Denis Kolbasov**, National Institute of Vet. Virol. and Microbiol., Pokrov, Russia	IS-5: Foot-and–mouth disease vaccine matching by liquid phase blocking ELISA **Tom Willems**, VAR-CODA, Belgium
12:05 - 12:20	ASF-6: Experimental characterization of the Caucasian African swine fever virus isolate in adult wild boar – confirmation of high virulence **Sandra Blome**, FLI, Germany	IS-6: Morbillivirus V proteins targets multiple components of IFN signalling pathway to control IFN action **Senthil Chinnakannon**, IAH, UK
12:20 - 12:35	ASF -7: African swine fever virus entry by endocytosis. Signaling and cellular targets required for a successful infection. **Covadonga Alonso**, CISA/INIA, Spain	IS-7: Evaluation of the protective potential in lambs of a recombinant MVA vaccine encoding the Rift Valley fever virus glycoproteins **Alejandro Brun**, CISA/INIA, Spain
12:35 - 13:50	**Lunch and poster viewing**	
13:50 - 14:00	**Poster prizes**	
14:00 - 14:40	**Keynote 6 . Carmina Gallardo**, CISA-INIA, Madrid, Spain “African swine fever (ASF) in Africa. The role of the African indigenous pigs in the transmission of the disease".	

	Session 5. African swine fever **Chairs: Chris Oura, Linda Dixon**	**Session 7. Risk Analysis** **Chairs: Elisabeth Erlacher, Franz Conraths**
14:40 - 14:55	ASF -8:African swine fever virus uses macropinocytosis to enter host cells **Elena Garcia**, CBMSO-CSIC-UAM, Madrid, Spain	RA-1: Highly Pathogenic Avian Influenza transmission risks: analysis of biosecurity measures and contact structure in Dutch poultry farming **Amos Ssematimba**, CVI, Netherlands
14:55 - 15:10	ASF -9: DNA immunization as a tool to design and develop future vaccines against ASFV **Fernando Rodriguez,** CReSA, Barcelona, Spain	RA-2: Assessing state and federal responses in USA to simulated introductions of Rift Valley Fever virus **Paul Gibbs**, University of Florida, USA
15:10 - 15:25	ASF -10: ASFV genes interfering with host cellular pathways: Actors for ASFV vaccine development **Yolanda Revilla**, CBMSO-CSIC-UAM, Madrid, Spain	RA-3: Contribution of Foot-and-Mouth Disease virus contaminated environment to the transmission of the disease in calves **Carla Bravo de Rueda**, CVI, Netherlands
15:25 - 16:00	**Coffee**	
16:00 - 16:15	ASF -11: Deletion of two virulence associated genes, DP71L and DP96R, from the genome of the attenuated ASFV strain OURT88/3 reduces protection induced in pigs. **Charles Abrams**, IAH, UK	RA-4: Horizontal transmission of Hepatitis E virus from experimentally infected wild boars to domestic pigs **Josephine Schlosser**, FLI, Germany
16:15 - 16:30	ASF -12: EPIZONE Partial protection of pigs inoculated with an attenuated ASFV strain, OURT88/3, against the highly virulent Georgia 2007/1 isolate **Marie-Frederique le Potier**, Anses	RA-5: EPIZONE – Impact of climate change on risk of incursion of Crimean-Congo Haemorrhagic Fever Virus in livestock in Europe through migratory birds **Paul Gale**, AHVLA, UK
16:30 - 16:45	**Summing up sessions**	
17:00 - 17:15	**Meeting Close** **Drinks reception Schmallenberg Symposium, Thistle Hotel 18.00-20.00**	

KEYNOTE SPEAKERS

KEYNOTE 1 : Identifying virus reservoirs in complex ecosystems: new tools for an old problem

Cleaveland S., Biek R., Matthews L., Hampson K., Lembo T., Haydon D.

Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G12 8QQ

Identifying virus reservoirs is beset by conceptual and practical difficulties that can act as a serious impediment to the rational and efficient management of several major diseases. Even a technical definition of a reservoir is problematic, and developing a more operational framework for the study of reservoirs is a challenge that has been largely neglected. In this presentation, we build from earlier reservoir definitions and frameworks to explore how the advent of new technologies and analytical approaches have re-defined the questions, and provided new opportunities for understanding reservoir dynamics through the analysis of virus sequence data, the application of ecological theory and the implementation of adaptive management approaches. We argue that, in complex ecosystems, reservoir dynamics can best be investigated by drawing on multiple strands of evidence to progressively build an understanding of how different populations contribute to pathogen persistence and transmission. In the face of an incomplete understanding, we discuss how interventions can be designed as part of adaptive management approaches, which can provide health benefits while simultaneously contributing to on-going investigations. The presentation draws on data generated from field epidemiological studies of viral pathogens in East Africa, including rabies, foot-and-mouth disease virus and malignant catarrhal fever.

KEYNOTE 2 : Rift Valley fever: hitting a moving target with a One Health approach

Stuart Nichol

Rift Valley fever is a mosquito-borne RNA virus (family *Bunyaviridae*) which can be associated with outbreaks of severe disease in livestock and humans in areas throughout much of Africa and the Arabian Peninsula. The primary vector is *Aedes* floodwater mosquitoes which can pass the virus transstadially to deposit virus infected eggs ready to hatch on the arrival of wet conditions. Large explosive epizootics usually occur in arid or semi-arid regions experiencing abnormally high rainfall and are often characterized by abortion storms, neonatal animal mortality approaching 100%, and significant mortality (~10 to 20%) among adult ruminant livestock, especially sheep and cattle. Human infection results in self-limiting febrile disease that in ~1-2% of patients progresses to more serious complications including hepatitis, encephalitis, retinitis or a hemorrhagic syndrome with high fatality. Livestock disease usually precedes human disease by a month or more due to the need for virus amplification and high viremias in livestock to facilitate human infections by 1) direct contact with virus infected animals or their tissues or fluids or 2) bite by virus infected secondary mosquito vectors. Epizootic and virus genetic data indicate that the virus has the ability to move over considerable distances, and several outbreaks have occurred outside of the main enzootic sub-Saharan zone. These have included leaps up into Egypt, across the Red Sea into Saudi Arabia and Yemen, and several introductions from the mainland into Madagascar. These observations, together with data demonstrating existence of susceptible mosquitoes in Europe and North America, raise concern about the possible natural or deliberate introduction of the virus into new regions. A live attenuated virus vaccine has been rationally designed and generated using reverse genetics, and has been shown to be safe and highly efficacious in several species including pregnant sheep. Such vaccines have considerable potential for use in a One Health approach to vaccinate livestock to prevent economically devastating disease and at the same time thereby reduce or eliminate human disease – basically hitting two birds with the one vaccine stone.

KEYNOTE 3: Within host evolution of mammalian influenza viruses

James L. N. Wood,

University of Cambridge, Department of Veterinary Medicine , Madingley Road Cambridge

Influenza viruses are characterized by an ability to cross species boundaries and evade host immunity, sometimes with devastating consequences. The 2009 pandemic of H1N1 influenza A virus highlights the importance of pigs in influenza emergence, particularly as intermediate hosts by which avian viruses adapt to mammals before emerging in humans. Although segment reassortment has commonly been associated with influenza emergence, an expanded host-range is also likely to be associated with the accumulation of specific beneficial point mutations. Determining the evolutionary basis of cross-species transmission and immune evasion is key to understanding the mechanisms that control the emergence of either new viruses or novel antigenic variants with pandemic potential. The hemagglutinin glycoprotein of influenza A viruses is a critical host range determinant and a major target of neutralizing antibodies.

We have studied the evolutionary dynamics of two viruses both associated with recent inter-specific transmission. Equine influenza virus (EIV) is a significant pathogen of the horse that causes periodical outbreaks of disease even in populations with high vaccination coverage. EIV has also recently jumped the species barrier and emerged as a novel respiratory pathogen in dogs, canine influenza virus. We studied the dynamics of equine influenza virus evolution in horses at the intrahost level and how this evolutionary process is affected by interhost transmission in a natural setting. To this end, we performed clonal sequencing of the hemagglutinin 1 gene derived from individual animals at different times postinfection. Our results show that despite the population consensus sequence remaining invariant, genetically distinct subpopulations persist during the course of infection and are also transmitted, with some variants likely to change antigenicity. Our data suggest that transmission bottlenecks may not always be as narrow as originally perceived and that the genetic diversity required to adapt to new host species may be partially present in the donor host and potentially transmitted to the recipient host.

Following on from this, and to understand better the mechanisms that shape the genetic diversity of avian-like viruses in pigs, we studied the evolutionary dynamics of an Eurasian Avian-like swine influenza virus (EA-SIV) in naïve and vaccinated pigs also linked by natural transmission. We analyzed multiple clones of the hemagglutinin 1 (HA1) gene derived from consecutive daily viral populations. Strikingly, we observed both transient and fixed changes in the consensus sequence along the transmission chain. Hence, the mutational spectrum of intra-host EA-SIV populations is highly dynamic and allele fixation can occur with extreme rapidity. In addition, mutations that could potentially alter host-range and antigenicity were transmitted between animals and mixed infections were commonplace, even in vaccinated pigs. We also repeatedly detected distinct stop codons in virus samples from co-housed pigs, suggesting that they persisted within hosts and were transmitted among them. This implies that mutations that reduce viral fitness in one host, but which could lead to fitness benefits in a novel host, can circulate at low frequencies.

References:

- Murcia*, P.R., Baillie*, G.J. et al (2010) The intra- and inter-host evolutionary dynamics of equine influenza virus. *Journal of Virology* **84** 6943-6954
- Murcia, P.R. et al (2012) Evolution of an Eurasian avian-like influenza virus in naïve and vaccinated pigs. *PLoS Pathogens* **in press**

KEYNOTE 4: The Evolution and Adaptation of Influenza Viruses in Swine

Oliver Pybus

Swine influenza A viruses cause significant economic losses in animal husbandry, and occasionally give rise to human pandemics, including that caused by the H1N1/2009 virus. Prior to the 2009 pandemic there was a lack of systematic and long-term longitudinal surveillance of influenza in pigs, and a poor understanding of the global evolutionary dynamics of swine influenza, especially in comparison to avian and human influenza viruses. I will outline the results of recent surveys conducted in Europe and Asia that have attempted to address these deficits. Additionally, I will show how new population genetic methods can be used to measure the process of influenza adaptation after cross species transmission of the virus from birds to swine.

KEYNOTE 5: Bats as Virus Reservoirs

Christian Drosten

Institut of Virology, University of Bonn Medical Centre, Bonn, Germany

Contact: drosten@virology-bonn.de

Bats have been found to harbor a number of viruses of significance for other mammals. The cumulative picture obtained from available studies has triggered speculations whether bats might act as viral reservoirs in a in more general sense. I my talk I will try to give examples for some of the main issues under investigation, including the role of population size, population immunity, as well as properties of particular bat-borne viruses focusing on recent studies on paramyxo- and coronaviruses.

KEYNOTE 6: "African swine fever (ASF) in Africa. The role of the African indigenous pigs in the transmission of the disease"

Carmina Gallardo[1], Raquel Nieto[1], Lina Mur[2], Alejandro Soler[1], Virginia Pelayo[1], Richard Bishop[3], Pedro Sánchez-Cordón[4], Carlos Martins[5], José Manuel Sánchez-Vizcaíno[2] and Marisa Arias[1]

[1]EURL for ASF, Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid, Spain, [2]WRL for ASF, Universidad Complutense, Facultad de Veterinaria, Madrid, Spain, [3]International Livestock Research Institute (ILRI), Nairobi, Kenya, [4]Facultad de Medicina Veterinaria, Universidad de Córdoba, Córdoba, Spain, [5] FMVT , Lisbon, Portugal.

African swine fever (ASF) is a complex and lethal viral disease of swine with significant socio-economic impact in the developed and developing world. The disease has a major negative effect on national, regional and international trade and constrains pig production by livestock farmers in affected areas in Sub-Saharan Africa and in the Caucasus region, where the disease was first identified in 2007. Transmission of African swine fever virus (ASFV) can occur in a sylvatic or in a domestic pig cycle, with or without tick involvement. Depending on the presence or absence of wild suids and arthropod vectors and the type of pig production system, the epidemiology varies substantially between countries, regions and continents. In East Africa the disease is maintained by the concurrent existence of different transmission cycles involving asymptomatic wild African pigs (*Phacochoerus* and *Potamochoerus* spp.), soft ticks of the genus *Ornithodorus*, mainly *O. porcinus* and domestic pigs with all the 22 known ASFV genotypes circulating. Moreover, recent investigations in eastern Africa have demonstrated a complex epidemiological situation in porcine local breeds faced to tolerant against ASF combined with a lack of humoral response co-existing with a high viral load. The capacity of the available diagnostic assays to detect ASF specific antibodies with high specificity and sensitivity independent of the viral genotypes circulating in a particular region has been recently demonstrated. So, the complex epidemiology described in East African regions might be related to "tolerant" indigenous pigs and it might reside in the immunogenetics and genetic characteristics of the indigenous pig populations, rather than being due to owner properties of the ASFV strains. This hypothesis was supported by the results obtained in a comparative experimental infection by the inoculation of Kenyan "indigenous pigs" and European domestic pigs using selected ASFV Kenya ASFV isolate belonging to genotype X. A significant delay of onset of ASF in "indigenous pigs" was observed with an unclear, unspecific and non-patognomonic picture of the disease together with a delay in the detectable antibody response. There is evidence that domestic pigs from East African countries such as Kenya exhibit some introgression of genetic material from the Asian centres of wild boar domestication and are therefore not identical to European and West African pig breeds. Consistent with the hypothesis of differences in serological responses between domestic pig breeds, previous findings indicate that the viraemia in bush pigs generally lasted longer than in warthogs in absence of seroconversion. These facts attributable to the phenotype of 'indigenous' African pigs might increase the risk of the endemicity of ASF and virus spreading and thus, difficult the control and success of ASF eradication.

DAY 1

SESSION 1:

EPIDEMIOLOGY & EMERGING DISEASES

ORAL: EU FP7 project Anticipating the global onset of novel epidemics [ANTIGONE]: Workpackage 3: Interspecies Barriers for Vector-borne Zoonotic Pathogens

Johnson, Nicholas[1]; Keummerer, Beate[2]; Papa, Ana[3]; Rudenko, Natasha[4]; Junglen, Sandra[2]; Drosten, Christian[2]; Grubhoffer, Libor[4]

AHVLA[1]; University of Bonn Medical Centre[2]; Aristotle University of Thessaloniki[3]; Biologicke Centrum[4]

Key words: Arthropod-borne, transmission, mosquitoes, ticks, zoonosis

The ANTIGONE project (EU 278976) brings together researchers from across Europe to investigate the factors that contribute to the pandemic potential of zoonotic pathogens. This work is underpinned by a 'chain of emergence' model for the emergence of zoonotic pathogens with primary research being focused on the interspecies barriers, intrahuman barriers and interhuman barriers. One area of interspecies barriers are the environmental, genetic and pathogen-vector interactions of arthropod-borne diseases. This will be investigated by Workpackage 3 "Interspecies barriers for vector-borne zoonotic pathogens".
Diseases caused by arthropod-borne pathogens greatly impact human and animal health, accounting for over 20% of all emerging infectious diseases. Mosquitoes and ticks are considered to be the most important vectors of pathogens that cause human and livestock diseases. This includes a range of flaviviruses (Dengue virus, West Nile virus and Japanese encephalitis virus), bunyaviruses (Rift Valley fever virus) and zoonotic bacterial pathogens, including Borrelia burgdorferi (Lyme disease) and Anaplasma phagocytophilum (human granulocytic anaplasmosis). These are considered the most prevalent tick-borne pathogens in Europe and the USA. The characterization of vector related factors promoting the pandemic potential of vector borne zoonotic pathogens is essential to develop new control strategies for both vectors and transmitted pathogens.
By targeting a number of vector-pathogen models including Crimean-Congo haemorrhagic fever virus / hyalomma ticks, Borrelia burgdorferi / Ixodes spp. ticks, Anaplasma phagocytophilum / Ixodes spp. ticks and Rift-Valley fever virus / Aedes spp., we aim to demonstrate using a combination of field data, experimental infection and transcriptomics/proteomics techniques, identify critical factors that enhance pathogen replication in the vector. The results of this project will (a) expand our knowledge of vector-pathogen molecular interactions, (b) contribute to a better understanding of the emergence and transmission of pathogens with pandemic potential, (c) complement existing genomics resources by creating new databases of vector genes and proteins differentially expressed in response to pathogen infection, and (d) identify new targets for potential interventions for control of vector infestations and pathogen transmission.

ORAL: Emergence of three Mosquito-Borne Epornitic Flaviviruses (West Nile Virus, Usutu Virus and Bagaza Virus) in Spain: Challenges for Diagnosis and Control

Jimenez-Clavero, Miguel A.[1]; Fernandez-Pinero, Jovita [1]; Llorente, Francisco [1]; Villaba, Ruben [2]; San Miguel, Elena[2]; Sanchez, Azucena [2]; Perez-Ramirez, Elisa [1]; Del Amo, Javier[1]; Elizalde, Maia[1]; Aguero, Montserrat[2]

CISA-INIA[1]; Laboratorio Central de Veterinaria[2]

Key words: West Nile virus, Bagaza virus, Usutu virus, Flavivirus, Spain

Last decade has witnessed an upsurge in the incidence and geographic spread of a variety of diseases caused by flavivirus infections transmitted by mosquito bites having wild birds as reservoirs. Relevant examples of these are the worldwide spread of West Nile virus (WNV), Usutu virus (USUV) in Europe, Zika virus in Indian Ocean countries, Tembusu and Baiyangdian viruses in China. Their potential impact not only in animal health, but also as zoonotic agents, is of concern.

In Spain eco-climatic conditions prevailing in wetland areas, where large populations of resident birds and birds migrating between Europe and Africa rest and nest, coexisting with abundant mosquito populations, provide an optimal habitat for the emergence of epornitic flaviviruses. However, these viruses were not reported in the country until surveillance intensification in the early years of this century revealed low but certain flaviviral activity. First evidence of local circulation of one of these viruses came in 2004, when seroconversions to WNV were shown in common coots (Fulica atra) in Southern Spain (1). The first human case of WNV clinical infection also dated from that year (1). The first flavivirus isolation (WNV) came in 2007 from diseased golden eagles in Central Spain (1). In parallel to these sporadic WNV clinical cases, RNA sequences from USUV were first detected in mosquitoes in North-Eastern Spain in 2006 (2), followed in 2008-09 by 7 more WNV and 1 USUV RNA sequences detected in mosquitoes in Southern Spain (3).

In 2010 this situation changed dramatically with the co-ocurrence of two important flavivirus episodes: on the one hand, the first occurrence of WNV outbreaks affecting horses reported in Spain began in late summer in Cadiz (Southern Spain), concurrently with three WNV clinical cases in humans in the same area (1). On the other hand, starting in the same area and at the same time, unusual mortality was observed in game birds (partridges and pheasants), but, contrary to what was expected, WNV was not involved. Instead, a flavivirus new to Europe was detected and isolated in samples of the affected birds: Bagaza virus (BAGV)(4). This virus had only been reported in Subsaharan Africa and India, and is similar to Israel turkey meningoencephalitis virus (ITMEV), which was first described in Israel in late 1950s, and since then sporadically recurs in this country. Besides Israel, ITMEV had only been reported in South Africa in 1980.

The situation above described is of concern. Firstly, because it is completely new, since to our knowledge no concurrent circulation of WNV, USUV and BAGV has been reported before. For this reason, new diagnostic tools have to be developed to enable the differential diagnosis of these three closely related pathogens, not only at the molecular level, where rapid tests allowing an efficient detection and differentiation are needed, but also, and particularly, at the serological level, where existing cross-reactions between flaviviruses make differential diagnosis extremely difficult. Progress made in this regard by our group will be discussed. Secondly, lessons learnt from recent outbreaks of USUV in humans and horses in Italy indicate that the potential of BAGV to over spill from birds and become a pathogen in other species like horses and humans should not be underestimated.

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ORAL: An emerging flavivirus has a potential to move from duck to mouse in China

Huang, Xiaohui[1]; Liu, Zongliang[1]; Ji, Yanhong[1]; Yin, Hong[1]; Cai, Xuepeng[1]; Zhu, Qiyun[1]

LVRI[1]

Key words: duck flavivirus, inter-species transmission, ADE

In China, duck flavivirus (DFV) was first isolated and identified in April 2010 from several duck farms in which the affected ducks were observed a decline of feed uptake, severe ovarian hemorrhage, ovaritis, heavy egg-drop. Hereafter, the outbreak was rapidly spread in most duck-breeding areas in eastern and southern of China. Partial envelope gene alignment showed DFV had a high homology (90.9%) with Tembusu virus from chicken (Sitiawan strain). The phylogenetic analysis indicated that it also kept high correlation with Tembusu strain branch. Until now, it has experimentally proved that the new emerging flavivirus enables to infect different duck species, even geese. However, whether it has the capability to transmissible infect mammals remains largely unknown. Here, we employed a murine model to characterize the biology of DFV. Our data showed that in each infected group, 105TCID50 dosage can result in weight drop at the most extend, and the percentage weight loss was approximately 15%. Viruses were detected in brain, spleen, liver and kidney respectively at day 3 after infection was taken by the intracerebral route. On the other hand, the mice infected by interaperitoneal route were raised until 14 days with no clinical symptoms and detectable antibody, however, the obvious clinical symptoms were observed at day 3 after secondary infection with 104TCID50 dosage by intraperitoneal route. In conclusion, DFV circulating in China has a potential inter-species transmission and an antibody-dependent enhancement role of virus secondary infection.

ORAL: BTV8 infections in pregnant cows during late gestation

Savini, Giovanni[1]; Di Gennaro, Anna Pia[1]; Carvelli, Andrea[1]; Pisciella, Maura[1]; Marini, Valeria[1]; Lorusso, Alessio[1]; Portanti, Ottavio[1]; Scacchia, Massimo[1]

IZS "G. Caporale"[1]

Key words: BTV-8, late gestation, placentar barrier, calves

This study evaluates the ability of two different BTV-8 strains to cross the placental barrier and infect foetuses during late gestation. Two groups of 5 cows each at about 7-8 months pregnancy were infected with BTV-8 European strain and BTV-8 South African Republic strain, respectively. Four 8 month pregnant cows were kept uninfected and used as a control. The animals, stabled in a insect proof facility, were monitored daily for clinical signs and rectal temperature were recorded. Blood and serum samples were collected for six months. Viraemia and neutralising antibody titers were assessed by virus isolation, RT-PCR and serum neutralisation respectively. When abortion or stillbirths occurred, a comprehensive post mortem examination was carried out and colostrums, spleens, brains and foetal cotyledons were tested for the presence of BTV. Newborn calves were monitored serologically and virologically before colostrum intake and blood samples were collected to assess the length of viraemia. Virological and serological data were analysed by using suitable statistical methods. Following infection all infected cows had BTV-8 viraemia and BTV-8 neutralising titers whereas neither viraemia nor neutralising titers were detected in the uninfected cows. At the end of the gestation period 15 calves were delivered, 8 of them did not survive 10 days. The presence of BTV-8 was demonstrated in the blood of 4 calves, two derived from cows infected with the European strain and two from cows infected with the South African strain. All viruses isolated in calves were infectious and capable of growing in tissue culture. The longest viraemia detected in calves was 19 days. In a calf born from a cow infected with the European strain, the viraemia started three weeks after the birth and lasted 14 days. This experiment demonstrates for the first time the presence of infectious BTV in calves born from cows infected with the BTV-8 South African field strain which showed the same transmission rate of the European strain (10%). More studies are needed to explain the three weeks incubation period found in the calf born from a BTV-8 European strain infected cow.

ORAL: A model in sheep and cattle to study the effect of vaccination on vertical transmission of BTV-8

Van Der Sluijs, Mirjam [1]; Schroer-Joosten , Dianne[1]; Fid-Fourkour, Aicha[1]; Vrijenhoek, Mieke[1]; Dufe, Divine[1]; Moormann, Rob[2]; De Smit, Abraham[1]

MSD Animal Health[1]; CVI[2]

Key words: Bluetongue, transmission, sheep, cattle, vaccine

Introduction
Field cases demonstrated that the Bluetongue virus serotype 8 (BTV-8) strain that caused the 2006 European outbreak has an unusually high ability to cross the placenta and infect the foetus. The role of vertical transmission in the epidemiology of BTV-8 remains to be elucidated. Yet, the BTV-8 outbreak demonstrated that the economic importance of vertical transmission of BTV-8 was substantial, due to loss of fertility, abortions and birth of weak or malformed offspring. In previous experiments, a model was set up to study the vertical transmission of BTV-8. Using this model, two experiments were done to investigate the effect of vaccination with a commercial inactivated BTV-8 vaccine on the vertical transmission of virulent BTV-8.

Materials and Methods
Ten mid term pregnant ewes (Experiment 1) or heifers (Experiment 2) were vaccinated with Bovilis® BTV8 in accordance with the manufacturer's instructions. Three weeks after completion of the vaccination schedule, all vaccinated animals were infected with virulent BTV-8 together with ten non-vaccinated pregnant control animals. Four additional pregnant animals received a mock challenge at the same time point. The dams were bled at regular intervals to follow BTV-8 viraemia. Three weeks after the challenge, the foetuses were collected. Blood and organ samples of the foetuses were tested for the presence of BTV by means of virus isolation, RT-qPCR and/or immunohistochemistry.

Results
In the sheep experiment, the lambs of the vaccinated ewes and the mock challenged ewes were negative in the virus isolation, whereas BTV-8 could be isolated from 11/23 lambs of 6/10 ewes in the BTV-8 challenged control group. The incidence and severity of BTV associated lesions, such as haemorrhages, meningitis/encephalitis and necrosis in the placentomes was significantly higher in the BTV-8 challenged control group.
In the cattle experiment, BTV-8 could be detected in 2/10 calves in the BTV 8 challenged control group. The calves of the vaccinated and mock challenged dams were negative for BTV.Viraemia could neither be demonstrated in the vaccinated ewes nor in the vaccinated heifers.

Conclusion
Vaccination blocked vertical transmission of BTV-8 in the sheep experiment. In the cattle experiment, the incidence of transmission was too low to demonstrate a statistically significant reduction of transmission by vaccination. However, the vaccine very effectively blocked viraemia in the dams, which suggests that the vaccine might also prevent transmission in cattle. These experiments demonstrate that vaccination can be an important aid in the reduction of economic losses due to vertical transmission of BTV-8. The experiments also confirmed the usefulness of the model; a model that also could be used to study vertical transmission of other viruses such as border disease virus or Schmallenberg virus.

ORAL: Detection of Bluetongue outbreak in Smolensk region of Russia in 2011

Panferova, Agnesa[1]; Koltsov, Andrey[1]; Novikova, Marina[1]; Tsybanov, Sodnom[1]; Kolbasov, Denis[1]

National institute of vet virology & microbiology[1]

Key words: Bluetongue BTV-14 Smolensk Russia

In September 2011 bluetongue was detected on one farm in Smolenskaya region which is located in the west part of Russia near with the Belarus border. Cattle, imported from Jurgen Greiner Ginsterweg 1 24576 Hagen Germany 29.08.11., was tested in period from 08.08.11 to 20.08.11. by ELISA and PCR in Germany and gave negative results in both tests. Transportation route passed through Poland and Belarus. Since 29.08.11 cattle was quarantined on the farm in Russia. The first sampling was 02.09.11. then samples from 71 animals were tested in NRIVVaM Pokrov by ELISA and PCR with negative results in both tests. Second sampling was 28.09.11. Initially, 5 seropositive cattle were registered by ID Screen Bluetongue Competition ELISA kit (ID Vet, France) within them 4 animals were PCR-positive tested with in-house PCR kit with high Ct values. Then the confirmation of these results was performed using published protocols PCR (Shaw et al., 2007; Toussaint et al., 2007 for segment 5, and Orru et al., 2006) for different genome segments, samples also showed high Ct values in all tests. Sampling was performed periodically with different intervals. The last time samples were taken 26.01.12.Until that time, samples had shown PCR-positive results. Thus, BTV-genome had been present in blood from cattle at least for 4 months. Later we made an attempt to identify the serotype of BTV by serotype-specific PCR as with published protocols for known european serotypes 1, 2, 4, 6, 8, 9, 11 and 16 (Mertens et al., 2007, Vandenbussche et al, 2009, Hoffmann et al.,2009) and by in-house real-time RT- PCR for serotype 1, 2, 4, 8, 9, 16. All attempts to identify serotype virus were unsuccessful. Then the RNAs, extracted from blood, placed on filter-paper were sent to Institute of Diagnostic Virology, Friedrich-Loeffler-Institute, Germany. The results of serotype-specific PCR using primers published by Eschbaumer et al, 2011, and following nucleotide sequencing have shown that virus belongs to BTV-14.
Monitoring data (immediate notification on OIE, WAHID, 30 Dec 2011) based on PCR and ELISA analysis of local (cattle, sheep and goats) and imported cattle indicate that BTV14 circulated in Russia. In January virus was isolated in our institute and BTV-14 was confirm in VNT with reference serum.
Testing of more cattle and sheep samples from the neighboring regions is ongoing and results will be presented.
Up to now, the epidemiology of BTV14 infection is unclear and under investigation.

ORAL: Bluetongue virus serotype 26 (BTV-26) – evidence for direct contact transmission in goats.

Batten, Carrie[1]; Henstock, Mark[1]; Steedman, Hayley[1]; Waddington, Samantha[1]; Edwards, Lorraine[1]; Oura, Chris [1]

IAH[1]

Key words: Bluetongue; contact transmission

In early 2010, bluetongue (BT) was suspected in a sheep and goat flock in Kuwait. Samples were submitted to the Institute for Animal Health, Pirbright for diagnosis. BTV RNA was detected in two ovine blood samples by a segment 10 real time RT-PCR assay (Orru et al., 2006). Virus was isolated from one of the BTV positive blood samples and genome segments 2, 3 and 7, encoding structural proteins VP2, VP3 and VP7 of this virus isolate were sequenced and compared to other orbiviruses, including multiple isolates of BTV. The results of these analyses suggested that the virus was a novel 26th serotype of BTV and showed the closest similarity to BTV-25, which was isolated from goats in Switzerland (Maan et al., 2011). The BTV-26 strain was not however thought to be the primary cause of the clinical signs of disease seen in the sheep and goat flock in Kuwait. In order to investigate the virulence of BTV-26 in sheep and goats two experimental infection studies were carried out. In the first experiment six sheep were infected with BTV-26 and only very mild clinical signs of disease were observed. The levels of viral RNA detected in the samples were lower and of shorter duration than seen with other field strains of BTV (Batten et al, 2012)

In a second experiment 5 goats (GT1-5) were experimentally infected with BTV-26 and one goat (GT6) was left uninfected as an in-contact control. Clinical signs were monitored throughout the experiment and levels of both BTV RNA and antibodies were measured at various time-points using BTV specific real time RT-PCR, antibody-detection ELISA and SNT.

BTV RNA was detected in the blood of all 5 BTV-26 infected goats by real time RT-PCR by 2-7 dpi. BTV RNA levels peaked at high levels (Ct range 19-23) between 9 and 11dpi. Interestingly the levels of viral RNA detected in the goats were considerable higher than those seen in the sheep, indicating that goats may be the natural host. One of the goats (GT3) was euthanized and on post mortem examination no pathological signs were observed, although there was a generalised hypertrophy of lymph nodes indicating that the goat was fighting an infection. No clinical signs were observed in the goats although one goat (GT1) had a slight nasal discharge. Nasal and ocular swabs were taken at 7 and 9dpi from all 5 goats and a low level of viral RNA (Ct 38) was detected in the nasal swabs taken from two of the goats at 9 dpi. All 5 goats seroconverted between 7-11dpi and neutralising antibodies were detected from 14dpi. Interestingly 21 days after the 5 goats were infected with BTV-26 thein-contact control goat (GT6) tested positive for BTV RNA (Ct 38). Further blood samples from this goat tested positive by PCR 3 and 7 days later with low Cts, indicative of an active BTV infection. When the experiment was terminated 28 days after the goats were infected the in-contact control goat (GT6) had seroconverted and a high level of BTV RNA was detected in the blood (Ct 24). Virus isolation on BHK cells was performed throughout the experiment and BTV-26 was isolated from the blood of all 6 goats including the in-contact control goat, however virus was not isolated from the PCR positive nasal swabs.

This experiment provides not only the first evidence for the direct contact transmission of BTV-26, but also the first evidence for the direct contact transmission of any BTV. Future work will investigate further the possible excretion routes of this virus through an experimental infection study planned for May 2012. Preliminary results from this study, if available, will be presented.

SESSION 2:

DIAGNOSIS

ORAL: Validation and establishment of an optimized DNA-microarray system for the non targeted detection of viruses

Abendroth, Björn[1]; Hoffmann, Bernd[1]; Höper, Dirk[1]; Beer, Martin[1]; Dastjerdi, Akbar[2]

FLI[1]; AHVLA[2]

Key words: DNA-microarray, non targeted detection, R-package, Pan Viral Chip

1 Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Riems, Germany
2 Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, KT15 3NB, UK

DNA microarrays are highly parallel biosensors which allow a fast, standardized, simultaneous detection and identification of many different viruses in one or more samples. In this study we used an 8x15k Pan Viral Chip developed within the European Network of Excellence project "EPIZONE" and printed by Agilent. These arrays contain catcher oligonucleotides specifically detecting 1960 different virus families/species.
The array was validated and its sensitivity was determined using two different viruses. On the one hand, we used the classical swine fever virus (CSFV) strain "Kozlov", a positive stranded RNA virus. On the other hand, a double stranded DNA virus (modified Vaccinia Ankara; MVA) was used. The nucleic acid samples (DNA or RNA) were processed prior to hybridization onto the array using three different workflows in comparison.
In order to distinguish between positive and negative signals in the raw data, data were analysed with three different methods in comparison. First, data were processed using the "DetectiV" R-package, initially developed for analysis of the EPIZONE chip data. Second, the well-established "limma" R-package was used for data analysis. Third, Z-score transformation in combination with calculation of the accompanying P-values was done. The results of the different data analysis methods were compared.
With the established optimal combination of sample processing and subsequent data evaluation, a series of different RNA and DNA viruses, including the novel Schmallenberg virus, and numerous sample materials were analysed. It could be shown that the established workflow can successfully be used for the very broad virus detection in cell culture and diagnostic samples.

ORAL: Exclusion diagnostics for highly contagious diseases – a new tool for early detection

Thuer, Barbara[1]; Kaiser, Vanessa[2]; Hadorn, Daniela C[2]

Institute for Virology and Immunoprophylaxis[1]; Swiss Federal Veterinary Office[2]

Key words: highly contagious diseases, early detection, exclusion diagnostics, suspicion

In case of highly contagious animal diseases, early detection of an outbreak is crucial with regard to the prevention of large-area spread by taking sudden disease control measures. One of the critical factors for early disease detection is the time lag between recognition of symptoms in the field and the decision to take samples for laboratory investigations. The legal requirements (animal health ordinance) for the clarification of a suspect case for a highly contagious disease asks for restriction measures, i.e. isolating the holding, even before the case is confirmed through laboratory investigation. It is not surprising that in most cases veterinarians only pronounce a suspicion for a highly contagious disease in advanced or very typical cases. If the laboratory result will be negative, he/she will be blamed for this "wrong" decision and the disposed restriction measures. Consequently, a lot of highly contagious disease outbreaks were diagnosed with a delay of several weeks after the recognition of first symptoms.

A lot of highly contagious disease outbreaks may start with unspecific symptoms. Therefore, veterinarians should get the possibility to take samples for laboratory investigations to routinely exclude highly contagious diseases. One of the goals of the Swiss Animal Health Strategy 2010+ is to support early detection by decreasing the inhibition threshold for laboratory investigations for highly contagious diseases. To do this, Switzerland has implemented a split investigation system of suspicion and exclusion for foot-and-mouth disease (FMD), classical- (CSF) and African swine fever (ASF), avian influenza (AI) as well as Newcastle disease (ND) in spring 2011. If symptoms fulfill defined criteria for these highly contagious diseases, veterinarians are still obliged to inform the cantonal veterinary administration to implement restriction measures already during laboratory investigations. Within the scope of exclusion diagnostics, veterinarians have the possibility to take samples with the idea to exclude FMD, CSF, ASF, AI or ND if unspecific symptoms occur. In the process of exclusion diagnostics, veterinarians have to contact disease specialists from the reference laboratory to discuss the specific sampling. For the laboratory, the split investigation system also has advantages due to an agreement that the results for exclusions have to be available within 2 days after arriving of the samples in the laboratory, instead of 8 hours in case of suspicion. Also different investigation processes for suspicion and exclusions can be used.

Beside that testing is paid by the government, it is crucial for successful implementation of this system that veterinarians have a basic knowledge on highly contagious diseases and know this new possibility to order exclusion diagnostics. Therefore, information campaigns were conducted in spring 2011. Since then, the laboratory investigation for FMD increased from an average of 3 cases per year to already 11 exclusions for 2011. To assess the usefulness of this new system, a short questionnaire will be carried out to investigate the main reasons for the veterinarians having used this system and the critical points to sustain it. We also want to compare the differential diagnoses made and the final diagnoses of the cases to optimize the criteria list for suspect cases. The results of this study will be shown.

ORAL: A hand-held platform to detect trans-boundary livestock disease viruses using loop-mediated isothermal amplification (LAMP)

Madi, Mikidache[1]; Mioulet, Valerie[1]; King, Donald P. [1]; Andreou, Michael P.[2]; Millington, Steve[3]; Das, Priya[4]; Wakeley, Philip R.[4]; North, Sarah E.[4]

IAH[1]; OptiSense Limited[2]; OptiGene[3]; AHVLA[4]

Key words: LAMP; isothermal amplification; diagnostics; field detection; FMDV; ASFV; CSFV

Rapid and accurate diagnostic tools play an important role in monitoring trans-boundary livestock diseases that have high consequence to farming industries. Portable assay formats have the potential to be used by veterinarians during examination of the animals with suspect clinical signs; providing results that can directly contribute to decisions that impact upon local disease control. Real-time PCR (rPCR) or reverse transcription rPCR (rRT-PCR) is a reliable and efficient tool to detect nucleic acid of viruses that cause these diseases such as Foot-and-Mouth Disease (FMD), African Swine Fever (ASF) and Classical Swine Fever (CSF). However, the use of rPCR in the field is limited by the requirement for complex and expensive equipment. Isothermal assays such as Loop-mediated isothermal AMPlification (LAMP) offer a simple alternative to PCR-based methods. Detection of specific nucleic acid sequences occurs at a single temperature and DNA products can be detected using an intercalating dye. The aim of this study was to evaluate a prototype hand-held isothermal platform (Genie III from OptiGene) for the detection of viruses that cause FMD, ASF and CSF. The Genie III is a compact, easy-to-use and portable instrument that takes a single strip of eight tubes for the isothermal amplification of DNA or RNA with highly-sensitive fluorescence detection. The device is battery powered, sealed (enabling outdoor operation), incorporates GPS for positional sensing and has wireless connectivity through Bluetooth and WiFi. Previously developed LAMP (and RT-LAMP) assays for the detection of FMDV, ASFV and CSFV were tested using both the Genie III and a lab-based real-time machine (Mx3000P, Stratagene) and the results compared. Using a serial dilution series of a representative sample from a clinical case (FMDV and ASFV) or nucleic acid (CSFV), the limit of detection for these LAMP assays were equivalent between the hand-held and laboratory based platforms. The variability of results obtained on the Genie III was assessed by repeat testing of CSFV RNA samples (n=5). These data indicate that it is possible to detect both DNA (ASFV; Asfavirus) and RNA (FMDV: Picornavirus, CSFV; Flaviviruses) templates using a highly portable machine that can perform LAMP and RT-LAMP assays, respectively. This equipment offers a simple (and potentially low-cost) approach for rapid and sensitive detection of important pathogens that cause livestock diseases.

ORAL: EPITOPE MAPPING FOR MONOCLONAL ANTIBODIES TO THE STRUCTURAL PROTEIN N OF RIFT VALLEY FEVER VIRUS USING A PEPTIDE PHAGE DISPLAY APPROACH

G, Lorenzo[1]; E, Lopez[1]; A, Brun[1]

CISA-INIA[1]

Key words: epitope, phage display, antibodies, immunoassays

Rift Valley fever (RVF) is a viral zoonosis that affects domestic ruminants as well as humans and is transmitted mostly by Aedes sp and Culex mosquitoes. This virus is endemic in Africa and spread outside the continent to Arabian Peninsula, Madagascar and Comoros archipelago. Furthermore, the presence of numerous species of potentially competent mosquito vectors in North America and Europe, the susceptibility of many mammalian species to RVFV infection, the global changes in climate, travel and trade confirm the potential of this virus to emerge in other countries. Development of more effective methods for RVFV outbreak prevention and control remains a global health priority.

Serological diagnosis assays are based in the antibodies detection against the viral nucleoprotein through ELISA assays. N protein is the most abundant component of the virion and is highly immunogenic producing high titers of specific antibodies in the host. The role of these antibodies is still unknown; they could be involved in evasion mechanism against the host immune response.

In this work, we have produced monoclonal antibodies (mAbs) following different immunization strategies of mice against N protein and used them for epitope mapping by means of a peptide phage display approach. A region comprised between residues 60-80 was identified. Truncated recombinant proteins of N including or not this region were generated and expressed in insect cells infected with baculovirus. All mAbs recognized the truncated protein including this region confirming the previous results obtained by phage display peptide library.

The region identified in this work was also recognized by hyperimmune sera suggesting that this region includes immunodominant epitope(s) present in the N protein.

N mutants were generated by substitution of the most conserved residues located in this region. Immune sera and mAbs showed differential reactivity against these mutants allowing the fine mapping of critical residues influencing the immunogenicity of the protein. Thus, the epitope identified here could be helpful for designing new diagnostic immunoassays more specific, cheap and simple against RVFV.

ORAL: Typing of viral haemorrhagic septicaemia virus (VHSV) by monoclonal antibodies

Olesen, Niels Jorgen[1]

VET-DTU[1]

Key words: VHS, fish rhabdovirus, monoclonal antibodies, molecular tracing

Viral haemorrhagic septicaemia (VHS) is a serious disease occurring in wild and farmed fish in the Northern hemisphere. Until the nineteen eighties the disease was believe only to cause severe mortalities in farmed rainbow trout in Europe. In the last decades however the causative agent VHS virus (VHSV) have been isolated from more than 80 fresh- and seawater fish species in North America, North East Asia and Europe. Some of the findings were linked to severe die offs, especially in the pacific North America and in the Great Lakes in USA and Canada.
VHSV isolates can be divided into 4 major genotypes and 10 subtypes with an almost distinct geographical distributions .The host range and the pathogenicity appear, at least to some extent, to be linked to the genotype. The genotypes are identified, based on sequencing of full-length and/or truncated genes from the viral G- and N-gene. In order to develop more rapid and cost efficient methods 7 mAbs with specific reaction patterns against each of the 4 genotypes and 7 subtypes of VHSV were produced, aiming at establishing a complete immunoassay for typing VHSV isolates according to their genotype. The development and validation of immunoassays for discriminating between 4 genotypes and 10 subtypes of VHSV using a panel of 9 mAbs is thus described in this work. The epitope specificity was assessed for most of the mAbs and by combining pathogenicity traits with amino acid sequences and mAb reactions we were able to point at putative pathogenicity markers on the viral genome showing mAbs as strong tools for studies of functional genomics and molecular epidemiology.

ORAL: Rapid detection of goose circovirus by loop-mediated isothermal amplification (LAMP)

Wozniakowski, Grzegorz[1]; Samorek-Salamonowicz, Elzbieta[1]

NVRI[1]

Key words: goose circovirus, rapid detecion, LAMP

Goose circovirus (GCV) presents an immunosuppressive threat in massive production of geese and ducks. The clinical symptoms during GCV infection include growth retardation and feathering disorders but may also remain non-symptomatic what makes the infected birds more susceptible for secondary infections. Diagnosis of GCV include histopathological examination, dot blot hybridization, polymerase chain reaction (PCR) and real-time PCR. However since these techniques require application of thermocyclers and qualified staff they may be not accessible for small laboratories. The aim of this study was to develop loop-mediated isothermal amplification assay (LAMP) as a simple method of GCV detection.
The conducted study has shown LAMP as a rapid tool detecting DNA of GCV as soon in 30 min time. The results were analysed using SYBR Green dye and GelRedTM solutions. LAMP detected the presence of GCV DNA in thirty-seven examined samples. The obtained results have shown GCV-LAMP as a sensitive, rapid and specific assay. The developed technique due to its simplicity may be applied by any veterinary laboratory or even mobile diagnostic units.

ORAL: Multicenter study for the comparison of nucleic acid extraction methods

Hoffmann, Bernd[1]; Beer, Martin[1]

FLI[1]

Key words: nucleic acid, extraction, ring trial

In context with the deliverables in the work package 4.1 real-time PCR diagnostics a nucleic acid ring trial was performed. The aim of the ring trial was a multicenter study for the comparison of the different extraction methods used in the labs. For a standardized read out all extracted samples were analysed by real-time PCR at the Friedrich-Loeffler-Institut. In total 12 samples of a single stranded RNA virus (CSFV) in serum, 12 samples of a double-stranded RNA virus (BTV) in EDTA-blood and further 12 samples of a DNA virus (pCV-2) in organ homogenate were submitted.
The task of the participating labs was the extraction of the viral RNA/DNA by in maximum two different procedures in duplicates. In general automated or manual extraction methods as well as different systems could be used (e.g. silica membrane-based or magnetic-beads based methods). Thus, three deep-well plates with in total 48 eluats each were prepared by the participants and were transferred to the Friedrich-Loeffler-Institut on dry ice. Here the data about the different extraction methods used in the several labs were summarised and based on validated real-time PCR assays the absolute quantification of the extracted RNA/DNA was performed.
The results of the extraction procedures used in the 12 participating labs will be presented. More than 15 different extraction kits from 6 companies were used for the viral genome extraction of CSFV, BTV and pCV-2. Although the most kits produce very qualified and comparable results some methods delivered a reduced efficacy for the nucleic acid extraction of RNA virus or DNA virus or both. Suboptimal extraction kits could be identified for manual as well as automated procedures and kits. Furthermore not all extraction kits delivered optimal results in all sample matrices used. Based on the produced data of the multicenter study a ranking of the analysed extraction kits for the different matrices will be presented.

SESSION 3:

EPIDEMIOLOGY AND EMERGING DISEASES

ORAL: Italian H1N2 swine influenza viruses: different evolutionary trends compared to European viruses

Moreno, Ana[1]; Sozzi, Enrica[1]; Lelli, Davide [1]; Foni, Emanuela[1]; Chiapponi, Chiara[1]; Zehender, Gianguglielmo[2]; Alborali, Loris[1]; Cordioli, Paolo[1]

IZSLER[1]; Ospedale Luigi Sacco[2]

Key words: H1N2 swine influenza viruses; times of the most recent common ancestor; phylogenetic analysis; Italy

In the swine population, three subtypes (H1N1, H1N2 and H3N2) are currently worldwide spread. Unlike human influenza, swine influenza viruses (SIVs) differ depending on the continent of origin. The H1N2 SIVs currently circulating in Europe derive from multiple reassortant events involving human H1N1, "human-like" swine H3N2 and "avian-like swine" H1N1 influenza viruses (IVs). This subtype emerged in UK, spread subsequently to pigs on the European mainland and was first isolated in Italy in 1998. Genetic reassortment between different IVs is considered one of the generating mechanisms for novel virus strains with pandemic potential for humans. To better understand the genomic evolution of the European H1N2 SIVs, we investigated the genomic characterization of 26 Italian strains isolated from 1998 to 2010. Full-length sequences of genes encoding for HA and NA were characterised and phylogenetic analysis was carried out including sequences of swine, human and avian IVs retrieved in genBank. DNA sequences were edited using Lasergene software, multiple sequence alignments were made using ClustalW. and then phylogenetic trees were constructed with Maximum Likelihood method (GTR+I+Γ4 model of base substitution)using MEGA5 software. To estimate times of the most recent common ancestor (tMRCA) and rates of nucleotides per sites per year, we applied a relaxed-clock Bayesian Markov chain Monte Carlo method as implemented in BEAST v1.6.1 selecting the uncorrelated log-normal clock and a constant population size demographic model. Phylogenetic analysis of the HA and NA genes showed a clear difference between the older strains (1998-2003) and the more recent ones (2003-2010). The older isolates were closely related to the established European H1N2 lineage, whereas all the more recent isolates, except two, showed a new HA-NA combination. This was characterized by a HA gene showing two aa deletions at positions 146-147 and a completely different NA deriving from the human H3N2 IVs isolated in 1997. Up to now in Europe these new reassortant strains apparently have been reported only in Italy. Three other reassortant H1N2 strains have been detected: 1-A/sw/It/22530/02 with the HA gene closely related to H1N1 viruses; 2- A/sw/It/ 58769/10 an uncommon reassortant strain with a HA closely related to H1N1 and a NA similar to H3N2 SIVs; 3-A/sw/116114/2010 with all genes except NA belonging to the H1N1pandemic IVs and NA closely related to A/sw/It/ 58769/10.The highest rate of evolution was observed in the HA segment (4,17x103 substitutions/site/year; 3,14-5,26 x 103 95%HPD) whereas in NA it was 3,79x103 (3,14-5,26 x 103 95%HPD). Based on these evolutionary rates, our tMRCA estimations showed that a European H1N2 precursor acquired in a first reassortment event the NA gene from an old human H3N2 IVs in 1978 (1974-1982 95%HPD) and later the HA gene from the human H1N1 IVs in 1986 (1982-1990 95%HPD). The older Italian strains were closely related to the European H1N2 viruses and originated from the same common ancestor. Furthermore the tMRCAs estimated for the recent strains suggest a common ancestor circulating around 2000 (1998-2002 95%HPD - HA; 1999-2002 95%HPD - NA) having a HA gene derived from two Italian strains of 1998 (with the two aa deletions) and a NA of recent human H3N2 origin. These results highlighted the different evolutionary trend of the recent Italian strains compared to circulating European viruses, showing the presence and establishment of reassortant strains involving human viruses in pigs in Italy.

ORAL: A virulent East-European Porcine Reproductive and Respiratory Syndrome virus strain induces pathology and a pronounced immune response

Weesendorp, Eefke[1]; Stockhofe, Norbert[1]; Rebel, Annemarie[1]

CVI[1]

Key words: PRRSV, immunology, virulent European strain

Introduction and objectives
Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive losses in breeding pigs and respiratory disease in pigs of all ages. The disease is endemic in most parts of the world. PRRSV is difficult to control due to a high mutation rate and the emergence of virulent strains. The objective of this study, which is part of the EU PoRRSCon Project, is to analyse the immunological and pathological response after infection with a virulent East-European PRRSV strain in comparison to a lower virulent European strain.

Materials and Methods
An animal trial was performed with three groups of sixteen pigs. In group 1, pigs were infected with the virulent East-European type PRRSV strain Lena. In group 2, pigs were infected with the European reference strain Lelystad (LV), that gives mostly subclinical infections. In group 3, the control group, pigs were inoculated with PBS. At days 7 and 21 after inoculation (p.i.), 4 pigs per group were immunized with an killed Aujeszky disease (ADV) vaccine to study the immune competence of pigs after PRRSV infection. Samples were collected for antigen detection, antibody responses, IFN-y ELISPOT assay and FACS analysis for the identification of the lymphocyte sub-populations. At days 3 and 7 p.i., four pigs per group were euthanized for post-mortem examination. At day 35, eight pigs per group (four immunized and four non-immunized) were euthanized for post-mortem examination. Several tissues were collected for immunohistochemical analysis and cytokine detection by PCR.

Results
Infection with the Lena strain resulted in fever of 11 days and clinical symptoms. No fever or clinical symptoms were observed in the Lelystad-infected pigs. Infection with the Lena strain resulted in high infectious virus titres, low numbers of IFN-γ secreting cells, a distinct change in cell populations in blood and a delayed response to immunization with ADV compared to the LV strain. Pathology showed a high pneumonia score in the Lena-infected pigs at day 7 p.i,, which was reduced to the same level as LV at day 35 p.i Histopathology showed an peak in (broncho)interstitial inflammatory cell infiltration for the Lena-infected pigs at day 7 p.i, but in both strains at 35dpi. PCR analysis on lung and lymphoid tissue showed that the Lena-infected pigs cleared the infection from lung and lymph node at day 35 p.i., while viral RNA was still detected in the lung and lymph node of the LV-infected pigs. In the lung a higher induction of IL-1β, IL-10 and TNF-α mRNA of the Lena-infected pigs at day 7 p.i. was observed compared to LV-infected pigs.

Discussion and conclusion
In this study, the pathogenicity and immune response of two European type PRRSV strains were compared. The strains induced different effects on the immune response., Lena strain infection resulted in higher replication than LV in pigs and a pronounced effect on immune parameters which resulted in viral clearance in tissues at the end of the experiment. The LV strain induced less effect on the immunological parameters, but viral RNA was not cleared from the tissues at the end of the experiment while the lung pathology increased compared to initial phase of infection (day 7 p.i.). We hypothesized that the less virulent LV strain does not induce a high immune response and therefore virus persist.

ORAL: Survey of several infectious diseases of zoonotic and epizootic significance in Austrian wildlife

Steinrigl, Adolf[1]; Bago, Zoltan[1]; Revilla-Fernandez, Sandra[1]; Hofer, Erwin[1]; Duenser, Michael[2]; Wodak, Eveline[1]; Hofrichter, Johannes[3]; Deutz, Armin[4]; Schmoll, Friedrich[2]; Steinrigl, Adolf[5]; Bagó, Zoltán[5]; Revilla-Fernández, Sandra[5]; Hofer, Erwin[5]; Duenser, Michael[5]; Wodak, Eveline[5]; Hofrichter, Johannes[5]; Deutz, Armin[6]; Schmoll, Friedrich[5]

Institute for Veterinary Disease Control[1]; AGES Institute for Veterinary Disease Control[2]; AGES Data, Statistics and Risk Assessment[3]; Bezirkshauptmannschaft[4]; Austrian Agency for Health and Food Safety[5]; Bezirkshauptmannschaft Murau[6]

Key words: wlldlife, zoonoses, epizootic

Introduction: The significance of wildlife reservoirs for a number of infectious diseases is increasingly acknowledged. Possible zoonotic threats to humans may arise through direct or indirect contact with wild animals or their products, for example in the course of hunting, or by consumption of undercooked game meats. In addition, notifiable diseases of domestic animals may remain undetected in wildlife for extended periods, posing the risk of spillover to domestic animals. Finally, monitoring of infectious diseases in wildlife can be regarded as an early warning system for emerging infectious diseases, as wildlife is roaming uncontrolled and is naturally exposed to a number of arthropod vectors that can transmit infectious diseases. Despite their potential significance, infectious diseases in wildlife are poorly represented in the scientific literature, probably due to difficulties to sample wild animals in a representative manner. Thus, the aims of the work presented here were (1.) to implement a representative sampling strategy and (2.) to estimate prevalences of selected infectious diseases in several wild animal species in Austria.

Material and methods: A survey design was worked out and implemented in cooperation with the Austrian Hunters' Association, with the aim to representatively sample red fox (Vulpes vulpes), red deer (Cervus elaphus) and wild boar (Sus scrofa), based on recent hunting bag statistics for these species. Foxes and red deer were tested for Mycobacterium tuberculosis complex (MTBC), red deer was additionally tested for Mycobacterium avium ssp. paratuberculosis (MAP). Wild boar were tested for Brucella suis, Suid herpesvirus 1 (SuHV-1) and Classical swine fever virus (CSFV). In addition, wild boar sera were tested for the presence of antibodies to SuHV-1 and CSFV.

Results: In total, 318 red foxes, 275 red deer and 298 wild boars were sampled from April 2011 to January 2012, corresponding to a response rate of 84%, 72% and 78%, respectively. Prevalences for surveyed infectious disease agents were: MTBC in red fox: 0.0%; MTBC in red deer: 0.5%; MAP in red deer: 1.6%; Brucella suis in wild boar: 1.0%; SuHV-1 in wild boar: 1.0%; CSFV in wild boar: 0.0%. Prevalences of antibodies to SuHV-1 and CSFV were 22.8 and 0.0%, respectively.

Discussion: Despite mild weather conditions in autumn 2011 that complicated efficient hunting, response rates were close to (red deer) or exceeding (wild boar, red fox) 75% of the scheduled sample sizes for the whole country. However, locally, certain areas were clearly underrepresented, as some hunters refrained from submitting the requested number of samples. Nevertheless, this survey is the first reliable estimate of selected disease prevalence in Austrian wildlife and provides important information regarding possible transmission scenarios between wildlife and domestic animals.

ORAL: Use of veterinary databases within the EPIZONE Network: the metadata core group

Ferré, Nicola [1]; Manca, Grazia[1]; Meindl-Böhmer, Alexandra[2]; Staubach, Christoph [3]; Nativi, Stefano[4]; Cristalli, Alessandro[1]; van der Poel, Wim [5]

IZS-Ve[1]; HVS[2]; FLI[3]; Centro Nazionale Ricerche[4]; CVI[5]

Key words: metadata, INSPIRE, data harmonization, interoperability, data policy

Introduction: The EPIZONE metadata core group (EPIZONE-MD) is a group of scientists which originated under Theme 2 (Strategic Integration) to make a concrete contribution to the regulation and specification of information discovery and access in the veterinary field. In particular, the group intends to define good practices for the veterinary scientific community of the EPIZONE network and beyond on how to best describe, publish, discover and access veterinary datasets. This will improve proper data sharing within the veterinary community, contribute to multidisciplinary applications, and ensure the sustainability of the numerous databases related to research projects while avoiding duplication of information. Nowadays many datasets are indeed available online, but it is often difficult to discover and evaluate the most suitable ones for a specific application. Discovery, evaluation, and use are empowered by providing formal data description (i.e. "data about a data"), the so-called “metadata”. Metadata formally provides crucial information, such as data purpose, location, category, content, lineage etc.. The aim of the present paper is to describe the approach introduced by the EPIZONE-MD to define the discovery of metadata.
Methodology: The key strategy adopted by the EPIZONE network is to apply the process and guidelines of the INSPIRE Directive (2007/2/EC) to the veterinary community. The INSPIRE Directive establishes an infrastructure for sharing spatial information in Europe enabling interoperability and, where practicable, harmonisation of data sets and services within Europe. To develop a preliminary trial on discovery of metadata, implementation and publication, a pilot project was started. This was also useful to assess the suitability of the INSPIRE guidelines for veterinary data description. Two different tools for resource description (metadata editor) have been evaluated by two EPIZONE-MD members: the European Open Source Metadata Editor (EUOSME) and the INSPIRE Geoportal Metadata Editor.
Discussion: Metadata for discovery is the first step for description of a dataset and allows datasets to be found by relevant criteria (i.e. query clauses), to be identified and evaluated. Using the INSPIRE specification for discovery, some lessons were learned; for examples, some extensions must be clearly considered. In fact, the currently available semantic terms and keywords are unsuitable for the veterinary areas.
Conclusions: It is acknowledged that there are extensions to be considered before implementing an INSPIRE-based metadata approach for the EPIZONE community. For this reason EPIZONE-MD will promote the development of a white paper on the implementation of good practices for metadata. Another task of EPIZONE-MD is to outline the action plan necessary for full implementation of a metadata catalogue according to open standard specifications. Finally, it is important to take into account that metadata warrants data accountability: for consumers, it is the label required to assess available data, while, for producers, it is the official declaration of data ownership and formally clarifies the “responsibility chain” in data recording and management. This is crucial to ensure transparent re-use of data amongst the veterinary community and to bring data together for public health purposes (i.e. emergencies or surveillance networks, as foreseen by European legislation). It also requires appropriate data policies.

ORAL: Peste des petits ruminants in European sheep and goats experimentally infected with a highly virulent Kurdish strain

Wernike, Kerstin[1]; Eschbaumer, Michael[1]; Breithaupt, Angele[1]; Wiesner, Henning[2]; Maltzan, Julia[2]; Mustefa, Regir[3]; Arif, Fakher A.[4]; Beer, Martin[1]; Hoffmann, Bernd[1]

FLI[1]; Academy for the Protection of Zoo and Wild Animals[2]; Mirga Sur[3]; Herman Inst. for Research,Communication,Culture[4]

Key words: Peste des petits ruminants; Experimental infection; Emerging diseases

Peste des petits ruminants (PPR) is a contagious viral disease of sheep and goats that is characterized by high fever, thick discharge of eyes and nostrils, severe eye infections, ulcers in the oral mucosa as well as severe diarrhea. Like the rinderpest, distemper and measles viruses, PPR virus (PPRV) is a morbillivirus of the Paramyxoviridae family. PPRV occurs all over West and Central Africa, the Middle East and Southern Asia. Its high morbidity and mortality can have a devastating impact on agricultural communities in developing countries, and the disease can pose a threat to the survival of endangered species of wild ruminants. A lineage IV strain of PPRV was responsible for mass fatalities among wild goats in Kurdistan in 2010/11 (Hoffmann et al., Transbound Emerg Dis 59[2]:173-6); virus isolated from a dead Kurdish goat was used for this experiment.
Three sheep and three goats of German domestic breeds were subcutaneously inoculated with 10^4 TCID50 of virus (isolated from a swab sample on CV1/SLAM cells, passaged once on Vero/SLAM). Sheep and goats were kept in separate rooms. Three uninfected sheep and goats, respectively, were housed together with the inoculated animals. After inoculation, clinical signs and body temperature were recorded. Whole blood, serum, nasal, oral and fecal swabs were taken every other day. Seven days after inoculation one sheep and one goat were euthanized and necropsied. The remaining goats were euthanized on days 12, 17, 18, the sheep 20 days after infection.
The first clear clinical signs appeared within 4 to 5 days of inoculation. Eventually, all inoculated animals, all in-contact goats and two out of three in-contact sheep developed high fever (up to 41.7 °C) and depression, severe diarrhea, ocular and nasal discharge as well as ulcers on the gums, palate, cheeks and tongue. Clinical signs were more pronounced in goats; five out of six goats had to be euthanized for humane reasons.
The viral RNA content in samples was measured by real-time RT-PCR. In blood samples of the inoculated animals, viral RNA was most abundant between 4 and 6 days after inoculation, but it remained detectable until the end of the study. In the two in-contact goats PPRV RNA was found for the first time on day 10, in the third goat on day 12. Two in contact sheep were positive from days 12 and 16, respectively. In blood samples of the last sheep, PPRV RNA was not detected at any time. Viral RNA was also present in nasal, oral and fecal swabs of all animals whose blood was PCR positive. On average, the viral load in caprine samples was higher than in ovine. In the inoculated animals, viral RNA detection in blood preceded the detection in swabs, while the opposite was true for the in-contact animals.
The animal experiment showed that the 2011 Kurdish strain of PPRV is highly virulent in goats and spreads easily to in contact animals, while disease severity and contagiosity in sheep are slightly lower. PPR is a prominent transboundary disease with a high potential impact for small ruminants in the European Union. Further research into its epidemiology and prevention is of vital interest to European agriculture.

SESSION 4:

NEXT GENERATION SEQUENCING

ORAL: “What’s in a strain?” Random access next generation sequencing of virus isolates as a diagnostic and quality control tool.

Van Borm, Steven[1]; Lambrecht, Bénédicte[1]; van den Berg, Thierry[1]; Vandenbussche, Frank[1]; Rosseel, Toon[1]

VAR-CODA[1]

Key words: next generation sequencing. avian paramyxoviruses. random access amplification

Novel sequencing technologies allow high quality analysis of the variability of viral genomes, and are increasingly being implemented to study viral evolution, epidemiology, and genomics with a resolution allowing novel insights. In addition, these technologies may in the near future become embedded in the diagnostic toolbox of virology laboratories. Using two case studies, we illustrate the added value of random access deep sequencing approaches in the virology laboratory. First, unidentified hemagglutinating agents from wild ducks were characterized using random deep sequencing. Avian influenza and Newcastle disease were excluded using real-time RT-PCR and classical virology. Our approach identified three different avian paramyxoviruses (APMV). From one pooled sample, the complete genome sequence (15054 nucleotides) of an APMV-4 was assembled from the random sequences. From the second pooled sample, the nearly complete genome sequence of an APMV-6 (genome size of 16236 nucleotides) was determined, as well as a partial sequence for an APMV-4. This APMV-4 was closely related but not identical to the APMV-4 isolated from the first sample. In a second case, we used random deep sequencing for the characterization of complete genomes of 11 pigeon variants of Newcastle disease (pigeon paramyxovirus type 1; pPMV-1). The approach not only yielded high quality pPMV-1 genomic information (>90% of the genome sequenced for all isolates), but also identified a contamination of several laboratory isolate stocks with a variety of pigeon circoviruses and documented sequence polymorphisms in the pPMV-1 viral populations analyzed. These studies illustrate the value of randomly generated next generation sequencing information for quality control of reference virus isolates, generation of high quality genomic information allowing detection of minority sequence variants, and for the characterization of samples that remain unidentified using routine virus-specific diagnostic approaches.

ORAL: The first detection of Schmallenberg–virus by metagenomic sequencing and intelligent bioinformatics

Scheuch, Matthias[1]; Höper, Dirk[1]; Beer, Martin[1]

FLI[1]

Key words: Schmallenberg–virus, metagenomic sequencing, bioinformatics

The first detection of Schmallenberg–virus by metagenomic sequencing and intelligent bioinformatics

Matthias Scheuch, Dirk Höper, Martin Beer
Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Suedufer 10, 17498 Greifswald-Insel Riems

Next generation sequencing (NGS) enables the comprehensive sequencing of genomic material from diverse samples, regardless of source and composition. The output of the different NGS technologies differs in the mean read length and read quantity. Irrespective of the sequencing technology, the metagenomic datasets consist of some tens of thousands up to millions of single sequence reads. These large datasets pose a challenge for the diagnostic metagenomic approach because the decisive information may come from only a single read that must be identified amongst the information overload of non-pathogen related reads.
Therefore, we developed an automated workflow for reliable classification of every single read into the different taxa. For an optimized performance, i.e. for the fast and accurate processing of the huge datasets, we rely on a combination of different alignment algorithms such as assemblies and mappings implemented in the Genome Sequencer software and NCBI BLAST tools. First, the data quantity is reduced based on organisms detected within contigs assembled from data subsets. Secondly, different BLAST variants assign remaining sequences. Finally, all detected organisms are listed in the result protocol grouped according to the taxonomy. The complete analysis of a dataset of approximately 100.000 reads usually requires now only 30 to 120 minutes. The input file format of the raw data is not limited to standard flowgram files (sff) but fasta-formatted sequences (if available accompanied by the fasta-formatted base qualities) can also be analysed. The workflow will be made available as a web-service soon.
Using the workflow described above for the analysis of the metagenome data from cattle suffering from an undiagnosed disease last summer and fall, we detected seven orthobunyavirus reads with approximately 69% homology to Akabane virus amongst approximately 27.000 reads in the first analysis within one hour. These reads provided the initial hint for the identification of the Schmallenberg-virus.

ORAL: Deep sequencing of African swine fever virus (ASFV) from direct field samples in multiple outbreaks in Uganda: implications for the spread and control of ASF

Masembe, Charles[1]; Muhangi, Denis[1]; Ndyanabo, Suzan[1]; Ademun Okurut, Anna Rose[2]; Michuki, George[2]; Onyango, Maria[3]; Rumberia, Cecilia[3]; Bishop, Rchard[3]; Djikeng, Appolinaire[3]; Kemp, Steven[3]; Skilton, Robert[3]; Stahl, Karl[4]

Makerere University[1]; Ministry of Agriculture[2]; International Livestock Research Institute [3]; The Swedish University of Agricultural Sciences[4]

Key words: African swine fever virus, next-generation sequencing, pig, genotype IX

Uganda has the largest and most rapidly growing pig production in Eastern Africa, with the pig population at 3.2 million. This pig industry is threatened by African swine fever (ASF), which is a devastating viral disease that is endemic in Uganda. The disease periodically kills 90 – 100 percent of affected animals and has neither treatment nor vaccine. Molecular characterization of this virus is routinely done on cell-cultured material using conventional consensus sequencing. In this paper we use the 454 Roche Next-generation sequencing approach on clinical field materials to determine to determine viral diversity in an individual samples, and relationships between different ASF outbreaks based on the P54 and P72 genes. The clinical samples were collected from the districts of Gulu, Moyo, Mpigi and Mityana. The ASF sequences belonged to genotype IX. The number of sequence reads per individual sample per gene varied from 1000-5000. There were no significant variants within individual samples. However there were fewer (5%) nucleotide differences among Ugandan ASFV across time and space; and more (50%) when compared to the rest of ASF sequences from other parts of the world. These results show the absence of multiple strains within the various epidemics in the country and confirm the presence of a single genotype during recent ASF outbreaks in Uganda. The low genetic variation and lack of genetic differentiation in space and time between outbreaks has important implications on the spread and control of ASF.

ORAL: Use of Full Genome Sequences to Determine Transmission Pathways of Foot-and-Mouth Disease Virus During Animal-to-Animal Experimental Infection

Juleff, Nicholas[1]; Valdazo-González, Begoña[1]; Paton, David J. [1]; Wadsworth , Jemma [1]; Wright , Caroline[1]; Charleston , Bryan [1]; King , Donald P. [1]; Knowles, Nick J. [1]

IAH[1]

Key words: FMDV full-genome-sequencing animal-to-animal experimental infection

Generation and analysis of full genome sequences was used to trace the origin and transmission pathways of foot-and-mouth disease virus (FMDV) outbreaks in the United Kingdom in 2001 and 2007. This analysis was also used to predict undisclosed FMDV-infected premises. The aim of this current work was to determine the number and nature of nucleotide substitutions which occur during animal-to-animal transmission under controlled experimental conditions. For this purpose, two cattle were inoculated with a cell culture-adapted (heparin sulphate binding) virus strain (O1/BFS 1860/UK/67). Each animal was used to separately initiate a contact-infected transmission chain involving four subsequent cattle. In vivo virus replication rapidly selected for variants with wild type (integrin) cell binding, but no further evidence for pre-programmed patterns of genome change was found. The genetic diversity generated over the course of infection was sufficient to enable reliable tracing of transmission pathways at the level of the individual animal. Moreover, there was a clear divergence when FMDV was transmitted through parallel groups of cattle. However, oesophageal/pharyngeal scrapings taken from one animal in each group at various times post infection (2, 4, 6 and 32 days) contained more diverse virus populations which were not seen in samples taken from epithelial lesions. These data support the use of full genome sequence analysis for tracing future FMDV epidemics and have implications for the correct interpretation and resolution of the transmission pathways of the infection.

ORAL: Intra and Inter-Host Transmission of FMDV Studied at the Ultra-Deep level through Space and Time

Wright, Caroline[1]; Morelli, Marco[2]; Knowles, Nick[1]; Juleff, Nicholas[1]; Paton, David[1]; King, Donald[1]; Haydon, Daniel[3]

IAH[1]; Instituto Italiano di Tecnologia[2]; University of Glasgow[3]

Key words: FMDV Ultra-Deep Transmission

Given the potential for viral genetic variability generated at the intra-host scale to impact upon the long-term evolutionary dynamics of RNA viruses, the depth at which this variability is transmitted, at the inter-host scale, is poorly understood. This knowledge gap has primarily been a consequence of the limited resolution afforded by viral sequencing techniques to effectively dissect the heterogeneous nature of infection within a single host. This study exploited the greater resolution afforded by next-generation sequencing (NGS) (1) to investigate the evolutionary dynamic between intra and inter-host transmission of foot-and-mouth disease virus (FMDV), both 'horizontally' through time and 'vertically' between sample types. The samples were collected during an infection experiment, which included three direct contact transmission events between bovine hosts, and analysed on the Genome Analyzer platform (Illumina). A protocol that identified artefacts introduced during amplification and sequencing was developed, which enabled the validation and quantification of the minority sequence variants that were detected. By identifying not only consensus level mutations but minority variants present at frequencies greater than 0.5%, NGS was able to reveal patterns of genetic changes over time within an infected host as well as evidence for variation in bottleneck size between the intra and inter-host scales. Ultra-deep sequence data gathered from multiple sample types (serum, oesophageal-pharyngeal scraping and epithelium) also elucidated to the potential presence of sub-populations within infected individuals. By linking viral populations observed within hosts to those observed between hosts, NGS has shown itself to be a valuable tool in working towards the development of a more sophisticated understanding of how viral genetic differences accumulate with time.

1. Wright CF, Morelli MJ, Thebaud G, Knowles NJ, Herzyk P, Paton DJ, Haydon DT, King DP. 2011. Beyond the consensus: dissecting within-host viral population diversity of foot-and-mouth disease virus by using next-generation genome sequencing. J. Virol. 85:2266-2275.

DAY 2

SESSION 5:

AFRICAN SWINE FEVER

ORAL: MOLECULAR CHARACTERISATION OF AFRICAN SWINE FEVER OUTBREAK VIRUSES IN AFRICA CAN ASSIST IN ASF CONTROL.

van Heerden, Juanita[1]; Malan, Kerstin[1]; Heath, Livio[1]

Agricultural Research Council[1]

Key words: ASF, sequencing, p72, p30

African swine fever was first described in Kenya in the 1920s as an acute haemorrhagic fever which causes mortality approaching 100% in domestic pigs, but subsequently identified in southern, central and West Africa. There is no vaccine available for the control of ASF, there does not seem to be any prospect of having one in the short term and perhaps not even in the medium term, therefore the conventional control relies on declaration of an infected area, imposing quarantine, a movement ban and stamping out all the pigs in the infected area with destruction of the carcasses by deep burial or burning. Reasons why the disease is difficult to control after it spreads in certain areas include late detection, insufficient funds to compensate owners of pigs, political instability, free-roaming animals, uncooked swill feed, illegal movements due to desperation of poor people who would do anything to get money for their remaining pigs, selling of infected meat, ticks and wild pig reservoirs, co-circulation of various genotypes and failure to identify risk factors. In Africa, ASF has potentially devastating effects on the commercial and subsistence pig production sectors, but the greatest losses are usually inflicted on the poorer pig producers who are less likely to implement effective prevention and control strategies or basic bio-security. Uncontrolled ASF occur in most countries where up to 90% of pigs are farmed at subsistence level. Eradication of wildlife is not acceptable and eradication of tampans is impossible, therefore control of ASF is important. The presentation of different clinical forms of ASF and similarity with other pig diseases, including classical swine fever virus (CSFV), means that diagnosis based on clinical signs is not straightforward and laboratory confirmation is recommended. Differentiation of virus strains is done by nucleotide sequencing corresponding to a 478 bp region of the C-terminal end of the p72 gene, encoding for the major capsid protein, VP72. ASF is divided into 21 genotypes suggesting that isolates originating from East and southern Africa may, to some extent, differ antigenically from isolates found in West Africa and Europe. The ARC-OVI-Transboundary Animal Diseases Programme (TADP) is the designated OIE reference laboratory for ASF in southern Africa. As part of the mandate of the OIE reference laboratory, diagnosis and characterisation of ASF outbreaks are performed for SADC member states, and other African countries. TADP obtained samples since 1954 from various countries including South Africa, Kenya, Portugal, Malawi, Mozambique, Zimbabwe, Spain, Zaire, Angola, Malta, Dominican Republic, Brazil, Namibia, Cameroon, Zambia, Burundi, Belgian, Holland, Botswana, Benin, Burkino Faso, Gambia, Ghana, Mauritius, Nigeria and Senigal. The phylogenetic analysis based on the CP204L gene sequences of various isolates indicated that the variability between maximum divergent East African isolates is significantly higher than that of the southern or West African isolates. Characterisation of the ASF viruses has indicated the transboundary nature of the disease. It has also demonstrated the importance of molecular characterisation in tracing the origins of ASF outbreaks which could assist in regional disease control using p72. However, p30 sequences do not always cluster according to the same genotypes indicated by p72. The p30 sequences can indicate possible recombination between strains from different regions. To be able to understand the origin of ASF and the nature of ASF infectivity better, more research are needed on virulent and avirulent genotypes of ASF.

ORAL: Analysis of low input pig farming in western Kenya in relation to prevalence of infection by an African swine fever virus genotype that does not induce apparent clinical symptoms or a detectable serological response.

Bishop, Richard[1]; Gallardo, Carmina[2]; Lekolool, Isaac[3]; Macharia, Joseph[4]; Arias, Marisa [2]; Bulimo, Wallace[5]; Okoth, Edward[1]

ILRI[1]; CISA-INIA[2]; KWS[3]; Department ofVeterinary services[4]; University of Nairobi[5]

Key words: ASF prevalence; p72 genotype X; Antibody response; Bushpig

We describe a study conducted in Homa bay district in Western Kenya in which key parameters of a free-range,low input pig management system were characterised in relation to viral prevalence Rapid Rural Appraisal techniques were used to collect data from 44 and 39 randomly selected pig-rearing households, respectively. In addition, serum, blood and tissue samples were collected from 74 and 69 domestic pigs from the farms at two sampling points, respectively and 8 bush pigs from the neighbouring Ruma National Park. The samples were analysed using the OIE serological and nucleic acid-based prescribed diagnostic techniques. All farms practised free range/tethering feed management, but supplementary feed was also provided in the form of crop residues and household food waste. The animals sampled exhibited no detectable clinical signs of ASF. Analysis of blood and serum samples using a PCR assay demonstrated 24%(±10) and 25%(±11) PCR positivity to ASFV, in the two independent samplings but no animals were sero-positive using the OIE indirect ELISA. The farms that contained ASFV positive pigs were located in divisions bordering the park from which bush pig-farmer conflict had been reported. Pigs from farms in the divisions located further away from the park were PCR negative. One of the bush pigs sampled from the park, from which tissues were obtained was PCR positive. Dendrograms based on the sequence of the p72 and p54-genes of domestic pig and bushpig viruses indicated that domestic and bush pig viruses clustered genetically. Similarly, the predicted amino acid sequence of the tetrameric repeats that constitute the central variable region (CVR) of the B602L gene also exhibited a high degree of identity between the two species. These results suggest a common source of infection or viral transmission between the two pig species in this study.

ORAL: Spatio-temporal dynamics of African Swine Fever in Gulu district, northern Uganda

Aliro, Tony[1]; Tejler, Emma[2]; Muhangi, Denis[1]; Ndyanabo, Suzan[1]; Boqvist, Sofia[2]; Ademun Okurut, Anna Rose[3]; Masembe, Charles[1]; Emanuelson, Ulf[2]; Stahl, Karl[2]

Makerere University[1]; Swedish University of Agricultural Sciences [2]; Ministry of Agriculture[3]

Key words: African swine fever, spatio-temporal, virus, pig, outbreak

Uganda has the largest and most rapidly growing pig industry in eastern Africa, with the pig population currently at 3.2 million. This pig industry, however, is threatened by African swine fever (ASF), a devastating viral disease that is endemic in the country, with high morbidity and mortality, and with no treatment or vaccine. Gulu district in northern Uganda is slowly recovering after 25 years of rebel insurgency. Pig farming is among the fastest growing livestock activities as communities resettle back to their villages, and pigs have become an immediate source of cheap protein and income for families. However, uncontrolled influx of pigs into the district and the free range system of management have predisposed to outbreaks of infectious diseases, and during the last 15 months we have confirmed ASF as cause of high mortalities in 14 out of the 16 subcounties in the district with very sever impact on the poor rural communities. To better understand the dynamics of ASF and to clarify major modes of transmission between the domestic pigs in the district, we carried out a study of the spatial and temporal dynamics of the disease during the current outbreak. All villages where ASF had been reported and confirmed were visited, affected farmers were identified through local leaders, farms were geo referenced and outbreak data was collected through questionnaires, and surviving pigs were sampled. Forty-one villages with history of confirmed outbreaks of ASF were visited and 211 affected farmers were identified and interviewed. Estimated outbreak duration ranged between a few weeks to a couple of months, possibly with multiple outbreaks in some villages. 1134 deaths and 251 survivors were recorded, giving an average estimated mortality in the affected villages of 82%. The interviews revealed movement of live infected pigs and trade of meat from diseased pigs as the major routes of introduction to naïve villages, and several chains of transmission were identified. For within village spread the free-range system was considered a major factor, as well as the tradition to sell meat at local butchers from animals that have died from disease. This paper will present the results from the study, the implications thereof, and discuss the way forward.

ORAL: Incidence of African Swine Fever infection in Sardinia during the 2011: a new epidemic or an old epiphenomenon?

Feliziani, Francesco[1]; Rolesu, Sandro[2]; De Mia, Gian Mario[1]; Patta, Cristiana[2]

Istituto Zooprofilattico Umbria e Marche[1]; Istituto Zooprofilattico Sardegna[2]

Key words: ASF, Sardinia,

African swine fever (ASF) is a devastating viral disease of pigs. Described since 1921 in Sub-Saharan African regions, ASF virus (ASFV) moved first to Europe, Central and South America, and, more recently (2007), into Caucasian countries and the Russian Federation. ASF was successfully eradicated from the Americas and Western Europe, but not in Sardinia, where the disease has been notified since 1978. Although a rigorous EU-supported eradication program is carried out in Sardinia since 1993, ASF outbreaks are still reported on annual basis in the island. This program has probably failed because of certain factors which assured the persistence of infection such as extensive farming system with nil or insufficient biosecurity measures, contact between wild boars and domestic pigs, illegal production/trade of pigs, and use of waste as feed.

On the basis of ASF incidence trend, we can distinguish 4 different phases of the disease:

1. first phase (1978-1995); the measures applied were ineffective and the infection spread in wide regional territory
2. endemic phase (1996–2003); because the application of an efficient surveillance plan the outbreaks number/year decreased
3. epidemic phase (2004–2005); two epidemic waves occurred: the first one (248 outbreaks) mainly involved the Nuoro province, whereas in second one (198 outbreaks) the Oristano province was the territory most affected
4. ipo-endemic phase (2006–2010); after the emergency faced in the period 2004-2005, during the 2006 no outbreaks were registered and, in the following years a very low number of outbreaks occurred; only sporadic cases were described in wild boar population.

During the second semester of the 2011, 34 outbreaks occurred, not only in the Nuoro province, considered as the actual reservoir of the infection, but scattered in the rest of the regional territory. This changing appears as a new crucial shift and it seems as the beginning of a new phase of the disease. Considering these data few questions are rising: how to consider the current epidemiological situation? Is the increasing number of cases an indicator of a new epidemic evolution of the disease? Moreover, are the provided measures adequate to control the spread of the infection?

In this epidemiological situation, the first evidence is that the 2011 outbreaks are not only concentrated in the historical high risk area, because the infection was notified in different part of the region. Furthermore it seems that the outbreaks were reported without evident link in spatial sense as well as in the temporal one and, in fact, the majority of them should be considered as primary case of infection.

In general, the epidemiological investigations, conducted by the public veterinary service during the eradication of ASF outbreaks, are inconclusive (in terms of tracing back investigations) and the source of infection is frequently classified as unknown. This means that the primary surveillance is not completely effective in Sardinia and not able to stem the spreading of the infection. Conversely the secondary surveillance, considered as the measures carried out to avoid the spread of infection from a primary outbreak, seems more efficient.

ORAL: Detection of specific antibodies in the organs of dead domestic pigs and wild boars after an experimental acute ASF infection and at the infection outbreaks in Russia in 2008 to 2011

Kurinnov, Viktor[1]; Belyanin, Sergey[1]; Vasiliev, Alexander[1]; Strizhackova, Olga[1]; Lyska, Valentina[1]; Nogina, Irina[1]; Balyshev, Vladimir[1]; Tsybanov, Sodnom[1]; Mironova, Ludmila[2]; Chernuikh, Oleg[3]; Kolbasov, Denis[1]

National institute of vet virology & microbiology[1]; Rostov regional laboratory[2]; Krasnodar regional laboratory[3]

Key words: ASF antibodies Russia

Detection of specific antibodies against ASF virus in porcine organ tissues and/or sera has been recommended by OIE for detection of the infection at its chronic or subclinical course. As a result of experimental infections of both domestic swine and wild boar with field isolates that had caused ASF outbreaks in Russia in 2008, 2009, 2010 and 2011, an acute form of the infection course was determined. On days 8 and 13 p.i. in parallel with detection of virus-specific DNAs using a polymerase chain reaction (PCR) and antigens with a Fluorescent Antibody Test (FAT), in the same organs of the dead animals (namely, lymph nodes and spleen) some specific antibodies against ASF virus were found using Indirect Fluorescent Antibody (IFA), with the titers ranging from 5.3 (2.8 to7.8) to 8.0 (6.5 to 9.4) log2, respectively.

To investigate the specific antibodies in the field, some pools of the extracts of 20% suspensions of samples collected from 178 dead domestic pigs at ASF foci on some small, medium size, or large industrial farms), and also from 51 wild boar animals found dead or (in rare cases) hunted in the Caucasus and/or in the Southern, the Central, and the North-West Federal Districts, were analyzed using IFA. From the epizootological data obtained on the farms, the time periods from a suspected case to its death was 5 days (ranging from 3 to 7 days), symptoms and post-mortem alterations being characteristic of acute or subacute forms of the disease. The virus presence in these samples was confirmed using two methods (i.e., PCR and FAT). For the animals examined, the specific antibodies were observed in 49 (40 to 57)% of the domestic pigs, and in 34 (2 to 66)% of the wild boars. The annual fluctuations for the amounts of pigs having specific antibodies to ASF virus in organs (with the denominator showing the number of pigs tested due to outbreaks per year) were 55% in 2008, 43% in 2009, 45% in 2010, and 51% in 2011 for domestic swine, and 13% in 2008, with an increase in the rate to 22% in 2009, 56% in 2010, and 46% in 2011 for wild boar. The specific antibody levels both in domestic swine and in wild boar ranged within 4.3 to 9.0 log2. However, the analyses of as many as 5056 domestic swine serum samples collected in the infected zone (namely, the Southern Federal District) failed to detect any ASF virus specific antibody.

The detection of ASF virus antibodies in significant amounts of infected & dead animals, both domestic swine and wild boar (presumably following an acute or subacute) course of the infection), should be not only attributed to the mechanisms of the infection natural development, but also be regarded as a risk factor for selection of a circulating virus followed by a subsequent spread of a virus having a moderate virulence. Also, permanent estimations and understanding of the diagnostic significance of virological and serological tests in the future remain important.

ORAL: Experimental characterization of the Caucasian African swine fever virus isolate in adult wild boar – confirmation of high virulence

Blome, Sandra[1]; Gabriel, Claudia[1]; Dietze, Klaas[2]; Breithaupt, Angele[1]; Beer, Martin[1]

FLI[1]; FAO[2]

Key words: African swine fever virus, Caucasian isolates, adult wild boar, experimental characterization

African Swine Fever (ASF) is one of the most important and complex diseases affecting pigs. The causative agent, African swine fever virus (ASFV), is a large, complex DNA virus of the genus Asfivirus within the Asfarviridae family. In 2007, ASF was reported in the Caucasus region where it concerned both domestic pigs and wild boar. Up to now, ASF keeps occurring on the territory of the Russian Federation, and the constant spread imposes a growing risk of introduction into free areas with a high pig and/or wild boar density. Recently, it could be shown that the Caucasian ASFV isolates are highly virulent for wild boar piglets and sub-adults (Gabriel et al., 2011). An almost peracute disease with severe but unspecific clinical signs was accompanied by 100 % mortality within approximately one week post inoculation. Based on these data, an endemic scenario driven by chronically diseased animals or carriers seemed unlikely. Nevertheless, ASF keeps occurring also in wild boar. As several infectious diseases show an age dependence of clinical courses with less severe signs in older animals, the above mentioned studies were supplemented with a limited study in adult wild boar to help clarifying their role in ASF epidemiology (Blome et al, 2012, in press).
To this means, one boar (10 years), two sows (4 and 5 years, respectively), and an additional boar piglet (0.5 years) were orally infected with 3 x 106 TCID50 of the Caucasian ASFV isolate (spleen homogenate in cell culture medium). All animals developed severe, unspecific clinical signs (fever, depression, anorexia, respiratory distress, ataxia) starting three to four days post inoculation. Infection was confirmed by real-time polymerase chain reaction of blood and swab (oronasal and fecal) samples taken at day 6 post infection and at the day of euthanasia. All inoculated animals died or were euthanized in a moribund state between days 8 and 9 post infection confirming high virulence and 100% mortality also for adult European wild boar. No antibodies were detectable by commercially available antibody enzyme-linked immunosorbent assays in serum samples throughout the experiment. Based on the available data, there is no indication that adult wild boar could show chronic infections or carrier states, potentially contributing to long-term persistence in an affected region. Nevertheless, factors like dose dependence of clinical courses need further attention. In terms of a risk assessment, spillover from infected domestic pigs, carcasses left under favorable climate conditions and feeding with contaminated swill remain most likely important suspects introducing ASFV into wild boar populations.

ORAL: African swine fever virus entry by endocytosis. Signaling and cellular targets required for a successful infection.

Cuesta-Geijo, Miguel Angel[1]; Galindo, Inmaculada[1]; Hernaez, Bruno[1]; Munoz-Moreno, Raquel[1]; Quetglas, Jose I.[2]; Alonso, Covadonga[1]

CISA-INIA[1]; University of Navarra[2]

Key words: African swine fever virus; Entry; Endocytosis; Cellular targets; Unfolded protein Response

African swine fever virus (ASFV) enters the cells by receptor-mediated endocytosis. This process has been shown to be clathrin-mediated and dependent on the GTPase dynamin. Once the virus has entered the cell, it hijacks the endocytic machinery for infection. We have analyzed the dependence of ASFV infection on the integrity of the endosomal pathway to determine that incoming viral particles traverse the early endosome (EE) to enter the cytosol during the first minutes of infection. Endosomal acidification in the first hour after virus entry was essential for successful infection but not thereafter. We characterized the relevance of small GTPases of Ras superfamily, especially Rho, Rac1 and Rab GTPases in these early infection stages. Also, infection was dependent on specialized endosomal membrane lipids involved in the signaling and maintenance of the endocytic pathway. Phosphoinositides turnover was essential both for early endosome and multivesicular body (MVB) maturation. We found that processes related to late endosomal (LE) compartment physiology were crucial at early infection. Also, other cellular targets related with unfolded protein response were relevant for subsequent infection steps. Our data demonstrate that the EE, MVB and LE compartments and the integrity of the endosomal maturation pathway play a central role during early stages of ASFV infection while transcription factors overexpressed at the unfolded protein response are crucial during late infection. Inhibition of some of these targets severely impairs ASFV infection.

ORAL: AFRICAN SWINE FEVER VIRUS USES MACROPINOCYTOSIS TO ENTER HOST CELLS

Sanchez, E.G.[1]; Quintas, A.[1]; Perez-Nuñez, D.[1]; Nogal, M.[1]; Barroso, S.[1]; Revilla, Y.[1]; G.Sanchez, Elena [2]; Quintas, Ana[2]; Perez-Nuñez, Daniel[2]; Nogal, Marisa[2]; Barroso, Susana[2]; Revilla, Yolanda[2]

Centro de Biología Molecular Severo Ochoa[1]; Centro de Biología Molecular Severo Ochoa-CSIC[2]

Key words: ASFV, entry, macropinocytosis, kinases

ASF is caused by a large and highly pathogenic DNA virus, African swine fever virus (ASFV), which causes severe economic losses and environmental threats. No vaccine against ASF is available so far, despite of the high hazard that the continued occurrence of ASF in Africa, the recent outbreak in the Caucasus, and potential dissemination represents.

Although virus entry is a remarkable target for development of protection tools, knowledge of the ASFV entry mechanism is still very limited. Whereas early studies have proposed that ASFV enters to cells through receptor-mediated endocytosis, what the specific mechanism is used by ASFV remains uncertain. Here we used the isolate Ba71, adapted to grow in Vero cells (Ba71V) and the virulent strain E70, to demonstrate that entry and internalization of ASFV includes most of the features of macropinocytosis. We show that after addition to the cells, the virus causes cytoplasm membrane perturbation, blebbing and ruffles. We also find that internalization of ASFV virions depends on actin reorganization, activity of Na+/H+ exchangers, and signaling events typical for macropinocytic mechanism of endocytosis. The entry of virus into cells directly stimulates dextran uptake and actin polarization, likewise PI3K-Akt, Pak1 and Rac1 activation. Inhibition of these key regulators of macropinocytosis, as well as treatment with the drug EIPA, results in considerable decrease in ASFV entry and infection.

In conclusion, this study identifies for the first time the whole pathway for ASFV entry, including the key cellular factors required for the uptake of the virus and the cell signaling involved.

ORAL: DNA immunization as a tool to design and develop future vaccines against ASFV

Lacasta, Anna[1]; Ballester, Maria[2]; Accensi, Francesc[1]; Argilaguet, Jordi M[2]; López-Monteagudo, Paula[2]; Gallardo, Carmina[3]; Salas, Maria Luisa[4]; Escribano, Jose M[5]; Lepotier, Marie-Frederique[6]; Rodriguez, Fernando[7]

CReSA[1]; CReSA (UAB-IRTA)[2]; CISA-INIA[3]; CBMSO[4]; INIA[5]; Anses[6]; CReSA (UAB-IRTA[7]

Key words: EPIZONE, ASFV, VACCINE, CTL

African swine fever (ASF) is a highly infectious disease affecting domestic pigs, which has to be immediately reported to the OIE. ASF virus (ASFV) remains endemic in Sardinia and in many Sub-Saharan countries, where it causes tremendous economical lost. The most recent reintroduction of the virus in Georgia from Eastern Africa and its spreading toward Russian counties, Iran and EU borders has opened new concerns about the risk of ASFV re-entrance to the rest of Europe and Asian countries, including China, the major swine producer and consumer in the world. The situation becomes aggravated by the fact that there is no vaccine available against ASFV, limiting the control measures to an efficient and rapid diagnosis of the disease and culling of the infected animals. Therefore, developing safe and efficient vaccines against ASF is a must. With this objective in mind and using DNA immunization as a tool, we have been able so far to obtain the following results:

i) Fusion of the extracellular domain of the ASFV Hemagglutinin (sHA) to p54 and p30, two immunodominant structural viral antigens, exponentially improved both the humoral and the cellular responses induced in pigs after DNA immunization.

ii) However, immunization with the resulting plasmid (pCMV-sHAPQ) did not confer protection against the lethal challenge with the virulent E75 ASFV-strain.

iii) Immunization with pCMV-UbsHAPQ, a plamid encoding the three viral determinants above mentioned (sHA, p54 and p30) fused to ubiquitin, specifically improved Class I antigen presentation, enhanced the CTL responses induced and more important, protected a proportion of immunized-pigs from lethal challenge with ASFV.

iv) The protection was afforded in the absence of antibodies, correlating with the expansion of CD8+ T-cells, specific against two SLAI restricted CD8 epitopes mapping within sHA and with demonstrated protective capabilities

v) DNA immunization with a cocktail of plasmids (around 4,000 individual clones) containing random fragments of the ASFV genome (covering 80% of the total size but excluding clones encoding p30, p54 and HA fragments) as fusions with ubiquitin, protected 60% of the immunized pigs from lethal challenge with ASFV; Protection rates that were also maintained when immunizing SPF-pigs

vi) Immunization with seven individual clones identified from the central region of the ASFV genome increased the protection afforded by pCMV-UbsHAPQ against the lethal challenge from 33 to 50% of the immunized pigs.

Our results definitively confirm the relevance of T-cell responses in protection against ASF and open new expectations for the future development of more efficient recombinant vaccines against this disease.

ORAL: ASFV GENES INTERFERING WITH HOST CELLULAR PATHWAYS: ACTORS FOR ASFV VACCINE DEVELOPMENT

G. Sánchez, Elena[1]; Nogal, Marisa[1]; Gallardo, Carmina[2]; G. Granja, Aitor[1]; deLeon, Patricia[1]; Bustos, MJose[1]; Hurtado, Carola[1]; Quintas, Ana[1]; Soler, Alejandro[2]; Martins, Carlos[3]; Arias, Marisa[2]; L.Carrascosa, Angel[1]; Revilla, Yolanda[1]

CBMSO-CSIC-UAM[1]; CISA-INIA[2]; Faculdade de Medicina Veterinária[3]

Key words: ASFV, viral genes, vaccine

ASFV is a large DNA virus that infects monocytes/macrophages of different species of suids, causing the acute and frequently fatal ASF. Infection by ASFV is characterized by the absence of a neutralizing immune response, which has so far hampered the development of a conventional vaccine. The absence of vaccine is likely due to the fact of the virus deploys a variety of strategies to evade the host's defence systems, such as inflammatory and immune responses and cell death. In this regard, we have previously shown that ASFV protein A238L inhibits the expression of the inflammatory genes cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and tumour necrosis factor alpha (TNF-α). We have reported that serine 384, in the amino-terminal transactivation domain of the transcription coactivator p300, is essential in the A238L-mediated control of the inflammatory response, thus indicating that this signalling pathway is disrupted by A238L and opening the possibility of manipulate ASFV-mediated cell activation. We have also shown that ASFV displays other sophisticated mechanisms of regulation, such as the control of apoptosis through the expression of specific anti apoptotic and regulatory viral genes, such as A224L and EP153R (two anti apoptotic genes), p53 activation and host translational machinery. Besides, EP153R has been shown to inhibit the expression of MHC-I antigens in the plasma membrane, thus modulating the host immune response against ASFV.

A number of attenuated models lacking of these specific genes have been generated to be used "in vivo" as attenuated viruses with vaccine potential. Groups of 4 pigs were infected with the corresponding ASFV, parental attenuated (NHV) or deletion mutant (DM-A238L, DM-A224L or DM-EP153R strains) and one group were maintained as control. Clinical signs (daily), viremia, antibody response and virus isolation were checked at different times during one month, and animals challenged with the virulent (genotype I) isolate L60. A 100% of survival rate was scored with all of the attenuated virus models generated in this study except those belonging to the pig group used as control of the L60 infection. A second virulent challenge was performed in a small number of animals with the heterologous virulent (genotype II) Arm07 isolate. The observations indicated that a high or partial protection was achieved in the pigs inoculated with parental NHV or DM-A224L and DM-EP153R respectively, while an acute form of ASF was observed in the DM-A238L that was apparently more virulent than that scored in the control Arm07-infected pigs that all die. These results indicate that A238L gene is relevant to induce an in vivo protection mechanism. Thus, not all of the selected ASFV genes should be manipulated by deletion to obtain an attenuated strain, but, as in the case of A238L, it is likely that an increased expression of the virus gene should be preferred in order to reduce the virulence of the parental ASFV.

ORAL: Deletion of two virulence associated genes, DP71L and DP96R, from the genome of the attenuated ASFV strain OURT88/3 reduces protection induced in pigs.

Abrams, Charles[1]; Chapman, Dave[1]; Goatley, Lynnette[1]; Netherton, Chris[1]; Golding, Josephine[1]; Fishbourne, Emma[1]; Cooke, Lyndsay[1]; Takamatsu, Haru[1]; Dixon, Linda[1]

IAH[1]

Key words: African swine fever virus, vaccine, virulence genes

African swine fever virus causes an economically important haemorrhagic fever in pigs which can result in very high mortality. The lack of a vaccine limits options for control. The genotype I attenuated ASFV strain OURT88/3 can induce protection against closely related -ASFV strains in pigs without causing significant clinical signs. However in some pigs adverse clinical signs are induced including swelling of joints and transient fever.
To attempt to reduce induction of these adverse clinical signs, an additional two genes were deleted from the genome of the OURT88/3 genome and replaced with a GUS gene reporter. Deletion of these genes, DP71L and DP96R, individually has previously been shown to reduce virus virulence in pigs. Groups of pigs were immunised intramuscularly with 10 e 4 TCID50/ml with parental OURT88/3 isolate (Group 1) or with this deletion mutant (Group 3). Three weeks post-immunisation, pigs were challenged with the closely related virulent strain OURT88/1. All 6 pigs from Group 1 were protected from challenge showing few clinical or post-mortem signs. In contrast 2 of the 6 pigs from group 3 developed clinical signs typical of ASFV and were euthanized 5 days post-challenge. Two pigs from each of groups 1 and 3 developed adverse post-vaccination reactions. The replication of vaccine and challenge viruses and host responses are currently being analysed.
The results show that deletion of the genes DP71L and DP96R from the OURT88/3 genome did not reduce adverse post-vaccination reactions and reduced levels of protection achieved in pigs. In future different combinations of gene deletions will be tested to optimise the induction of protection in absence of adverse clinical signs.

ORAL: Partial protection of pigs inoculated with an attenuated ASFV strain, OURT88/3, against the highly virulent Georgia 2007/1 isolate

Le Potier, Marie-Frédérique[1]; Goatley, Lynnette[2]; Hutet, Evelyne[1]; Cariolet, Roland[1]; Fishbourne, Emma[2]; Hawes, Pippa[2]; Chapman, Dave[2]; Cooke, Lyndsay[2]; Dixon, Linda[2]; Takamatsu, Haru-H[2]

Anses[1]; IAH[2]

Key words: African swine fever, vaccination, Georgia

African swine fever was introduced to Georgia in the Caucasus region in 2007 and spread to neighbouring countries including the Russian Federation. The situation has not been resolved and ASF has been reported from areas close to eastern European countries and threatens further cross-boundary spread. There is no vaccine available against ASFV and this limits options for control.

We have previously demonstrated that immunisation with an attenuated ASFV strain, OURT88/3, from genotype I can protect pigs against related genotype I isolates and also against a genotype X isolate from Uganda (King et al., 2011). Complete genome sequencing indicated that the Georgia 2007/1 isolate sequence is more closely related to the OURT88/3 isolate sequence than the genotype X isolates (Chapman et al., 2011). We therefore tested whether the immunisation of pigs with the OURT88/3 isolate could protect pigs against challenge with the Georgia 2007/1 isolate.

Groups of pigs were immunised intra-muscularly with 10e4 TCID 50/pig of attenuated strain OURT88/3 and boosted 3 weeks later with the same dose of OURT88/3 or primed with 10e3 TCID50/pig and boosted 3 weeks alter with closely related virulent isolate OURT88/1 (10e4 TCID 50/pig). After a further 3 weeks, pigs were challenged with Georgia 2007/1 isolate or with a virulent genotype I isolate from W. Africa Benin 97/1 with 10e4 TCID 50/pig. The results showed that 50% of the immune pigs challenged with Georgia 2007/1 isolate survived in comparison to 66% of pigs which survived challenge with Benin 97/1 isolate. Several of the immunised pigs challenged with Georgia 2007/1 isolate developed clinical signs earlier than the group challenged with Benin 97/1 and the control non-immune group. This indicates an immune enhancement of disease might have occurred. On the other hand, no virus in blood or spleen was detected by qPCR at the end of experiment in the immune pigs that survived from challenge. This suggests that OURT88/3 immunisation could induce protective immunity against Georgian isolate. Results from analysis of levels of virus replication in tissue and blood, and host responses including antibody and T cell responses, cytokine and chemokine responses will be presented.

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SESSION 6:

INTERVENTION STRATEGIES

ORAL: Rescued 'serotyped' bluetongue viruses based on vaccine-related BTV6\net08 confer full protection in sheep against virulent BTV8\net06

van Gennip, René GP[1]; van de Water, Sandra GP[1]; Maris-Veldhuis, Mieke A[1]; van Rijn, Piet A.[1]

CVI[1]

Key words: Bluetongue vaccine serotype

European countries suffering by circulation of bluetongue virus (BTV) serotypes 1, 2, 4, 9, and 16. In 2006, a huge BT-outbreak has started after incursion of BTV serotype 8 (BTV8\net06) in N-W Europe resulting in the largest BT-outbreak ever recorded. A mass vaccination campaign could only be launched in 2008, after inactivated BTV8 vaccine became available. More recently, BTV6 (BTV6\net08) and BTV11 were reported in N-W Europe in 2008. In addition, many other serotypes are reported at the borders of Europe, and new serotypes were recognized inside and outside Europe; BTV25 in Swiss goats, and BTV26 in goats in Kuwait. The (re-)emergency of known and unknown BTV serotypes needs a rapid response to supply effective vaccines.

Bluetongue is an arthropod-borne disease in ruminants. BTV transmission occurs by blood-feeding biting midges of the species of Culicoides. BTV, family Reoviridae, genus Orbivirus, contains ten dsRNA genome segments encoding at least 11 viral proteins. The serotype is dominantly represented by the highly variable S2[VP2] and to less extent by S6[VP5]. These form the outer shell and interact with the underlying VP7, the target for BTV ELISAs. In contrast to VP7, the outer shell induce serotype-specific neutralizing antibodies, and are supposed to be important for the protection against the respective BTV serotype.

We have developed reverse genetics for virulent BTV8\net06 and non-virulent BTV6\net08 (IAH-collection: BTV-8 NET2006/04, BTV-6 NET2008/05 [1,2]). Rescued 'synthetic' BTVs are genotypically different due to silent mutations, but phenotypically indistinguishable from their ancestor virus, including virulence and non-virulence for synthetic BTV8 and BTV6, respectively [3].

Here, we used reverse genetics to rescue 'synthetic' reassortants with eight genome segments of vaccine-related BTV6\net08 [4], and genome segments S2[VP2] and S6[VP5] of serotypes 1 or 8 to complete a set of ten. Thus, synthetic 'serotyped' vaccine viruses shared eight genome segments of BTV6\net08 and only differ in genes for VP2 and VP5. These serotyped vaccines were solely used or in a cocktail vaccine. All vaccinated sheep were completely protected against virulent BTV8\net06 at 21 days post vaccination. Remarkably, protection was irrespective of the serotyped vaccine. Furthermore, virulent BTV8\net06 could not be detected in either of the vaccinates, but induced disease and viraemia in the non-vaccinated group. Results of monitoring of the body temperature and other clinical signs, monitoring by several PCR tests (panBTV, S2-specific, and S10 specific), and monitoring by serological tests (ELISA and VNT) will be presented and discussed.

This approach opens the way to develop serotyped vaccine viruses in the view of preparedness on the incursion of other, i.e. (non-)European or newly recognized serotypes. In order to meet the highest safety assurance, these serotyped vaccine viruses could also be used as master seeds for the fast production of inactivated vaccines. Whichever application, these serotyped vaccine viruses will significantly reduce the time between introduction and the start of emergency vaccination.

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ORAL: Bluetongue virus induces type I IFN in primary plasmacytoid dendritic cells via a MyD88 dependent TLR7/8 independent signalling pathway

Suzana, Ruscanu[1]; Pascale, Florentina[1]; Hemati, Behzad [2]; Mertens, Peter [3]; Meyer, Gilles[4]; Roy, Polly [5]; Zientara, Stephan[6]; Breard, Emmanuel [6]; Sailleau, Corinne [6]; Urien, Céline [1]; Chauveau, Emilie [6]; Charley, Bernard[1]; Schwartz Cornil, Isabelle [1]

INRA[1]; Islamic Azad University Karaj [2]; Department of Institute for Animal Health [3]; ENVT[4]; LSHTM[5]; Anses[6]

Key words: BTV, plasmacytoid dendritic cells, type I IFN,

Innate antiviral defense largely depends on type I interferon (IFN-α/β). Dendritic cells and especially plasmacytoid dendritic cells (pDCs) are innate immune cells that secrete large amounts of IFN-α/β in response to viral infection. RNA and DNA viruses stimulate pDCs preferentially through TLR7 and TLR9 respectively. However, little is known about the mechanism(s) of pDCs activation by dsRNA viruses such as Reoviridae and Rotaviridae families. Here we studied the interaction between Bluetongue virus (BTV) an insect-borne dsRNA virus and primary pDCs from its natural host - the sheep in order to bring insights into the mechanisms involved in IFN-α/β induction in response to dsRNA viruses. We found that following in vivo injection, BTV induced IFN-α/β in skin lymph and in blood. Whereas BTV replicated in a substantial fraction of the conventional DCs (cDCs) and pDCs in vitro, only pDCs, that were isolated from both lymph and blood, responded to BTV by producing important amounts of IFN-α/β. BTV replication in pDCs was not mandatory for IFN-α/β production that was still induced by UV-BTV and not by heat-inactivated BTV. Other inflammatory cytokines, including TNF-α, IL-6 and IL-12p40, were also induced by UV-BTV in primary pDCs. The induction of IFN-α/β required endo/lysosomal acidification and maturation. However despite being an RNA virus, UV-BTV did not signal through TLR7 for IFN-α/β induction. In contrast, pathways involving the MyD88 adaptor, and the kinases PKR and SAPK/JNK were implicated in UV-BTV signaling for IFN-α/β production by primary pDCs. Altogether, this work highlights the importance of pDCs for the production of innate immunity cytokines induced by a dsRNA virus and it unravels that a dsRNA virus uses a novel TLR-independent and Myd88-dependent pathway for IFN-α/β induction in pDCs. These findings have impact for the design of efficient vaccines against dsRNA viruses and for understanding the immune parameters involved in the antitumor properties of some of the reoviridae members.

ORAL: Sensing and control of Bluetongue virus infection in epithelial cells via RIG-I and MDA5 helicases

Doceul, Virginie[1]; Chauveau, Emilie[1]; Lara, Estelle[1]; Adam, Micheline[1]; Breard, Emmanuel[1]; Sailleau, Corinne[1]; Schwartz-Cornil, Isabelle[2]; Zientara, Stéphan[1]; Vitour, Damien[1]

UMR1161 Virologie ANSES-INRA-ENVA[1]; INRA[2]

Key words: BTV, Innate immunity, Interferon

Background: Bluetongue virus (BTV), an arthropod-borne member of the Reoviridae family, is a double-stranded RNA segmented virus that causes an economically important livestock disease which has spread across Europe in recent decades. It can infect many species of domestic and wild ruminants including sheep, deer, cattle and goats. Type I interferon (alpha/beta interferon [IFN-α/β]) production was reported in vivo and in vitro upon BTV infection. However the cellular sensors and signalling pathways involved in this process remain unknown.
Methods: The effect of BTV strains and replication on IFN-β production during a kinetic of infection was assessed at the mRNA level by real-time quantitative RT-PCR (RT-q-PCR) and at the protein level by IFN-β ELISA. The involvement of the IRF3 and NF-kB transcriptional factors in the production of IFN-β was determined by immunoblotting and immunofluorescence. SiRNA-mediated knockdown of several pattern-recognition receptors (PRRs) was used to determine their contribution in the production of IFN-β upon BTV infection.
Results: The expression of IFN-β and other pro-inflammatory cytokines was induced strongly in A549 cells infected with BTV, at both protein and mRNA levels. This production appeared to be dependent on virus replication, since infection with UV-inactivated virus could no longer induce IFN-β. We could also demonstrate that BTV infection activated the IRF3 and NF-kB pathways. Interestingly, the expression of IFN-β mRNA was greatly reduced after siRNA-mediated knockdown of the RNA helicases retinoic acid-inducible gene-I (RIG-I) or melanoma differentiation-associated gene 5 (MDA5), or their common adaptor protein mitochondrial antiviral signaling protein (MAVS). In contrast, silencing of MyD88, Toll-like receptor-3 (TLR-3) or the recently described DexD/H-box helicase DDX1 sensor had no effect on IFN-β mRNA induction. Finally, we found that overexpression of either RIG-I or MDA5 severely impaired BTV expression in infected A549 cells.
Conclusion: These results suggest that the RIG-I-like (RLR) pathway is specifically engaged for IFN-β production following BTV infection and that RIG-I and MDA5 can both contribute to its recognition and control.

ORAL: Potential application of FMDV non-coding RNAs as immune adjuvants on FMDV vaccination

Borrego, Belen[1]; Rodriguez-Pulido, Miguel[2]; de la Losa, Nuria[1]; Mateos, Francisco[1]; Sobrino, Francisco[2]; Saiz, Margarita[2]

CISA-INIA[1]; CBMSO Cantoblanco[2]

Key words: FMDV vaccination, adjuvants, antivirals, type-I IFN

Foot-and-mouth disease virus, FMDV, is the causative agent of a highly contagious vesicular disease of livestock considered as a major animal health problem worldwide. In areas where FMD is enzootic, the disease is controlled by vaccination with inactivated virus, but due to the short-term immunity induced periodic revaccinations are needed. On the other hand, in many countries free of disease preventive vaccination is not allowed because of the economical cost of commercial restrictions associated, and only emergency vaccination is accepted in case of an outbreak. However, the onset of protective immunity takes about 5-7 days, time more than enough for FMDV to replicate and spread from infected to susceptible, non-immune animals. Therefore many efforts are being focussed on the development of new control strategies that can induce an earlier and long-lasting protective immunity.

FMDV is very sensitive to interferon. Therefore, the delivery of IFN or IFN-inducers in combination with an inactivated vaccine stands as a good approach for vaccine improvement, since that could combine the rapid antiviral activity of IFN together with its role stimulating the adaptive immune response. We have recently described the ability of in vitro-transcribed RNAs, mimicking structural domains in the non-coding regions of the FMDV genome (ncRNAs), to stimulate the production of type-I IFN in suckling mice and induce protection against lethal doses of infectious virus for short time intervals (8-48 hrs) after RNA inoculation. In this work we aimed to study the potential of one of these synthetic non-infectious antiviral molecules as immune adjuvant for conventional FMDV vaccination in adult Swiss mice.

Groups of mice were inoculated with a BEI-inactivated FMDV vaccine alone or in combination with the ncRNA, and the immune response induced after one single vaccination at different times post immunization was analysed. Our results show that co-inoculation of the ncRNA with the FMD vaccine improved the immunity induced, in terms of promptness, titre and duration of the specific antibodies developed. The correlation between this immune response and protection against viral infection will be discussed.

ORAL: Foot-and–mouth disease vaccine matching by liquid phase blocking ELISA

Willems, Tom[1]; Robiolo, Blanca[2]; Li, Yanmin[3]; Haas, Bernd[4]; Beascoechea, Claudia Perez[5]; Smitsaart, Eliana[6]; Perez-Filgueira, Mariano[7]; De Clercq, Kris[1]

VAR-CODA[1]; CEVAN-CONICET[2]; IAH[3]; FLI[4]; SENASA[5]; Biogénesis S.A.[6]; INTA[7]

Key words: Foot-and–mouth disease, vaccine matching, ELISA

Introduction
Foot-and-mouth disease virus (FMDV) is a highly variable RNA virus existing in seven different serotypes. The antigenic variability within one serotype can limit the cross-reactivity and therefore the probable cross-protection of vaccine strains within this serotype. Serological assays are routinely used during the process of selecting the most appropriate vaccine strain for protection against given field isolates. This study presents the results of a small scale vaccine matching ring test for an FMD A24 Cruzeiro vaccine using a liquid phase blocking ELISA (LPBE). The goal was to improve the comparability of r1 values from different laboratories.

Methods
Vaccine matching is performed by determining the log10 antibody titer (ti) of a bovine post vaccinal serum (BVS) to the homologous virus strain (tiho) and by comparing this to a ti against a heterologous strain (tihe). An r1 value is calculated as the antilog of {tihe- tiho} what results in a ratio between 0 and 1. Values above 0.4 are considered cross-reactive and possibly cross-protective, values between 0.2 and 0.4 are weak cross-reactive and values below 0.2 are non-cross-reactive.
The selected FMD viruses were the homologous A24 strain and 3 heterologous strains (A1: A/Argentina/01, A2:A81/Arg/87, A3:A/Arg/00). In vivo data indicates that the A24 vaccine does not cross-protect against the heterologous strains. Three BVS pools were created: Pool A and B consisted of 5 BVS with high and medium log10 antibody titers (ti) to A24 (3.1 and 2.5, respectively). Pool C was a mixture of pool A and B and had an intermediate ti of 2.8. The pools were divided over 9 samples (3 samples per pool).

Results
Four laboratories received all 9 samples and inactivated virus of all 4 FMDV strains to perform the vaccine matching tests. All 9 samples were tested against all 4 strains and this was repeated 3 times. First, all labs tested the samples with their in-house LPBE for detecting the homologous ti to A24. Subsequently, all labs found low optical density (OD) values for the heterologous strains and correctly concluded that there is no cross reactivity for the A24 against the 3 heterologous strains. Since this study aimed to compare r1 values, the LPBE protocols were optimized to determine a ti against the heterologous strains. For this purpose, lab 1 used unspecific trapper and detector monoclonal antibodies. Lab 2 switched their trapper and detector reagents, and lab 3 and 4 adapted the concentrations of their in-house LPBE reagents until they obtained sufficient OD values. As a result, lab 1 scored all samples correctly with r1 values below 0.4 and did so with a high reproducibility. Lab 2 had highly reproducible results but misclassified all samples as all r1 values were above 0.4. Lab 3 misclassified sample n°9 against all 3 strains and lab 4 misclassified all 9 samples against strain A3.

Conclusion
In conclusion, the LPBE can be used for vaccine matching but caution is needed when comparing r1 values determined with LPBE assays from different labs. Standardization of protocols and techniques is needed and each lab may have to re-evaluate their cut-off points for cross-reaction and possible cross-protection.

Acknowledgements
This research was funded by the European Community's 6th and 7th Framework Programmes (EC-EPIZONE FOOD-CT-2006-016236; FP7/2007-2013, grant nr 226556, FMD-DISCONVAC), the Belgian Federal Public Service of Health, Food Chain Safety and Environment (RT-05/06-ALTANDI-2) and the RP-PJ of the CODA-CERVA.

ORAL: Morbillivirus V proteins targets multiple components of IFN signalling pathway to control IFN action

Chinnakannan, Senthil Kumar[1]; Baron, Michael[1]

IAH[1]

Key words: V proteins, Morbilliviruses, Interferon signalling pathway, Jak1/Tyk2 phosphorylation, STAT1/STAT2 phosphorylation

Morbillivirus infections are responsible for serious diseases in humans and several species of animals; Measles in humans, Rinderpest in cattle, Peste des petits ruminants in sheep and goats, Canine distemper in dogs and carnivores, Phocine distemper in seals, and Cetacean morbillivirus infection in porpoises and dolphins. The P gene of morbilliviruses, in addition to the P protein also encodes three non-structural proteins V, W and C. Previous studies with rinderpest virus (RPV) and measles virus (MeV) have demonstrated that the V protein of these viruses play a crucial role in blocking type I (IFNα/β) and type II (IFNγ) interferon action. In order to understand the molecular mechanism underlying the ability of morbillivirus V proteins to block type I and type II IFN action we performed comparative studies with the V proteins of four important morbilliviruses: RPV, MeV, peste des petits ruminants virus (PPRV) and canine distemper virus (CDV). Results showed that all the morbillivirus V proteins studied, to a certain extent, are effective blockers of type I IFN action, however, they show varied abilities to block type II IFN action. The ability to block the type II IFN induced gene transcription correlated with STAT1 binding, but there was no correlation between STAT1/STAT2 binding and their abilities to block the type I IFN induced gene transcription or the antiviral state. This suggested that the morbillivirus V proteins could employ an additional mechanism to efficiently block type I IFN action. Further study revealed that the V proteins of RPV, MeV, PPRV and CDV could all interfere with Tyk2 phosphorylation, an early event of the type I IFN signalling pathway that is necessary for the phosphorylation of STAT2. In addition, the RPV V protein also showed an ability to block Jak1 phosphorylation, explaining the reason behind this protein's ability to efficiently block type II IFN induced antiviral state. Co-precipitation study revealed that morbillivirus V proteins have the ability to bind Tyk2 and Jak1. This study highlights the ability of morbillivirus V proteins to target multiple components of the IFN signalling pathway (Jak1/Tyk2, STAT1/STAT2) to control IFN action. Our results also suggest that morbillivirus V protein association with the receptor associated janus kinases Jak1/Tyk2 and the STAT1/STAT2 might result in the formation of a complex at the IFN receptor, interfering with the activation of IFN signalling pathway.

ORAL: Evaluation of the protective potential in lambs of a recombinant MVA vaccine encoding the Rift Valley fever virus glycoproteins

N, Busquets[1]; E, Lopez[2]; G, Lorenzo[2]; F.X., Abad[1]; I, Galindo[1]; R, Rivas[1]; D, Solanes[1]; A, Bensaid[1]; F, Rodriguez[1]; S, Gilbert[3]; M, Domingo[4]; A, Brun[2]

Centre de Recerca en Sanitat Animal, CReSA[1]; CISA-INIA[2]; The Jenner Institute[3]; de Recerca en Sanitat Animal, CReSA, [4]

Key words: vaccination, modified vaccinia ankara MVA, lambs

Rift Valley Fever virus (RVFV) is a mosquito-borne bunyavirus widely distributed in Sub-Saharan countries, Egypt and the Arabian Peninsula, causing disease in both human and livestock. RVF is now considered an emerging threat for European countries due to globalization. It is desirable to improve virus detection as well as improving the current vaccines against RVFV in susceptible species. Recently, we observed protection in mice against lethal RVFV challenge by means of a recombinant MVA encoding the RVF glycoproteins Gn and Gc (rMVA-GnGc).The present study has evaluated the protection conferred by the same vaccine in lambs, one of the most RVFV susceptible species.Three groups of 6 lambs were used, one group was immunized subcutaneously once with rMVA-GnGc, a second group was immunized with rMVA encoding GFP (vaccination control) and a third one was inoculated with saline solution (infection control). Fourteen days later, all animals were subcutaneously inoculated with 105 TCID50 of the 56/74 virulent RVFV isolate. The vaccine efficacy was assessed in terms of clinical signs, pyrexia, viremia, virus shedding by RT-qPCR and detection of specific antibodies by cELISA and sero-neutralization tests. Clinical signs associated with RVF were observed only in one lamb from the rMVA-GnGc vaccinated group and in two animals from the vaccination control group. One lamb from each vaccinated and vaccinated control groups died, showing RVF characteristic liver lesions. Sheep from both control groups showed pyrexia from days 2 to 5 post challenge (pc) while rMVA-GnGc vaccinated animals showed a single peak of pyrexia at day 3 pc. RVFV RNA was detected in both nasal and oral swabs from days 3 to 7 pc in some sheep from both control groups, while no sheep receiving the rMVA-GnGc vaccine showed viral shedding (with the only exception of the deceased lamb). Finally, the rMVA-GnGc vaccinated group raised serum neutralizing antibodies earlier and to higher levels than both control groups. The present study shows that a single dose of the rMVA GnGc vaccine was sufficient to reduce virus replication and shedding but did not provide sterile immunity. Therefore, further optimization of this vaccine approach is still required.

SESSION 7:

RISK ANALYSIS

ORAL: Highly Pathogenic Avian Influenza transmission risks: analysis of biosecurity measures and contact structure in Dutch poultry farming

Ssematimba, Amos[1]; Hagenaars, Thomas[1]; de Wit, Sjaak[2]; Ruiterkamp, Floris[2]; Fabri, Teun[2]; Stegeman, Arjan[3]; de Jong, Mart[4]

CVI[1]; Animal Health Service[2]; Utrecht University[3]; Wageningen University[4]

Key words: biosecurity; contact and neighbourhood structure; highly pathogenic avian influenza; transmission pathways, exposure-risk assessment

Gaining more insight into the mechanisms of virus spread between farms is necessary for the possible development of better control strategies. We carried out an in-depth interview study aiming to systematically explore all the poultry production activities to identify the activities that could potentially be related to virus introduction and transmission. One of the between-farm contact risks that were identified is birds that were moved between farms during thinning with violations of on-farm biosecurity protocols. In addition, several other risky managerial practices, risky visitor behaviours and biosecurity practices were identified. They include human and fomite contacts that occurred without observing biosecurity protocols, poor waste management practices, presence of other animal species on poultry farms, and poor biosecurity against risks from farm neighbourhood activities. Among the detailed practices identified, taking cell phones and jewellery into poultry houses, presence of showers that were never used and the exchange of unclean farm equipment were common. We also found that poultry-related companies are often located in a closer neighbourhood of poultry farms and they present a significant part of the neighbourhood risks. Farmers had divergent opinions about the visitor- and neighbourhood-associated risks. This knowledge is important for communicating about biosecurity to the farmers. We considered the most risky contact types to be; animal movements during thinning and restocking, almost all human movements that accessed poultry houses and proximity to other poultry farms. The overall risk posed by persons and equipment accessing storage rooms and the premises-only contacts was considered to be medium. Apart from the restocking and thinning contacts posing a high risk on only the broiler farms, most of the other exposure-risks are considered to be similar for layer and broiler farms. The identified obstacles to proper biosecurity practices include absence of facilities, non-adherence or inconsistent application and the non-exhaustive protocols.

ORAL: Assessing state and federal responses in USA to simulated introductions of Rift Valley Fever virus

Gibbs, Paul[1]

College of Vet Medicine, University of Florida[1]

Key words: Rift-valley-fever; interagency, disease response, exercise, USA

The introduction of West Nile (WN) virus into New York in 1999 and the number of cases of encephalitis in people, horses and indigenous birds came as a surprise to the USA. West Nile virus had certainly not been “on the radar” as a highly feared virus. The efficient transmission of the virus by many native species of mosquitoes allowed the rapid spread of this virus in subsequent years throughout the contiguous states of the USA, parts of Canada and the Caribbean. The virulence of the virus in causing extensive death in people, horses, and birds was unexpected and certainly out of character when compared with its history in other parts of the world. The similarity of the epidemiology of WN virus and Rift Valley fever (RVF) viruses indicates that, were RVF virus to be introduced to the USA, the ensuing epidemic could have a greater impact on human and livestock health than that following the introduction of WN virus to North America. Experimental infections of North American species of mosquito with RVF virus have indicated that several common and widely distributed species could transmit the virus.

To test the ability of state and federal agencies to respond to an introduction of RVF virus into the USA, three separate “table top” exercises have been held in Florida, Puerto Rico, and St Croix. The timeline of the exercises was real-time for the first 1½ days of the scenario and accelerated on the second half of day 2 into the following 3 weeks. Situation updates were given 3 times a day with extensive use of video and news reports.

The exercises provided the participants with the opportunity to plan, initiate and evaluate current response concepts and capabilities to simulated introductions of RVF virus into the USA.
Specific objectives were
• to bring together the key state regulatory/emergency response agencies that would operate together in a real disease outbreak situation
• to have participants explore issues surrounding current diagnostic and response capabilities to RVF currently available in the USA
• to have participants explore issues surrounding multi-agency crisis communication involving a vector-borne zoonotic disease incident

Each exercise simulated a different route by which the virus was introduced. As an example, in Florida, the outbreak was characterized by an initial recognition of increased calf mortality followed by mild to severe human cases in other parts of the state. The outbreaks occurred in the late fall, were of limited extent, and "died out". While under the scenario RVF virus did not become established in any wildlife populations in Florida, incidents were incorporated into the scenario that involved wildlife. The introduction of the virus was attributed to bioterrorism in Florida, through a viraemic traveler in Puerto Rico, and through infected mosquito egg rafts in St Croix.

From observations during the exercises and through subsequent questionnaires, the participants found the exercises realistic, but challenging. Since little is known of the susceptibility of US wildlife species to RVF virus, the wildlife specialists found it was difficult to speculate on the epidemiology of RVF in wildlife.

These exercises can be considered an example for future multi-agency exercises dealing with a vector-borne disease with a) a zoonotic component and b) involving ruminant wildlife. The exercises highlighted a) that RVF might be very difficult to control and b) the need for more information on the susceptibility of North American wildlife species to Rift Valley fever virus.

ORAL: Contribution of Foot-and-Mouth Disease virus contaminated environment to the transmission of the disease in calves

Bravo de Rueda, Carla[1]; de Jong, Mart[2]; Eblé, Phaedra[1]; van Hemert-Kluitenberg, Froukje[1]; Dekker, Aldo[1]

CVI[1]; Wageningen University[2]

Key words: FMD, virus, calves, environment, transmission

Foot-and-Mouth Disease (FMD) infected animals can contaminate the environment with their secretions and excretions. There is a lack of knowledge on the contribution of a FMD virus contaminated environment to the transmission of the disease. The aim of this study was to quantify direct and indirect transmission in naïve calves. Indirect transmission was studied by using pens that had previously housed infected calves and by introducing infection-free calves to these facilities. Oropharyngeal fluid swabs, urine, faeces and blood samples were collected and it was analysed whether they were positive for FMD virus. We used a modified SIR model to calculate the transmission rate parameter caused by direct contact, which is the average number of secondary infections caused by one infectious animal per day and, to calculate the transmission rate parameter caused by indirect contact, which is the average number of new infections caused by the environment contaminated by one infectious animal one or more days ago. The modified SIR model includes virus decay (estimated from laboratory experiments) and accumulation of virus in the environment (because of the presence of an infected animal the preceding day). Both direct and indirect transmission occurred. Using the estimates for the transmission parameters and the infectious periods, we calculated the reproduction ratio's R direct and indirect. Our findings show that a contaminated environment with FMD virus has an important role in FMD virus transmission, because even after removal of infected cattle, contact cattle can still become infected.

ORAL: Horizontal transmission of Hepatitis E virus from experimentally infected wild boars to domestic pigs

Schlosser, Josephine[1]; Eiden, Martin[1]; Vina-Rodriguez, Ariel[1]; Fast, Christine[1]; Lange, Elke[1]; Groschup, Martin H.[1]

FLI[1]

Key words: Hepatitis E virus, HEV, pathogenesis, wild boars, pigs

Hepatitis E virus (HEV) is an emerging pathogen and a major causative agent of acute viral hepatitis in humans, primarily in developing countries. However, an increasing number of autochthonous HEV infections have been found in industrialized countries recently. Of note, HEV infection is not always self-limited, as cases of chronic hepatitis E have frequently been described in immunocompromised patients. Four genotypes have been identified in mammalian species. Human isolates belong to HEV genotypes 1, 2, 3 and 4. Genotype 1 and 2 are exclusively detected in humans, whereas genotypes 3 and 4 seem to represent also zoonotic viruses with domestic pig and wild boar being reservoir hosts. Several cases of hepatitis E in humans have been linked to the consumption of undercooked meat products of the infected animals by HEV genotype 3.

The aim of the present study was to investigate the propagation of wild boar-derived HEV (genotype 3) in pigs and wild boars after intravenous inoculation. Moreover, sentinel animals were included to detect horizontal transmissions. Data were used to identify differences in the outcome and pathogenesis of HEV infection in wild boars and domestic pigs after intravenous or faecal-oral inoculation. Blood and faecal samples of infected animals and sentinels were collected periodically and analyzed to determine the onset of viremia, faecal HEV excretion and seroconversion. After necropsy (28/29 dpi) tissue samples were taken for the detection of HEV RNA using an in-house qRT-PCR as well as for histopathological and immunohistochemical examination.

HEV RNA was detected in all intravenously infected swine and also in most of the sentinels. Additionally, we were able to demonstrate viral antigens by immunohistochemistry. Results demonstrate for first time that wild boar-derived genotype 3 HEV can be transmitted to domestic pigs by intravenous inoculation. Furthermore, we describe the horizontal HEV transmission among wild boars and also from wild boar to domestic pigs. These findings clearly underline the role of wild and domestic suids as a potential source for HEV transmission. However, to be able to estimate the currently unknown public health risk by contact with pigs and by consumption of pork meat, transmission routes and dynamics of infection need to be further clarified.

ORAL: IMPACT OF CLIMATE CHANGE ON RISK OF INCURSION OF CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS IN LIVESTOCK IN EUROPE THROUGH MIGRATORY BIRDS

Gale, Paul[1]; Stephenson, Ben[1]; Brouwer, Adam[1]

AHVLA[1]

Key words: Crimean-Congo haemorrhagic fever virus, climate change, risk assessment, wild birds, ticks,

Crimean-Congo haemorrhagic fever virus (CCHFV) is transmitted by ticks of the genus Hyalomma and infection in humans may result in severe clinical illness and even death. Although infection is generally subclinical in livestock, domestic ruminants may serve as a reservoir for infection of feeding adult ticks and the virus may be spread to butchers and abattoir workers through contact with blood or tissues from viraemic livestock. CCHFV is endemic in several Balkan countries and has recently been isolated from Hyalomma ticks in Spain. The objectives of the work presented here are to predict the risk of incursion of CCHFV in livestock in Europe introduced through immature Hyalomma marginatum ticks on migratory birds under current conditions and in the decade 2075-2084 under a climate change scenario.

CCHFV is endemic in parts of sub-Saharan Africa where many species of European breeding birds over-winter. Four species of migrant bird namely Willow warbler (Phylloscopus trochilus), Northern wheatear (Oenanthe oenanthe), Tree pipit (Anthus trivialis) and Common quail (Coturnix coturnix) were chosen because they have high abundance in Europe, migrate from sub-Saharan Africa to Europe each spring and have contact with the ground in arid, shrub or grassland-type habitats where H. marginatum ticks may be present. A spatial risk map of Europe was constructed using three data sources: 1) breeding ranges and abundances for these four species of bird, 2) UK Met Office HadRM3 spring temperatures for prediction of moulting success of nymphal H. marginatum ticks into adults; and 3) livestock densities. The model accommodates almost 120 million individual birds entering Europe each year over the months of March, April and May under current conditions.

It is concluded that the absolute risk of incursion of CCHFV in livestock through ticks introduced by the four species of migratory bird is very low and that this risk is little affected by climate change due to opposing effects (Gale et al. 2012). Thus, although higher temperatures predicted in March and April increase the probability of the success of moult of the nymphal ticks into adults in the future climate scenario, there is a decrease in the projected abundance of birds (and hence numbers of imported immature ticks) by 34% in this model.

This presentation focuses on the methodology and in particular the risk equations developed to predict the probability of one or more livestock becoming infected in each 25 km x 25 km grid cell. Uncertainty in some model parameters including the probability of an adult tick finding a livestock animal is also discussed.

Acknowledgements
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POSTERS

Diagnosis

Epidemiology and Emerging Diseases

Next generation sequencing

Intervention Strategies

African swine fever

Risk Assessment

Attendees: EPIZONE 6th Annual Meeting

Institute	Surname	First Name	Country
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The University of Nottingham	Abu-Median	Abu-Bakr	United Kingdom
National institute of vet virology & microbiology	Agnesa	Panferova	Russia
Istituto "G.Caporale"	Alessio	Lorusso	Italy
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VET-DTU	Bøtner	Anette	Denmark
IAH	Baron	Michael	United Kingdom
FLI	Beer	Martin	Germany
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Pfizer Animal Health	Helena	Paradell	Spain
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Norwegian Veterinary Institute	Hopp	Petter	Norway
MERIAL	Hudelet	Pascal	FRANCE
INGENASA	Jacques	Delbecque	SPAIN
CISA-INIA	Jimenez-Clavero	Miguel Angel	Spain
IAH	Kalita	Dhruba Jyoti	United Kingdom
MSD Animal Health	King	Alasdair	Netherlands
VET-DTU	Kirkeby	Carsten	Denmark
Virology Laboratory, EMAI	Kirkland	Peter	Australia
IVI Switzerland	Kochinger	Stefanie	Switzerland

National institute of vet virology & microbiology	Kolbasov	Denis	Russia
CVI	Kooi	Bart	Netherlands
AFSSA	LARA	Estelle	France
AFSSA	Le Potier	Marie-Frédérique	France
Univ. of Veterinary Medicine Vienna	Lebl	Karin	Austria
IDvet	Lecoq	Anna	France
IZSLER	Lelli	Davide	Italy
SVA	Lindström	Anders	Sweden
CISA-INIA	Lopez	Elena	Spain
Carl von Ossietzky University	Lühken	Renke	Germany
CISA-INIA	Mª Luisa	Arias	Spain
LSI	Magnee	Damien	France
IZS-Ve	Manca	Grazia	Italy
Agri-Food and Biosciences Institute	McKillen	John	UK
Pfize Animal Health	Mercedes	Mouriño	Spain
IZSLER	Michele	Dottori	Italy
Choose an institute...	Mino	Olympia Danae	Greece
VET-DTU	Mogens	Madsen	Denmark
IAH	Morecroft	Lizzie	United Kingdom
IZSLER	Moreno Martin	Ana Maria	Italy
IZS -LT	Nardini	Roberto	ITALY
VLA	Nick	Johnson	United Kingdom
University of the West Indies	Oura	Chris	Trinidad
GLA	Palmarini	Massimo	Scotland
CISA-INIA	Paloma	Fernández-Pacheco	Spain
IZSLER	Papetti	Alice	Italy
IAH	Paton	David	United Kingdom
VLA	Paul	Gale	United Kingdom
IZSLER	Pezzoni	Giulia	Italy
Royal Veterinary College	Pfeiffer	Dirk	United Kingdom
Oxford University	Pybus	Oliver	UK
AHVLA	Rajan	Dr Ravi	United Kingdom
AGES Institute for Veterinary Disease Control	Revilla-Fernandez	Sandra	Austria
IZS PLV	Rizzo	Francesca	Italia
VAR-CODA	Rosseel	Toon	Belgium
ADIAGENE	Rouillard	Tony	FRANCE
INRA Jouy en Josas	Ruscanu	Suzana	France

IAH	Sanz Bernardo	Beatriz	United Kingdom
Glasgow University	Sarah	Cleaveland	UK
Istituto G. Caporale	Savini	Giovanni	Italy
IDEXX Switzerland AG	Schelp	Christian	Switzerland
FLI	Scheuch	Matthias	Germany
AHVLA	Scott	Sellers	UK
Evira Finnish Food Safety Authority	Sihvonen	Liisa	Finland
CVI	Ssematimba	Amos	Netherlands
AGES Institute for Veterinary Disease Control	Steinrigl	Adolf	Austria
VET-DTU	Rasmussen	Thomas Bruun	Denmark
Illumina	Taylor Lawley	Cindy	USA
Institute of Virology and Immunoprophylaxis	Thür	Barbara	Switzerland
VAR-CODA	Tignon	Marylène	czech Republic
VET-DTU	Uttenthal	Åse	Denmark
SVI Jihlava (State Veterinary Institute)	Vaclavek	Petr	Czech Republic
IAH	Valdazo-González	Begoña	United Kingdom
VAR-CODA	van den Berg	Thierry	Belgium
CVI	Van der Poel	Wim	Netherlands
CVI	van Rijn	Piet A.	Netherlands
AFSSA	Vitour	Damien	France
QIA	Jee Yong	Park	Republic of Korea
IAH	Waddington	Samantha	United Kingdom
VLA	Wakeley	Philip	United Kingdom
Agri-Food and Biosciences Institute	Welsh	Michael	UK
FLI	Wernike	Kerstin	Germany
AHVLA	Woolaway	Kathryn	UK
AFSSA	Zientara	Stéphan	France

DIAGNOSTICS

D1: African horse sickness antibody detection by indirect immunofluorescence

Chaignat, Valérie[1]; Thür, Barbara[1]

Institute for Virology and Immunoprphylaxis[1]

Key words: African Horse sickness, indirect immunofluorescence, serology

African Horse Sickness (AHS) is a vector-borne disease caused by an orbivirus of the family Reoviridae. AHS-Virus (AHSV) is transmitted by several Culicoides spp. The nine known serotypes circulate in Africa and serotype 4 and 9 have also circulated sporadically outside this continent.
All equidae can be infected. Four clinical forms are distinguished. Horsesickness fever, the mildest form, is mainly seen in vaccinated horses and in mules, donkeys or zebras. The most severe forms, the peracute (or pulmonary) and subacute edematous (or cardiac) ones are mainly observed in horses and lead to a morbidity of over 70%. A mixed form, where symptoms of both the pulmonary and cardiac forms are observed, is rarely clinically diagnosed.
The screening methods for antibody (Ab) detection are ELISAs (either indirect or competitive) homemade or commercial; and as confirmatory tests, immunoblotting and virus neutralisation (VNT) as well as complement fixation are described. Also indirect immunofluorescence (IIF) assays are published but the proposed protocols need highly skilled laboratory personal for accurate interpretation. Providing that a laboratory is able to work with cells, this method is technically easy to perform, inexpensive and fast. We therefore designed an improved, highly sensitive and easier to evaluate IIF, and compared it with a commercially available blocking ELISA (INEGZIM AHSV COMPAC PLUS).
In our assay, AHSV-9 infected Vero cells were mixed with the same amount of uninfected cells in each well of 96 or 24 well cell culture plates. The plates were fixed with aceton and stored either for few days at room temperature or for months at -20 °C.
Sera were incubated (diluted 1:10 in PBS, 45 min at 37 °C) and after a washing step, a secondary Ab (rabbit polyclonal Ab to horse IgG+IgM+IgA or IgG, 1:100 in PBS, 45 min at 37 °C) was added. After washing with PBS, the plates were rinsed with deionised water. Glycerol with 10% v/v of Tris-HCl, (0.01 mol/L, pH 8), was added to the wells to avoid drying and to achieve a brilliant staining. Plates were kept in dark at 4 °C until microscopic examination. The presence of typical intracellular inclusions bodies with a bright apple-green fluorescence indicated the presence of AHS-specific Ab in the tested sera. The evaluation was eased thank the mix of AHSV serotype 9-infected and uninfected Vero cells in the same well.
By analysing horse sera from AHS-vaccinated, experimentally infected, as well as non-infected and non-vaccinated animals, the relative diagnostic sensitivity and specificity of the IIF were 95.2% and 100% respectively, compared to the blocking ELISA. The IIF showed the same analytical sensitivity than the blocking ELISA. Although cells were infected only with AHSV-9, the test could detect antibodies against all the different AHSV serotypes. The method can be reliably used to detect Ab against all nine AHS serotypes, is highly specific and demonstrates a very high analytical and a good diagnostic sensitivity.
As the fixed plates are not infectious and can be stored without lost of quality at room temperature for several days, they can be shipped to laboratories that do not work with cells or AHSV.

D2: EPIZONE: Development of a loop-mediated isothermal amplification assay (LAMP) for the detection of capripoxvirus DNA

Murray, Lee[1]; Edwards, Lorraine[1]; Tuppurainen, Eeva[1]; Oura, Chris[1]; Mioulet, Valerie[1]; King, Donald[1]

IAH[1]

Key words: capripoxvirus, isothermal amplification, LAMP, diagnostics, nucleic acid

Sheep poxvirus (SPPV), Goat poxvirus (GTPV) and Lumpy skin disease virus (LSDV) are the most serious poxviruses of cattle sheep and goats. They are double stranded DNA viruses of the genus Capripoxviridae, (subfamily Chordopoxviridae) within the family Poxviridae. Lumpy skin disease (LSD) is endemic in most African countries and also causes sporadic outbreaks in the Middle East, whereas sheep pox (SPP) and goat pox (GTP) occur in Africa (north of the Equator) as well as in many Asian countries. Tentative diagnosis of LSD, SPP and GTP can be achieved by the recognition of characteristic clinical signs in livestock. However, rapid and accurate laboratory tests that can be used for diagnosis are essential to support programmes to successfully control and eradicate these diseases. This study reports the development of a Loop-mediated isothermal AMPlification (LAMP) assay for the detection of Capripoxvirus (CPV) DNA. A single LAMP assay targeting a conserved region of the CPV P32 gene was selected from 6 pilot LAMP assays that were designed and evaluated in initial experiments (other assays also targeted RNA Polymerase RPO30 Subunit and DNA Topoisomerase I). After optimisation, the addition of loop primers was used to accelerate this LAMP reaction; decreasing the time to generate a positive signal by ~10 minutes. This LAMP assay successfully detected 47 out of 49 DNA samples that were representative CPV isolates (SPPV, GTPV and LSDV) and did not cross-react with DNA extracted from six other mammalian poxviruses (Orf, Cow Pox, Swine Pox, Buffalo Pox, Camel Pox or Cheetah Pox). The analytical sensitivity of the assay was determined to be at least 163 DNA copies which is equivalent to the performance reported for diagnostic real-time PCR currently used for the detection of CPV. In addition to the detection of the LAMP reaction products using an intercalating dye and a real-time PCR machine, or by agaraose-gel electrophoresis, dual labelled LAMP products (generated using internal LAMP primers that were conjugated with either biotin or fluoroscein) could be readily visualised using a lateral-flow device (Forsite Diagnostics, York). These results demonstrate that this LAMP assay is a rapid and sensitive method to detect CPV DNA that may have utility for use in the field, or in non-specialised laboratories where expensive equipment is not available.

D3: Rapid molecular HA subtyping of Avian Influenza isolates by specific real-time RT-PCR tests

Elizalde, M[1]; Agüero, M[2]; Buitrago, D[2]; Arias, M[1]; Muñoz, M.J[1]; Lelli, D[3]; Moreno, A[3]; Fernández-Pinero, J[1]

CISA-INIA[1]; Laboratorio Central de Veterinaria[2]; IZSLER[3]

Key words: AIV; HA subtyping; RRT-PCR

Avian influenza (AI) is a highly contagious disease caused by influenza virus type A, member of Orthomyxoviridae family. To date, 16 AIV different hemagglutinin (HA) subtypes have been described (H1-H16). Reference H5 (Slomka et al 2007 Avian Dis. 51:373-377; Monne et al 2008 J.Clin.Microbiol. 46:1769-1773) and H9 (Monne et al 2008) specific real-time RT-PCR (RRT-PCR) methods are running in the lab providing suitable results with field isolates; however, recommended EU RRT-PCR test for H7 subtype (Monne et al 2008) gives negative results with some recent Italian AIV H7 isolates. There is a need to develop rapid, accurate and reliable methods to identify the different AIV subtypes directly in clinical specimens. The particularly high variability of AI isolates, even within the same HA subtype, is well known and the availability of specific molecular tests is very complex. This work describes the development and validation of a set of subtype-specific RRT-PCR methods for the rapid identification of AIV HA-subtypes, H5 and H9 excluded.

Initially, a comprehensive selection of each HA subtype full-length gene sequences available from GenBank was aligned. Primers and TaqMan probes specific for single HA subtype (H1-H4, H6-H8, H10-H16), hybridizing to all European published sequences, were selected. A collection of 16 reference AIV HA-subtypes from the EU Reference Laboratory (VLA, UK) was used to develop each designed RRT-PCR assay. A total of 35 inactivated isolates corresponding to three annual EU AI PCR proficieny panels were employed to firstly evaluate the competence of the new assays. Finally, two panels of AIV isolates (n=73), including reference strains and recent field Spanish and Italian isolates, were tested for validation studies performed at LCV, Spain and IZSLER, Italy, respectively. RRT-PCR assays were optimised separately for each HA-subtype using commercial AgPath-ID one-step RT-PCR kit (Applied Biosystems).

The analytical sensitivity of each developed HA subtype-specific RRT-PCR was assessed testing replicates of ten-fold serial dilutions of the corresponding AIV reference subtype. Recommended EU RRT-PCR assay targeting AIV M gene (Spackman et al 2002 J.Clin.Microbiol. 40:3256-3260) was performed for comparison, showing similar or even higher sensitivity for each subtype assay. Specificity studies were carried out in separate reactions with AIV reference isolates representing all 16 HA-subtypes, and using a collection of known AIV negative clinical samples from different bird species. Fluorescence signal was obtained exclusively for homologous HA-subtype, while remained under background fluorescence level in negative clinical material. Validation studies were then performed analysing the three annual EU AI PCR proficiency panels and the two sets of AIV isolates, including field isolates belonging to the different HA-subtypes detected in Spain and Italy. AI isolates were correctly identified by the corresponding specific HA RRT-PCR test, remaining undetected by the heterologous subtypes assays.

The presented RRT-PCR assays proved to be highly specific and revealed a satisfactory sensitivity. Additional evaluation studies would be valuable to prove the usefulness of these tests for HA identification of AIV isolates from other European areas, as well as its capability for AIV subtyping directly in clinical positive samples.

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D4: Detection of African horse sickness virus in experimental infected animal by real-time RT-PCR.

Titov, Ilya[1]; Zhivoderov, Sergey[1]; Malogolovkin, Alexander[1]; Lunitsin, Andrey[1]; Tsybanov, Sodnom[1]; Kolbasov, Denis[1]

National Research Institute of Veterinary Virology[1]

Key words: AHSV;PCR; real-time PCR, experimental infection

African horse sickness (AHSV), – is a non-contagious, arthropod borne disease of one-hoofed animals, characterized by fever, swelling of the subcutaneous tissue, hemorrhagic diathesis, respiratory lesions and high mortality. Clinical signs vary but in horses it causes a rapidly progressing disease that is fatal in most cases. (J.J.O. Koekemoer,2008).

The causal agent is an double-stranded RNA-containing virus family Reoviridae, genus Orbivirus, the size of viral particle is 70-80 nm. Nine distinct serotypes of AHSV have been identified. The main carriers of the virus are blood-sucking biting midges. The disease occurs in Africa, the Near and Middle East, as well as in India and Spain. (M. Quan, C.V. Lourens 2010).

The aim of our work was to study the clinical signs of AHSV and pathological changes in the artificial reproduction of the disease, and also indicate fragments of the genome of the virus in blood samples, organs and tissue samples from an infected animal by real-time RT- PCR. In our work we used real-time RT-PCR, described by (Aguero M. et al., 2008). The primers and probe set were directed to a highly conserved sequence within the VP7 (segment 7) region of the AHSV genome. The object of the molecular - genetic studies were blood, internal organs and tissues of the animal. To study the pathological changes used method of complete post-mortem necropsy. In primary study one horse was subcutaneously inoculated by AHS virus strain Uttar Pradesh (9 serotype) in 1000 LD50. Clinical examination, thermometry and blood samples taking was performed daily.

At 4 dpi observed an increase in temperature to 38.5 C°. Reaction of animal to external stimuli is weakened. On the 6th dpi, indicated a temperature rise to 40.3 C°. Oedema is appear under the skin of the head and neck. On day 7 observed a decrease in temperature to 39.7 C°; anorexia to complete refusal of food. Breathing was heavy, irregular, rapid. On the 8th dpi horse was died. Necropsy showed typical for AHS pathological lesions.

In blood samples AHS virus genome by real-time RT-PCR was detected since 3 dpi to animal death. AHS virus genome also was identified in organ samples such as spleen, kidney, heart, lymph nodes, lung and blood clot. And except brain and phibrin from pharynx. Virus was successfully isolated from blood samples since 3dpi. Virus titer present 3 lg LD50/ml, 5 lg LD50/ml and 6 lg LD50/ml on 3, 5, 7 dpi respectively.

In our work we have seen typically clinical signs and post mortem lesions of AHS. Also these study shown a results related with a possibility to detect virus genome by RT-PCR since 3 days post inoculation.

D5: Evaluation of recombinant Haemorhagic enteritis virus hexon protein as a tool for serological diagnostic

Lobova, D[1]; Jarosova, V[1]; Celer, V[1]

University of Veterinary & Pharmaceutical Science[1]

Key words: hexon protein, immunoblot, turkey

Hemorrhagic enteritis virus (HEV) is group 2 of genera Aviadenovirus , family Adenoviridae which infects avian species. The HEV is a well-established cause of an economically important enteric disease in turkeys characterized by depression, hemorrhagic diarrhea, splenomegaly and immunosuppression in turkeys in four or more weeks of age. Serologic examinations indicate a high incidence of HEV in the most turkey-producing areas of the world as Canada, England, Germany, Australia, India, Israel and Japan. The aim of this study was to express recombinant hexon protein (102 kDa), the immunodominant protein of virus capsid and to establish diagnostic value of this protein for the detection of antibodies in turkey serum samples and to assess seroprevalence of the disease in the Czech Republic. The gene coding hexon protein was PCR amplified as three independent fragments and each gene fragment was cloned into pTrcHis2-TOPO Expression kit (Invitrogen). Expression of recombinant protein was performed in E. coli TOP 10 cells and monitored by SDS-PAGE and immunoblotting with monoclonal anti-polyhistidine antibody. The yield of purified proteins reached 1-5 mg from 1L of bacterial culture. Only HET1 region of hexon protein reacted with control positive serum in immunoblot, hence we can conclude on the presence of antigenic determinants in N´ terminal part of hexon protein.
The reactivity of turkey sera with HET1 protein was checked by immunoblotting and the results were compared with ELISA kit (Synbiotics). The total of 100 serum samples was examined. Both immunoblot and commercial ELISA kit gave similar results with tested sera. From 100 serum samples 66 serum samples were positive (66%). Immunoblot using recombinant hexon protein proved useful for detecting turkey flocks infected with Heamorhagic enteritis virus.

D6: Identification of porcine circovirus type 2 by immunohistochemistry and in situ hybridization – a comparison of methods.

SZCZOTKA, ANNA[1]; PEJSAK, ZYGMUNT[1]; STADEJEK, TOMASZ [1]

NVRI[1]

Key words: swine, PCV2, immunohistochemistry, in situ hybridization

Introduction

Porcine circovirus type 2 (PCV2) is an etiological agent of postweaning multisystemic wasting syndrome (PMWS) (1). To confirm PMWS the specific criteria must be fulfilled (4). The aim of the study was to develop and to optimize immunohistochemistry (IHC) method for PCV2 identification and to compare it with in situ hybridization (ISH) method.

Material and methods

The study was conducted on formalin-fixed, paraffin embedded samples of internal organs collected from 2008 to 2010 from wasted swine suspected of PMWS. Overall, 44 sections (38 lymph nodes, 5 intestines and 1 thymus) previously analyzed by ISH, were tested by IHC for the presence of PCV2.

Avidin-biotin complex immunoperoxidase method was developed for PCV2 identification. ISH was performed on corresponding slides as described before (3; 5). All the samples were also hematoxylin-eosin (HE) stained.

Results

PCV2 was detected in most tissues subjected to the analysis. After IHC positive staining was observed in cytoplasm of macrophages and multinucleated giant cells in lymph nodes. In small intestine the staining was observed in gut-associated lymphoid tissue and in villous epithelium.

All 4 sections identified by ISH as highly positive gave similar results by IHC. Also, all experimental samples negative in ISH showed no staining in IHC. However, there were differences in scoring of the sections after staining with the two compared methods. In 21 slides (47,7%) stronger staining was found in IHC than in ISH. Of these, 6 slides that scored doubtful in ISH, were found clearly positive in IHC.

Discussion

According to Sorden's criteria, IHC and ISH are the only laboratory methods fulfilling the requirements of PMWS diagnosis (4).

In this study IHC protocol was developed for detection of PCV2 antigen in tissues from pigs with PMWS. The results demonstrate that both ISH and IHC successfully detected PCV2 viral antigens or nucleic acid in examined tissues. However, our results indicate that IHC has higher sensitivity and specificity than ISH. This is in agreement with other with observations by other authors (2; 3). In addition, IHC results are easier to interpret due to better image quality after staining. Summarizing, IHC is reliable and useful technique for PMWS diagnosis and is likely to substitute ISH in our diagnostic laboratory.

Acknowlegements

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D7: Application of a VP7-Specific Blocking ELISA for the Serological Diagnosis of African Horse Sickness

Bailey, L[1]; Mertens, P.P.C[1]; Castillo-Olivares, J[1]

IAH[1]

Key words: AHSV, VP7 ELISA, antibody kinetics, virus neutralising, DIVA

African horse sickness is the most lethal disease of equids. In association with the changing climate and growth in the movement of equids there is the potential for African horse sickness to follow in Bluetongue's footsteps and extend its distribution northward into Europe. Diagnostic resources need to be in place to assist in the control of the disease in the event of such an incursion. This study investigates the usefulness of the VP7 ELISA and explores its potential as a DIVA (differentiating infected from vaccinated animals) assay in conjunction with recombinant vaccines based on other African horse sickness virus (AHSV) antigens, such as VP2. The kinetics of VP7-specific and virus neutralising (VN) antibody responses were studied in the context of controlled African horse sickness virus infections. The study characterised the kinetics of VP7 -specific antibody responses in horses and donkeys infected with different AHSV strains and serotypes, indicating when VP7 antibodies can be detected after infection, which can assist in the interpretation of single results from an unknown source. Furthermore, the study highlighted the potential for the VP7 ELISA to be used as a DIVA assay in conjunction with recombinant VP2 vaccines. Vaccination with a modified Vaccinia Ankara virus expressing AHSV-VP2 (MVA VP2) induced a strong VN antibody response against AHSV-4. In contrast, the VP7 ELISA detected no VP7 specific antibodies in response to vaccination. The assay's potential in conjunction with other new generation vaccines needs to be explored further.

D8: Development of multiplex real-time PCR assay for the detection of RHDV and Myxoma virus

Burmakina, Galina[1]; Lunitsin, Andrey[1]; Tsybanov, Sodnom[1]; Malogolovkina, Nina[1]; Morgunov, Sergey[1]; Gluhareva, Ekaterina[1]; Bobrovskaya, Nina[1]; Kolbasov, Denis[1]

National Research Institute of Veterinary Virology[1]

Key words: RHDV; myxoma virus, multiplex real-time PCR

During the past 50 years two readily distinguishable rabbit-specific diseases caused by Myxoma virus (MYXV) and Rabbit haemorrhagic disease virus (RHDV) respectively, have been decimated wild rabbit populations worldwide. Rabbit haemorrhagic disease (RHD) is a highly contagious disease, characterised by high rate of morbidity and mortality in both wild and domestic adult rabbits (Oryctolagus cuniculus). The genome of RHDV an identified member of the family Caliciviridae (V. Ohlinger et al., 1990), consists of a single plus-stranded RNA. Myxoma virus (MYXV; family Poxviridae, subfamily Chordopoxvirinae, genus Leporipoxvirus) (R.M. Buller et al., 2005) is a linear double stranded DNA virus that specifically infects rabbits and hares.

Since 2008 till 2012 outbreaks of the RHDV and Myxomatosis have been reported in more than 30 regions of Russian Federation. Considering the current epidemic situation development and subsequent validation of RHDV and MYXV real-time PCR assays appears to be an actual task. In this regard, the main goal of our study was to elaborate a highly sensitive and specific multiplex real-time PCR using TaqMan probes as a new diagnostic tool for one-stage rapid identification of Rabbit haemorrhagic disease virus and Myxoma virus.

18 RHDV and myxoma virus isolates from diseases outbreaks during 2003-2012, 12 different German RHDV strains kindly provided by Dr. H. Schirrmeier (Friedrich-Loeffler-Institut, Germany) and in addition bacterial strains of Pasteurella multocida,Pseuomonas aeruginosa, Staphylococcus aureus were used. Primers and probes were designed to span a 150-bp fragment within the M022L gene of MYXV and 105-bp fragment within the VP60 gene of RHDV respectively.

The probe for RHDV detection was labeled with the reporter dye FAM, while the probe for identification of Myxoma virus was labeled with the reporter dye ROX. To control the efficiency of NA isolation in individual sample a heterologous IC-specific (EGFP) real-time PCR was included into the multiplex assay as was previously described by (Hoffmann et al., 2005).

In order to estimate the specificity of newly designed assay a variety of RHDV and MYXV isolates, 3 bacterial strains (see above) and different tissue samples obtained from negative rabbits were tasted. All RHDV samples have shown clearly defined positive signal above the threshold on FAM channel, where as MYXV samples have been revealed properly according to the threshold value of ROX-fluorescence signals. Pasteurella multocida, Pseuomonas aeruginosa, Staphylococcus aureus and different tissue samples of negative rabbits were not detected by the assay.

The multiplex real-time PCR was shown to be able to detect these viruses in blood and different tissues of infected rabbits in the early stages of infection. The use an internal control allowed to evaluate the efficiency of nucleic acids isolation and to exclude false negative results.

The major advantage of this assay is that it can be use for simultaneous identification and differentiation of Myxoma virus and RHDV in single step of analysis. Thus, the newly developed assay will be helpful in generating a fast primary diagnosis for notification of these diseases.

D9: Development of a biosafe Peste des petits ruminants virus antigen for diagnostic assays

Baron, Jana[1]; Baron, Michael D.[1]

IAH[1]

Key words: recombinant virus; diagnostics; PPR

Peste des petits ruminants virus (PPRV) is a morbillivirus in the family Paramyxoviridae. It causes a moderate to severe disease in sheep, goats and a number of wild life species. The virus has been spreading extensively in the last 10 years, becoming a major threat to livelihoods in many developing-world countries, and is endemic in countries bordering Europe to the east. Continued serosurveillance is required in areas suffering or liable to outbreaks of disease, both to monitor for infections and to assess the efficiency of vaccination campaigns. The surface proteins of the virus are glycoproteins, and correct formation of many epitopes are dependent on synthesis in mammalian cells, and so several of the diagnostic tests in use require virus preparations to act as antigens for, e.g. competition ELISA. This therefore requires the growth of virus, which must be carried out under high containment, and shipment of material which contains or may contain live virus. We have sought to improve the safety of these systems by determining if it is possible to develop an entirely helper cell-dependent system to grow morbilliviruses.
In the absence of a system for creating recombinant PPRV, we have used a previously developed chimeric virus containing the surface glycoproteins (F, H) and matrix protein (M) of PPRV and the core proteins (N, P and L) of rinderpest virus (RP-PPR-MFH). We chose to remove the P protein, as it forms part of the viral polymerase and is therefore completely essential for virus replication. In addition, it is known that morbillivirus P proteins are not interchangeable, so co-infection with, e.g. measles virus, would not support the growth of a P-deleted PPRV. We created a cell line (VDS-P) that expresses the P protein, using Vero cells expressing dog SLAM (VDS) as the base: SLAM is the natural morbillivirus receptor, and these cells have proven a good host for PPRV in our hands. We modified the RP-PPR-MFH genome to remove large parts of the P protein coding sequence, while retaining expression of the V and C accessory proteins that are also expressed from the P gene. We were able to rescue this P-deleted virus in VDS-P cells, where it grew to reasonably high titres (10^4.5 TCID50/ml) compared to the parental virus. The P-deleted virus was completely unable to grow in normal VDS cells, showing that the virus is now completely helper-cell dependent and can be handled and transported under low containment. Viral antigen, prepared by standard techniques, could be used in the competition ELISA that detects antibodies to the PPRV H protein.

D10: Perspectives on molecular detection methods of Lyssaviruses

Fischer, Melina[1]; Hoffmann, Bernd[1]; Freuling, Conrad[1]; Müller, Thomas[1]; Beer, Martin[1]

FLI[1]

Key words: Lyssavirus, rabies, real-time RT-PCR, molecular diagnostics

Abstract

Lyssaviruses belong to the family Rhabdoviridae within the order Mononegavirales. Their genomes consist of a negative stranded linear RNA. Although dog transmitted rabies causes the majority of the estimated 55 000 worldwide fatalities per year, bat lyssaviruses have also caused human cases. Based on a broad range of different host species, their geographical distribution and the evolutionary age lyssaviruses display a high degree of genetic variability. Thus the development of molecular diagnostics for the reliable detection and identification of members of the genus Lyssavirus (pan-Lyssavirus assay) is a challenge, but is valuable for the fight against rabies worldwide. In this study different published PCR-systems or primers for the detection of a wide range of lyssaviruses were tested as real-time RT-PCRs with different one-step and two-step assays. Comparison of different two-step assays by varying the RT-chemistry revealed differences in sensitivity. Nevertheless, most of the tested one-step systems provided an improved performance, including a reduced assay time and a reduced risk of cross-contaminations, when compared to the optimized two-step assay. Finally, we also provide an overview of additional state-of-the-art molecular methods to detect and differentiate Lyssavirus species in general.

D11: Neutralizing antibodies correlate closely with the presence of antibodies to Rift Valley Fever Virus glycoprotein Gn in small ruminants

Jaeckel, Susanne[1]; Eiden, Martin[1]; Balkema-Buschmann, Anne[1]; Ziller, Mario[1]; Paweska, Janusz T.[2]; Groschup, Martin H.[1]

FLI[1]; National Institute for Communicable Diseases[2]

Key words: Rift Valley Fever Virus; ELISA; glycoprotein; small ruminants; field samples

Rift Valley Fever Virus (RVFV) is an arthropod-borne virus that infects a wide range of vertebrate hosts and can cause significant morbidity and mortality in domestic ruminants. The virus has a potential to spread from Africa to Europe and establish itself in the latter region, where endemic mosquito species are competent RVFV vectors. The threat for emergence of RVFV in Europe requires efforts to develop safe and standardized diagnostic assays for both human and veterinary surveillance.

All currently available ELISA assays using recombinant antigens are based on the detection of antibodies against the RVFV nucleocapsid protein (NP). To determine the immunogenicity and relevance of other RVFV structural proteins we developed an indirect IgG ELISA based on bacterially expressed and biochemically characterised RVFV glycoprotein Gn for the screening of small ruminant sera. The validation of the Gn based assay was carried out by testing a panel of about 2000 field samples from sheep and goats in African countries; these sera were dichotomised by virus neutralisation test (VNT).

The results show that bacterially expressed Gn-protein, although lacking post translational modifications, was able to specifically detect IgG antibodies. Sensitivity and specificity of the Gn-based ELISA was determined in correlation to the VNT results by ROC analysis. Results show a good correlation between the presence of neutralizing and anti-RVFV glycoprotein Gn antibodies.

In conclusion, the Gn-based indirect IgG ELISA represents a novel, safe and highly accurate diagnostic assay and will aid assessing the status of the neutralizing antibody response in small ruminants.

D12: Generation and characterisation of monoclonal antibodies against Rift Valley Fever Virus nucleocapsid protein and glycoprotein Gn and Gc

Jaeckel, Susanne[1]; Eiden, Martin[1]; Balkema-Buschmann, Anne[1]; Dauber, Malte[1]; Brun, Alejandro[1]; Groschup, Martin H.[1]

FLI[1]

Key words: Rift Valley Fever Virus; monoclonal antibodies; recombinant proteins

Rift Valley Fever Virus (RVFV) is a mosquito-borne virus that causes high neonatal mortality in livestock and deadly haemorrhagic fever in humans. It belongs to the genus Phlebovirus in the family Bunyaviridae and infects a wide range of vertebrate hosts like sheep, goats and cattle. RVFV can survive for many years in dried out regions in mosquito eggs. For this reason, large epidemics can eventually develop following massive rain falls. RVFV is considered to have a clear potential to spread from Africa to Europe as numerous vector competent mosquito species can also be found there.

Aim of this study was the generation of monoclonal antibodies (mabs) for diagnostic purposes and for studying the RVFV pathogenesis in infected individuals. For this reason, structural proteins of RVFV (glycoproteins Gn and Gc and nucleocapsid protein NP) were bacterially expressed, successfully purified and biochemically characterised. After immunization of Balb/c mice with these recombinant proteins, 691 clones in total were screened for the production of antibodies against NP, Gn and Gc epitopes by using indirect ELISAs. 12 positive clones to NP, 28 to Gn and 5 to Gc were obtained.

All mabs displayed specific binding to RVFV strain MP12 proteins in western blot as well as in indirect immunofluorescence assay when RVFV antigens in infected cells were probed. The specific epitopes and potential virus neutralizing activities will be revealed in future studies. The availability of these mabs will allow the development of competitive ELISAs and of immunohistochemistry protocols to enable studies on the tissue tropism and route of RVFV spread in infected animals.

D13: REAL-TIME FLUOROGENIC REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION ASSAY FOR THE SPECIFIC DETECTION OF BAGAZA VIRUS

Buitrago, Dolores [1]; Rocha, Ana [1]; Tena-Tomás, Cristina[1]; Vigo, Marta[1]; Agüero, Montserrat[1]; Jiménez-Clavero, Miguel Angel[2]

Laboratorio Central de Veterinaria[1]; CISA-INIA[2]

Key words: Bagaza virus; Diagnostics; Flavivirus; Real time; RT-PCR

In September 2010 an outbreak of disease in wild birds (partridges and pheasants) occurred in Southern Spain. Samples from the affected birds were analysed by pan-flaviviral heminested RT-PCR, followed by nucleotide sequencing of the amplified cDNAs obtained, allowing the identification of the aethiological agent of the disease as Bagaza virus (BAGV), a member of the Flavivirus genus (family Flaviviridae)1.
BAGV was first isolated in 1966 in Bagaza, Central African Republic, and, to date, besides the mentioned outbreak in Spain, this virus, as such, has only been identified in Western Africa (Central African Republic, Senegal) and in India. Nucleotide sequence comparison suggests that BAGV is synonymous to Israel turkey meningoencephalitis virus (TMEV), an arthropod-borne flavivirus affecting turkeys, first described in Israel in 1958, and reported only in Israel and South Africa.
The first occurrence of BAGV in Spain urged to develop rapid, reliable and efficacious diagnostic methods to facilitate the surveillance of this disease in the field. This work describes the development of the first real-time RT-PCR specific method, based on a 5'-Taq nuclease-3' minor groove binder DNA probe (Taqman MGB) and primers targeting the Bagaza NS5 gene, addressed to Bagaza virus. The method allowed the detection of BAGV with a high sensitivity, whereas other closely related flaviviruses (Usutu virus, West Nile virus, Japanese encephalitis virus) analyzed in parallel were not detected. The assay was evaluated using field samples of red-legged partridges dead during the outbreak, as well as samples from surveillance programs. This new method proved to be valid for the detection of BAGV genome either in virus isolates or in clinical samples from affected animals, providing a sensitivity equivalent to the gel-based pan-flavivirus RT-PCR method previously employed, while reducing the risk of false-negative results due to crosscontamination, which is a well-known drawback of nested PCR protocols.
In summary, this newly developed method provides the diagnostic laboratory with an effective and rapid analytical tool, useful in differential diagnosis and surveillance of this flaviviral disease of birds, which could represent an emerging animal health risk in Europe.

References:

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D14: ACUTE PHASE PROTEIN RESPONSE IN PIGS WITH SUBCLINICAL SWINE INFLUENZA

Pejsak, Zygmunt[1]; Pomorska-Mol, Malgorzata[1]

NVRI[1]

Key words: Acute phase proteins, pigs, swine influenza, subclinical infection

The acute phase response is an early response of the organism caused by various factors (1). Whilst APP responses have been observed for a large range of infections (1,2) whether subclinical swine influenza (subSI) induces an APPs response is not known. The aim of study was to assess whether experimentally induces subSI, caused by H1N1 SIV, evokes an C-reactive protein (CRP), haptoglobin (Hp), serum amyloid A (SAA) or/and pig major acute phase protein (Pig-MAP) responses in pigs. Fourteen 5-week-old piglets were sourced from high health status herd and were shown to be both influenza A virus and antibody (Ab) (subtypes H1N1, H1N2, H3N2) negative. SIV A/sw/Poland/KPR9/2004 (SwH1N1) was used for the intranasal infection. On day 0, ten piglets were inoculated with 107.3TCID50 SwH1N1. Four pigs served as controls. Blood were collected on -7, 0, 1, 2, 3, 5, 7, 10 dpi. Nasal swabs were taken at 2, 3, 4, 5, 10 dpi. Two infected and 1 control pigs were euthanized on 2 and 4 dpi. Remaining pigs were euthanized on 10 dpi. Ab against SIVs were measured using a HI assay. For determination of APP commercial ELISAs were used. The RRT-PCR method was used for detection of SIV in nasal swabs, tracheas and lungs, as described previously (3).

No relevant respiratory or systemic clinical sings were observed in pigs from both groups. All infected pigs exhibited Ab against H1 between 7 and 10 dpi. M gene RRT-PCR assay revealed positive results in nasal swabs from all infected pigs between 2 and 5 dpi. In all infected pigs, euthanized at 2- 4 dpi, the presence of SIV were confirmed for tracheas and lungs. No viral RNA was detected in lungs on 10 dpi. Postmortem examination revealed typical lesions deriving from SIV infection in the lungs of infected pigs. Only levels of Hp and SAA were significantly induced after infection, with mean maximum values from day 1 to 2 ($p<0.05$). The mean peaked level of Hp was over 3.5-fold higher compared to controls. The levels of Hp and SAA were significantly elevated from 1 to 2 dpi, as compared to the control pigs ($p<0.05$). Mean peak level of SAA reached 43.26 µg/ml (almost 15-fold higher compared to day 0-level). The concentrations of CRP and Pig-MAP did not differ significantly from those observed in control pigs. In control pigs levels of APP remained relatively constant. Strong, positive correlation was found between maximum concentration of SAA in serum and changes in the lungs (R-Spearman = 0.65, $p<0.05$).

The most common problem of diagnosing SI remains the timing of sample collection. Influenza virus titer peaks about 2 dpi and there is little virus shed 6 to 8 dpi. Since concentration of serum Hp or SAA increased at 1-2 dpi and concentration of Pig-MAP remaining unchanged, the monitoring of serum level of these APPs may be helpful in identification of pigs most suitable for specific diagnostic of SIV. Furthermore, the positive correlation (R-Spearman = 0.65, $p<0.05$) found between maximum concentrations of SAA in serum and changes observed in the lungs, makes this APP potentially important indicator for disease evolution or estimation of treatment strategy. Though, to confirm this hypothesis more studies are needed, especially under field conditions.

Acknowledgments

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D15: DEVELOPMENT OF MULTISPECIE SEROLOGICAL ASSAYS TO DETECT SPECIFIC ANTIBODIES TO Mycobacterium bovis IN SERUM SAMPLES

López, Lisset[1]; Ranz, Ana[1]; Venteo, Angel[1]; Pérez, Teresa[1]; Ruiz, Tamara[1]; DeJuan, L[2]; Romero, Beatriz[2]; Boadella, M[3]; Gortázar, C[3]; Boniotti, B[4]; Pasquali, P[5]; Rueda, Paloma[1]

INGENASA[1]; VISAVET[2]; IREC[3]; IZS della Lombardia e dell'Emilia[4]; Instituto Superiore di Sanitá di Roma[5]

Key words: diagnosis, bovine tuberculosis

Bovine tuberculosis (TB) is a chronic bacterial disease of animals and humans caused by M. bovis. In a large number of countries bovine tuberculosis is a major infectious disease among cattle, other domesticated animals, and certain wildlife populations. Transmission to humans constitutes a public health problem. For years eradication programmes have been carried out. Traditional mycobacterium culture remains the gold standard method for routine confirmation of infection but Delayed Hypersensitivity test and gamma-interferon test are the ones used in these programmes. Detection of antibodies specific could be a useful alternative for TB diagnosis. Different antibodies detection assays have been described although all of them show sensitivity troubles.

The aim of this study has been to develop and validate multispecie assays based on Double Recognition ELISA (ELISA-DR) and Immunochromatography (IC) techniques for detection of antibodies specific of M. bovis using the recombinant protein MPB83, expressed in insect cells.

To validate these assays, sera from different species previously catalogued by gamma interferon assay (13 bovine sera) or by necropsy (22 deer, 13 buck and wild boar experimental sera: 51 negative and 58 positive) were used. Moreover, cross reactivity with M. avium subsp. paratuberculosis was determined. For this propose sera and plasma of 9 cows and 9 goats positive to Paratuberculosis and negative to TB were used.

Preliminary results show that both assays detect antibodies specific of TB. None of them showed cross-reaction with antibodies to PTB. Depending on the specie analyzed, sensitivity and specificity of ELISA-DR range between 80-100% and 86-100%, respectively. Concerning to IC, values of 99% specificity in comparison with necropsy are remarkable for deer.

Samples from different species such as alpaca, elephant and American bison are going to be tested. In addition sera collected from wild boar and deer are being evaluated.

Although higher number of samples is necessary, results indicate that both diagnostic approaches are useful tools for control and surveillance of TB infection.

(KBBE-2007-1-3-04) Strategies for the eradication of bovine tuberculosis (TB-STEP)

D16: Receptor-phenotyping of influenza A virus isolates by using an impedimetric biosensor

Martin Del Burgo, MA[1]; Wicklein, B[2]; Yuste, M[1]; Carregal, E[3]; Llobera, A[3]; Ortin, J[4]; Darder, M[2]; Aranda, P[2]; Fernandez, C[3]; Ruiz-Hitzky, E[2]; Real, G[1]

CISA-INIA[1]; ICMM.CSIC[2]; IMB-CNM.CSIC[3]; CNB.CSIC[4]

Key words: influenza A, receptor phenotyping, biosensor

We present a novel impedimetric sensing approach for discrimination of receptor-binding phenotypes of influenza A virus isolates. The sensing device is based on a nanostructured, biomimetic architecture inspired by the membrane of target cells. In contrast to immunosensors, which are based on antibodies as bioreceptor, we introduce receptor molecules that mimic those of target cells as sensing entity. In a bottom-up approach, the artificial receptors are built on a gold electrode surface by sequential assembly of a 1-octanethiol/octyl-galactoside hybrid bilayer, followed by an enzyme-mediated functionalization of the terminal galactoside groups with sialic acid molecules. The detection mechanism relies hence on the specific affinity between the sialic acid-galactose receptor moieties anchored on the modified electrode surface and the hemagglutinin (HA) viral surface protein. By using the appropriate type of sialyltransferase enzyme, sialylation of galactose residues is made through alpha-2,3 or alpha-2,6 linkages, which permit the envisaged detector to discriminate rapidly between avian vs. human strains of influenza A virus with the absence of elaborate sample preparation steps as in case of other selective influenza sensors. This relevant feature could make the here presented detector a reagentless, label-free diagnostic device for influenza phenotyping. Genetic and phenotypic characterization of novel re-assorted viruses with pandemic potential is of utmost importance for rapid implementation of control measures and to understand the molecular mechanisms underlying inter-specific transmission of influenza viruses. Therefore, discrimination of pathogenic phenotypes of emerging influenza virus isolates is an important issue in epidemiology studies and surveillance of pandemics.

D17: A duplex real-time RT-PCR assay for sensitive and specific detection of Peste des petits ruminants virus

Monaco, Federica[1]; Sebhatu, Tesfaalem[2]; Polci, Andrea[1]; Cosseddu, Gian Mario[1]; Scacchia, Massimo[1]; Lelli, Rossella[1]

Istituto G. Caporale[1]; National Animal and Plant Health Laboratory[2]

Key words: Peste des petits ruminants, diagnosis, qRT-PCR, Eritrea

A duplex real-time reverse transcription-polymerase chain reaction (RT-PCR) assay for a simple and rapid diagnosis of Peste des petits ruminants (PPR) was developed. Nucleotide sequences available on public databases (GenBank) were aligned and a conserved region was identified in the sequence coding for nucleocapsid protein (Np) where primers and FAM-labelled TaqMan-MGB probe were designed. For the determination of the analytical sensitivity, an in vitro transcript (PPR_Np T7) of the target region was constructed and tested. Furthermore, the PPR_Np T7 transcript was used as positive control and standard for quantifying target copies. A commercial heterologous Armored RNA was used as an internal positive control (IPC) for either RNA isolation or RT-PCR steps. A strong linear correlation (R2>0.99) was observed between Ct values and the corresponding amount of sRNA PPRV_Np; the amplification efficiency was close to 99%. The newly designed duplex real-time RT-PCR proved to have a sensitivity of approximately twenty copies/µl. The selected primer-probe combination was strictly PPRV-specific in fact no signals of amplification (Ct>40 cycles) were recorded neither when the qRT-PCR PPR_Np was applied on viruses closely related or clinically similar to PPR nor when applied on 30 PPR-negative blood samples. A preliminary evaluation of its diagnostic performances was assessed by testing 41 tissue samples collected from sheep and goats during several outbreaks of PPRV occurred in Eritrea from 2001 to 2011. The test identified the PPRV in 36 of the samples without amplifying any close related viruses.

D18: Consequences of diagnostic methods with pooled samples

Froehlich, Andreas[1]

FLI[1]

Key words: imperfect diagnostic methods, pooled samples, sensitivity, specificity

Introduction:
Due to the argument of financial limitations on surveillance and control programs for diseases reasonably small sample sizes are taken in consideration. Still known that low-cost diagnostic methods usually do not represent the state of the art science and technology. Consequently the question occurs, if required knowledge can be achieved.

Considered strategy on a diagnostic method:
Example given: A certain disease is sought-after or a very small prevalence is to be examined or roughly to be determined. As an option pooling of individual samples would minimize the effort in diagnostic processing. Based on the theory from this strategy of the diagnostic method the following consequence appears: With a cumulative pool volume the sensitivity increases, whereas the apparent specificity drops away at the same time for the diagnostic method.

The problem und the general task:
The described theoretical facts lead to the following observable effect: Due to a growing pool size even more true positive individual samples cover with an increasing number of false positive individual samples, whereby the probability of a positive pool result grows strongly. Above a certain number of individual samples in a pool it almost surely obtains a positive diagnostic pool result. Thus a strongly diminished or no gain of knowledge is to be achieved by doing a reduced diagnostic effort. The problem with this diagnostic procedure depends on finding a suitable pool size that meets the desired amount of knowledge.

Aim of the presentation:
For selected situations it is to be shown comparatively wherein and how one and the same diagnostic method differs in its entirety with fixed minimum requirements.

D19: Detection of six endemic and notifiable porcine diseases by multiplex real time RT PCR

Wernike, Kerstin[1]; Hoffmann, Bernd[1]; Beer, Martin[1]

FLI[1]

Key words: multiplex real-time PCR

Natural co-infection with Porcine reproductive and respiratory syndrome virus (PRRSV) and Porcine circovirus type 2 (PCV2), two of the most economically important diseases of pigs worldwide, are common in countries with intensive swine production. A reverse transcription real-time polymerase chain reaction (RT-qPCR) assay for the simultaneous detection of PRRSV and PCV2, combined with an internal control (IC) based on the beta-actin gene, was developed.

In addition, a screening system for diseases notifiable to the Office International des Epizooties was integrated in the multiplex RT-qPCR. The probes specific for the genome detection of Classical and African swine fever virus (CSFV, ASFV), Foot and mouth disease virus (FMDV) and Suid herpesvirus 1 (SuHV1) were labelled with an identical fluorophore, allowing the application of real-time PCR machines with four detection channels. A positive result in the screening system requires further differentiation using the PCR systems in single-target approaches to receive a final diagnosis. However, the multiplex PCR provided an indication of the presence of one of the viral genomes in the sample.

The analytical sensitivity of the multiplex RT-qPCR was determined using series of 10-fold dilutions of the individual RNA/DNA standards. In any case at least 200 genome copies per µl template were reliably detected.
A large panel of different body fluid and tissue samples from healthy as well as diseased swine, wild boars and other animal species was tested by the multiplex RT qPCR and, in excerpts, by the single-target approaches. Overall, good accordance was observed between multi- and singleplex PCR assays. Every sample was correctly identified in the multiplex approach. Moreover, the PCR systems for the detection of the notifiable diseases were hardly affected by the simultaneous amplification of high amounts of PRRSV- and PCV2-specific sequences.

So far, tests for pathogens whose occurrence is unlikely have been performed in a limited dimension, potentially resulting in retarded detection of notifiable diseases.
The newly developed RT-qPCR allows the rapid and reliable detection of CSFV, ASFV, FMDV and SuHV-1 in clinically diseased animals, even when additionally infected with PRRSV and PCV2 without additional cost, effort, amount of sample material and time required.

D20: IMPROVEMENT OF A ONE STEP REAL TIME RT-PCR FOR THE DETECTION OF ANY SWINE VESICULAR DISEASE VIRUS VARIANTS

Benedetti, Dennis[1]; Pezzoni, Giulia[1]; Grazioli, Santina[1]; Brocchi, Emiliana[1]

IZSLER[1]

Key words: real-time RT-PCR, Swine Vesicular Disease Virus, diagnosis, field samples

In the last decade, real-time PCR assays have been developed for the diagnosis of swine vesicular disease (SVD). Among these, the one adopted by the Community Reference Laboratory is a one-step real-time RT-PCR based on two primers/probe sets (2B-IR and 3-IR) designed within the conserved 5'untranslated region (5'UTR). This test was evaluated with a panel of reference isolates but it was not validated with field samples. In this study, the diagnostic performance of the 5'UTR real-time assay was evaluated using field samples and improved according to the Italian SVDV molecular epidemiology.
A phylogenetic analysis, based on a 3D gene fragment, showed the current presence in Italy of two genomic sub-lineages, one comprises viruses typically evolved in Italy since 1992 (Italian sub-lineage) and the other includes viruses detected in Portugal in 2003 and from 2004 also in Italy (Portuguese sub-lineage). SVD is controlled in Italy by a surveillance and eradication program. The virological surveillance performed in faecal samples is mainly based on the conventional one step RT-PCR that targets the 3D gene.
Performances of the 5'UTR real time RT-PCR and the 3D conventional RT-PCR were compared by using the same viral RNA extracted according to the OIE manual.
The assays were evaluated on 74 field faecal samples, originated from different SVD outbreaks occurred in Italy during the period 1997-2011 and found positive by the conventional RT-PCR. The two primers/probe sets of the real-time RT-PCR showed a significant difference in capability to detect isolates of the two SVDV sub-lineages. In fact, while the real time PCR based on the 2B-IR primers/probe set did not detect 7 out of 46 samples of the Italian sub-lineage, the other set failed to detect all the 26 samples belonging to the Portuguese sub-lineage, but all could be detected if results of both sets were combined. The sequencing of the 5'UTR region fragment could explain these failures, as it highlighted the presence of mismatching either within the primers sequence (set 2B-IR) or in the probe sequence (3-IR). On the basis of these results we degenerated the 2B-IR primers (2B-IR deg), as well as the 3-IR probe sequence (3-IR deg) in critical sites. The new degenerated primers/probe reactions were evaluated on serial dilutions of two SVDV Italian isolates, representing the Italian sub-lineage and the Portuguese sub-lineage respectively. The detection limit of the real-time PCR performed with the degenerated primers/probe sets was confirmed similar to that showed by the original sets and by the conventional RT-PCR (between 1 and 10 TCID50). However, the test conducted with 2B-IR deg primer/probe set was capable to reveal as positive the 7 feacal samples previously undetected and the 3-IR deg set showed the ability to detect the Portuguese sub-lineage samples.
A phylogenetic analysis based on a portion of the 5'UTR region was performed on 22 field samples. It partially confirmed the current co-circulation of distinct (Portuguese and Italian) lineages. However, it's noteworthy that four isolates belonging to the Italian sub-lineage according to the 3D region, were clustered within the Portuguese lineage taking into account a part of the 5'UTR region, suggesting that the two regions are evolving independently.
This modified real-Time PCR provides a reliable and simple diagnostic test with a maximal likelihood of detecting any SVDV clinical sample, while continued sequencing of new isolates would make available updated knowledge insight the SVD viruses evolution.

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D21: A reverse genetics approach to study the importance of neutralising antigenic site 1 and 2 of serotype O Foot-and-Mouth Disease virus

Kalita 1, Dhruba Jyoti[1]; Asorf, Amin [1]; Mahapatra1, Mana [1]

IAH[1]

Key words: Reverse genetics, Antigenic site, Serotype

The Foot-and-mouth disease virus (FMDV) is a single stranded positive sense RNA virus belonging to the genus Aphthovirus in the family Picornaviridae, cause economically devastating disease of cloven hoofed animals with a global distribution. The virus has a high mutation rate and exists as seven serotypes of FMDV (O, A, C, Asia 1, SAT1, SAT2 and SAT3) as well as numerous and constantly evolving subtypes showing a spectrum of antigenic diversity. Presently available foot-and-mouth disease (FMD) vaccines are capable of protecting animals from clinical disease, however they do not confer sterile immunity. We have recently observed that most of the antibodies are produced against neutralising antigenic site 2 followed by site 1 in polyclonal response of serotype O FMDV vaccinated animals (Mahapatra et al., 2012). However antigenic site 1 has been reported not to be essential for protection in serotype A FMDV (Fowler et al., 2008, 2010). In order to study the importance of neutralising antigenic site 1 and 2 of serotype O FMDV in protection, a reverse genetic approach was used for either deletion or replacement of antigenic site 1 and 2 in a serotype O cDNA clone. Plasmids harbouring the full length clone of FMDV with the desired changes have been obtained. Work is underway to recover viable virus that can be used for antigenic and serological characterisation.

D22: The study of breeding colony with reproductive failures associated with canine herpesvirus-1 (CHV-1)

Vankova, M[1]; Celer, V[1]

University of Veterinary & Pharmaceutical Sciences[1]

Key words: Canine herpesvirus 1, PCR, recombinant glykoprotein D, immunoblot, serology

Canine herpesvirus (CHV-1) is a member of Alphaherpesvirinae sub-family, with a host range restricted to the domestic and wild animals of the Canidae family. In the adult dog, CHV-1 infection causes usually subclinical infection or only mild clinical sings of the upper respiratory or genital tract. However, in neonatal puppies, the virus can cause acute and usually fatal infection in litters of puppies. Infection during pregnancy can also implicate infertility, abortion, stillbirth and birth of weak pups. Typically, the virus induces latent infecton.

In our study breeding colony of English and French Bulldogs displaying reproductive failures in the past was diagnosed with the respect to the presence of CHV1. The main described problems were infertility, birth of weak pups and puppies death a few days after birth.

The presence of CHV-1 in the colony was diagnosed by PCR; serological testing was based on immunoblot with recombinant gD as antigen.

Serological testing was performed on serum samples, for virus detection by PCR, vaginal swab samples were used. Examination was done on samples originating from eight bitches and four dogs. Two of these animals lost their litters in the previous pregnancy and CHV-1 was confirmed in parenchymatous organs of death puppy at least in one case. In mother of CHV-1 positive puppy, virus was detected also in vaginal swab one month after birth. Remaining animals were found CHV-1 negative.

Antibody testing proved elevated levels of CHV-1 specific antibodies in two bitches with reproductive failure and in four other animals. Remaining animals were serologically negative.

Our study demonstrated that shedding of virus on vaginal surface can persists for weeks following birth of affected puppies and is accompanied by the rise of CHV-1 specific antibody levels in affected mother.

D23: Development of isothermal amplification methods for pen-side detection of avian influenza virus, classical swine fever, and african swine fever

Greetham, H[1]; Piepenburg, O[1]; Brooks, D[1]; Hanna, A[2]; Couper, W[2]; Collins, S[2]; Amarasekera, G[2]; Reid, S[2]; Wakeley, P[2]; Crooke, H[2]; Slomka, M[2]

TwistDx Limited[1]; AHVLA[2]

Key words: Isothermal, detection, avian influenza, classical swine fever, african swine fever

Recombinase Polymerase Amplification (RPA) is an isothermal DNA amplification method proposed as a candidate technology for pen-side testing. RPA primers and fluorescent probes were designed for the nucleoprotein gene of avian influenza virus (AIV), the 5UTR of classical swine fever virus (CSFV), and the VP72 gene of African swine fever virus (ASFV) detection. RNA extracted in the laboratory from 73 diverse AIV isolates (including 47 H5 and H7 AIVs) and 48 H5 and H7 clinical specimens from poultry (comprising 33 swabs, 9 feathers and 6 tissues) were tested in parallel by AIV RPA and M gene RRT PCR assays. Clinical specimens were collected from both low and high pathogenicity poultry outbreaks (n=19) and experimental infections (n=29).

CSFV and ASFV sample extracts were tested with corresponding RPA assays for target specificity. A panel of 31 pestivirus RNAs including CSFV genotypes 1.1, 1.2, 2.1, 2.2, 2.3, 3.1 and 3.4, and 18 non-specific pestiviruses including BDV1,2,3,4, BVDV1 and BVDV2 were tested with a CSFV RPA assay. Twenty-two ASFV DNA extractions of differing genotypes were used to test specificity of an ASFV RPA assay. All tested CSFV and ASFV genotypes were successfully detected using RPA. No false positives were observed using either CSFV or ASFV RPA assays. Further assay optimisation is required prior to sensitivity testing for both swine fever assays.

All but one of the 121 AIV specimens were positive by M gene RRT PCR, while 68 of 73 isolates and 40 of 48 clinical specimens were successfully detected by AI RPA. The 13 RPA detection failures occurred among the lower AIV titre samples that registered Ct values of >30 by M gene RRT PCR. No AIV RPA false positives were observed in testing 40 swabs and 15 tissues from AIV-uninfected chickens plus 116 field clinical materials from AI-uninfected wild birds.

This study showed AIV RPA to be potentially at least 100 fold more sensitive than AIV lateral flow devices which are the currently available AIV pen-side tests. All three RPA assays demonstrated good assay specificity. Continuing rigorous validation is crucial to demonstrate the value of proposed molecular pen-side testing technologies as part of robust outbreak-management strategies.

EPIDEMIOLOGY AND EMERGING DISEASES

EED1: PTV-12, A NOVEL PORCINE TESCHOVIRUS SEROTYPE: MOLECULAR AND SEROLOGICAL CHARACTERIZATION, AND IN VIVO STUDIES ON ITS PATHOGENICITY, TROPISM AND CROSS PROTECTION IN MINIPIGS

Cano, Cristina[1]; García, María Ana[1]; Jiménez, Miguel Ángel[1]

CISA-INIA[1]

Key words: Porcine Teschovirus; Picornavirus; phylogenetic analysis; serology

Introduction

Porcine teschoviruses (PTVs) constitute a genus within the Picornaviridae family, comprising 11 known serotypes to date (PTV-1 to PTV-11). Although most PTVs are usually non-pathogenic, some viral variants, particularly within PTV-1, can be more virulent and cause severe disorders in the central nervous system (Teschen disease) or milder signs (Talfan disease) as well as reproductive disorders and skin lesions.

A previous survey in Spanish pig populations revealed a high diversity of teschoviruses (Cano-Gómez et al 2011). In this study, phylogenetic analysis based on VP1 capsid protein grouped all isolates into 12 clusters, 11 of which corresponded to the known PTV serotypes, and one additional group suggested the existence of a twelfth serotype. This group is new to science, and has been putatively named serotype 12.

The aim of the present study was to investigate the molecular and serological characteristics of a representative PTV-12 isolate (CC25), including its possible pathogenicity and tissue tropism in vivo in minipigs. In addition, using this experimental model, we have investigated if a pre-existing infection with this PTV-12 isolate could confer cross protection against infection with a heterotypic PTV-1 strain.

Materials & methods

CC25 complete genome amplification was performed using specific oligonucleotides designed for this work. Phylogenetic analysis was carried out using the polyprotein region (P1). Homology analyses were also performed with the whole sequence obtained.

Minipigs (n=6) were orally inoculated with a high dose (3 x 107.0 TCID50) of CC25. Control minipigs (n=3) were sham-inoculated with diluent (DMEM). Biological samples were collected at different times after inoculation. At 21 dpi, the infected CC25 group was orally challenged similarly (3 x 107.0 TCID50) of Tirol (PTV-1) strain and sampled in the same way. An equivalent group of minipigs (n=4) not inoculated previously with PTV-12 were also challenged with PTV-1. Detection of virus in the samples was done by RRT-PCR (Cano Gómez et al 2011) while neutralizing antibody titres were measured by seroneutralization test. Necropsy was carried out according to an established schedule.

Results

Sequence and homology analyses show that isolate CC25 represents a new genotype among the Teschovirus genus, since 1) its homology with the PTV genotypes already described (1-11) is as low as those existing between different serotypes and 2) it clusters separately in a P1-based phylogenetic tree. Moreover, serological data support that CC25 belong to a different serotype among Teschoviruses.

Regarding pathogenesis, no disease signs were observed in any PTV inoculated animal during this study, thus confirming the lack of pathogenicity of CC25 and Tirol strains in the experimental conditions employed.

Tissue tropism studies revealed the presence of the virus in both digestive tract and secondary lymphoid organs but not in the central nervous system.

Serologically, CC25-inoculated minipigs developed specific antibodies neutralizing CC25 in vitro (but not, or to a lower extent, other PTV serotypes), peaking at 7-10 dpi. Pre-existing CC25 infection did not cross-protect against Tirol strain infection in these working conditions.

This study corroborates the existence of a new Teschovirus serotype (PTV-12) represented by strain CC25, and describes its main characteristics. CC25 is apparently non-pathogenic and do not confer protection against further infection with a heterotypic PTV-1 strain.

Acknowledgements

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EED2: A global approach for the official controls management in the Veneto Region

Manca, Grazia[1]; Patumi, Ilenia[1]; Ferrè, Nicola[1]; Bortolotti, Laura[1]; Marangon, Stefano[1]

IZS-Ve[1]

Key words: information system, official controls, data quality, traceability

The achievement of the high level of protection of human life and health pursued by EC policies depends on animal health and welfare, as well as food and feed safety. The collection, recording and management of data play a key role in establishing epidemiological surveillance networks and traceability systems for animals and products as required by EC legislation (Council Directive 97/12/EC, EC Regulation 178/02).
Since 1994, the Veterinary service (VS) of the Veneto Region has been developing a Regional Information System (RIS) with the aim of creating and maintaining an effective and structured communication network amongst all people involved (health authorities, producers, consumers). The aim of this paper is to describe the results of the application at a regional level of Information Technologies to the official control management performed by both the VS and the Food Hygiene Medical services (MS).
Since 2007 two procedures for the management of the activities performed by the VS as well as the MS have been developed. One of the available functionalities for both the procedures is the official controls planning and management in the food industries. This tool allows the organisation and recording of the whole process (food industries selection, inspection, verification and audit, non-compliance and related corrective actions notification). The different criteria for the processing plants selection are based on a set of information about the production typology, coming from the Regional Data Bank (RDB) of animal farms and related industries, but also on the history of the controls and results of the analysis carried out by the official laboratories, in order to better direct the plants choice. Data related to the analyses performed by the official laboratories are generated by the procedure in use at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVE) and put in relation with inspection activity data through interoperability protocols. For the frequency definition of the official controls in the food industries, each plant has been previously categorised on a risk base, and scored following the criteria laid down in EC Regulation 854/2004: in this way it is possible to automatically define the numbers of controls based on a risk classification. Finally the tracing of any functional connection amongst companies is also available.
In the same way the VS use the regional procedure for the registration of the inspections performed for the national plan for animal welfare: in early 2012 the required reports have been automatically generated by the system, thus fulfilling the information debts towards the Italian Health Ministry and the European commission about 2011 activities. In addition these data are linked to the sanitary status of each farm, thanks to a specific tool for the outbreaks management and to the registration of the activities for the eradication programs activated in our Region. Geographical information about animal farms and related industries are also collected in the system. All loaded data are available and can be consulted in real time by VS and MS, thus ensuring a rapid and effective response if a sanitary event occurs. In the last year a new approach to metadata, catalogue creation and publication has been set up, and it will constitute the new frontier for a better standardisation of data semantics and a possible reuse of data in different areas and in an international context.

EED3: Isolation and identification of the BTV-14, detected in Smolensk region of Russia

Panferova, Agnesa[1]; Koltsov, Andrey[1]; Novikova, Marina[1]; Kurinnov, Viktor[1]; Lunitsyn, Andrey[1]; Kolbasov, Denis[1]

National institute of vet virology & microbiology[1]

Key words: bluetongue BTV-14 Smolensk Russia

The first step involved the isolation of the virus from blood of one sero- and PCR-positive cow from imported group with strongest positive results in real-time RT-PCR. Virus isolation was performed on EDTA-treated blood sample using intravenous inoculation of 10-12 day-old embryonated chicken eggs (two passages) followed by repeated passage in Vero cells. The virus amplified in Vero cell with a titre of 105,0 TCID50/ml. Finally, the viral isolate was typed by a virus neutralisation test with 24 reference serums and gave neutralisation titre 3,0 lg with reference serum against 14 serotype. Simultaneously for virus isolation 2,5 month lamb was used. Lamb was inoculated intradermally and subcutaneously with 1ml and 1 ml respectively of washed erythrocytes from pooled blood samples from 4 PCR-positive cows. During the period of observation infected lamb had a fever, whereas there were no typical for bluetongue clinical signs. Blood samples were collected at 3, 5, 7, 8, 9 days post inoculation and then twice a month. BTV RNA was detected in infected lamb blood up to the 70 dpi with a peak on 7-9 dpi (the experiment is still ongoing). Virus was isolated from the blood collected on 10 dpi and after one passage in ECE and two passages in Vero cell had titre - 106,75 TCID50/ml.

For characterisation of the BTV-RNA we disigned three primers sets for segment 2 and two primers sets for segment 5 to use it for sequence studies. Sequencing of parts of segment 2 showed that segment are genetically closely related to that of the BTV14 reference strain segment 2 the major representative of the serotype differs on one position from that of the reference BTV14 strain. Sequencing and comparison of parts of segment 5 showed 100 % gomology with segment 5 of FJ713346 strain prototype 600572 from USA. Genetic studies in other segments of the genome of the virus continues.

EED4: Molecular survey on Polish MDV field strains

Wozniakowski, Grzegorz[1]; Samorek-Salamonowicz, Elzbieta[1]

NVRI[1]

Key words: Marek's disease, molecular survey, meq oncogene

Marek’s disease (MD) presents a serious problem in mass production of poultry. MD is caused by Marek’s disease virus (MDV) which belongs to Mardivirus genus of Alphaherpesvirinae subfamily. In spite of prophylaxis of MD in chickens an emergence of new virulent MDV strains is still observed. The possible cause of this phenomenon is the continious evolution of MDV strains. The main oncoprotein of MDV is Meq encoded by MDV076 gene which is known to harbour different mutations in the nucleotide sequence that are associated with the virus pathogenicity. It is important because Meq is responsible for the transformation of infected T lymphocytes. The protein product of meq is 339 amino acid long its capable to form dimers with proapoptotic factors what impedes programmed cell death and non-controlled cell division of infected cells. Previously we have reported an occurrence of recombinant MDV strains with reticuloendotheliosis virus LTR region that influenced meq sequence. The presented study was conducted on 48 MDV strain isolated between 2009 and 2012 year. The field isolates were compared with reference vvMDV and vv+MDV strains: 584A, 648A, 595, 549A and TK. The detailed analysis has shown that eight recently isolated field strains have evident frame shift withlin MDV076 gene of MDV. However we found that the nucleotide sequence of the meq has not been changed since the similarity between these strains and reference MDV strains ranged from 98.2 to 100 percent. The most important sequence motifs found in 16 MDV field strains were deletion of thymine in 110 nt position and adenine in 147 nt position. Similarly the specific insertions of thymine, guanine and adenine and guanine were detected in positions 107, 129, 130 and 142, respecitvely. There were also four specific transitions observed in positions: 144, 206, 354 and 526. Other two transversions were found in positions 220 and 526. These mutations influenced aminoacid sequence since the common deletions of proline, glutamic acid and lysine were found in 33, 42, 49 aminoacid positions. There was single common substitution of phenyloalanine into serin at 32 aminoacid position. The specific was the presence of glycine and glutamic acid at 48 and 69 positions. The observed common mutations indicated on the common origin and features of reference vvMDV and vv+MDV and presently isolated Polsih field strains.

EED5: Genotyping of PCV2 strains in pig herds in the Czech Republic

Slaninkova, E[1]; Celer, V[1]

University of Veterinary & Pharmaceutical Sciences[1]

Key words: PCV2, pig, genotyping, sequencing

Porcine circovirus 2 is a causative agent of economically important diseases whose pathogenesis has not been fully elucidated yet. The aim of this work was the genetic typing of PCV2 strains circulating in the Czech Republic from different clinical forms of the disease. The relationships between the genotype of the virus and clinical manifestation were studied. ORF2 and ORF3 genes were selected for sequencing and phylogenetic analysis. We have amplified and sequenced a total of 42 ORF2 and 17 ORF3 genes. By comparing analysed sequences with published PCV2 genomes in GenBank, genotypes belonging to PCV2 group 1 and PCV2 group 2 were detected in swine herds in the Czech Republic.
After a genetic shift that took place no later than 2002 in the Czech Republic, the genotype PCV2 group 2 has been replaced by PCV2 group 1 and now occurs sporadically. We have detected only two samples of genotype PCV2 group 2 in years 2002 – 2010. Both samples originated from subclinical infections and were clustered as 2C and 2E. All samples of genotype PCV2 group 1 were classified to the cluster 1A/B. The genotype PCV2 group 1 can’t be regarded as more virulent, as it was proved in many subclinical infections. The protein encoded by ORF3 plays a role in the pathogenesis of the circovirus diseases. Its nucleotide sequence is very conserved and we haven´t found any molecular markers which could be linked to the virulence of sequenced strains. Nevertheless results of ORF3 gene sequencing have confirmed phylogenetic analysis based on ORF2 sequences.

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EED6: A dynamic compartmental model for the West Nile Virus vector Culex pipiens

Lebl, Karin[1]; Brugger, Katharina[1]; Rubel, Franz[1]

University of Veterinary Medicine Vienna[1]

Key words: Cx. pipiens, population model, vector born disease, mosquito, weather

Cx. pipiens mosquitoes are important vectors of a variety of arthropod born infectious diseases. Here we focus on West Nile virus (WNV), a zoonotic disease affecting birds, horses and humans. Cx. pipiens vectors play a key role in the natural transmission cycle of the virus and thus in the spreading of WNV. Therefore, a profound knowledge of the population dynamics of the vector is inevitable. We intended to generate a model valid for lager areas forced by environmental parameters such as temperature, precipitation and geographic latitude. It is designed to replace an existing, less sophisticated, mosquito model, which is part of the WNV model presented by Laperriere et al. (2011).
The mosquito model consists of 5 stages: (E) – eggs, (L) larvae, including the pupal stage, and the adult stages during host seeking (Ah), egg developing (Ae) and diapause (Ad). Functions describing the rates between the stages were estimated from literature, comprising birth rate, transition rates between the stages and stage specific mortality rates. As input parameters influencing the model rates we used mean daily temperature, daily accumulated precipitation and daytime length (a function of geographic latitude).
For model validation we used long term capture data from Cook County, Illinois (P. Geery, Desplaines Valley Mosquito Abatement District, pers. comm.); climatic data were obtained from the National Oceanic Atmospheric Administration (NOAA). To estimate the model fit we used the root mean square error.
The results show that our model is able to reproduce inter-annual variability in Cx. pipiens abundance as well as realistic daily time series for the period 1980-2010. Our model can therefore be implemented in SIR models on Cx. pipiens born diseases like WNV. Further, it provides a basic framework for future developments such as an evaluation of mosquito control strategies.

Laperriere, V., K. Brugger, and F. Rubel, 2011: Simulation of the seasonal cycles of bird, equine and human west nile virus cases. Preventive Veterinary Medicine 98: 99-110.

EED7: Preliminary results of virologic investigations on bats from Northern Italy

Lelli, Davide[1]; Moreno, Ana[1]; Lavazza, Antonio[1]; Barbieri, Ilaria[1]; Canelli, Elena[1]; Boniotti, Beatrice[1]; Bresaola, Marcello[1]; Cordioli, Paolo[1]

IZSLER[1]

Key words: bats, viruses

Bats are increasingly recognized as reservoir hosts for a large number of emerging viruses. We investigated the circulation of viruses in the Italian bat population, in particular Arboviruses (Arthropod-borne viruses), Coronaviruses and Lyssaviruses. Through a broad virologic diagnostic protocol, the study was also addressed to the detection of new, unknown or emerging viruses potentially zoonotic or dangerous for bats.
Faecal samples and dead individuals were collected by bat experts in Wild Animal Recovery Centres, and known reproductive, wintering or swarming sites. Brain, lung, spleen, liver, kidney and intestine were collected for virologic analysis. Brain samples were tested for the presence of lyssaviruses by the fluorescent antibody test and the mouse inoculation test to exclude positivity prior to perform any other analysis. We used three screening RT-PCRs for detection of viruses belonging to the genus flavivirus (2), alphavirus (3) and orthobunyavirus (4) and a new pan-coronavirus PCR. Samples were then cultured onto VERO and MARC-145 cells line and examined by negative staining electron microscopy (nsEM).
In total 112 carcasses and 44 faecal samples were collected from Pipistrellus khulii (48%), Pipistrellus spp. (38.5%) and others species (13.5%): Nyctalus noctula, Rhinolophus hipposideros, Tadarida teniotis, Vespertillio murinus, Plecotus auritus and Hypsugo savii.
Samples resulted negative for arboviruses and lyssaviruses. Seventeen samples caused cytopathic effect in cell cultures. The nsEM performed on both supernatants of infected cell cultures and tissue and faecal homogenates revealed the presence of many viral particles morphologically related to reoviruses. All 17 isolates were identified as Mammalian Orthoreovirus (MRV) by RT-PCRs specific for L1 gene (5). Fourteen isolates were typed as MRV3 whereas 3 resulted not typed by a multiplex PCR specific for the S1 gene (6). Partial sequencing of L1 and S1 MRV genes were performed and phylogenetic trees were constructed with the Neighbour-joining method using MEGA5 software. Phylogenetic analysis of L1 showed the presence of two different clusters: one formed by 13 isolates and the other with the remaining 4 isolates, which were closely related to T3/canine/It/Decaro/04 strain. In the phylogenetic tree of S1 gene the 14 typed strains were closely related to strain Decaro/04, and belong to the lineage III. MRV were not the sole virus identified; infact coronaviruses were found in twelve samples. More detailed data on these strains are described in a separate communication in this congress (7).
To our knowledge MRV has never been isolated from bats and no sequences data are available. However, in this case no cause-effect relationship between bat death and MRVs isolation was established. Indeed, the pathogenic role of MRV in all other mammals is still unclear. Based on this results MRV3 infection seems to be widespread in Italy among different bats species, in particular Pipistrellus khulii. This might be taken into account for public health, considering that these species are common in urban areas. Further surveillance on bats is needed to fully describe the ecology and evolution of viral agents in Italian bats.

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EED8: Influence of climatic condition on Usutu virus detection.

Calzolari, Mattia[1]; Bonilauri, Paolo[1]; Defilippo, Francesco[1]; Balzani, Agnese[1]; Maioli, Giulia[1]; Bellini, Romeo[2]; Dottori, Michele[1]

IZSLER[1]; CAA, G. Nobili[2]

Key words: Usutu, climatic data, mosquitoes, pcr

Since the first signalling of west Nile virus (WNV) in Italy in 2008, a surveillance system was set in Emilia-Romagna region. This system includes also an entomological part consisting in sampling mosquitoes by attractive traps (baited by carbon dioxide), identification, and arbovirus biomolecular screening in pooled mosquitoes. This mosquito based surveillance allowed the detection on WNV from 2008 to 2010 and of anther Flavivirus, such as Usutu virus (USUV), from 2009 to 2011. In 2010 a network of regularly working traps was set up, on a 10 km x 10 km grid operating across Emilia-Romagna region. The network was been confirmed also in 2011 and 83 out of 189 traps have worked in both years. These traps have collected 541,963 mosquitoes in the two years, 495,226 of which belonged to the species Culex pipiens. The sampled mosquitoes were sorted in 3856 pools, one C.x pipens pool was WNV positive in 2010 and 110 mosquito pools were USUV positive (107 were composed by Cx. pipiens species and 3 by Aedes albopictus species). For 81 of these traps, daily climatic data at 5x5 km grid resolution were provided by the Hydro-Meteorological Service of the Emilia-Romagna Regional Agency for Environmental Protection (ARPA-SIM). Obtained data were total rainfall (Tr), potential evapotranspiration obtained by Hargreaves formula (Tev), average temperature, minimum temperature (Tmin), maximum temperature (Tmax); from these data the temperature range (Tr=Tmax-Tmin) and the hydroclimatic balance (IB=Tr-Tev) were obtained. The monthly climatic data averages were calculated for both years. Traps were classified as positive or negative if at least in one pool USUV was been detected. Climatic data were compared in positive and negative traps using ANOVA test with $p<0.05$. The preliminary statistical analysis, interestingly, show that the monthly temperature range was highest in positive traps in 20 cases out of 24 and in 9 of them the difference was statistically significant. Regarding the hydroclimatic balance this climatic data was lowest in positive traps in 22 out of 24 months and in 6 of them the difference was statistically significant. These results seem to point out an association between drought conditions and wide temperature range and the probability of detection of USUV circulation in mosquitoes.

EED9: First reported low pathogenicity avian influenza virus subtype H9 infection of domestic fowl in England

Parker , C Daniel[1]; Reid, Scott M [2]; Ball, Allan[1]; Cox, William J[2]; Essen, Steve C[2]; Hanna, Amanda[2]; Slomka, Marek J[2]; Irvine, Richard M[2]; Brown, Ian H[2]

Slate Hall Veterinary Practice Ltd [1]; AHVLA[2]

Key words: avian influenza virus; H9 real-time RT-PCR; broiler breeders; UK poultry; egg drop in poultry

In December 2010, infection with a H9N1 low pathogenicity avian influenza (LPAI) virus was detected in a broiler breeder flock in East Anglia. Disease suspicion was based on acute drops in egg production in two of four sheds, poor egg shell quality and evidence of diarrhoea. H9N1 LPAI virus infection was confirmed by real-time reverse transcription polymerase chain reaction. Sequencing of the haemagglutinin and neuraminidase genes revealed high nucleotide identity of 93.6% and 97.9% with contemporary North American H9 and Eurasian N1 genes, respectively. Attempted virus isolation in embryonated SPF fowls' eggs was unsuccessful. Epidemiological investigations were conducted to identify the source of infection and any onward spread. These concluded that infection was restricted to the affected premises and no contacts or movements of poultry, people or fomites could be attributed as the source of infection. However, the infection followed a period of extremely cold weather and snow which impacted on the biosecurity protocols on site and also led to increased wild bird activity, including waterfowl around the farm buildings. Although H9 infection in poultry is not currently considered notifiable, H9N2 LPAI viruses have been associated with significant production and mortality episodes in poultry in Asia and the Middle East, as well as causing zoonotic infections. In the present H9N1 outbreak, clinical signs were relatively mild in the poultry with no mortality, transient impact on egg production and no indication of zoonotic spread. However, this first H9 UK poultry case for 40 years, and the first reported detection of H9 LPAI virus in chickens in England, vindicates the need for continued vigilance and surveillance of AI viruses in poultry populations.

EED10: Genetic Characterization of APMV-1 isolated from wild captive birds

Munir, Muhammad[1]; Goraya, Mohsin Ulla[1]; Zubair Shabir, Muhammad [1]; Abbas, Muhammad[1]; Tanveer, Muhammad[1]; Zohari, Siamak[1]; Berg, Mikael[1]

SVA[1]

Key words: Wild captive birds, NDV, PCR, characterization

Introduction.
Newcastle disease is a highly contagious disease affecting domestic poultry, caged pet birds and wild birds with large economical consequences. Out of total eight genes in APMV-1 genome, comparison of the nucleotide sequences of F gene among the different strains of NDV is convienent and relaible method to devide APMV-1 into groups, which could be further sub-divided into five lineages or twelve genotypes. There have been several outbreaks of Newcastle disease in both commercial and backyard poultry in Pakistan, despite of extensive vaccination. The purpose of this study was to screen and to overview the genome characteristics of APMV-1 circulating in wild captive birds in Pakistan.

Methods.
Samples suspected for APMV-1 were collected from Quails (Coturnix ypsilophora), Guineafowl (Numida meleagris), Peacock (Pavo cristatus), Himalayan Monal (Lophophorus impeyanus) and from backyard poultry birds (Gallus gallus domesticus). The pathogenicity of all the isolates was determined in embryonated eggs. The F gene was amplified from viral RNA using OneStep PCR kit. The PCR products were purified and sequenced using the same primers as for the PCR.

Results.
The resultant sequences were aligned and a phylogenetic tree was constructed using BioEdit software, MEGA4 and DNAstar. Phylogenetic analyses showed that all the isolates clustered with viruses from genotype VII. However, APMV-1 from wild captive birds belonged to genotypes VIIa whereas from backyard poultry birds clustered inh VIId. The our previously characterized isolates from Pakistan belong to either VIId or have established a novel genetic cluster. All the isolates were found to be virulent based on their F protein cleavage sites and their pathogenicity indices.

Conclusions.
Wild captive birds are highly susceptible for APMV-1 and genotypes more than one are circulating in Pakistan, which warrant further investigations to screen the wild birds and to determine their role in the epizootiology of the APMV-1.

EED11: Detection of IBV QX in commercial poultry flocks in the United Kingdom

Irvine, Richard M[1]; Jones, Rebecca M[1]; Ellis, Richard J[1]; Cork, Jennifer L[1]; Errington, Jane[1]; Cox, William J[1]; Ceeraz, Vanessa[1]; Wood, Alisdair M[1]; Reid, Scott M[1]; Brown, Ian H[1]

AHVLA[1]

Key words: Infectious bronchitis virus; IBV QX; commercial poultry; IBV S1 genotyping; IBV real-time RT-PCR

Strains of infectious bronchitis virus (IBV) showing a close relationship with the so-called Chinese QX strain of IBV (IBV QX) have recently been detected in the UK. Following the initial isolation of a QX-like variant strain serologically unrelated to the major UK contemporary IBV strains from a small backyard flock during 2007, European IBV QX-like strains have been detected in backyard and hobby flocks. Since 2009, such strains have also been detected in samples submitted for diagnostic investigation from commercial poultry flocks (Irvine et al., 2010). Samples testing positive by IBV real-time reverse transcription polymerase chain reaction were subject to partial sequencing of the S1 gene (Jones et al., 2011). Whilst the European IBV QX-like genotypes detected from commercial poultry flocks in Great Britain (GB) and Northern Ireland are closely related, differences are apparent in the S1 sequence, revealing separate sub-lineages. The greatest differences occur between European IBV QX-like strains detected from backyard poultry and commercial flocks. Furthermore, strains from some commercial poultry flocks in GB share a higher degree of S1 sequence homology with each other and a strain detected from poultry in The Netherlands. Together, these data suggest separate routes of introduction may have occurred. Emergence of a novel variant such as IBV QX might threaten commercial poultry production as currently-used vaccines might not be fully protective and the severity of disease caused by novel strains may differ from that caused by extant strains. Further work is required to fully understand the epidemiology of IBV QX in UK poultry populations.

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EED12: Genetic and serologic characterization of Infectious Bronchitis virus strains circulating in poultry in north western Italy.

Rizzo, Francesca[1]; Ameri, Dania[1]; David, Moshè[2]; Longoni, Corrado[3]; Giammarino, Mauro[2]; Grattarola, Carla[1]; Mandola, Maria Lucia[1]

Istituto zooprofilattico PLV[1]; ASL CN1[2]; freelance veterinarian[3]

Key words: infectious bronchitis, poultry, genetic characterization

IBV, single-stranded RNA virus, Coronavirinae Family, Gamma Coronavirus Genus, is responsible of great economic losses in the poultry industry. This agent has a well-documented capacity to modify its genome by spontaneous mutation/recombination, driven also by selective pressure, caused by the extensive use of vaccines. Several IB variants have been identified worldwide so far, some being unique in a specific area, others with a wider geographic distribution (de Wit J.J., 2011). IBV control is focused on vaccination strategies, but, due to its great antigenic variability, a lack of cross-protection against the wild strains is observed (Muradrasoli S., 2010). The present study aim to define the IBV circulation through the monitoring of a small area (Cuneo province-Piedmont region), where respiratory symptoms were recurring in some poultry farms, to detect the emersion, diffusion and mean of new variants, as potential risk factors. Tracheal swabs and serum were collected during 2011 from 47 poultry farms; vaccination data were also collected at sampling time. Diagnostics involved viro/serological analysis as direct virus identification with a One-step RT-PCR assay (Cavanagh 1999), targeting a portion of S1 protein gene, on RNA extracted from tracheal swabs and virus isolation (VI) on embryonated chicken eggs, attempted only from RT-PCR positive/doubtful samples. Genotyping was achieved by sequencing the S1 gene portion. Indirect ELISA was performed on 30 sera per farm and hemagglutination inhibition (HI) test used for serotyping. 20 out of 47 poultry farms were RT-PCR positive, but VI was successful only from samples of 7 RT-PCR positive farms. IBV S1 gene sequence was obtained from 13 farms. The genotypes detected in the area are: QX type in 2 farms, M41 type in 6 farms, D274 type in one farm, Q1 type in 4 farms. Sequences showed a 97% to 100% rate of nucleotide homology with the respective reference ones. ELISA results showed the recurrence of 4 typical serological profiles in the farms tested: seronegative farms, seropositive farms with ELISA titles distributed homogeneously between high, medium and low positivity values, seropositive farms with a strong majority of high seropositives and farms with a patchy distribution of positive values, with at least the 30% of seronegatives. On RT-PCR negative farms, HI test revealed the presence of antibodies vs serotypes usually applied in vaccination schemes (M41, D274, 793/b). The virological evidence of QX strain as first finding in Piedmont area and Q1 strain as second relief in Italy, points out the need for identifying the epidemiologic connections between infected farms and the possible introduction routes, probably due to acquisition of infected chick batches from extra-regional/national breeders. The considerable high number of RT-PCR positive flocks, all declared as vaccinated, could be due to failure in vaccination, bad vaccine doses management, use of variants not sufficiently cross-protective against heterologous field strains. The correct approach to the different diagnostic tools at our disposal, is an important outcome of this study. Emergence of new strains/variants (QX/Q1) in previously free territories, stress the need of a constant monitoring of field situation, to early detect both introduction of known IBV genotypes from other areas and emersion of new variants. Moreover a geographically based review of vaccine composition (Terregino, 2008), is essential to increase cross-protection and efficacy against this fast evolving pathogen.

EED13: Analysis of Kobuvirus in farm animals from Northern Ireland

McMenamy, Michael John[1]; McClintock, Julie[1]; Lagan-Tregaskis, Paula[1]; Welsh, Michael[1]; McKillen, John[1]

Agri-Food and Biosciences Institute[1]

Key words: Kobuvirus, Northern Ireland, Cattle, Pigs, Sheep

Kobuviruses are non-enveloped viruses from the family Picornaviridae with (+ve)ssRNA genomes, ~8Kbps in length (1). Until recently the only member of the Kobuvirus genus was Aichi virus discovered in Japanese patients suffering from gastroenteritis (1). Subsequently bovine and porcine kobuviruses were identified. Viruses from species including dogs, mice, sheep, goats and even bats have been tentatively suggested as new members of the kobuvirus genus. The majority of non-human kobuvirus research has been carried out in cattle and swine. There may be some association between kobuvirus and enteric disease in these species although many studies demonstrating a correlation between kobuvirus prevalence and diarrhoea did not routinely test for the presence of other common enteric pathogens. To date kobuvirus has been characterised in animals throughout Asia, northern Europe and in Brazil. In this study we detail the investigation of the prevalence of kobuvirus in cattle, sheep and pig samples Northern Ireland.

Lung and mesenteric lymph node (MLN) samples, each were recovered from cattle, pigs and sheep from animals exhibiting symptoms of enteric disease from Northern Ireland, between 2009 & 2011. All samples were homogenised and viral nucleic acids extracted. Viral nucleic acids were assayed for the presence of kobuvirus by real-time PCR using a set of generic kobuvirus primers (2) to amplify a 216bp region of the 3D-gene. Where possible those putatively positive samples were analysed by gel electrophoresis and subject to sequence analysis.

Kobuvirus was detected in cattle, pigs and sheep from Northern Ireland. Cattle: 15/112; pigs: 5/100 & sheep 1/99. Advanced sequence analysis was carried out on selected cattle and pig kobuvirus sequences. These exhibited >90% nucleotide similarity to existing kobuvirus sequences.

The levels of kobuvirus detected in farm animals from Northern Ireland was low, especially in those sheep populations tested. Although quantitative real-time PCR was not carried out CT values suggested that kobuviruses were present at very low concentrations in those species tested. In the vast majority of studies faeces from animals suffering from diarrhoea has been tested. This might be one reason for the levels of detection and that testing was carried out on lung and MLN samples. In future more appropriate samples will be sought for study.

Although kobuviruses have been detected in healthy and diseased animals, particularly those with enteric problems, the clinical significance is not yet fully known. Recently porcine kobuvirus was identified as a potential emerging virus within pig populations that could have subclinical implications (3). It is possible that these kobuviruses identified in farm animals, and those now appearing in companion and wild animals could potentially pose a zoonotic risk to humans, particularly those in regular contact with animals e.g. veterinarians and producers. Further testing of diseased and healthy farm animals for the presence of kobuvirus would therefore be prudent.

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EED14: First isolation of salmonid alphavirus type 2 (SAV2) in Poland

Borzym, Ewa[1]; Maj, Joanna[1]; Matras, Marek[1]; Bigarré, Laurent[2]

NVRI[1]; Anses[2]

Key words: salmonid alphaviruses (SAV), rainbow trout, phylogeny, subtypes

Salmonid alphaviruses (SAV) are recognized as serious pathogens of farmed Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss) in Europe. SAV (family: Togaviridae, genus alphavirus) isolates have been grouped in six distinct subtypes SAV1-6 (Fringuelli 2008). SAV2 was initially isolated in France where it caused the sleeping disease (SD) of rainbow trout. Later on, it has also been isolated in England, Spain, Germany and Italy.

For the first time, SD provoked severe losses in rainbow trout farms in Northern Poland during the summer 2011. The typical sleeping behavior was observed on juveniles of rainbow trout (~35 g). Mortalities ranged from 5 to 20 %. The objective of this work was to identify the genotype of the SAV and try to trace the origin of the virus.

Samples for virus isolation consisted of kidney, spleen and liver pooled from 10 fish. After inoculation on rainbow trout gonad (RTG-2) cells, a typical cytopathic effect was observed. Total RNA was extracted from cell culture supernatant and submitted to RT-PCR with primers amplifying two informative regions of the genome: a conserved region in the E2 gene and a variable region in the nsP3 gene.

The sequences revealed that the isolate from Poland was a strain of SAV 2, sharing a very strong genetic identity (>99 %) with isolates from France and Italy. Since the trout eggs were imported from France, our hypothesis favors the introduction of the virus in Poland from France with contaminated eggs. This emphasizes the need of more controls in the exchange of living material between countries.

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EED15: EXPERIMENTAL INOCULATION OF A RECENT PATHOGENIC ISOLATE OF SWINE VESICULAR DISEASE VIRUS (POR 1/04) IN PIGS

Paloma, Fernández-Pacheco[1]; Fernandez-Pinero, Jovita [1]; Fernandez, Raul[1]; Cano-Gomez, Cristina[1]; Sanchez, Miguel Angel[1]; Del Pozo, Mateo [1]; Arias, Marisa[1]; Jimenez-Clavero, Miguel A.[1]

CISA-INIA[1]

Key words: Swine vesicular disease; SVDV; diagnostics; pathogenesis; pigs

Swine vesicular disease virus (SVDV) (Family Picornaviridae, genus Enterovirus) causes a disease of pigs characterized by the development of vesicular lesions in mouth and hooves, which can be confused with those caused by foot-and-mouth disease virus and other pathogens producing vesicular diseases. For this reason it is included in the OIE list of obligatory declaration diseases. Currently in Europe the disease remains active only in Italy, despite intense efforts pursuing its eradication.

Most SVDV infections are subclinical, making its detection difficult, and thus increasing the risk of the disease to remain unnoticed, unless efficient laboratory-based surveillance is put in place. One of the most recent SVD outbreaks observed in the EU (outside Italy) occurred in Portugal in 2003-04, where, interestingly, typical vesicular lesions were observed in the affected pigs. The present work aimed at studying the pathogenicity and contact transmission of one of the viral isolates (POR 1/04) obtained during that outbreak.

Six 9-week-old pigs (Landrace-Large White) were kept in biosecurity level 3 animal facilities, and divided into two 3-pig groups, housed in separated, isolated boxes. 2 pigs of each group were inoculated with 2x105 TCID50 of SVDV (isolate POR 1/04) each, through the coronary band. The remaining 2 pigs were maintained as contact control animals. All animals were monitored from 0 to 146 days post inoculation (dpi). Monitoring consisted of daily exploration for clinical signs, and periodical collection of samples, including faeces, serum, skin biopsy and vesicular fluid. Standardized diagnostic techniques were used for virus isolation using IB-RS2 cell line, SVDV RNA detection by RRT-PCR (Reid et al, 2004 J Virol Methods 116:169-76), and serum antibody (Ab) detection, including in-house liquid-phase blocking ELISA (LPBE) and IgM-ELISA, and virus neutralization test (VNT).

All inoculated pigs showed clinical signs of varying severity, starting at 5 dpi, mostly consisting of lameness and vesicles on the feet (interdigital spaces and coronary band), tongue and snout. Two of them, one in each box, died at 8 and 14 dpi, respectively, due to undetermined causes, but not related to the inoculation. The remaining pigs recovered completely within 5 weeks. Viral RNA was weakly detected in serum just at 2 dpi, while viral detection in faeces was achieved between 2-5 and 27 dpi. Virus isolation was inconsistently achieved from faecal samples of all inoculated pigs, even testing four replicates of selected samples. All inoculated pigs developed SVDV-specific Abs, detectable by LPBE. Neutralizing Abs were first detected at 5 dpi and persisted throughout the study with high titers. IgM also started at 5 dpi, while declining at 13-20 dpi. Viral RNA was finally detected in all skin biopsy samples and vesicular fluids.

The two contact control pigs acquired the infection, as revealed by: 1) observation of disease symptoms at 5 and 7 days post-contact (dpc), respectively; 2) detectable viraemia at 7 and 9 dpc, respectively, and 3) seroconversions at 9 and 13 dpc, respectively. SVDV was isolated from fecal samples as early as 5 dpc.

As a conclusion, the pathogenicity of the recent SVDV isolate POR 1/04 has been experimentally assessed. The course of the infection, kinetics of antibodies, viraemia and contact transmission has been established for this particular isolate of SVDV.

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EED16: Complete coding sequences and genetic analysis of two novel bocavirus species isolated from swine in Northern Ireland

McMenamy, Michael John[1]; McKillen, John[1]; McNair, Irene[1]; Duffy, Catherine[2]; Charreyre, Catherine[3]; Welsh, Michael[1]; Allan, Gordon[2]

Agri-Food and Biosciences Institute[1]; Queen's University Belfast[2]; Merial SAS[3]

Key words: Porcine bocavirus, full coding sequence

Parvoviruses are small non-enveloped ssDNA viruses with a ~5Kb genome. The genus bocavirus includes bovine parvovirus 1 (BPV-1), canine minute virus (CMV), human bocaviruses (HBoV), gorilla bocavirus (GBoV1) and porcine bocaviruses (PBoV). Research carried out in Northern Ireland resulted in the isolation in cell culture of 2 novel PBoVs designated as PBoV 3 & 4 (1). At present there are a myriad of PBoV species and variants (2-5), each with a 3 open reading frame (ORF) genetic structure not typical of most parvoviruses. In this study we briefly discuss the full genetic organisation of PBoV 3 & 4 and analyse their genetic relatedness to other bocaviruses.

PBoV3 & 4 were recovered from postweaning multisystemic wasting syndrome (PMWS)-affected farms. Both were passaged in primary pig kidney cells. Virus pools were purified using sucrose and CsCl gradient purification and nucleic acids extracted. Extracted material was fragmented using restriction enzymes and cloned. PBoV3 (JF512472) & 4 (JF512473) were partially sequenced (1) leaving the 3' end of both viruses incomplete. Subsequent cloning resulted in 5228bp sequence data for PBoV3 & 5131bp for PBoV4. ORF analysis of these sequences suggested the full coding sequence had been completed including the VP1/2 coding regions of both viruses. Putative full coding sequence was compared using ClustalW 2.1 sequence analysis software with current PBoVs and those from other species including BPV-1, CMV, HBoV and GBoV1.

Clustal analysis indicated that at nucleotide level PBoV 3 & 4 are 80% similar. PBoV3 & 4 exhibited 38-89% nucleotide homology with other PBoVs; whereas homology with non-porcine bocaviruses ranged from 41-47%. Analysis of the ORFs of PBoV 3 & 4 suggested a NS1, NP1 and VP1/2 genetic organisation as demonstrated by other bocaviruses. However, the NS1 coding ORF1 of PBoV3 is relatively longer than that of PBoV4 and other currently sequenced PBoVs by ~100 amino acids warranting further investigation.

We are the first laboratory to report that PBoVs have been adapted to grow in tissue culture (1). Although the pathogenicity of PBoV 3 & 4 has not yet been determined related PBoVs have been linked to respiratory illness (5) and PBoV3 was isolated from a piglet suffering from diarrhoea and pneumonia. Sequence analysis has demonstrated the ever expanding diversity exhibited between PBoVs. The possibility remains, that PBoVs could be emerging pathogens, if not as primary pathogens then as co-factors in complex porcine disease scenarios. Therefore it is essential that further research is carried out to determine the prevalence and pathogenic potential of these novel PBoVs.

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EED17: Genetic Variability of Foot-and-Mouth Disease Viruses Isolated from Free-Living African Buffalo (Syncerus Caffer) in Southern Africa

Wadsworth, J[1]; Anderson, EC[2]; Ferris, NP[1]; Knowles, NJ[1]

IAH[1]; Veterinary Research Laboratory[2]

Key words: Foot-and-mouth disease, buffalo, VP1, sequencing

African buffalo (Syncerus caffer) can be infected with foot-and-mouth disease virus (FMDV) without clinical signs and may become long term carriers. These buffalo are only known to harbour the three Southern African Territories (SAT) serotypes. It is thought that young buffalo usually become infected within the first year of life, but little is known about the transmission dynamics and virus genetic diversity within buffalo herds. It is suspected that African buffalo sometimes initiate outbreaks of FMD in domesticated species, either directly by an unknown mechanism(s) or indirectly via other wildlife species in which clinical disease is apparent (e.g. impala). In this study scrapings from the upper oesophagus and pharynx area were taken from free-living African buffalo in multiple herds in six areas around Zimbabwe between 1990 and 1998, viz. from i) the Gona-re-zhou National Park and adjoining Hippo Valley Game Ranch, the Lone Star Ranch, Nuanetsi Ranch and Save Conservancy; ii) the Doma and Dande Safari Areas in the lower Zambezi valley; iii) the Urungwe Safari Area in the Zambezi valley east of Lake Kariba; iv) the Matusadona National Park on the southern shores of Lake Kariba, the Bumi Hills Nature Reserve, on the east shore of Lake Kariba; v) the Chirisa Safari Area south of Lake Kariba; and vi) the Hwange and Victoria Falls National Parks. Viruses were isolated on primary bovine thyroid cells and were typed by ELISA as SAT 1 (n=89), SAT 2 (n=26) or SAT 3 (n=42), although five samples contained mixtures of two serotypes. The complete VP1 gene sequence was determined for the majority of the virus isolates and compared with each other and with other FMDV SAT sequences (including some from African buffalo located in neighbouring countries). Genetically most viruses grouped together in accordance with the buffalo herd from which they were sampled; however, some herds contained animals with genetically diverse virus lineages. The very close relationship between viruses, isolated from some young animals within a herd, suggested the possibility of acquired infection during “mini-outbreaks” rather than multiple transmission events from older animals. This is a unique collection of viruses and although they were collected in the 1990s, they still have relevance to tracing the origins of outbreaks today.

EED18: PASSIVE SURVEILLANCE OF BATS IN THE NETHERLANDS. MOLECULAR EPIDEMIOLOGY AND EVOLUTION OF EBLV-1.

Kooi, Bart[1]

CVI[1]

Key words: EBLV-1, bats, phylogeny, surveillance

Introduction. Since 1987 a reactive surveillance program is running in the Netherlands to investigate the presence of European Bat Lyssavirusses (EBLV-1 and EBLV-2) in bats. In this program all contact cases, with human or pets, are send to the laboratory in Lelystad and tested for EBLV with a EU prescribed immune fluorescence test (IFT). Up to date almost 5000 samples have been tested. The main reservoir for EBLV is Eptesicus serotinus, 334 positive cases so far which is about 22% of the samples tested, second species is Myotis dasycneme, with almost 4% positive thus far. EBLV-2 has not been detected in the Netherlands since 1993.

We performed a molecular epidemiology study on a collection of more then 40 EBLV-1 viruses that currently circulate in the Netherlands. Based on the sequences of the coding regions of the N and G genes a phylogenetic tree was generated in which the two distinct lineages or subgroups EBLV-1a and EBLV-1b were clearly visible. Surprisingly the larger EBLV-1a group could be further subdivided into three phylogenetic groups that were consistent with topology. Two groups were predominantly found in the northern provinces whereas the third group was spread from east to west in the middle of the country.

A most recent common ancestor analysis was performed which confirmed a distinct difference in origin of the two EBLV-1 subgroups. Whereas the EBLV-1a subgroup migrated into Europe via the east-west route, EBLV-1b apparently entered the continent from the south, most likely through the Iberian Peninsula.

EED19: EPIZONE: DETECTION AND GENETIC CHARACTERIZATION OF CORONAVIRUS IN BATS FROM NORTHERN ITALY

Papetti, Alice[1]; Lelli, Davide[1]; Moreno, Ana [1]; Cordioli, Paolo[1]; Boniotti, Maria Beatrice[1]

IZSLER[1]

Key words: Coronavirus; CoV; Bat; Italy; Phylogenetic analysis

Recent studies have identified bats as the most likely source of all coronavirus (CoV).
In 2005, bats were recognised as the likely natural host of Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) (1). Since then, an increasing number of novel Bat-CoVs has been identified from several continents (China, north and south America, Africa and Europe) (2-6). In contrast, their presence in Italy is still poorly studied (7).
CoVs are enveloped viruses with a positive-sense single-stranded RNA genome of 26-32 kb and are subdivided into three genera.
CoVs are considered as a group of viruses with a high potential for interspecies transmission, both for their large genome susceptible to mutation and recombination events as for their diffusion in a wide variety of animals.
The aim of this study is to determine the presence of CoVs in Northern Italian bats and it is a part of a larger survey to investigate the diffusion of viral agents in Italian bat populations, explained in detail in the work presented in this congress by Lelli et al. (8).
In this work we analysed 25 feces and 84 carcasses from 8 different bat species collected from summer 2010 to summer 2011.
The samples were subjected to RNA extraction and screened for the presence of CoVs using a one-step reverse transcription (RT) – polymerase chain reaction (PCR) with pan-coronavirus degenerate primers that amplify a small fragment (180 bp) of the RNA-dependent RNA polymerase (RdRp) gene. The PCR products were sequenced to confirm the presence of CoVs. Phylogenetic analysis was performed by alignment of sequences obtained by a second RT-PCR amplification of a 440 bp region of the same gene. The sequences were compared to the available CoV sequences in the GenBank database, using the Basic Local Alignment Search Tool (BLAST) program and phylogenetic trees were drawn using the neighbor-joining method with the MEGA 5 software.
CoVs were detected in 2 out of 25 fecal samples and in 10 out of 84 intestine. We found CoV RNA in 3 different bat species: Pipistrellus Khulii, Hypsugo Savii and Nyctalus Noctula.
Phylogenetic analysis of the RNA polymerase sequence fragment showed the presence of a major cluster including 9 strains related to the group Betacoronavirus. None of them were closely genetically related to SARS-CoVs. The remaining 3 strains clustered in the group of Alphacoronavirus.
This study underlines the diffusion and genetic diversity of CoVs in Italian bat populations and reinforces the idea that a surveillance activity is likely necessary to discover possible new zoonotic agents and eventually to prevent the spread of bat-associated zoonosis.

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EED20: Entomological survey for Rift Valley Fever in Namibia in 2011

Fares, Fabrizio[1]; Bishi, Alec[2]; Goffredo, Maria[1]; Polci, Andrea[1]; Pinoni, Chiara[1]; Molini, Umberto[1]; Monaco, Federica[1]; Scacchia, Massimo[1]

Istituto G. Caporale[1]; Directorate of Veterinary Services, MAWF[2]

Key words: Rift Valley Fever, entomological surveillance, Namibia, Culex theileri

Rift valley Fever (RVF) is an acute arthropod-borne zoonotic disease caused by a Phlebovirus in the family Bunyaviridae.
Even if direct transmission through contact with infected tissue might occur and could play an important role in human infection, mosquitoes still represent the most common way to spread the virus. Several mosquito species have been considered as vectors and reservoirs of the virus. Mosquitoes of the genera Aedes and Culex are known to play respectively a complementary role of “vector-reservoir” and “vector-amplifier”. Transmission occurs to a wide range of vertebrates including humans. It can cause high mortality rates in newborn ruminants, especially sheep and goats, and abortion in pregnant animals.
RVF is endemic in most of the sub-Saharan territories with different cyclic reoccurrence causing severe epidemics with many human and animal fatalities. The same devastating consequences are known to occur once the virus circulate outside the traditional endemic territories as reported in Egypt, in Saudi Arabia and Yemen. There is a strong association between outbreaks and extensive rainfall which is thought to favour infected mosquito breeding.
Between May and June 2010, RVF outbreaks were confirmed in sheep farms in southern Namibia. Clusters of infection were identified in the regions of Hardap and Karas. In seven farms located in the same regions an entomological survey was performed in 2011. During the field activity the survey was extended to the Northern region of Oshikoto (2 farms), where outbreaks occurred in the meantime. The aim of the survey was to identify the possible mosquito vector species involved in the virus transmission to humans and animals. Mosquitoes have been collected using BG-Sentinel traps and light traps, in order to collect diurnal and nocturnal species. The adult insects were killed by freezing for a few minutes, identified at species level, divided in pools and submitted for the virus detection by real time RT-PCR.
A total of 1430 mosquitoes have been collected, belonging to 6 genera and 11 species: Aedes (Stegomya) metallicus (Edwards, 1912), Anopheles (Cellia) demeilloni (Evans, 1933), Anopheles (Cellia) listeri (de Meillon, 1931), Anopheles (Cellia) marshallii (Theobald, 1903), Coquillettidia (Coquillettidia) chrysosoma (Edwards, 1915), Culex (Culiciomyia) nebulosus (Theobald, 1901), Culex (Culex) pipiens (Linnaeus, 1758), Culex (Culex) theileri (Theobald, 1903), Culex (Culex) univittatus (Theobald, 1901), Culex (Culex) quinquefasciatus (Say, 1823) and Culiseta (Allotheobaldia) longiareolata (Macquart, 1838).
The most abundant species resulted Cx. pipiens (22.7%), Cx. theileri (12,5%), An. demeilloni (11,5%) and An. listeri (11,2%).
A total of 132 mosquito pools have been examined for virus detection by RT-PCR, and one pool of 42 not engorged females of Culex theileri, collected in the Oshikoto region, was found positive.

EED21: Full genome sequencing and phylogenetic analyses identify California mosquito pool virus as a new member of the species Corriparta virus (genus Orbivirus, family Reoviridae)

Belaganahalli, Manjunatha N[1]; Maan, Sushila[1]; Maan, Narender[2]; Nomikou, Kyriaki [1]; Guimera, Marc[1]; Attoui, Houssam[1]; Mertens, Peter P C[1]

IAH[1]; LLR University of Veterinary and Animal Sciences[2]

Key words: Corriparta virus, Orbivirus, California mosquito pool virus, Sequencing, Phylogenetic analysis

The genus Orbivirus is the largest genera of the family Reoviridae, containing 22 recognized virus species as well as 15 unclassified 'orbiviruses', some of which may represent novel virus species. The orbiviruses are arboviruses that are transmitted by both ticks and/or hematophagous insect vectors (phlebotomine flies, mosquitoes or Culicoides). The orbiviruses have wide host range that includes domestic and wild ruminants, equines, marsupials, sloths, bats, birds, humans, etc. The emergence of multiple bluetongue virus (BTV) serotypes in Europe, USA, Australia as well as the detection of BTV-25, BTV-26 in Switzerland and Kuwait, and the emergence of other orbiviruses (Wallal, Warrego and Eubenangee viruses) in Australia, may be linked with climate change, illustrating the continuing and possibly increasing threat posed by these viruses.

BTV, African horse sickness virus (AHSV) and Epizootic haemorrhagic disease virus (EHDV) are all transmitted by Culicoides and currently are the most economically important orbiviruses. Sequences are available in public databases for multiple isolates of these three virus species / serogroups. Further sequence data is also available for another 3 Culicoides borne Orbivirus species (Palyam virus, Eubenangee virus and Equine encephalosis virus). However, full genome data are available for only three mosquito borne orbivirus isolates (Umatilla virus, Peruvian horse sickness virus and Yunnan orbivirus), one tick borne orbivirus, Great Island Virus (GIV), and one distantly related tick virus, St Croix River virus (SCRV). The lack of a comprehensive data set representing all Orbivirus species has made phylogenetic comparisons, molecular diagnosis (e.g. by RT-PCR assays) and taxonomic classification of other novel virus isolates, more difficult.

Corriparta virus (CORV) is a mosquito borne orbivirus belongs to the Corriparta virus species, which contains a total of 6 serotypes/isolates. CORV was isolated from birds, and neutralizing antibodies were found in both domesticated animals and human. The virus has therefore been recognised as zoonotic, although no clinical manifestations have been identified in humans. Partial genome sequences of the sub-core T2 protein of CORV are available in GenBank but these data are not sufficient for the conclusive identification and taxonomical classification of CORV isolates. We report full-genome sequence-data of Corriparta virus, as part of a project to generate data for the entire genomes from representative isolates of each Orbivirus species.

Comparisons of CORV sequences to the partial sequences available for California mosquito pool virus (CMPV) indicate that these two viruses share >90% aa identity in their conserved T2 and outer core (T13) proteins. Phylogenetic analysis of orbiviruses identified three distinct grouping of orbiviruses corresponding to their transmission vectors suggesting orbiviruses are evolving along with their vectors through the co-speciation process. A wider phylogenetic analysis of the T2 and T13 protein sequences (which determines the size and shape of the virus core) also grouped CORV and CMPV with other mosquito borne orbiviruses.

In conclusion, identity levels in aa sequence and phylogenetic grouping indicates that CMPV is a member of the existing Corriparta virus species and not a new species, as was proposed earlier (Victoria et al., 2008).

Victoria, J.G., Kapoor, A., Dupuis, K., Schnurr, D.P., Delwart, E.L., 2008, Rapid identification of known and new RNA viruses from animal tissues. PLoS Pathog 4, e1000163.

EED22: Bluetongue virus detection in Culicoides biting midges in Italy

Goffredo, Maria[1]; Quaglia, Michela[1]; Ippoliti, Carla[1]; Federici, Valentina[1]; Di Gennaro, Annapia[1]; Casaccia, Claudia[1]; Savini, Giovanni[1]

IZSAM Teramo[1]

Key words: Bluetongue, Culicoides, C. imicola, Obsoletus Complex

Since 2000, Italy has been experiencing the incursion of several serotypes of bluetongue virus (BTV1, 2, 4, 8, 9,16). Besides the clinical outbreaks, BTV circulation has been monitoring through serological and entomological surveillance program which includes sentinel animals and black light traps located all over the country. Collections of Culicoides biting midges were made on a regular basis in permanent collection sites. In case of outbreak/seroconversion, further traps were placed in those farms with seroconverted or BT clinically suspected animals, in order to identify potential vector species. All the collections were analyzed at species or complex level, and ad hoc pools were sorted and tested by RT-PCR and, in some cases, by virus isolation to assess the presence of BTV.

The RT-PCR was able to detect BTV RNA in 188 of the 2.741 Culicoides pools examined. Positive midges included C. imicola, C. obsoletus/scoticus and C. pulicaris. It was also possible to isolate in cell culture strains of BTV2 and BTV9 from C. obsoletus/scoticus and strains of BTV1 and BTV4 from C. imicola.

This study correlates the distributions of the different BTV serotypes which have circulated/are circulating in Italy with those of the various vector species flying in the same areas and discusses the possible role played by each Culicoides species in transmitting BTV in Italy.

EED23: Centralized Database for poultry farms: a public-private synergy

Bortolotti, Laura[1]; Cobianchi, Mario[1]; Rostellato, Daniele[1]; Dalla Pozza, Manuela[1]; Favero, Laura[2]; Marangon, Stefano[1]

IZS-Ve[1]; Veneto Region[2]

Key words: Database, poultry companies, birds movements

Since 1997, when the first Highly Pathogenic Avian Influenza (HPAI) outbreak occurred in Northern Italy, the Veneto Region has implemented a centralized database to register industrial poultry farms. This has been considered an essential tool for the appropriate management of both surveillance and monitoring plans and disease emergencies in the poultry sector. The system has been gradually implemented in order to acquire more detailed and reliable information on animal population and on its trade flows, involving poultry companies in the process of the information development.

For each poultry holding, the database includes: i) farmer personal information and role (owner, keeper of animals), ii) holding data such as location (address, geographical coordinates), number of poultry sheds, number of birds to be housed per production/cycle, farming methods, and iii) data on birds such as number, species, type of production, date of restocking, date of loading for slaughter and health status.

At the very beginning data was stored and updated by the Veterinary Services (VS) exclusively. However this system didn' t allow the continuous update of data, since the information regarding commercial links between poultry Companies and farmers and data on types of birds produced in each farm change rapidly and is market determined.

Since 2006, farmers and poultry companies have had the possibility to directly manage and update data on the movements of birds in the regional database, by means of a software which uses a web services technology, according to the HTTP SOAP/XML standards. This choice has optimized the overall efficiency of the system.

In 2011 over 200 million birds were housed, corresponding to 5,382 records, of which 2,413 were transmitted directly by the breeders' management software, without any manual intervention.

A web-oriented reporting service has also been developed in Ajax to inform the VS about the birds' arrival in each farm a week before and its compliance with the sanitary regulations and welfare conditions. Data are submitted to an automated validation process, the discrepancies between the data reported by the Companies and those registered in the Database by VS are outlined and their correction is monitored regularly. Similarly the holdings without any birds restocking are identified and reported to VS in order to control the real situation of the farm. As well the system offers a specific tool for farmers and VS to verify the cage area per laying hen according to Council Directive 1999/74/EC and 2002/4/EC.

The automated data transmission has decreased the workload for operators and the errors tied to data entry with a clear improvement of data quality and reliability. The automatic comparison between data collected from different sources has improved the consistency of the data set, whilst the reports on data quality have increased the awareness of operators and the accuracy of data. Thus the number of anomalies has progressively decreased over time.

The system offers a real-time knowledge of the industrial poultry sector in a densely populated poultry area and is an essential tool to generate reliable information to be used for disease control in case of an epidemic (immediate identification of at risk farms and areas, rapid definition of restriction zones, etc.). Moreover the dataset, which has a historical depth of more than 6 years, generates data and information to be used for the correct planning of monitoring programs, epidemiological studies and risk assessment.

EED24: West Nile disease in Sardinian wild birds

Savini, Giovanni[1]; Monaco, Federica[1]; Briguglio, Paolo[1]; Pinto, Stefano[1]; Pinoni, Chiara[1]; Polci, Andrea[1]; Di Gennaro, Annapia[1]; Pais, Monca[1]; Teodori, Liana[1]; Bruno, Rossana[1]; Ruiu, Angelo[1]; Lelli, Rossella[1]

IZS "G. Caporale"[1]

Key words: west nile virus, wild birds, neurological signs, NS3

In the last decade West Nile virus (WNV) has significantly extended its historical geographical range by affecting more countries than before. In Italy the virus first appeared in 1998. After 10 years of silence, it re-appeared in 2008 and it has been continuously circulating since. WNV started initially in the North Eastern part of Italy and then it spread to the Centre-South invading Sicily in 2010. Infections have been characterised generally by mild clinical signs. However severe disease and deaths were observed only occasionally in humans and horses. Several species and genera of mosquitoes can act as vectors for WNV while several species of birds can act as amplifying hosts. In some species of birds infection is associated with mortality and disease, in others infection can be unapparent. In Italy, in the recent years infection has never been associated to clinical signs in wild birds until the last September, coincident with the appearance of WNV in Sardinia. During this epidemic, several horses became infected, some of them died or were euthanised because of the severity of the clinical signs. Most of the horses recovered after showing typical neurological symptoms. In the same period and in the same area an unusual number of wild birds dying after showing neurologic illness, were noted. Between September and November 2011, a total of 52 birds were admitted to a local rehabilitation clinic. Out of them, 13 showed neurological signs including lethargy, depression, ruffle feathers, flaccid paralysis of the wing muscles thus implying incapability of flying, pectoral atrophy, absence of the flight instinct, weak legs, haemorrhagic nostril discharge. All of them died within two or three days. A post mortem examination was carried out and brain, lung, spleen and kidney samples were tested for the presence of WNV by real time RT-PCR and classical virus isolation. WNV was detected in the tissues of one owl (Athene noctua), two buzzards (Buteo buteo), two eurasian jays (Garrulus glandarius) and one wild duck (Anas platyrhynchos). All strains belonged to lineage 1 WNV. In four occasions it was also possible to isolate the virus in cell culture. A 975bp fragment of the NS3 encoding gene of the isolate detected in the owl, shared high nt homology with the other WNV strains which have recently circulated in the Mediterranean Basin. However, when the deduced aminoacid sequences were compared, that of the owl WNV strain was similar to the other Italian strains showing a threonine residue in position 249 whereas the others exhibited a proline in the same place. In the owl isolate an alanine at position 436 and an arginine residue at position 476 replaced threonine and glycine respectively. Whether the alanine at position 436 was found in all the Sardinian strains the arginine residue at position 476 was peculiar of the owl strain. This report describes for the first time the potential role of WNV in determining neurological clinical signs in Italian wild birds. Whether the atypical aminoacid costellation of the NS3 of the owl isolate might have played a role in virulence needs to be further investigated by reverse genetics.

EED25: West Nile and Usutu virus vectors in Italy

Fares, Fabrizio[1]; Savini, Giovanni[1]; Goffredo, Maria[1]; Cipriani, Micaela[1]; Di Gennaro, Annapia[1]; Marini, Valeria[1]; Santilli, Adriana[1]; Ippoliti, Carla[1]; Monaco, Federica[1]; Lelli, Rossella[1]

IZSAM Teramo[1]

Key words: West Nile, Usutu, mosquitoes

West Nile (WNV) and Usutu viruses (USUV) have been circulating in Italy since many years. The activities described in this report were part of the extensive National surveillance plan for monitoring Flavivirus which is in place since 2002 and is lead by the National Reference Centre for Foreign Diseases (CESME). The program involved horses, insects as well as wild and domestic birds.
The entomological surveillance is based on a net of collection sites selected in risky areas and in areas with virus circulation. In order to identify possible vector species a comprehensive mosquito collection was further set up during outbreaks(i.e. Emilia Romagna, Sardinia, Sicily).

Between 2008-2011, a total of 2415 mosquito collections have been performed all over Italy, using CDC light-traps, BG Sentinel traps, gravid traps, aspiration and larval collection. More than 27,000 mosquitoes have been collected and identified. They belonged to 24 species and 7 genera including either Culicinae and Anophelinae subfamilies. Culex pipiens and Ochlerotatus caspius resulted the dominant species, representing together more than 80% of the total collected mosquitoes. In the areas with active circulation, WNV was detected in Cx. pipiens, Oc. caspius and Cx. modestus.
Similarly, when the mosquito pools were tested by RT-PCR and virus isolation for the presence of Usutu virus, it was detected in Cx. pipiens, Oc. detritus and Culiseta annulata.

In 2010-2011 winter season an overwintering study was also carried out in the Molise Region. It was observed that mosquitoes although at low abundance, were still present during the winter months in the studying area, even with temperatures below zero. The most abundant species found was Cx. pipiens, followed by Cs. annulata, Oc. detritus and Oc. caspius. As described before, all these species were involved in the WNV and/or USUV life cycles and, thus, the possibility of these viruses to overwinter through adult mosquitoes cannot be excluded.

EED26: Genetic diversity of bovine viral diarrhea virus (BVDV) detected in cattle in Turkey

Yilmaz, Huseyin[1]; Altan, Eda[1]; Turan, Nuri[1]; Ridpath, Julia[2]

University of Istanbul[1]; United States Department of Agriculture[2]

Key words: BVDV, Cattle, ELISA, real time RT-PCR, E gene, 5UTR, diversity, Turkey

Abstract

Rapid detection, culling and strains used for vaccination are key factors to control Bovine viral diarrhea (BVD) in cattle. The aim of this study was to investigate frequency and diversity of bovine viral diarrhea virus in cattle in Turkey. For this, 1124 blood samples were analysed by antigen-ELISA. BVDV was detected in 13 of 19 farms. When 20 of 26 ELISA positive samples retested by ELISA and real-time RT-PCR, 6 of them were found to be positive by both assay. The regions on glycoprotein E2 (Gp53) and 5'UTR of BVDV were sequenced in 19 positive samples. The alignment indicated that the 17 Turkish BVDVs clustered with the BVDV-1 and 2 Turkish BVDVs with the BVDV-2. Based on phylogenetic analysis of the 5' UTR, 8 strains (5, 6, 10, 11, 12, 13, 17 and 19) clustered with BVDV1f sub-genotype. One strain (4) was closer to the BVDV1f sub-genotype than to other sub-genotypes, but is relatively distant from other BVDV1f sub-genotypes. Strain 8 appears to be a BVDV1i and 14 is a BVDV1d. The remaining 6 strains (1, 2, 3, 7, 9, 18) belong to a novel sub-genotype. Similar results were obtained on the bases of comparison of E2 sequences with one exception. Strain 5 clusters as a BVDV1f based on 5' UTR comparison but is grouped with the novel sub-genotype based on E2 sequences. In conclusion, results of phylogenetic analyses indicate that diverse strains were found in this study and this point should be considered in diagnosis and preventive measurements. Strict eradication programme is necessary to control BVDV infections in Turkish cattle.

EED27: The integrated surveillance system on Mosquito Borne Diseases in the Piedmont region (Northwestern Italy) in an evolving epidemiological scenario

Bertolini, Silvia[1]; Radaelli, Maria Cristina[1]; Vitale, Nicoletta[1]; Desiato, Rosanna[1]; Pautasso, Alessandra[1]; Casalone, Cristina[1]; Maurella, Cristiana[1]; Ru, Giuseppe[1]; Rizzo, Francesca[1]; Mandola, Maria Lucia[1]; Prearo, Marino[1]; Mosca, Andrea[2]; Masoero, Loretta[1]; Chiavacci, Laura[1]

Istituto Zooprofilattico Sperimentale PLVDA[1]; IPLA[2]

Key words: Mosquito Borne Diseases, integrated surveillance, Northwestern Italy

During last years, there has been a worldwide increasing of Mosquito Borne Diseases (MBD) becoming a dramatic health threat for both humans and domestic animals. West Nile Virus (WNV) is the most widespread MBD in the Mediterranean Basin. Recently it has been evidenced its capability of being endemic in Italy.

Piedmont Region is next to two areas where WNV outbreaks have occurred (Camargue and Emilia Romagna). Usutu virus (USUV), a closely related second MBD, was identified in northern Italy since 2007 (Savini et al., 2011) and caused two cases of human encephalitis in 2009 (Pecorari et al., 2009; Cavrini et al., 2009). The possibility of MBD introduction and spread in Piedmont lead to the need of planning an integrated surveillance system in order to identify early virus circulation even in absence of clinical signs in susceptible hosts.

The aim of our work is to present the integrated surveillance system on MBD enforced in 2011 in Piedmont. Based on evidences in literature, the knowledge of the epidemiological cycle of MBD and the geographical features of our region, we identified different surveillance systems worth to be applied on different segments of the epidemiological cycle of the disease:

1. entomological active surveillance, in order to identify the presence of potential pathogen vectors;
2. virological/serological active surveillance on:
2.1 wild migratory birds, for an early virus introduction identification;
2.2 mosquitoes, for an early individuation of virus circulation;
2.3 sinantropic birds, for highlighting areas where virus may become endemic;
2.4 sentinels, for an early identification of virus transmission to the mammals;
3. passive clinical surveillance on animals and humans, for detecting clinical cases.

On those assumptions an integrated regional surveillance system has been implemented since 2011 in Piedmont from May to September. The system includes: (1) Entomological active surveillance. The Piedmont area under 600 m a.s.l was divided in 42 20X20Km squares where 50 georeferenced traps were placed and sampled twice a month. (2) Wild migratory and sinantropic birds surveillance. Enforced in 2009, under the coordination of the National Reference Centre for Exotic Diseases of animals, consists of a monthly sample of wild birds caught or shot in area of 1400 Km2 in the southeastern part of the region. (3) Virological mosquitoes active surveillance. Mosquito collections to detect the presence of RNA belonging to Flavivirus genus were conducted in 16 sites selected on risk based factors. (4) Sentinel active surveillance in urban context. Settled by the Ministry of Health in 2011 in the province of Turin a sample of 607 horses and 338 bovines were screened by a competitive ELISA. (5) Passive surveillance is being carried out over the entire region.

Our approach demonstrates the applicability of an integrated and multidisciplinary surveillance system with the involvement of many experts in different topics (veterinarians, epidemiologists, entomologists, statisticians, biologists).

2011 entomological surveillance was designed in order to know the real vector distribution in the whole region. The maintenance of this activity for the next two years will provide stronger data to design future risk-based surveillance. Moreover the active surveillance on sentinels in urban context should allow to identify new indicators of the human exposure to MBD.

EED28: Introduction rate of a low pathogenic avian influenza virus infection in different Dutch poultry sectors

Gonzales, Jose L.[1]; Bouwstra, Ruth J.[1]; Koch, Guus[1]; Elbers, Armin R.W.[1]; de Wit, Sjaak J.[2]; Stegeman, Jan A.[3]

CVI[1]; Animal Health Service[2]; Utrecht University[3]

Key words: low pathogenic avian influenza,outdoor farming, poultry, relative risk

Low Pathogenic Avian Influenza (LPAI) viruses of the H5 and H7 subtype have the potential to evolute to Highly Pathogenic Avian Influenza (HPAI) viruses in poultry and therefore infections with these subtypes are notifiable. Consequently, member states of the European Union have implemented surveillance programmes. In the Netherlands a syndromic surveillance and serological monitoring programme is in place. In the monitoring programme, all poultry farms are tested 1-4 times a year. Frequency differs between the different poultry types and housing systems (indoor and outdoor layer chickens, broilers, ducks, turkeys, etc) based on the supposed differences in the risk of introduction of LPAI infections. However, quantitative information regarding the possible differences in risk between these poultry types is sparse. In this study the rate of introduction of LPAI in different poultry types was quantified. Data from the Dutch LPAI surveillance programme(2007–2010) were analysed using a generalised linear mixed and spatial model. Results showed that outdoor-layer farms had a 11, turkey 8, duck-breeder 24 and meat-duck 13 times higher rate of introduction of LPAI than indoor-layer farms. Differences in the rate of introduction of LPAI could be used to (re)design a targeted risk-based surveillance programme.

EED29: Sequencing and phylogenetic analysis of the Eubenangee viruses identifies Pata virus as new species in the genus Orbivirus

Maan, Sushila[1]; Belaganahalli, Manjunatha N. [1]; Maan, Narender S. [2]; Nomikou, Kyriaki [1]; Pritchard, Ian [3]; Lunt, Ross [3]; Kirkland, Peter D. [4]; Attoui, Houssam [1]; Brownlie, Joe [5]; Mertens, Peter P.C.[1]

IAH[1]; LLR University of Veterinary and Animal Sciences[2]; Australian Animal Health Laboratory, CSIRO, [3]; Elizabeth Macarthur Agriculture Institute[4]; Royal Veterinary College, [5]

Key words: Orbivirus, Eubenangee virus, sequence, phylogenetic analysis, species

The Eubenangee viruses are icosahedral, 10 segmented dsRNA viruses belonging to the genus Orbivirus within the family Reoviridae. Since 1998 multiple exotic strains / serotypes of bluetongue virus (BTV) (the Orbivirus type species) have emerged in Europe, North America, and Australia. Novel BTV serotypes (BTV- 25 and BTV-26) have also been identified in Switzerland and Kuwait, and other orbiviruses (e.g. Stretch Lagoon Orbivirus and Minacu virus) have emerged in Australia and Brazil respectively [1,2], illustrating the continuing threat posed by the orbiviruses to both domesticated and wild animals.
The Eubenangee viruses are transmitted by hematophagous-insect-vectors, infecting both domesticated animals (cattle) and marsupials (wallabies and kangaroos). They currently include 4 serotypes (Eubenangee virus (EUBV), Tilligerry virus (TILV), Pata virus (PATAV) and Ngoupe virus (NGOV)), and have been implicated as the cause of Tammar Wallaby Sudden Death syndrome (TSDS), a disease that resembles peracute bluetongue in sheep. The Eubenangee serogroup orbiviruses have shown partial or low level cross-reaction with bluetongue virus in both complement-fixation and agar gel immune-precipitation tests, but are considered to represent a distinct serogroup / virus species.
Although sequence data are available for multiple isolates of BTV and some other orbiviruses (including epizootic haemorrhagic disease virus (EHDV)), no full genome sequences were available for the Eubenangee viruses, making molecular-diagnosis (e.g. by RT-PCR) and unequivocal identification of novel virus-isolates, very difficult.
We report full-genome sequence-data for 3 Eubenangee viruses (EUBV, TILV and PATAV) as part of a project to generate representative data for each of the Orbivirus species [3]. These sequence analyses confirm that all of the Eubenangee viruses are related to BTV and EHDV but do not belong to either of these virus species. These data also indicate that PATAV is not a member of Eubenangee virus species but represents a distinct virus species that is more closely related to EHDV and BTV than to the Eubenangee viruses.
We have proposed to the Reoviridae Study Group of the International Committee for the Taxonomy of Viruses (ICTV) that PATAV should be re-classified as a new species within the genus Orbivirus. Full genome sequence data have revealed the evolutionary relationships of these viruses and will provide a basis for the development of novel diagnostic assays to identify and distinguish them, as well as for the classification of existing and novel isolates.

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EED30: Survey of several infectious diseases of zoonotic and epizootic significance in Austrian wildlife

Steinrigl, Adolf[1]; Bago, Zoltan[1]; Revilla-Fernandez, Sandra[1]; Hofer, Erwin[1]; Duenser, Michael[2]; Wodak, Eveline[1]; Hofrichter, Johannes[3]; Deutz, Armin[4]; Schmoll, Friedrich[2]; Steinrigl, Adolf[5]; Bagó, Zoltán[5]; Revilla-Fernández, Sandra[5]; Hofer, Erwin[5]; Duenser, Michael[5]; Wodak, Eveline[5]; Hofrichter, Johannes[5]; Deutz, Armin[6]; Schmoll, Friedrich[5]

Institute for Veterinary Disease Control[1]; AGES Institute for Veterinary Disease Control[2]; AGES Data, Statistics and Risk Assessment[3]; Bezirkshauptmannschaft[4]; Austrian Agency for Health and Food Safety[5]; Bezirkshauptmannschaft Murau[6]

Key words: wildlife, notifiable infectious diseases

Introduction: The significance of wildlife reservoirs for a number of infectious diseases is increasingly acknowledged. Possible zoonotic threats to humans may arise through direct or indirect contact with wild animals or their products, for example in the course of hunting, or by consumption of undercooked game meats. In addition, notifiable diseases of domestic animals may remain undetected in wildlife for extended periods, posing the risk of spillover to domestic animals. Finally, monitoring of infectious diseases in wildlife can be regarded as an early warning system for emerging infectious diseases, as wildlife is roaming uncontrolled and is naturally exposed to a number of arthropod vectors that can transmit infectious diseases. Despite their potential significance, infectious diseases in wildlife are poorly represented in the scientific literature, probably due to difficulties to sample wild animals in a representative manner. Thus, the aims of the work presented here were (1.) to implement a representative sampling strategy and (2.) to estimate prevalences of selected infectious diseases in several wild animal species in Austria.

Material and methods: A survey design was worked out and implemented in cooperation with the Austrian Hunters' Association, with the aim to representatively sample red fox (Vulpes vulpes), red deer (Cervus elaphus) and wild boar (Sus scrofa), based on recent hunting bag statistics for these species. Foxes and red deer were tested for Mycobacterium tuberculosis complex (MTBC), red deer was additionally tested for Mycobacterium avium ssp. paratuberculosis (MAP). Wild boar were tested for Brucella suis, Suid herpesvirus 1 (SuHV-1) and Classical swine fever virus (CSFV). In addition, wild boar sera were tested for the presence of antibodies to SuHV-1 and CSFV.

Results: In total, 318 red foxes, 275 red deer and 298 wild boars were sampled from April 2011 to January 2012, corresponding to a response rate of 84%, 72% and 78%, respectively. Prevalences for surveyed infectious disease agents were: MTBC in red fox: 0.0%; MTBC in red deer: 0.5%; MAP in red deer: 1.6%; Brucella suis in wild boar: 1.0%; SuHV-1 in wild boar: 1.0%; CSFV in wild boar: 0.0%. Prevalences of antibodies to SuHV-1 and CSFV were 22.8 and 0.0%, respectively.

Discussion: Despite mild weather conditions in autumn 2011 that complicated efficient hunting, response rates were close to (red deer) or exceeding (wild boar, red fox) 75% of the scheduled sample sizes for the whole country. However, locally, certain areas were clearly underrepresented, as some hunters refrained from submitting the requested number of samples. Nevertheless, this survey is the first reliable estimate of selected disease prevalence in Austrian wildlife and provides important information regarding possible transmission scenarios between wildlife and domestic animals.

EED31: Epidemic spread of Usutu virus in Germany in year 2011

Ziegler, Ute[1]; Jöst, Hanna[2]; Becker, Norbert[2]; Eiden, Martin[1]; Rodriguez, Ariel V.[1]; Angenvoort, Joke[1]; Höper, Dirk[1]; Hoffmann, Bernd[1]; Emmerich, Petra[3]; Bosch, Stefan[4]; Konrad, Armin[5]; Schmidt-Chanasit, Jonas[3]; Groschup, Martin H.[1]

FLI[1]; German Mosquito Control Association (KABS)[2]; Bernhard-Nocht-Institute, Department of Virology[3]; Nature and Biodiversity Conservation Union (NABU) [4]; Avifauna-Nordbaden.de[5]

Key words: Usutu virus, Arbovirus, Emerging Diseases, Flavivirus, Germany

Usutu virus (USUV) is an arthropod-borne (arbo), single-stranded RNA virus belonging to the Japanese encephalitis virus group within the family Flaviviridae.
In June 2011, Blackbirds (Turdus merula) were frequently found dead in areas close to the cities of Mannheim and Heidelberg, Germany, which cumulated in a massive die off in these cities and neighboring Rhine valley areas. Moreover, several dead Great Grey Owls (Strix nebulosa) were reported from the zoological gardens of Heidelberg and Mannheim. These mysterious losses were eventually explained by the real-time PCR detection of USUV in an affected animal. Until end of 2011 a total number of 223 dead birds were collected and tested for the presence of viral pathogens. USUV RNA was detected by real-time RT-PCR in 86 birds representing 6 species. Virus was isolated in cell culture from different organs of the infected Blackbirds. The complete polyprotein coding sequence was obtained by deep sequencing of liver and spleen samples of a dead Blackbird. Phylogenetic analysis of the USUV isolate revealed a close relationship with a strain from Vienna that caused mass mortality among birds in Austria in 2001. Apart from wild birds also birds kept in aviaries were affected.
USUV had already been detected in 2010 in a mosquito-based surveillance program in Germany. However, no USUV-specific antibodies or specific nucleic acid sequences were found in serological monitoring studies of wild and resident birds which were sampled between 2007 and 2011. However, some of the migratory birds carried neutralizing antibodies against West Nile virus (WNV) as discriminated by USUV and WNV cross-neutralization tests.
The appearance of USUV in the river Rhine valley provides proof of principle for the possibility that epidemics with other arboviruses may arise as well. Therefore a state-of-the-art monitoring system for such emerging viruses seems mandatory.

EED32: Comparison of three surveillance systems for the determination of West Nile virus enzootic circulation during the 2010-2011 epidemics in Greece

Dovas, Chrysostomos I.[1]; Chaintoutis, Serafeim C.[1]; Chaskopoulou, Alexandra[2]; Gewehr, Sandra[3]; Papanastassopoulou, Maria[1]; Mourelatos, Spiros[3]; Bouzalas, Ilias[1]; Zientara, Stephan[4]; Lecollinet, Sylvie [4]; Danis, Kostas[5]

Faculty of Vet. Medicine, Aristotle University[1]; United States Department of Agriculture[2]; Ecodevelopment S.A.[3]; European Reference Laboratory of equine diseases[4]; Hellenic Cent. for Disease Control and Prevention[5]

Key words: West Nile virus, surveillance, early warning, chickens, pigeons

During 2010, a large outbreak of West Nile virus (WNV) infections occurred in Greece with 262 reported human cases. Among them, 197 were diagnosed with West Nile neuroinvasive disease (WNND), whereas 35 patients died. WNV infection was also confirmed in 17 horses with neurological sings. The epidemic reoccurred in 2011. A study was conducted aiming to compare three surveillance systems' capability (domestic pigeons, free-range chicken, and captive chicken) of providing data on WNV enzootic circulation, prior to the onset of human cases in Central Macedonia, Greece. The spread of WNV in pigeons was also determined, after the 2010 and 2011 epidemics, and pigeon seropositivity was associated to incidence rates of human WNND.

Between October 2010-February 2011 and in October 2011, blood samplings were conducted from 131 and 42 coops, 5 birds younger than one year old per coop, respectively. Sera were assayed by ELISA for the detection of WNV specific antibodies and positive results were confirmed with seroneutralization test. Pigeon seropositivity correlated with the incidence of human WNND by municipality. The determined pigeon seropositivity in the epicentre of the epidemic (Imathia and Pella prefectures) was high regarding 2010 (68-84%) whereas it was lower in 2011 (29-35%).

In June-July 2011, pigeon sera were obtained from 54 coops, born after the November of 2010 or tested as seronegative the previous winter. Antibodies were detected in 20 birds, while the first two were sampled on June 16 in Pella municipality, 1.5 months before the onset of human cases in the region (July 29).

Between 16-27 June 2011, blood was taken from free-range chickens younger than 5 months old belonging to 46 coops. 23 seroconverted birds were identified, while the first 3 were sampled in Veria municipality, 1.5 months before the onset of human cases.

Finally, in May 2011, 7 cages of 6 chickens each were placed at the edges of Thessaloniki City and blood samples were collected weekly until October. Mosquitoes were also sampled in the vicinity of the cages using CO2-baited CDC light traps. In total, 11 chickens seroconverted throughout the summer. The first seroconversion was detected on June 29, 1 month before the onset of human cases. WNV was detected in 2/11 seropositive chickens with RT-PCR from samples taken one week prior to seroconversion. WNV was isolated in Vero cell culture. Part of the NS3 gene was determined to be identical to the strain "Nea Santa-Greece-2010", concluding that the strain became endemic for Greece. Culex mosquito populations peaked in mid-June, ~10 days prior to the first chicken seroconversion, remained high throughout the summer and showed a significant drop in September.

Consequently, all three surveillance systems can successfully be used, in order to detect enzootic circulation of WNV and predict the increased risk for human transmission in Greece. It is important that WNV enzootic transmission was detected 1.5 months prior to human cases. As a result, health authorities were informed in a timely manner and were facilitated the successful implementation of preparedness plans to protect public health and minimize the impact of the epidemic of 2011. Domestic pigeons and free-range chicken surveillance were proved to be a low cost and convenient system for early warning and the determination of transmission foci, whereas repetitive sampling in captive chickens was more informative regarding the determination of the epidemic development and the isolation and molecular identification of WNV circulating strain.

EED33: DISCONTOOLS - Prioritizing Research

Declan, O'Brien[1]

IFAH-Europe[1]

Key words: Prioritization, emerging diseases, technology, zoonoses, Public-private partnerships

On the initiative of the European Commission, a range of technology platforms have been established, led by the respective industries, to drive technological development, resulting amongst others in the creation of the European Technology Platform for Global Animal Health (ETPGAH). Its focus is on threats to the European animal and human population from emerging diseases, also taking a global perspective. It looks at availability of tools for diagnosis, prevention (vaccines) and treatment (pharmaceuticals) bringing researchers in the relevant areas to one table and facilitating exchange of experience and development of new projects.

By identifying the most critical gaps and focusing research, progress will be made more quickly in developing our capacity to control diseases. ETPGAH succeeded in stimulating cross border research collaboration and in the follow-on project DISCONTOOLS is working on a detailed model to identify gaps and prioritise diseases. DISCONTOOLS, a joint initiative of industry and a wide range of stakeholders including the research community, regulators, users and others, provides a mechanism for focusing and prioritising research that ultimately delivers new and improved vaccines, pharmaceuticals and diagnostic tests.

The project has developed a model, based on 52 diseases, which includes the collection of critical data, identification of gaps in knowledge, assessment of impacts on animal health and welfare, public health, wider society, trade and assessment of gaps in existing control tools.

The data is available on a public website www.discontools.eu such that comments can be received from the public ensuring that the data remains up-to-date.

The output should guide all parties funding research in terms of focusing the research effort on the most critical gaps in our ability to control the most damaging diseases.

EED34: Novel Rhadinovirus (Gammaherpesvirus) DNA detected in a wild Muntjac deer in Northern Ireland

Lagan-Tregaskis, Paula [1]; McMenamy, Michael [2]; Ball, Cheryl [3]; McCabe, Lorna [2]; McClintock, Julie [4]; Welsh, Michael [5]; Reid, Neil [6]; Dick, Jaimie [6]; McKillen, John : [5]

Agri-Food Biosciences [1]; AFBI [2]; AFBI [3]; Agri-Food and Biosciences Institute, [4]; AFBI[5]; Quercus, The Queen's University, [6]

Key words: Rhadinovirus, gamma herpesvirus, deer,

Herpesviruses are enveloped dsDNA viruses found in the animal kingdom. There are 4 genera in the subfamily Gammaherpesvirinae including Rhadinovirus. Rhadinoviruses have been associated with the development of Kaposi's sarcoma tumors in humans, which presents with cutaneous lesions. Rhadinoviruses have been detected in other species including cattle, mice, sheep and horses. Rhadinoviruses are unique in that they incorporate the host cellular genes into their genome. As part of a wider virological study DNA with a close homology to a known Rhadinovirus was detected in a Muntjac deer culled in Northern Ireland.

Deer, especially introduced species, are a major concern with regards to impacts on biodiversity, forestry and agriculture (1). The invasive Reeves' muntjac deer (Muntiacus reevesi) is native to Asia but established in Great Britain. Muntjac have recently appeared in the wild in Ireland. With no natural predators, the deer in Great Britain are designated as a pest by farmers and foresters. The first verified record in the wild in N. Ireland was confirmed in June 2009. The animal in this study was culled in 2010.

Twenty four different tissues were sampled from the Muntjac carcass. The tissues were homogenised and had nucleic acids extracted. PCR was carried out on the viral nucleic acids with nested primers (2). Weak amplicons of the expected size (215-315bp) were detected in the colon, mediastinal lymph node, lung and blood. Amplicons were purified in advance of sequence analysis. Resultant sequence data was analysed by BlastX. The translated protein sequence was 88% similar to a sequence of type 2 ruminant rhadinovirus from mule deer (Accession no. ADY80543). The sequence matched with lower homology to a wide range of Gammaherpesvirus from the Rhadinovirus and Macavirus genera, including ovine herpesvirus 2, genus Macavirus, which causes sheep-associated malignant catarrhal fever (3), and caprine herpesvirus 2, genus Macavirus, which is known to cause malignant catarrhal fever in some species of deer.

The discovery of a novel Gammaherpesvirus is notable but its significance as a pathogen of deer is unknown at this time or the potential risk to native deer, farmed deer and domesticated ruminant populations. There is no evidence to suggest that this virus is pathogenic, and while this taxonomic group contains non-pathogenic viruses of ruminants, the relatedness of the virus to known pathogens of ruminants is of some concern as there is a possibility of a benign virus evolving to become more pathogenic or expand its host range. In addition apparently harmless viruses can prove pathogenic when introduced to immunologically naive or susceptible populations. So while the risks associated with this virus are probably small the fact that DNA from a novel virus has been identified suggests that there is significant value in future virological work involving invasive species, particularly Muntjac. Further sequencing of the viral genome through standard molecular methods would allow definitive classification of the virus and metagenomic analysis of other samples using next generation sequencing would be a powerful method of further virus discovery.

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NEXT GENERATION SEQUENCING

NGS1: Next Generation Sequencing of Classical Swine Fever Virus and Border Disease virus cloned in Bacterial Artificial Chromosomes

Fahnøe, Ulrik [1]; Höper, Dirk[2]; Beer, Martin[2]; Bruun Rasmussen, Thomas[1]

VET-DTU[1]; FLI[2]

Key words: Next Generation Sequencing, Pestivirus, Bacterial artificial chromosome

Next generation sequencing is a powerful tool for complete sequencing of large amounts of DNA. We have recently cloned full genome cDNA copies (obtained by long-range RT-PCR) of entire genomes of classical swine fever virus (CSFV) strain Koslov and Border disease virus strain Gifhorn into bacterial artificial chromosomes (BACs). From these BACs, RNA copies of the viral genomes can be transcribed in vitro and upon transfection of these RNAs into mammalian cells, autonomous replication of the viral genome occurs and infectious progeny can be rescued. However, we have observed that virus progeny can be rescued only from some of our BAC constructs whereas others are not replication competent. To further analyze this discrepancy we have completely sequenced selected pestivirus BAC DNAs using a 454 Genome Sequencer FLX to evaluate the number/kind of deviations in the cloned genome sequences. In addition, we have sequenced the full genome cDNA fragments used for the BACs by the same approach. This enables us to evaluate in more detail the nature of nucleotide changes in the pestivirus BACs that lead to lack of replication-competence and/or virus rescue. Additionally, detailed knowledge of the genomic sequence can aid the attempts to create new infectious BAC clones. The quality and the depth of the sequence data will be carefully analyzed, compared and presented.

NGS2: epiSEQ - Molecular epidemiology of epizootic diseases using next generation sequencing technology

Van Borm, Steven[1]; Beer, Martin[2]; King, Donald[3]; Cattoli, Giovanni[4]; Haydon, Daniel[5]; Höper, Dirk[2]; Hoffmann, Bernd[2]; De Clercq, Kris[1]; Lambrecht, Bénédicte[1]; Knowles, Nick[3]; Granberg, Fredrik[6]; Monne, Isabella[4]; Fusaro, Alice[4]; Reeve, Richard[5]; Rosseel, Toon[1]; Belak, Sandor[7]

VAR-CODA[1]; FLI[2]; IAH[3]; IZS-Ve[4]; University of Glasgow[5]; Swedish University of Agricultural Sciences[6]; SVA[7]

Key words: next generation sequencing; molecular epidemiology; collaborative research project

This poster presents a novel research consortium supported under the 2nd joint call for transnational research projects by EMIDA ERA-NET.
Epi-SEQ aims to exploit Next Generation Sequencing (NGS) technologies to generate improved tools that can be used during epidemics of viral diseases threatening livestock industries in Europe. A multidisciplinary team with expertise in molecular virology, bioinformatics, modeling, and evolutionary biology will streamline and validate pipelines for sample preparation, data generation and sequence analysis. The project will target important RNA viruses that cause sporadic epidemics in Europe (FMDV, AIV, NDV), or are endemic in wildlife populations in some EU member states (CSFV), as well as DNA viruses that pose a threat to the EU (ASFV and poxviruses). The project will produce high-resolution sequence data from sample collections held in partner laboratory archives, as well as undertake new in vitro experiments to test specific selection pressures that influence viral evolution. These integrative studies encompassing this spectrum of epidemiology will stimulate the development of innovative tools and provide broad insights into the comparative evolutionary ecology and epidemiology of viruses of human and veterinary importance.

INTERVENTION STRATEGIES

IS1: ONE YEAR EFFICACY OF BTVPUR ALSAP® VACCINE AGAINST A VIRULENT BTV-1 OR BTV-8 CHALLENGE IN CATTLE

HAMERS, Claude[1]; GALLEAU, Stéphanie[1]; COUDIER, Yannick[1]; BIBARD, Amandine[1]; DUBOEUF, Michelle[1]; BESANCON, Laure[1]; GOUTEBROZE, Sylvain[1]; HUDELET, Pascal[1]

MERIAL S.A.S.[1]

Key words: Bluetongue, cattle, Vaccine, Duration of Immunity

INTRODUCTION

Bluetongue virus (BTV) can cause an infectious, non-contagious, disease in wild and domestic ruminants. It is transmitted between ruminant hosts through the bites of certain species of Culicoides midges. Transmission occurs mainly in late summer and fall in Europe, when climatic conditions are favorable and adult insects are active. However, the recent BTV outbreaks in Northern Europe have demonstrated that transmission can occur throughout the year.
Herein, we demonstrate in vaccination / challenge studies in cattle that vaccination with inactivated vaccines containing purified BTV serotype 1 (BTV-1) and serotype 8 (BTV-8) provided a full protection for a period of 1 year.

MATERIAL AND METHODS

Four groups of cattle were maintained in an insect-proof facility throughout the experiment.
Group A was vaccinated twice (3 weeks apart) with 1 mL of a BTV-1 vaccine formulated at low antigen dose.
Group B was vaccinated twice (3 weeks apart) with 1 mL of a BTV-8 vaccine formulated at low antigen dose
Groups C and D were not vaccinated and served as controls.
Twelve months after completion of the vaccination, Groups A and C underwent a BTV-1 virulent challenge.
Twelve months after completion of the vaccination, Groups B and D underwent a BTV-8 virulent challenge.
After the challenges, all animals were monitored for rectal temperature, clinical signs and viraemia (qRT-PCR) over 4 weeks.

RESULTS

Maximal rectal temperatures were significantly lower in the vaccinated groups as compared to the control groups .
Clinical scores were lower in the vaccinated groups as compared to the control groups.

Viraemia (qRT-PCR) was detected in all controls (moderate to high copy numbers) at almost all time points. None of the vaccinated animals was ever detected positive.

CONCLUSION

These results demonstrate that vaccination of cattle with BTVPUR ALSAP® provide full clinical and virological protections against a BTV-1 or a BTV-8 challenge for at least 12 months.

IS2: One year efficacy of BTVPUR ALSAP® 1-8 vaccine against a virulent BTV-1 or BTV-8 challenge in sheep

GALLEAU, Stéphanie[1]; HAMERS, Claude[1]; BLANCHET, Michel[1]; COUZEREAU, Marjorie[1]; BESANCON, Laure[1]; DUBOEUF, Michelle[1]; GOUTEBROZE, Sylvain[1]; HUDELET, Pascal[1]

MERIAL[1]

Key words: BTV, sheep, vaccine, duration of immunity

INTRODUCTION

Bluetongue virus (BTV) causes an infectious, non contagious, disease in wild and domestic ruminants. It is transmitted between ruminant hosts through the bites of certain species of Culicoides midges. Transmission occurs mainly in late summer and fall in Europe, when climatic conditions are favourable and adult insects are active. However, the recent BTV outbreaks in Northern Europe have demonstrated that transmission can occur throughout the year.

Herein we demonstrate in vaccination/challenge studies that vaccination with a bivalent inactivated vaccine containing purified BTV serotype 1 (BTV-1) and purified BTV serotype 8 (BTV-8) provided a full protection of sheep for a period of one year.

MATERIAL AND METHODS

Twenty-eight BTV seronegative sheep were randomly allocated to 4 groups of 7 animals each. They were maintained in an insect-proof facility throughout the experiment.
Groups A and B were vaccinated twice (3 weeks apart) with 1 mL of a bivalent vaccine formulated at BTV-1 and BTV-8 antigen titres lower than standard product release levels (BTVPUR ALSAP® 1-8).
Groups C and D were not vaccinated and served as controls.
Twelve months after vaccination, groups A and C were challenged with a virulent BTV-1 strain while groups B and D were challenged with a virulent BTV-8 strain.
Following the challenges, all sheep were monitored for rectal temperatures, specific clinical signs and viraemia (qRT-PCR) over 2 weeks.

RESULTS

The challenge results demonstrated that immunization with BTVPUR ALSAP® 1-8 provides:
- highly significant reduction of hyperthermia following BTV-1 and BTV-8 challenges,
- highly significant reduction of clinical signs following BTV-1 and BTV-8 challenges,
- complete and highly significant prevention of viraemia following BTV-1 and BTV-8 challenges.

CONCLUSION

The study results demonstrated that vaccination with BTVPUR ALSAP® 1-8 provide a full protection against virulent BTV-1 and BTV-8 challenges for at least one year in sheep.

IS3: EPIZONE Time-course of antibody and cell-mediated immune responses to Porcine Reproductive and Respiratory Syndrome virus under field conditions

Dotti, Silvia[1]; Guadagnini, Giovanni[1]; Salvini, Francesco[1]; Razzuoli, Elisabetta[1]; Ferrari, Maura [1]; Alborali, Giovanni Loris[1]; Amadori, Massimo[1]

IZSLER[1]

Key words: PRRS; IFN-γ; ELISA; ELISPOT; field infection

Major discrepancies are often observed between experimental trials of Porcine Reproductive and Respiratory Syndrome (PRRS)-virus infection under controlled conditions in isolation facilities and observations made in the field. Owing to the above, a cohort study was carried out in a PRRS virus-infected, farrow-to-finish pig farm to characterize the time-course of the virus-specific immune response, and the possible repercussions thereof on the vaccination / conditioning programmes on farm. Two groups of replacement gilts were introduced into the farm nurseries and weaned at a mean age of 30 days. At weaning (day 0), they were ear-tagged and blood samples for PCR and assays of antibody and cell-mediated immunity were collected over six months at 4-week intervals. Despite the occurrence of three and two distinct waves of infection in group 1 and 2, respectively, the large majority of animals showed little if any cell-mediated immune response using an interferon-gamma release assay on whole blood. In particular, the interferon-gamma response to the cell lysate control antigen was usually greater than the PRRS virus-specific one. A tendency to a higher prevalence of positive / dubious responses was observed toward the end of the trial. To rule out any possible bias of our test procedure, this was used along with an ELISPOT assay for interferon-gamma-secreting cells on a group of PRRS-virus infected pigs in our isolation facilities. A very good agreement was shown between the two sets of results. As a further control, Pseudorabies-vaccinated pigs under field conditions scored positive in our interferon-gamma release assay. Therefore, under field conditions, no development rather than delayed development of the PRRS virus-specific interferon-gamma response could be the rule for a long time, as also shown in non-adult pigs in a study on US strains of PRRS virus (Klinge K.L. et al., Virology Journal 2009, 6:177). Housing and hygiene conditions, as well as heavy exposure to environmental microbial payloads and airborne LPS in intensive pig farms could adversely affect the host's immune response to PRRS virus and partly account for the discrepancies between experimental and field studies.

IS4: Protection of IFNAR (−/−) mice against Bluetongue virus serotype 8, by heterologous (DNA-rMVA) and homologous (rMVA- rMVA) vaccination, expressing outer-capsid protein VP2

Jabbar, Tamara[1]; Calvo-Pinilla, Eva[2]; Mateos, Francisco [2]; Gubbins, Simon [1]; Bin-Tarif, Abdelghani [1]; Bachanek-Bankowska, Katarzyna [1]; Takamatsu, Haru-Hisa [1]; Ortego, Javier [2]; Mertens , Peter Paul Clement [1]; Castillo-Olivares, Javier[1]

IAH[1]; CISA-INIA[2]

Key words: BTV-8, MVA, VP2, Mouse model, protection

The protective efficacy of vaccination against bluetongue virus (BTV) was tested using expressed serotype 8 (BTV-8) capsid proteins in a mouse model. Two vaccination strategies were evaluated: either by priming with plasmid DNA containing cDNA copies of BTV capsid-genes, expressing proteins VP2, VP5 and/or VP7, followed by vaccination with recombinant Modified Vaccinia Ankara (rMVA) expressing the same proteins; or a prime-boost regime using rMVA (expressing these proteins) on both occasions. The DNA-rMVA, or rMVA-rMVA prime-boost were administered at a three week interval and all of the animals that received VP2 generated neutralising antibodies. The vaccinated and non-vaccinated-control mice were subsequently challenged with a lethal dose (10pfu) of BTV-8. Mice vaccinated with VP7 alone were not protected. However, mice vaccinated with DNA-rMVA or rMVA-rMVA expressing VP2, or VP2, VP5 and VP7 were all protected, with VP2-alone generating the highest level of protection.

IS5: Preliminary study on the construction of recombinant vaccine of goose parvovirus

Tarasiuk, Karolina[1]; Wozniakowski, Grzegorz[1]; Samorek-Salamonowicz, Elzbieta[1]

NVRI[1]

Key words: Derzsy's disease, goose parvovirus, recombinant protein, VP3, vaccine

Derzsy's disease (DD) is a contagious disease, which causes serious losses in mass waterfowl production. The infectious agent of Derzsy's disease is goose parvovirus (GPV), about 20-22 nm in diameter, non-enveloped and assembled from 32 capsomers. The virus belongs to Dependovirus genus. The only means of the disease prevention is properly performed vaccination practice. Due to the high variability of the virus new vaccines containing high amounts of immunogenic and pure proteins of GPV capsid are desired. The outer surface of GPV capsid is formed by both VP2 and VP3 proteins. The one possibility of obtaining GPV capsid recombinant proteins is their in vitro expression in E.coli.
Briefly, during our studies 24/03 vaccine strain and 4 field strains from the department' strain collection were used. The viruses were amplified in goose embryo fibroblasts (GEF) with the Eagle's minimum essential medium with 0.01% antibiotic mixture supplemented with 10% foetal bovine serum. The GEFs were inoculated at the density of 1.0 x 106 cells per ml with the homogenisates of livers and spleens taken from infected geese. After 7 days of cell cultrues incubation the presence of cytopathic effect specific for goose parvovirus was observed. The viral material harvested after three serial passages of GEFs was used for the further investigations. The PCR primers complementary to the VP3 encoding sequences were designed on the basis of the complete genome sequence of GPV. The used primers had introduced restriction sites on the 5' ends of the sequence. Two complete encoding regions were amplified using Pfu polymerase (Qiagen) using Touch-Down PCR protocol. The PCR products were purified by gel extraction then sequenced. Subsequently the PCR products and pT7RS vector were digested with BamHI and SaII then both products were ligated using ligase enzyme mix (Epicentre). BL21 cells were transformed with recombinant pT7RS-VP3 plasmid. The purity of the recombined clones were tested on Luria Bertani plates supplemented with X-Gal and IPTG. The recombined proteins will be purified using 6-His tag then their immunogenity will be analysed. The protein will be used for the construction of vaccine against GPV and ELISA test for the determination of anti-GPV antibody titer.

IS6: Serological cross-reactivity of MVA based vaccines expressing VP2, VP5 and VP7 of African Horsesickness virus serotype 4.

Manning, N[1]; Bachanek-Bankowska, K[1]; Smith, K[2]; Mertens, P[1]; Castillo-Olivares, J[1]

IAH[1]; The Royal Veterinary College[2]

Key words: African Horsesickness, VP2, vaccine, cross protection, MVA

African Horse Sickness (AHS) is an Orbivirus that is spread by Culicoides vectors, causing > 90% mortality in naïve horse populations, with devastating consequences to the equine industry. Current 'live' vaccines do not fulfil safety requirements for use in non-endemic countries. For this reason, modified vaccinia Ankara (MVA) strains expressing AHSV subunit (capsid) proteins are being investigated as alternative vaccines for AHS.
An MVA expressing VP2 from AHSV serotype 4 not only induces virus neutralising antibodies (VNAb) in ponies, but also provides homologous protection in a mouse model. The major neutralising antigen, VP2, is antigenically variable and is distinct between the 9 different AHSV serotypes. Other capsid proteins, including VP5 and VP7, are more conserved between the AHSV serotypes but are thought to exert some conformational influence on the antigenic structure of VP2, potentially enhancing the immunity of VP2 based AHSV vaccines.
We have explored whether the inclusion of MVA viruses expressing VP5 and VP7, in MVA-VP2 based vaccine formulations, increases the cross-reactivity or titre of virus neutralising antibodies, after vaccination in ponies. During the course of these studies we serologically characterised a new set of monotypic AHSV reference strains. The development of monotypic viruses for use in vaccine development is crucial following the recent discovery of AHS reference virus contamination with heterologous serotypes.
Our results indicate that AHSV4-VP2 alone induced a VNAb response that was serotype specific for all of the vaccine combinations tested. The use of VP5 alone did not induce detectable levels of VNAbs, and inclusion of MVA-VP5 or MVA-VP5+MVA-VP7 to MVA VP2 did not enhance the VNAb levels or enhance cross reactivity to heterologous serotypes.

IS7: 'EPIZONE:' Type I and type II interferon responses to infection by attenuated and non-attenuated PRRS virus strains

Razzuoli, Elisabetta[1]; Dotti, Silvia[1]; Villa, Riccardo [1]; Martinelli, Nicola[1]; Ferrari, Maura[1]; Amadori, Massimo[1]

IZSLER[1]

Key words: PRRSV, pig, IFN-alpha, IFN-gamma

The critical issues of PRRS virus (PRRSV) pathogenicity and virus/host interaction are still ill-defined, which may jeopardize the development of effective disease control measures. Also, immunization strategies are of concern since innate and cell-mediated immune responses are delayed and or somewhat suppressed in PRRSV-infected pigs, let alone in the PRRS-vaccinated ones. In particular, there is evidence that some strains can inhibit or dampen IFN-alpha as well as IFN-gamma responses. This could pave the way to prolonged virus persistence in the host. Owing to the above, we decided to perform an experimental infection in naïve pigs to investigate different features of the innate and adaptive immune response, related to the early control of PRRS virus infection. Two different field isolates of genotype I PRRSV were employed in as many experiments: BS114 / 2000 (attenuated in clinical signs, by seven passages in animals) and BSAL / 2011 (non-attenuated, first inoculation in animals), administered by the endonasal route (105 TCID50/ml). On the whole, 6 pigs (group 1) were infected with BSAL / 2011, 7 pigs (group 2) were infected with BS114 / 2000, 5 animals (group 3, controls) were inoculated with placebo (PBS). All pigs were checked until post infection day (PID) 35. No dullness, anorexia and respiratory symptoms were shown in the first two weeks after infection with both viruses; 3 animals out of 6 in group 1 had fever between 7 and 14 DPI and two of them died on PID 23-25, one of them showing mild respiratory signs (sneezing and cough).
All sera of PRRS-virus infected animals were PCR-positive on PID 7; interestingly, all BS114 – infected animals were PCR-negative (with one exception) on PID14, whereas sera of group 1 pigs were positive until PID 21 (3 out of 6), with a nice correlation between fever and duration of viremia. Concerning antibody production, no differences were shown between the two strains; in fact, all infected pigs of groups 1 and 2 showed specific antibodies by PID 14, in agreement with previous studies. Very different were the IFN-alpha and gamma responses in the two groups. Little if any in vivo IFN-alpha response was detected in sera of group 2 animals, as opposed to IFN-gamma, which showed a major peak on PID 7 associated to low IFN-alpha titres (1 UI/ml on average). On the contrary, with one exception, no PRRS virus-specific IFN-gamma response was shown in vitro. The animals of group 1 showed instead a moderate IFN-alpha response: all animals were positive on PID 3 (22±10.4 UI/ml), 7 (4.1±1.5 UI/ml) and 14 (5±1.6 UI/ml). Concerning the IFN-gamma response, no PRRSV-specific production was evidenced in group 1 and only 2 out of 6 animals (on PID 7 and 21) showed a non-specific response.
Our results indicate that early control of PRRS virus infection was not due in our study to a PRRS virus-specific immune response. A peculiar form of innate IFN-gamma response to the BS114 (group 2) was detectable on PID 7, which may have contributed to the subsequent decay of viremia. On the other hand , the lack of type II IFN response could be correlated with a much longer viremia in group 1 animals. Therefore, the active suppression of an early, innate IFN-gamma response of pigs might be a crucial pathogenicity factor of several genotype I PRRSV strains.

IS8: Ganjam/Nairobi sheep disease virus induces a strong pro-inflammatory response in infected sheep

bin Tarif, Abid[1]; Holzer, Barbara[1]; Baron, Michael D.[1]

IAH[1]

Key words: emerging virus; diagnostics

Ganjam virus (GV) and Nairobi sheep disease virus (NSDV), once considered two separate bunyaviruses of the genus Nairovirus, are now recognised as strains of the same virus, although whether the virus originates in India (where it is called GV) or in East Africa (where it is called NSDV) is not yet known. The virus is transmitted by Ixodid ticks, and causes a severe hemorrhagic disease in sheep or goats. The geographical spread of the virus is limited by the availability of suitable tick hosts, and so changes in the distribution of the tick vectors as a result of climate change may bring viruses such as GV/NSDV into European flocks. The virus also provides a useful model for the closely related human pathogen Crimean Congo hemorrhagic fever virus (CCHFV).

We have been studying the replication and pathogenesis of the virus in vitro and in vivo. As part of this project, we have developed a specific and highly sensitive real-time PCR assay for viral genome, which may be useful as a diagnostic tool. The assay detects as few as 5 copies of the genome target, and has no cross-reaction with Dugbe virus, the related nairovirus found primarily in cattle. We have evaluated the assay in animal samples by using it on samples from a small infection study in which the pathogenicity of two GV/NSDV isolates was studied. We found that virus genome could be detected in infected animals as little as 2 days post infection, and that the more pathogenic isolate was associated with higher levels of viraemia. Viral genome RNA could easily be detected in RNA extracted from whole blood or plasma. No particular tissue-specific distribution of virus was observed by real-time PCR.

Further analysis of cytokine responses was carried out by developing a number of real-time PCR assays for ovine cytokine mRNAs. These showed that infection was associated with the increased transcription of a number of inflammatory cytokines (IL-1B, IL-8, IL-12, IFN-γ) in blood leukocytes, and a reduction in transcription of IL-4. Pathogenesis was associated with strong increases in IL-6, IL-10 and TNFα, but a decrease in transcription of TGFβ.

IS9: Characterization of the cellular immunity provided by MVA and DNA vaccines against RVFV in mice

E, Lopez[1]; G, Lorenzo[1]; A, Brun[1]

CISA-INIA[1]

Key words: vaccines, cellular response, ICCS, ELISPOT, cytotoxicity, interferon-γ

In this work we studied the induction of T-cell responses upon rMVA and DNA vaccination against RVFV, since both vaccine strategies showed good protection levels upon lethal RVFV challenge in Balb/c mice. A collection of class-I MHC predicted 9-mer peptides derived from the glycoprotein (GnGc) and nucleoprotein N primary sequences were tested by an interferon-γ ELISPOTassay. Whilenone of the N related peptides were able to induce the secretion of significant levels of interferon-γ, three 9-mer peptides derived from the glycoprotein sequence showed specific stimulation of spleen cells in mice vaccinated with MVA encoding GnGc but not in mice vaccinated with a DNA-GnGc vaccine. Intracellular cytokine staining (ICCS) assays indicated that CD8+ cells from MVA vaccinated mice were stimulated in the presence of these peptides. In these mice a percentage of peptide specific cytotoxic lymphocytes were also observed. These data suggest that immunization with MVA GnGc vaccine is able to induce significant levels of specific CTLs that could contribute to the observed virus clearance in mice challenged by RVFV. Identification of the full set of CTL epitopes on the GnGc sequence will help to design more rationale vaccines against RVF.

IS10: Induction of Cytokines in Pigs co-infected with Swine Influenza Virus and Bordetella bronchiseptica*

Pejsak, Zygmunt[1]; Pomorska-Mol, M[1]; Markowska-Daniel, I[1]; Kowalczyk, A[1]

NVRI[1]

Key words: swine influenza virus, coinfection, interleukin response

Forty 4-week-old pigs were randomly divided into 4 experimental groups (n=9) and control group (n=4). Experimental pigs were i.n. infected with swine influenza virus (SIV) (Sw/Poland/KPR9/2004 H1N1 strain, dose 10^7.3 TCID50), Bordetella bronchiseptica (Bbr) (dose 3.4x10^8 CFU), SIV and Bbr or PBS. At day post infection (DPI) 2, 4 and 10 3 pigs from each group were necropsied. The amount of virus and bacteria in broncho-alveolar lavage fluid (BALF) and the macroscopic changes in the lungs were determined. To test the hypothesis that early production of cytokines might play a role in the induction of lung lesions at the early stage of infection, the level of mRNA of cytokines genes in BALF were determined using RT-PCR.

On DPI 2, the level of IFNα transcript was significantly higher only in the BALF of co-infected pigs, as compared to both infected and control groups. Transcript levels of IL-10, an anti-inflammatory cytokine, did not increase. It was diminished to the BALF of all experimental pigs and detected only at DPI 4 and 10.Transcripts for the proinflammatory cytokines IL-1, IL-6, and TNFα were relatively stable ($P>0.05$) or undetectable. Only mRNA of IL-8 increased significantly ($P<0.05$) on DPI 2 and 4 in BALF of co-infected pigs, as compared to pigs infected with either pathogen alone.

* Prepared within Project NoN308097537

IS11: Monitoring the determinants of efficient viral replication using Classical Swine Fever Virus-reporter replicons

Risager, Peter Christian[1]; Everett, Helen[2]; Crooke, Helen[2]; Belsham, Graham J[1]; Bruun Rasmussen, Thomas [1]

VET-DTU[1]; AHVLA[2]

Key words: CSFV, Replicons, Luciferase, homologous recombination, LongPCR

Classical swine fever virus (CSFV) is the etiological agent of the severe porcine disease, classical swine fever. Unraveling the molecular determinants of efficient replication is crucial for gaining improved knowledge of the pathogenic features of this virus.
Monitoring the replication competence of the CSFV genome within cells can be achieved using autonomously replicating constructs (replicons) containing a reporter gene that expresses a readily quantifiable enzyme.
Here, a newly implemented cloning technique was applied to genome modification of the full-length CSFV cDNA previously inserted into a single-copy bacterial artificial chromosome (BAC). This technique, the Red/ET counter-selection method, is based upon homologous recombination, thus obviating the need for internal restriction sites or complex cloning strategies.
Several CSFV replicons with deletions in regions encoding virus structural proteins considered non-essential for RNA replication were constructed and these deletions were replaced with an in-frame insertion of the Renilla luciferase (Rluc) sequence. RNA transcripts from these replicons should be translated as a single functional open reading frame. Full-genome cDNAs (~10-12,3 kb) were amplified from the BACs using a stable long-PCR method and in vitro transcripts were assayed in permissive cells. The CSFV-Rluc replicons were evaluated for their ability to replicate using immunofluorescence staining (α-NS3 and α-E2), and the Renilla luciferase assay.
We conclude that Rluc expression is an efficient way of monitoring replication of these constructs.

IS12: Immunohistochemical characterisation of virus specific cytokine expression induced by Classical Swine Fever Virus Strains with variations in the cell binding epitope.

Crudgington, B[1]; Everett, H[1]; Richard-Londt, A.[1]; Hicks, D[1]; Crooke, H[1]; Salguero, F.J.[1]

AHVLA[1]

Key words: CSFV, Viral distribution, Cytokine profile

Epitopes of CSFV envelope proteins, such as the E2 protein, facilitate cell surface binding and entry into host cells. The E2 epitope, TAVSPTTLR, is of particular interest as it is a determinant of virulence; amino acid substitutions in this region have been shown to produce milder clinical signs. The CSFV strain CBR/93 has a serine replacing a proline in this sequence. Immunohistological techniques were used to assess the impact of antigenic variance on host immune response and viral distribution.

Six animals were challenged either with UK2000/7.1 or CBR/93 strains. Clinical signs, gross pathology and histological features were recorded and immunohistochemical labelling of cytokines, IFN-γ, IL-1α and IL-12p40 was conducted for each animal. Differential immunohistochemical detection of viral E2 was conducted with antibodies WH303 (UK2000/7.1) and WH304 (CBR/93).

CBR/93 induced milder and delayed clinical signs before a rapid disease progression when compared to UK2000/7.1. Both strains induced pathological damage typically associated with CSFV infection and stimulated an immune response including the upregulation and expression of IFN-γ, IL-1α and IL-12p40. Upregulated cytokine expression coincided with viral antigen detection. UK2000/7.1 induced higher cytokine expression in all tissues in comparison with CBR/93. Monoclonal WH304 specifically detected CBR/93 viral E2 antigens.

The dissimilar clinical course of these two strains may not be a result of differential viral dissemination but the varying levels of stimulation of the anti-viral immune response in the host.

IS13: Application of reverse transcription Real – Time PCR to estimate the efficiency of the vaccine against Nairobi sheep disease

Salnikov, Nikolai[1]; Zhivoderov, Sergei[1]; Malogolovkina, Nina[1]; Tsybanov, Sodnom[1]; Kolbasov, Denis[1]; Lunitsyn, Andrei[1]

SRI NRIVV&M of the RAAS[1]

Key words: Nairobi sheep disease, vaccine, PCR

Nairobi sheep disease - anthropozoonotic transmissible disease of sheep, goats and humans, manifested by recurrent fevers, hemorrhagic gastroenteritis, glomerulonephritis, muco - purulent nasal discharge and diarrhea, is characterized by a mortality rate that can vary between 40 and 90%. The causative agent of Nairobi sheep disease - RNA virus belonging to the genus Nairovirus family Bunyaviridae. The disease is widespread in south-eastern Africa and Asia. For the prevention of disease using inactivated and attenuated vaccines. The aim of our work was to test the possibility of using PCR to evaluate the effectiveness of the vaccine.

In the experiment used four sheep. The first and second sheep immunized by a vaccine prepared from the attenuated strain "MM" of Nairobi sheep disease virus, the third and fourth sheep was the controls. In 14 days after immunization carried out infection of the vaccinated and control sheep by Nairobi sheep disease virus (virulent strain "NSD-X") at a dose of 100 LD50/cm3. Sheep № № 1,2,3 were inoculated the virus by intramuscular injection, and the fourth sheep – intravenously. For animals during 14 days clinical observation was conducted, daily blood samples were taken, which was investigated by reverse transcription Real – Time PCR.

Starting from 3-4 days after infection in the sheep № № 3 and 4 developed a fever, the genome of Nairobi sheep disease virus was detected in the samples of its blood. Later in sheep № 4 revealed the characteristic symptoms of the disease (diarrhea, depression, feed refusal) and on the 6th day it died. In the third sheep the disease was in a sub acute form, and after the 8th day of fever the animal recovered.

Vaccinated sheep throughout the observation period remained healthy. In blood samples from these sheep disease virus genome was not detected, indicating the absence of viral replication in their body. This can be explained by the presence of large amounts of neutralizing antibodies raised in response to imposing the vaccine strain of the virus at the time of immunization.

Thus, these studies indicate the possibility of testing the efficiency of an attenuated vaccine against Nairobi sheep disease by identifying viral genome in the blood of vaccinated animals.

IS14: Comparative microarray analysis between CP7_E2alf and C-strain vaccination after CSFV early challenge

Renson, P[1]; Quenault, H[1]; Le Dimna, M[1]; Bougeard, S[1]; Keranflec'h, A[1]; Cariolet, R[1]; Blanchard, Y[1]; Le Potier, MF[1]

Anses[1]

Key words: CSFV, C-strain, CP7_E2alf vaccine, microarray, inflammatory response

Introduction: The conventional C-strain live vaccine for Classical Swine Fever (CSF) induces an early protection but does not allow the serological differentiation of infected animals from the vaccinated ones. Since 2004, the CP7_E2alf recombinant chimera of BVDV with CSFV E2 protein is identified as a good candidate for DIVA vaccination against CSF. We have demonstrated in a previous study that, similar to an immunization using the C-strain, the application of the CP7_E2alf vaccine displayed an onset of protection against an early CSFV challenge at day 2 post-vaccination. As the mechanisms of this early protection are still poorly understood, a comparative microarray analysis has been realized post-challenge using the C-strain and the CP7_E2alf vaccine in order to identify potential similarities and dissemblances in the transcriptionnal response during this process. Gene expression modulations have been evaluated in tonsils, the primary replication site for both vaccines and challenge strain.
Materiel and methods: SPF pigs were orally vaccinated with either the C-strain or the CP7_E2alf vaccine and then challenged 2 days post-vaccination with the moderately virulent CSFV strain Bas-Rhin. A control group, not vaccinated, was infected with the same strain. Sequential slaughtering has been performed on 5 pigs per day during 3 days post-challenge or post-infection (pi) to collect tonsils. Total RNA was isolated from tonsils, converted to aminoallyl-RNA, Cy3- or Cy5-labelled using the Low Input QuickAmp Labeling kit, then hybridized on 4x44K Porcine Gene Expression Microarray from Agilent. After scanning of the microarray and normalization of the raw data, variant genes were identified using the non-parametric Wilcoxon-Mann-Whitney test. Using the Ingenuity Pathway Analysis software, the sets of differentially expressed genes were then characterized according to the Gene Ontology annotations and organized in networks for biological functions or signaling pathways.
Results: The transcritpome analysis of tonsils from vaccinated versus unvaccinated pigs revealed variations in cellular growth and proliferation genes expression and also in inflammatory responses. The results also highlighted the signalling pathways for IL6 and IL2 as differently modulated with vaccinated pigs. In between the CP7_E2alf and the C-strain vaccinated pigs, some differences were observed in the expression of genes involved in the inflammatory response, especially in pathways for IFN, IL10 and IL17. All the differences between CP7_E2alf and C_strain vaccines were essentially observed at D1 pi.
Conclusion: These results suggests that protection mechanisms induced by these live vaccines could be related to a better stimulation of leukocytes differentiation, proliferation and survival, especially mediated by IL2 which is an important live factor for T-cells during clonal expansion. Moreover, these data supports the hypothesis inferred in our previous study that both vaccines interfere with acute IFN response induction, as well as inflammatory reactions mediated by IL6 and IL10, observed during the CSFV infection. Microarray results also pointed out a difference at D1 pi between vaccines, suggesting that the C-strain vaccine induces a slightly better protection than the CP7_E2alf vaccine with relation to an earlier onset of the different mechanisms mentionned.
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IS15: Early onset of protection against lethal HPAIV H5N1 infection – what is possible?

Röhrs, Susanne[1]; Kalthoff, Donata[1]; Beer, Martin[1]

FLI[1]

Key words: Avian Influenza, vaccination, Neuraminidase, immune response

In light of the widespread occurrence of highly pathogenic avian influenza (HPAIV) H5N1 and the consistent failure of eradication attempts in many countries in Asia and Africa, additional tools for its prevention are indispensable. Most important tools are safe and efficacious vaccines. We therefore used a novel neuraminidase-deleted apathogenic variant of the HPAIV H5N1 strain R65 ("H5N1del") for a series of immunization/challenge experiments. In a first trial, it could be demonstrated, that a complete protection from lethal HPAIV H5N1 challenge infection was induced after a single intramuscular (i.m.) application of 104.5 TCID50 per chicken. The animals developed high titres of hemagglutinin-specific antibodies shortly after immunization, but no neuraminidase-specific reactivity was seen in an N1-specific ELISA test. Furthermore, there was no indication of a generalized infection, and no viral RNAs were detected in brain and lung samples or cloacal swabs, and only tracheal samples revealed a limited replication of challenge virus. Interestingly, the same level of protection could be achieved in a second trial with the H5N1del strain, when six-week-old chickens were immunized seven and three days before challenge infection, respectively. In contrast, immunization only one day before HPAIV H5N1 challenge infection could not prevent clinical symptoms and death, but resulted in a delay in the onset of disease. In addition, the mutant allowed a neuraminidase-based marker strategy.
In conclusion, a very early onset of immunity against HPAIV seems feasible using a modified live vaccine prototype and should be taken into consideration for future developments and control strategies.

AFRICAN SWINE FEVER

ASF1: Replication of the African swine fever virus Georgia 2007/1 isolate in Ornithodoros erraticus ticks

Diaz, Adriana[1]; Netherton, Christopher L[1]; Dixon, Linda K[1]

IAH[1]

Key words: African swine fever virus, vector, replication

African swine fever (ASF) is a lethal viral disease caused by African swine fever virus (ASFV), with virulent isolates generating up to 100% mortality in domestic pigs. ASFV also causes persistent infections in argasid ticks of the genus Ornithodoros, making it the only known arthropod-borne DNA virus. To evaluate the ability of the Georgia 2007/1 ASFV isolate to replicate in the European species Ornithodoros erraticus, we orally infected ticks and titrated the virus recovered from whole tick homogenates at up to 12 weeks post-feeding by haemadsorption (HAD) assay. The OUR T88/1 isolate, known to replicate in O. erraticus ticks, was used as a positive control.

Ticks became infected after feeding on blood containing both low (4 log10 HAD50) and high (6 log10 HAD50) titres of each isolate. Statistical analysis suggested that ticks infected with the Georgia 2007/1 strain showed a lower initial dose than the OUR T88/1 isolate but that there was no significant difference in the rate of replication after initial infection, suggesting that the Georgia 2007/1 isolate may be less efficient at attaching to tick gut cells during the initial stages of infection. The titre in the blood meal and the time since infection were also statistically significant.

These results indicate that the Georgia 2007/1 isolate is able to replicate in Ornithodoros species, which would make the control of ASFV outbreaks considerably more difficult in areas where the vectors become infected. This should be considered in future assessments of the threat represented.

ASF2: Application of a Rapid Vaccine Discovery System to African swine fever virus.

Dixon, Linda[1]; Takamatsu, Haru[1]; Goatley, Lynnette[1]; Chapman, Dave[1]; Netherton, Chris[1]; Cooke, Lyndsay[1]; Jankovich, James[2]; Robida, Mark[2]; Trevino, Troy[2]; Jacobs, Bert[2]; Sykes, Kathy[2]

IAH[1]; Biodesign Institute, Arizona State University[2]

Key words: African swine fever virus, vaccine, recombinant vaccinia, DNA vaccine

African swine fever limits pig production and causes major economic losses in many African countries. The recent spread through the Trans Caucasus and Russian Federation threatens further spread to Europe. The lack of a vaccine limits options for disease control.
Protection against lethal challenge with virulent isolates can be achieved by immunisation of pigs with attenuated ASFV. Studies have shown that depletion of CD8+ T cells abrogates this protection, and passive transfer of antibodies from immune pigs confers protection against some lethal challenges. However there is little knowledge about the protective antigens.
The aim of the work was to screen all ASFV encoded antigens for their potential to induce different types of immune responses and to select the most immunogenic antigens from each response type to include in immunisation and challenge experiments in pigs. To achieve this individual ASFV ORFs were cloned in a plasmid library for DNA priming and a recombinant vaccinia virus library for boosting immune responses in pigs. An expression library was also constructed to generate antigens to be used to measure antibody and cellular responses to individual antigens.
The procedure for delivery of pools of ASFV genes to pigs by DNA prime by gene gun and recombinant vaccinia virus boost was optimised. This included testing preparation of pools for gene gun delivery as mixed or individual slurries, testing pool sizes to confirm that immune interference did not occur and optimising dose and number of immunisations.
Currently 47 antigens have been tested for induction of antibody responses, and cellular responses by proliferation and Interferon gamma ELISPOT assays. Antigens were ranked by these assays for their immunogenicity. Results of an immunisation and challenge experiment will be presented.

ASF3: A comparison of diagnostic assays for African Swine Fever virus

Edwards, Lorraine[1]; Waddington, Samantha[1]; Henstock, Mark[1]; Batten, Carrie[1]; Oura, Chris[1]

IAH[1]

Key words: diagnosis, pigs, ASFV,

African Swine fever virus (ASFV) is a large enveloped double stranded DNA virus which is transmitted by soft ticks (family Argasidae, genera Ornithodoros) to wild cervids such as warthog and bush pigs. These species of wild pigs act as reservoir hosts of ASFV in Africa. Outside of Africa, ASFV has been spread to domesticated pigs through contaminated material from the environment and the feeding of infected swill or meat scraps. Infection in domesticated pigs is highly contagious and difficult to control. The economic losses can be large as ASFV infection can result in high morbidity and mortality. In Europe, diagnostic laboratories can diagnose ASF infection rapidly using real-time PCR; many laboratories also require the use of a secondary ASFV diagnostic assay to meet legislative requirements or for quality assurance purposes. In the developing world the cost of the real-time PCR consumables and equipment are often beyond economical reach, therefore requiring a cheaper alternative. Recently, a new technique of loop-mediated isothermal amplification (LAMP) was developed for ASF diagnosis. The LAMP assay has the potential to be further developed into a penside test. The ASF antigen ELISA is recommended for the detection of ASF antigen from blood, spleen or lymphatic nodes. This study compares the techniques of real-time PCR, LAMP and antigen ELISA for ASFV detection in diagnostic laboratories. Twenty one tissue samples and three blood samples collected at the peak of infection from experimentally infected pigs (housed in insect free accommodation) were tested using the three assays. The results of the real-time PCR and the LAMP were comparable (data to be presented). The results from the antigen ELISA did not correlate for four of the 24 samples. These four samples (one spleen and three mesenteric lymph nodes) gave an inconclusive or negative result in the antigen ELISA, but a positive result using real-time PCR and LAMP, these results will be discussed. In addition, 47 field samples collected from several regions in Ghana from wild cervids at different stages of infection were also compared using the real-time PCR and LAMP, the results of which will be discussed.

ASF4: Effect of porcine IFNα on replication of African Swine Fever Virus (ASFV)

Golding, Josephine[1]; Netherton, Christopher[1]; Dixon, Linda [1]; Taylor, Geraldine[1]

IAH[1]

Key words: EPIZONE ASFV

ASFV is a large dsDNA virus which causes a lethal haemorrhagic disease in domestic and wild pigs. ASFV is a major threat to the pig industry world-wide as there is no effective vaccine, and there is currently an extensive outbreak in European Russia. The ASFV genome contains large length variations which are associated with the loss or gain of genes within multigene families (MGFs). Members of MGF 360 and 530 have been implicated in suppressing the induction of interferon-α/β (IFNα/β) [1]. A recent report showed that virulent ASFV induces IFN in vivo [2]. However, it has been reported that virulent ASFV is sensitive to IFNα in vitro [3].
We have confirmed that virulent ASFV induces IFNα/β in vivo and have demonstrated that replication of virulent ASFV isolates in porcine alveolar macrophages are not inhibited by porcine IFNα. However, the replication of avirulent isolates containing MGF 360/530 deletions was reduced by IFNα. This suggests a role for members of MGF 360/530 in overcoming the IFNα-induced antiviral state.

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[2] Karalyan, Z., et al., (2012). Vet Immunol Immunopathol. 145, 551
[3] Esparza, I., et a;., (1988). J Gen Virol 69, 2973

ASF5: DETECTION AND QUANTIFICATION OF AIRBORNE AFRICAN SWINE FEVER VIRUS

Ferreira, Helena[1]; Weesendorp, Eefke[1]; Quak, Sjaak[1]; Stegeman, Arjan[2]; Loeffen, Willie[1]

CVI[1]; Utrecht University[2]

Key words: African Swine Fever, airborne, detection, animal experiment

Introduction

In order to improve control measures against the spread of African Swine Fever (ASF), knowledge on the specific contribution of the different routes of transmission can be useful. Up to this date, only a few studies (Wilkinson and Donaldson, 1977; Wilkinson et al., 1977) have been done on airborne ASF virus detection.

Objectives

The aims of this study were to validate an air sampling technique for ASF virus (ASFv) and assess the biological and physical decay of airborne ASFv under in vitro conditions. Furthermore, to detect and quantify virus excreted in the air after experimental infection of pigs with different ASFv strains.

Materials and methods

Three moderately virulent virus isolates were used: the Brazil'78, the Malta'78 and Netherlands'86. Samples were taken using the MD8 air scan sampling device. For validation of the air sampling method, 10 ml of virus suspension, containing approximately 105 TCID50/ml, was aerosolized in an empty isolator with a volume of 0.87 m3. This was repeated twice for each virus strain. To determine the half-life of airborne virus, air samples were taken every 15 minutes, up to 45 minutes after aerosol production. In order to determine the sedimentation rate, Petri dishes with medium were placed on the floor of the isolator and collected at the same time points. All the samples were tested in a virus titration (VT) and a quantitative PCR (qPCR).

During animal infection experiments with the Brazil'78, Malta'78 or Netherlands'86 isolates, air samples were collected at several time points to detect and quantify virus in the air. Each group was housed in a separate room, containing ten pigs. Air samples were collected for 10 min, using a sampling speed of 8 m3/h. Until day post inoculation 27 (dpi 27), air samples were collected three times per week. From dpi 28 until the end of the experiment at dpi 70, air samples were collected twice per week. Oropharyngeal swabs and faeces were collected simultaneously. All animal experiment samples were tested in the qPCR.

Results and discussion

Air sampling with the MD8 scanner, using gelatine filters to catch aerosols and other particles in the air, is suitable to detect ASFv under both in vitro and in vivo condition. This is the first time that ASFv was detected from the air in rooms housing infected pigs. Under in vitro conditions, the half-life of virus analysed by PCR was on average 19 min and by virus titration on average14 min. This suggests that virus does not only disappear from the air by sedimentation, but that also inactivation of airborne virus occurs. In rooms with infected pigs, virus could be detected in air samples by PCR from dpi 4 until the end of the experiments at dpi 70. A correlation was seen with the number of pigs that excreted virus in the faeces.

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ASF6: Not seeing the trees for the woods: Whole cell population MHC class I responses to African swine fever virus infection may not reflect responses at the level of individual cells

Saward Arav, Derah[1]; Chapman, Dave[1]; Skinner, Mike[2]; Dixon, Linda[1]

IAH[1]; Imperial College London[2]

Key words: ASFV MHC-I expression viral immunomodulation

African swine fever virus (ASFV) is a large double-stranded DNA virus which causes fatal haemorrhagic fever in domestic swine. The ASFV C-type lectin protein EP153R has been implicated in the modulation of surface major histocompatibility complex class I (MHC-I) expression. Previous investigations have shown that surface levels of MHC-I molecules decrease in ASFV-infected cell populations in comparison to levels in uninfected cell populations, while internal levels either increase or remain constant. These studies did not differentiate between expression levels in infected and uninfected cells within a population.

In the current study, confocal microscopy has revealed lower levels of detectable internal MHC-I in infected cells than uninfected cells within the same population, although comparable expression was detected at the cell surface. Investigations are ongoing to determine if this lack of staining of internal MHC-I is due to a conformational change which prevents the antibody from binding to MHC-I molecules, or if levels of MHC-I are reduced by changes in transcription or translation.

Dual-stained flow cytometry will quantify variation in internal levels of MHC-I between infected and uninfected cells within an infected cell population and RT qPCR will elucidate changes in transcription of SLA-I mRNA. The role of EP153R in the modulation of MHC-I expression is being investigated using ASFV mutants which do not express EP153R and by expression of tagged EP153R.

ASF7: Serological Surveillance of African Swine Fever in Domestic Pigs in Korea

Park, Jee Yong[1]; Yeh, Jung Yong[1]; Lee, Ji Hye[1]; Seo, Hyun Ji[1]; Cho, Yun Sang[1]; Kim, Hyun Joo[1]; Kim, Yong Joo[1]; Ko, Young Joon[1]; Lee, Hyang Shim[1]; Cho, In Soo[1]

Animal Quarantine and Inspection Agency[1]

Key words: African Swine Fever, Surveillance

Introduction

African swine fever (ASF) is an OIE listed viral disease of domestic and wild pigs causing haemorrhagic fever with high mortality. Recent outbreaks of ASF outside Africa has raised concern of increased risk of spread to previously free countries, requiring sufficient level of surveillance to be conducted to quickly identify new introductions and also to provide sufficient evidence of disease free status. For this purpose, a statistically designed serological surveillance was conducted to demonstrate freedom from ASF in domestic pigs in Korea.

Materials and Methods

For the surveillance, two-stage random sampling strategy was selected with first stage sampling unit being farms and second stage being pigs within the selected farms. For the calculation of the required sample size, method proposed by Garner et al. was used, and among herd prevalence of 2% and within herd prevalence of 30% were applied. No stratifications were deemed necessary. For laboratory tests, ELISA with assumed sensitivity of 98% and specificity of 98% was used. By using the above parameters and collecting 8 samples sample per farm, a total of 1,272 samples from 159 farms were required to provide 95% probability of detection of ASF outbreak with the above defined prevalence. Surveillance strategy was assessed using a stochastic model based on methods proposed by Audige et al.

Results and Discussion

For this study, serum samples were collected from farms across the country from mid 2010 to mid 2011. A total of 1,347 samples from 169 farms were collected, which was in excess of the required sample size. All samples were tested by ELISA as prescribed by the OIE manual of diagnostic tests and were all shown to be negative for antibodies to ASF. Assuming that the selected farms and animals was representative of the situation in the country, this testing regime would be able to provide with 95% confidence that the country is free from ASF during the study period.

From this surveillance, no evidence of antibodies to ASF were detected in domestic pigs in Korea, which support the view that Korea is free from ASF.

ASF8: PHARMACOKINETICS AND SAFETY OF AN EXPERIMENTAL MOLECULE WITH IN VITRO ANTIVIRAL ACTIVITY AGAINST THE AFRICAN SWINE FEVER VIRUS

Tignon, Marylene[1]; Roels, Stefan[1]; Van der Heyden , Sara[1]; Neyts , Johan[2]; Krečmerová , Marcela[3]; Koenen , Frank[1]; Cay , Ann Brigitte[1]

VAR-CODA[1]; Rega Institute for Medical Research, K.U. Leuven[2]; Institute of Organic Chemistry and Biochemistry[3]

Key words: African swine fever, antiviral, pharmacokinetics, safety

Several nucleoside and nucleotide analogues have been identified and approved as potent antiviral agents. HPMPDAP (9-(S)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine) is part of the group of acyclic nucleoside phosphonates (ANPs) that have proven their efficiency with convincing activity against poxviruses, cytomegalovirus retinitis in AIDS patients, chronic hepatitis B virus (HBV) infections, and human immunodeficiency virus (HIV) infections. HPMPDAP has demonstrated in vitro antiviral activity against the African Swine Fever Virus (ASFV), the causative agent of African swine fever (ASF). This hemorrhagic disease affecting domestic and wild Suidae can cause devastating outbreaks in pigs, with important economic impact. Currently it is endemic in most of the African countries and since 2007 has spread dramatically from the Caucasus region to neighbor countries including Russian Federation. Up to now conventional approaches to develop a vaccine have remains unsuccessful and control strategies to prevent spread of the virus are limited to prophylactic measures and pre-emptive culling. In this context, antiviral approach could provide an alternative solution in epidemic situations.
Pharmacokinetics and toxicity studies were performed in domestic pigs as a pre-required of the in vivo efficacy assessment of HPMPDA.
For pharmacokinetic study two large white pigs weighting 23-24 kg equipped with deep catheter in jugularis vein were administrated during 4 days intramuscularly (IM) with 20 mg/Kg HPMPDAP. Plasma samples were collected before and 15, 30, 45, 60 min., 2, 3, 4, 5, 6, 8, 12 hours post administration during 4 days and analysed by MS-HPLC (Synovo). For toxicity evaluation, four groups of two large white pigs weighting 20-30 kg were IM injected with 4 different doses of the compound (7 (0.5 mg/kg for 14 days) – 20 (5 mg/kg for 4 days) – 25 (2.5 mg/kg for 10 days – 80 (20 mg/kg for 4 days) mg/kg). There was daily clinical observation and blood collection. The blood parameters that were controlled included hematology, ionograms, renal and liver parameters. Autopsy was performed 3 to 4 days after the last administration. During autopsy, samples of liver and kidneys were taken for further histopathological examination.
High concentration of HPMPDAP was observed in plasma as soon as 15 min post IM administration that decreased rapidly to reach concentration below EC50 (1,3 µM) 4 hours post administration.
Based on daily clinical observations there was apathy, anorexia and vomiting noticed in the groups with the 3 highest doses. In the highest dose group 1 animal died on day 5. The observed toxicity was confirmed by both the blood parameters and histopathological findings (liver & kidney). At the lowest dose no adverse effect was observed and this was confirmed by hematological and histopathological findings.
HPMPDAP has previously been shown to exert potent antiviral activity in mouse models for various DNA virus infections and was also demonstrated to be very well tolerated in mice. A close analogue of HMPDAP, ie HPMPC or Cidofovir is successfully used in humans for the treatment of life-threatening infections with the cytomegalovirus. However, much to our surprise, HPMPDAP was not well tolerated in pigs. Further study will show if the tolerated dose will be sufficient to induce protective antiviral effect in challenge in vivo experiment.

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ASF9: Preliminary validation of the ID Screen African Swine Fever indirect ELISA based on three recombinant ASF proteins

Pourquier, Philippe[1]; Comtet, Loic[1]

IDvet[1]

Key words: African Swine Fever ELISA validation

INTRODUCTION

African swine fever (ASF) is a highly contagious, viral haemorrhagic disease of pigs, warthogs, European wild boar and American wild pigs caused by the African Swine Fever Virus (ASFV). There is neither treatment nor vaccine for ASF. To prevent introduction and spread of the disease, countries implement strict surveillance, control and eradication programs which require accurate and reliable diagnostic tests.
In this context, IDvet has launched the ID Screen® African Swine Fever Indirect ELISA. This test detects anti-AFSV antibodies in domestic or wild pigs, in serum, plasma or blood filter paper samples.
Unique features of the ID Screen African Swine Fever Indirect ELISA include the coating of three recombinant ASFV antigens (P32, P62, and P72), and the ability to use the test with blood filter paper samples as well as serum and plasma.
This study presents the preliminary validation data obtained for this ELISA kit on both serum and filter paper samples.

METHOD AND RESULTS

Specificity

- 556 disease-free sera from domestic pigs (France and Norway), wild boars (France), and Iberian pigs (Spain) were tested. All samples were found negative.
- 90 negative animals were tested by both the serum and filter paper protocols. All sera were found negative by both protocols.

Sensitivity

- 3 sera were tested from pigs vaccinated on day 0 and day 24 with the ASF strain Ourt88/3, challenged on day 42 with a mild ASF strain (ANSES, Ploufragan, France), and bled on day 62 or 63. After challenge, all three animals gave strong positive results with the ID Screen® ASF Indirect ELISA.
- 8 reference sera provided by the Community Reference Laboratory (CRL), CISA-INIA, Spain were analysed. The ID Screen® ASF Indirect ELISA accurately identified all sera.
- 92 sera from infected herds (Sassari, Sardinia, Italy) were tested in parallel by the ID Screen® ELISA and the CRL ELISA reagents (CISA-INIA, Spain). Test correlation was found to be 95.7%. The relative sensitivity and specificity were 97.67% and 97.83%, respectively.
- 3 sera were titrated and tested by both the serum and filter paper protocols. The measured analytical sensitivity was similar regardless of the sample type tested (serum, Whatman 1 or Whatman 3 filter paper).

CONCLUSION

The ID Screen® African Swine Fever Indirect ELISA is an efficient and reliable tool for the diagnosis of ASF in wild and domestic species.
It is the only commercial ELISA based on 3 recombinant antigens. Both the serum and filter paper test protocols shown excellent sensitivity and specificity
Collecting blood using filter paper facilitates sample collection in field. With the ID Screen protocol, filter paper samples may be tested in a 96-well deep-well plate, making sample processing faster and less prone to mix-ups.

ASF10: Conservability of ASFV in pork products

Gazaev, Ismail[1]; Malogolovkin, Aleksander[1]; Tcibanov, Sodnom[1]; Kolbasov, Denis[1]

Institute of Veterinary Virology and Microbiology[1]

Key words: ASFV, Conservability, pork products

In consideration of occurrence of different clinical forms of the disease, especially an symptomatic one, a high probability of killing of visually healthy animals – virus carriers and further distribution of the disease with contaminated pork products appears.
According to epidemiological analysis of sources of ASFV introduction into free areas of Russia and further distribution of the disease, it was settled that the main routes of ASFV transmission are contaminated food wastes and pork products.
The main idea of current work is studies of ASFV conservability in samples from pork products.
For this purpose, 3 clinically healthy piglets were infected by ASFV “Stavropol” strain with a dose of 5.0 lg HAD 50/ml.
Animals were slaughtered on second, forth, sixth days post infection. Meat and fat obtained from killed animals were preserved by salting.
For the purpose of studying of influence of temperature on ASFV inactivation in pork products, samples were kept under different temperature conditions: +22+27oC; +4+6oC; -18-20oC.
Samples of stored meat and pork fat at different temperatures were used for virus isolation.
As a result, that ASFV strain “Stavropol 2009” has been detected in samples of meat and pork fat, stored at + 22+27oC, for 16 days with titer of 1,5-2,0 lg HAD 50/ml, however on the twentieth day the virus wasn’t detected. Virus from samples stored at +4+6oC was detected during 84 days with titer of 2,0-2,5 lg HAD 50/ml (observation period), and from samples stored at -18 - 20oC - during 118 days with titer of 2,5-3,0 lg HAD 50/ml (observation period).
Thus, according to current investigations, infectious ASF in tissues and pork products from pigs killed before the appearance of clinical signs is conserved for long periods of time, so this may be an essential factor of the disease distribution.

ASF11: NEW IMMUNOASSAYS FOR DIAGNOSIS OF ASFV BASED ON VP72: CAPTURE ELISA FOR THE DETECTION OF SPECIFIC IgM AND PEN-SIDE TEST FOR BLOOD SAMPLES

Pérez, Teresa[1]; Gallardo, Carmina[2]; Venteo, Angel[1]; Ranz, Ana[1]; Nieto, Raquel[2]; Simón, A[2]; Rueda, Paloma[1]; Sastre, Patricia[1]; Arias, Marisa[2]; Sanz, Antonio[1]

INGENASA[1]; CISA-INIA[2]

Key words: ASFV, DIAGNOSIS, PEN-SIDE

1. Introduction and Objectives.

African swine fever (ASF) is a complex and lethal disease of swine caused by a large double-stranded DNA virus belonging to the Asfaviridae family. The acute forms of the disease are devastating and result in very high mortality that may reach 100% producing a major negative effect on national, regional and international trade. ASF it has been endemic in most of sub-Saharan African countries and in Sardinia (Italy) until 2007. In April 2007 its remarkable potential for trans-boundary spread was amply demonstrated appearing in the Republic of Georgia with subsequent outbreaks to Armenia, Azerbaijan and Russia. Currently ASF is considered as being established in the Russia Federation threatens to other regions of Europe, central Asia and even China, which has the largest pig population in the world. Since there is no vaccine available, rapid and specific diagnostic procedures are an essential component in affected countries. The early appearance and subsequent long-term persistence of antibodies make antibody detection techniques a key factor in the control of the disease. To this end, INGENASA in collaboration with the European Union Reference Laboratory for ASF (CISA-INIA), have been working in the development an initial standardization of a Capture ELISA for detection of specific IgM. In addition has been improved a rapid, one-step immunochromatographic strip (pen-side test) PPA CROM (Ref 11.PPA.K41 WB) for its applicability in the field to detect specific anti-ASF antibodies directly in blood specimens besides serum.

2. Material and Methods

Three different ASFV proteins were used as antigen for the IgM-ELISAs which comprised the fused p30-p15 protein, the p72 protein and the uncharacterized protein of 33kDa encodes by the K205R ORF. For initial standardization and validation of the IgM-ELISAs, a panel of experimental serum samples was used. The samples were obtained from four independent experimental infections using the Kenyan ASF viruses belonging to genotype IX and X, the current East Europe circulating virus of genotype II and the attenuated and non haemadsorbing Portugal ASFV strain NH/P68 (NHV) belonging to p72 genotype I.

To test the applicability of the immunochromatographic assay INGEZIM PPA CROM to detect antibodies in blood samples, in this study were included a wide panel of paired experimental and field porcine serum and blood samples available at CISA-INIA.

The results were compared to those obtained using the OIE serological prescribed assays (OIE 2008) as gold standards.

3. Results and Conclusions.

From the results obtained in the initial standardization and validation of the IgM-ELISAs, we can conclude that the new developed IgM capture ELISA, using p72 as antigen, detected specific IgM to ASFV at early times post infection. These positive samples remained negative using HT and K205R based IgM-ELISAs, as well as using the OIE prescribed serological assays. Although more studies are required for further validation, these preliminary results indicate that the p72 based IgM-capture-ELISA could be very useful tool for early detection of ASFV infection.

In addition, the analyses of blood samples using the immunochromatographic assay INGEZIM PPA CROM allow us to detect specific antibodies against ASF with appropriate values of sensitivity and specificity showing the capability of the assay for rapid diagnosis of ASF in the field and where laboratory support and skilled personnel are limited.

ASF12: Ring trial on ASFV novel molecular diagnostic techniques.

Iscaro, Carmen[1]; Giammarioli, Monica[1]; Duarte, Margarida[2]; Fernandez-Pinero, Jovita[3]; Gunter Keil, Michael[4]; Health, Livio Edward [5]; Leblanc, Neil[6]; McKillen, John [7]; Mur Gil, Lina[8]; Oggiano, Annalisa[9]; Sanz, Antonio[10]; Stahl, Karl[11]; Severi, Giulio[1]; De Mia, Gian Mario[1]

Ist. Zoopr. Sperimentale Umbria e Marche[1]; Lab. Nacional de Investigacao Veterinaria[2]; CISA-INIA[3]; FLI[4]; Agricultural Research Council[5]; SVA[6]; Agrifood & Bioscencies Inst. Northern Ireland[7]; Universidad Complutense de Madrid[8]; Ist. Zoopr. Sperimentale della Sardegna[9]; INGENASA, Immunologia y Genetica Applicada S.A.[10]; Makerere University, Inst. Env. & Nat. Res.[11]

Key words: African swine fever virus, UPL-real time PCR, LAMP, Ring Trial

Several molecular procedures for ASFV DNA detection are adopted routinely and appear appropriate for diagnosis of ASF. In addition to these, some innovative methods have been recently implemented for the molecular diagnosis.
The following novel diagnostic methods, developed within the FP7 European Project ASFRISK, have been selected for a Ring Trial that has been organized by IZS-UM: the UPL-PCR method (developed by CISA-INIA) and the LAMP assay (developed by SVA).
The Ring Trial was organised by the National Reference Laboratory for African Swine Fever, IZSUM, Perugia (Italy). A total of 11 labs took part in the Ring Trial, representing ASF National Reference Laboratories of EU member countries (CISA-INIA, FLI, SVA) and of African countries (OVI), and some other partners working on ASF (Universidad Complutense de Madrid, INGENASA, LNIV, AFBI, IZS Sardegna).
The panel comprised a collection of 24 blind frozen DNA samples, consisting of 18 positive and 6 negative samples. The positive samples are represented by tenfold dilutions of two different ASFV genotypes: (i) genotype I derives from an outbreak in Cagliari (Italy) in 1979; (ii) genotype IX derives from an outbreak in Kenya in 2005. Virus samples were amplified, purified and extracted. The positive DNA samples were diluted in healthy donor pig material in serial form and distributed as ten to the minus 2, 3, 4, for genotype I, and as ten to the minus 1, 3, 5 for genotype IX. In total, 6 groups of samples have been prepared, 3 for each genotype; each group included the same DNA sample in triplicate, to evaluate the intra-laboratory repeatability. The seventh group includes 6 negative DNA samples from organs of local pigs, not ASFV infected. Therefore, reagents for UPL real-time PCR and for LAMP PCR (wet and dry assay), as well as the relative protocols, were provided to the participants.
All the participants performed the two methods (UPL-PCR and LAMP) by applying the recommended protocols on their own instruments and sent the results to the organizer.
Both UPL and LAMP showed good performances detecting correctly the positive and the negative samples. All the labs obtained the same features and found the same critical points. Both the methods were considered by the participants as easy, handful and fast assays. UPL gave similar performances by all the participants with similar Ct values. The LAMP protocol had to be previously adapted to the real time machine, resulting laborious and time consuming. Therefore, a difficult interpretation of results has been reported in some cases. LAMP assay gave negative results with some weak positive samples. Nevertheless its lower sensitivity does not compromise the performances of the method, considering that it should be used for ready on-site diagnosis.
The Ring Trial results showed effectiveness of both techniques for ASFV molecular diagnosis. In particular, LAMP is recommended for field diagnosis and/or for not well equipped laboratories. The Ring Trial allowed us to test two novel molecular techniques inter-labs, with a remarkable concordance between results. In conclusion the results showed that these molecular techniques represent a rapid and effective tool for ASFV diagnosis.

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ASF13: Development of ready to use PCR and real-time PCR commercial kits for reliable detection of ASFV.

Redondo, Elena[1]; Fernández Pinero, Jovita[2]; Sanz, Antonio[1]; Gallardo, Carmina[2]; Arias, Marisa[2]; Rodríguez, María José[1]

INGENASA[1]; CISA-INIA[2]

Key words: ASFV, Diagnosis, Real Time PCR

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 very 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.
Commercial diagnostic kits are of great value because of their rapid acquisition, simplicity, and reproducibility. The main object of this work was to develop ready to use PCR kits for the accurate detection of ASFV based on two previously described and validated PCR methods [1, 2].

A duplex conventional PCR prototype was designed by selecting specific primers for ASFV detection [1] and primers annealing within porcine β-actin gene. Optimal reaction conditions were established and PCR DNA products were properly identified by agarose gel electrophoresis.
A primer set and a UPL-probe (Universal Probe Library/Roche) specific for ASFV detection were selected for real-time PCR [2]. An endogenous internal control based on a specific VIC hydrolysis LNA-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.
In order to have the possibility to use the PCR kits in regions with limited resources, jellification of the PCR mixes was tested to preserve them during transport at room temperature and for storage at 4ºC. Jellified mixes were assayed in collaboration with Biotools. Mixes providing the most robust results were selected.

Using appropriate primer concentrations for the internal control in balance with those corresponding to ASFV, no adverse effects on the sensitivity was observed and the duplex assays proved to have similar results to that of the simplex PCR tests. Evaluation of both prototypes was carried out in collaboration with CISA-INIA by testing a reference collection of ASFV samples.
One-reaction tube jellification, including all reagents required, proved to be very useful for end-point PCR kit. Real-time PCR jellified prototype was finally prepared in two tubes comprising the complete mixes, due to the lack of universal plastic consumables for all real-time equipments.

In order to obtain ready to use PCR kits for ASFV detection, two fully validated methods were improved in two ways. On one hand, an internal endogenous control was incorporated to each conventional and real-time PCR assay to prevent the appearance of false negative results. This did not affect the sensitivity of any of the assays. Furthermore, this control could be used in other pig diagnostic assays. On the other hand, we have prepared ready to use PCR prototypes that allow transport at room temperature and preservation at 4ºC, which can be very useful in restricted situations. The two presented PCR kits give promising results and could be of great value for rapid and reliable detection of ASFV.

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ASF14: Surveillance for African swine fever in Masaka and Rakai, Uganda

Muhangi, Denis[1]; Ocaido, Michael[1]; Masembe, Charles[1]; Stahl, Karl[2]; Ndyanabo, Susan[1]

Makerere University[1]; SVA[2]

Key words: African swine fever (ASF), epidemiology, pigs, small-holder farmers, management systems, Uganda.

African swine fever (ASF) is a haemorrhagic fever infecting domestic pigs with a high mortality, and affects the socio-economic status of small-holder farmers. In Uganda, the disease is endemic but little has been studied about its epidemiology in the country. If the disease is to be controlled and prevented, there is need to understand the role played by the different factors in spreading African swine fever virus (ASFV) in the country. In this regard, we carried out a longitudinal study in two districts in Central Uganda in which we sampled 725 pigs from 244 pig farms. We sampled apparently healthy-looking pigs for blood and serum to investigate presence of virus antigen and antibodies against the virus and we sampled the farms twice (June to October 2010 and March, May to June 2011). We also carried out a questionnaire based survey on those farms focusing on management practices. The main objective of the study was to identify factors related to disease outbreaks and virus persistence. During this period of the study, 17 farms (7.3%) reported an ASF disease outbreak. On management, there were a number of practices identified as common on farms like feeding swill, borrowing or lending their boars to neighboring for breeding and non-housed pigs and piglets (tethering and free-range). 8 (1.1%) of all the pigs were positive on ELISA and 15 (2.1%) were positive on Real-time PCR during the first sampling; on second sampling, the results were 0 (0%) and 10 (1.4%) respectively. Notably, the Ct values were too high (weakly positive) (mean Ct value=35.6) except in one case where the Ct value was very low (strongly positive) in an ASF clinically sick pig (Ct value=21.8). We suggest that if the current measures of quarantine have to be effective, farmers have to be sensitized on good practices in management and biosecurity.

ASF15: EPIZONE Portable platforms for the pen-side detection of African swine fever virus tested in field conditions in Northern Uganda

LeBlanc, Neil[1]; Okoth, Edward[2]; Hakhverdyan, Mikhayil[1]; Masembe, Charles[3]; Bishop, Richard[2]; Belák, Sándor[1]; Ståhl, Karl[4]

SVA[1]; International Livestock Research Institute[2]; Makerere University[3]; Swedish University of Agricultural Sciences[4]

Key words: ASFV, pen-side, WP 4.4, Uganda

This work represents a culmination of the research performed in Epizone Work Package 4.4: Pen-side Tests and partnerships in Uganda in 2012. The purpose of work package 4.4 was to investigate methods for the detection of pathogens in the field or modestly equipped/temporary laboratories. In this work, testing of ASFV portable platforms took place in a field setting in the Gulu district in Northern Uganda. Two detection platforms, the TCOR 4 portable real-time PCR (Tetracore Inc.) and Genie II isothermal amplification device (Optigene Ltd.) were brought as part of the field laboratory. In addition, material for three methods of DNA extraction from whole blood were brought in for testing. They included FTA elute indicating cards, Tego sample cards and a magnetic bead extraction method adapted for the field from the National Veterinary Institute's routine robotic nucleic acid extraction kit (Nordiag AB). In addition, three assays were brought for testing, two real-time PCR assays and one LAMP assay. The methods and equipment tested represented the most promising equipment and methods for the pen-side detection of ASFV investigated to date. The primary field location was in an area near Koch Goma, Gulu District, Uganda; which had no electricity or running water. The laboratory was run three times over an eight day period, twice in Koch Goma and once in Kampala. The results indicate that platforms now can be established at pen-side or in field laboratories that perform at a level comparable to sophisticated molecular laboratories. The results have led to further collaborative efforts planned with the International Livestock Research Institute in Kenya; another field trial is being planned to facilitate the use of a portable ASFV platform in a major field study.

ASF16: Risk of African swine fever introduction into the European Union (EU) through wild boar pathway

De la Torre, Ana[1]; Bosch, Jaime[1]; Iglesias, Irene[1]; Martínez, Marta[1]; Mur, Lina[2]; Martínez-López, Beatriz[2]; Sánchez-Vizcaíno, Jose Manuel[2]; Muñoz, Maria Jesús[1]

CISA-INIA[1]; UCM[2]

Key words: Wildboar, Risk assessment, European Union, African swine fever

Many viral diseases affecting domestic pig populations are also transmitted by wild boar (WB), such as African Swine Fever (ASF). Although the role of the surviving WB in the spread of African swine fever (ASF) is yet not well understood, they may act as disease reservoirs, as demonstrated the previous experience in Spain and Portugal. This fact together with the current ASF spread North-West across the Caucasus region and Russian Federation, has increased the concerns about the potential role that WB may have in the potential ASF introduction into the EU. As a consequence, the estimation and quantification of the potential risk of ASF introduction into EU though WB would be certainly useful to identify high risk areas, target surveillance and risk-reduction measures. This study presents a novel GIS-based semi-quantitative approach to evaluate the risk of introduction of ASF into the European Union (EU) through WB from non-EU countries situated on the EU border. The semi-quantitative model considered 6 risk estimators: (1,2) WB/domestic pig outbreak density in the origin countries, using data from OIE and applying a kernel density method; (3,4) WB/domestic pig population density in the origin countries (FAO EMPRES WATCH); (5) Surface of shared WB suitable habitat along border; WB estimated distribution in Europe was based on the approach developed by Bosch et al (2012) using CORINE Land Cover; (6) Distance to the nearest outbreak to EU countries, using ArcGIS 9.3.1. The study included those EU and non-EU countries situated in the EU border. Data values for each risk estimator were normalized and categorized into scores from 0 to 5. Risk estimators were combined in a final risk score using linear combination. Finally, a Jackknife sensitivity analysis was performed which consists in repeating the analysis as many times as factors have been included, discarding one different factor each time. Results identified the highly influent factors that mean the factors which remain significant in all or most of the combinations.

Model results revealed that the highest risk of ASF introduction into EU through wild boar pathway is concentrated in Romania, Poland, Latvia, Finland and Latvia. EU authorities in those countries should be aware of this potential risk and communicate with wild boar hunters and pig farmers, particularly back-yard farmers, in order to rapidly detect and control any suspicion of ASF.

This work was financially supported by the research program FP7-KBBE-2007-1-211691 and S2009/AGR-1489.

ASF17: Development of a Suspension Microarray for the Genotyping of African Swine Fever Virus Targeting the SNPs in the C-Terminal End of the p72 Gene Region of the Genome

LeBlanc, Neil[1]; Cortey, Martí[1]; Fernandez Pinero, Jovita[2]; Gallardo, Carmina[2]; Masembe, Charles[3]; Okurut, Ademun Rose[4]; Heath, Livio[5]; Van Heerden , Juanita[5]; Sánchez-Vizcaino, José Manuel [6]; Ståhl, Karl[7]; Belák, Sándor[1]

SVA[1]; CISA-INIA[2]; Makerere University[3]; Ministry of Agriculture[4]; Onderstepoort Veterinary [5]; Universidad Complutense [6]; Swedish University of Agricultural Sciences [7]

Key words: African swine fever virus, Genotyping, p72, microarray, Luminex

African swine fever virus (ASFV) causes catastrophic disease in domestic pigs, with high rates of morbidity and mortality, which can reach 100%. To date, the serological diagnosis has the drawback of not being able to differentiate variants of this virus. Previous studies have identified 22 genotypes based on sequence variation in the C-terminal region of the p72 gene, which has become the standard for categorizing ASFVs. A genotyping assay has been developed using a segment of PCR-amplified genomic DNA of approximately 450 bp, which encompasses the C-terminal end of the p72 gene. Paired DNA probes which are identical except for a single nucleotide polymorphism (SNP) at the centre position, were designed to either individually or in combination differentiate between the 22 genotypes. The assay was developed using Luminex xMAP technology. Characterization of the sample was performed by comparing fluorescence of the paired SNP probes, i.e. the probe with higher fluorescence in a pair identified the SNP that a particular sample possessed. In the final assay, a total of 52 probes were employed, 24 SNP pairs and 4 general detection. One or more samples from each of the 22 genotypes were tested. The assay was able to detect and distinguish all 22 genotypes. Thus, the novel assay provides a powerful tool for the complex and rapid diagnosis of African swine fever.

RISK ASSESSMENT

RA1: A quantitative analysis of secretion and excretion of Foot-and-Mouth-Disease Virus

Bravo de Rueda, Carla[1]; Dekker, Aldo[1]; Eblé, Phaedra[1]; de Jong, Mart[2]

CVI[1]; Wageningen University[2]

Key words: FMD, virus, quantitative, secretion, transmission

Indirect transmission of FMD can occur when infected animals have contaminated the environment with their secretions and excretions. Most of the secretions and excretions from infected animals can contain the virus. In the current study we analysed quantitatively the virus quantities in secretions and excretions from FMDV infected animals. For this purpose we collected data from publications in which FMDV experiments were described. The occurrence of the highest titres of FMDV in different secretions and excretions and, the experimental conditions in which they occurred were recorded and analysed. This data allowed us to extract possible explanatory variables which could predict the major secretion or excretion of FMDV in a multivariate model. In a separate analysis of laboratory data, it was shown that the total amount of secreted and excreted FMDV was highly correlated with the maximum amount of secreted and excreted FMDV. Therefore a regression analysis was done to identify the factors that determine the maximum amount of secreted or excreted FMDV. Type of secretion and excretion, animal species, FMDV serotype and time after infection , had all significant influence on the maximum amount of secreted and excreted virus in the model. The highest amounts of virus were found in oral pharyngeal fluid (OPF swabs of all the studied species) and cow's milk. Animals that were infected with FMDV serotype O secreted and/or excreted higher amounts of virus than animals infected with other serotypes. In most of the secretions and excretions, considerable amounts of FMDV were already present before 2 days post infection, at which the animals did not show clinical signs or when only the first clinical signs were apparent. We also show that depending on the animal species, there is a variation on the occurrence of maximum viral titres in the different secretions or excretions. We suggest that cow's milk, OPF of all the here reported species, airborne excretion from swine and blood from swine are major sources for FMDV indirect transmission to occur.

RA2: HORIZON SCANNING FOR EMERGENCE OF NEW VIRUSES IN ANIMAL AND PUBLIC HEALTH

Gale, Paul[1]

AHVLA[1]

Key words: horizon scanning, futures, emerging viruses, routes, scenarios

Viruses yet to emerge are, by definition, not in the scientific literature, while other known viruses may re-emerge in unexpected ways. This presentation considers how proactive horizon scanning techniques can be developed to identify novel route(s) of transmission and origins (i.e. country and source reservoir) for the emergence of viruses in the medium to long term. The emergence of a virus generally involves a combination of events together with a change in key drivers, typically socio-economic, environmental, climatic and/or zoological factors. Central to horizon scanning, therefore, is the construction of "complex scenarios". An example of such is the use of a veterinary drug (diclofenac) ultimately leading to increased rabies transmission to humans (Markandya et al. 2008). In a novel approach developed at a European Science Foundation-funded workshop, complex scenarios in the form of "spidergrams" were produced by randomly linking factors which may directly or indirectly affect the emergence of diseases (Gale and Jansen 2010). The factors were chosen from a database under eight header categories (as defined by the workshop's participants). Many thousands of scenario chains can be produced by this method and most may be irrational. However, the approach enables the testing of combinations not previously considered but which would be tested in nature. Spidergrams also generate novel discussion points which could lead to new perspectives. Talking to scientists in different and diverse fields is clearly central to horizon scanning. In this respect, spidergrams could provide a focus for facilitating discussion between scientists across a range of disciplines. The resource and ingenuity of the on-line community also needs to be tapped for ideas and in this respect on-line games could be developed for generating novel scenarios and transmission pathways.

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RA3: Mapping the risk for the bluetongue virus disease in Austria

Brugger, Katharina[1]; Rubel, Franz[1]

University of Veterinary Medicine Vienna[1]

Key words: Bluetongue virus, Culicoides, season

Veterinary authorities need to estimate the risk and establish efficient protection and control measures when facing diseases with economical impact. These risk assessments are often based on one common epidemiological tool, the basic reproduction number R0. Here we applied a risk assessment for the bluetongue virus (BTV) in Austria to identify regions at risk. The commonly used R0 estimate for BTV is based on temperature dependent rates like biting rate, extrinsic incubation period and vector mortality rate. Further the host population, cattle and small ruminants, and the vector population (Culicoides spp.) are considered. Based on our daily Culicoides monitoring at the University of Veterinary Medicine Vienna, Austria, we quantified the seasonal abundance of the vector population by a statistical model using cross-correlation maps and Poisson regression. The seasonal cycle was well described by temperature, precipitation and soil moisture, respectively. Daily surface soil moisture was derived from ASCAT satellite data, while temperature and precipitation were obtained from weather forecast models. The results comprise daily risk maps with approx. 10 km spatial resolution. Using this method will give us the opportunity to make predictions up to one single month and will help policy makers in the agricultural field.

RA4: Impact of frost on overwintering larvae of biting midges in cattle manure

Lühken, Renke[1]; Kiel, Ellen[1]

Carl von Ossietzky University[1]

Key words: biting midge, Culicoides, breeding sites, frost, winter

As demonstrated by many studies, several species of biting midges have the ability to overwinter as larvae. The development of the overwintering larval population is expected to have a strong impact on the first generation in the year resulting in a synchronization and extended emergence of the biting midge population peaking in spring or early summer. However, until now the impact of frost on the survival of overwintering larvae is not known. Therefore, this study aimed on the colonization of cattle manure during the winter period and the impact of temperatures below zero on the survival of immature biting midges.
Ten samples of cattle manure were taken every two weeks from December 2011 to February 2012 on two cattle farms in Northern Germany. All samples were directly transferred to the laboratory. Firstly, each sample was divided into two parts. One half was stored into ethanol (70%) and later investigated for larval biting midges. The other half was put under an emergence trap. Emerging biting midges were collected every second day. Temperatures in the core of ten cattle dungs were measured in the field.
Additionally, another ten samples of cattle manure were taken and divided into two halves. One half was put in a freezer for a short period. Temperatures were measured in the cattle dung reaching lowest temperatures between 0°C and -19°C. All samples from the freezer were accordingly put under emergence traps, whereupon the other half was directly put under emergence traps. Emerging biting midges were collected every second day.
Our results approved previous studies, which found overwintering larvae of biting midges. Larvae were found in the cattle manure during the whole study period between December 2011 and February 2012. Additionally, biting midges emerged from samples, which were previously exposed to different temperatures below zero in the field or in the freezer. Nevertheless, first results indicated lower emergence rates after exposure to frost. Interestingly, although the air temperatures in the field felt -10°C for several days, temperatures in the core of cattle dungs never were below -5°C.
These preliminary results demonstrated a high tolerance of immature biting midges against temperatures below 0°C, which maybe supported by an insulating effect of dung. A harsh winter period is not expected to inhibit the emergence of biting midges in spring or early summer, but may reduce the number of individuals.

RA5: Geographic distribution of potential vector of tick-borne viruses in Northern Italy

Maioli, Giulia[1]; Bonilauri, Paolo[1]; Calzolari, Mattia[1]; Defilippo, Francesco[1]; Cervi, Valentina[1]; Luppi, Andrea[1]; Dottori, Michele[1]

IZSLER[1]

Key words: tick-borne viruses, vector, maps

Tick-borne viruses can rise or appear in areas where diseases were not previously detected. In particular, novel tick species and associated tick-borne pathogens may be transported over long distances by migratory birds or the ecological features may change the epidemiology of these viruses. Crimean-Congo haemorrhagic fever (CCHFV, genus Nairovirus, family Bunyaviridae) is one of the most widely distributed tick-borne diseases in the world, affecting people in certain areas of Africa, Asia, Eastern Europe and the Middle East. CCHFV is transmitted by ticks of the genus Hyalomma, in particular Hyalomma marginatum. Tick-borne encephalitis (TBE) in Europe is a disease caused by tick-borne encephalitis virus (TBEV, genus Flavivirus, family Flaviviridae) and is maintained in cycles involving Ixodid ticks (Ixodes ricinus) and wild vertebrate hosts.
Ixodid ticks were collected from wild fauna, during 2008-2011, from two Italian region, Lombardia and Emilia-Romagna, and mapped using an open source software (QuantumGis) at municipality level.
A total of 5367 tick exemplars were collected from 212 municipalities in four years of surveillance.
If we consider the two main species potential vector of viruses, I. ricinus (3483 exemplars) and H. marginatum (20 exemplars) we could notice a great difference in their distribution. I. ricinus was found in 72% of the areas studied (154 municipalities of 212) whilst H. marginatum only in 2% of the municipalities studied.
I. ricinus is well distributed in all northern Italy, with different abundance between plain areas and mountain areas, while H. marginatum was not recorded above 45° north latitude.
Risk of introduction or spread of these viruses depend also from several others factors like host reservoirs, birds and mammals, human activities and environmental changes. Future risk assessment could be implemented by adding data on vertebrate host and ecological features at tick distribution maps.

RA6: Analysis of biotic and abiotic factors influencing the (re)occurrence of West Nile virus infection in Tunisia

Calistri, Paolo[1]; Ben Hassine, Thameur[2]; Ippoliti, Carla[3]; Conte, Annamaria[3]; Danzetta, Maria Luisa[3]; Bruno, Rossana[3]; Lelli, Rossella[3]; Hammami, Salah[4]

Istituto "G. Caporale", Teramo[1]; entre National de Veille Zoosanitaire[2]; Istituto “G. Caporale”, Teramo[3]; Centre National de Veille Zoosanitaire[4]

Key words: West Nile virus, remote sensing, environment, Tunisia

West Nile disease (WND) presence and circulation is mainly influenced by the presence of a sufficient number of bird populations able to sustain the virus amplification and a relative high abundance of mosquitoes to support the transmission between vertebrate hosts. Among the complex set of biotic and abiotic factors influencing the emergence and spread of this vector-borne disease, two main variables have been considered to have a great influence on the probability of WNV introduction and circulation in Tunisia:

• the presence of susceptible bird populations, and

• the existence of geographical areas where the environmental and climatic conditions are more favourable to mosquito multiplications. This second condition is mainly influenced by rainfall and availability of water pools, whereas temperature conditions may be considered relatively less important for Tunisia.

Satellite measurements and other remote sensing techniques can be used to characterise the environment in which the vectors proliferate.

The following geographical distribution of environmental and climatic variables has been considered: wetlands and humid areas, Normalised Difference Vegetation Index (NDVI), rainfalls. In addition, 64 locations of water bird populations in Tunisia have been geographically identified. Only bird species belonging to the Orders Charadriiformes and Passeriformes have been considered. Weighted sums in ESRI ArcGIS have been performed to integrate the risk factors.

Finally, a geographical comparison of the risk map produced and WN human cases reported in Tunisia have been made.

RA7: A qualitative risk assessment method to evaluate BVD eradication plans

Feliziani, Francesco[1]; Stifter, Ernst[2]; Maresca, Carmen[1]; Scoccia, Eleonora[1]; De Mia, Gian Mario[1]

Istituto Zooprofilattico Umbria e Marche[1]; Bolzano Province Veterinary Service[2]

Key words: risk assessment; BVD; eradication plan

Bovine Viral Diarrhoea (BVD) and Mucosal Disease (MD) are caused by a pestivirus. This infection is now well recognised as a significant disease in dairy herds, because it is capable of causing production losses of up to 50% in recently infected herds and insidious losses in endemically infected herds. Persistently infected (PI) carrier calves play a key role in spreading the virus as they can shed virus throughout their life, acting as a source of infection for other animals. For this reasons the BVD eradication plans are based on the identification and the removal of PI animals as well as on the application of other direct and indirect control measures such as vaccination campaign. Recently, several European countries implemented specific programmes to eradicate the BVD Virus (BVDV) infection and also in Italy some actions are already underway while others are still in draft.
The aim of this study was to provide an evaluation tool able to individuate critical points of BVD eradication strategies and to analyze the applicability of corrective actions.
A logic flow based on major risk factors associated with the persistence and the diffusion of BVDV infection was assessed. Afterwards the method was applied to the data regarding the BVD eradication plan running in the province of Bolzano (Italy).
A backward model risk assessment and two fault tree, the one based on the probability of the BVDV introduction and the other based on the probability of BVDV spreading within the territory, were carried out. The probability to introduce the BVD infection was evaluated by the product of the potential risk of infected animals being imported and the potential risk of being born infected.
Two parameters were examined in order to determine the probability of the infected animals being imported into the province: the BVD prevalence level of exporting countries and the volume of trade.
Two additional parameters were examined in order to determine the probability of animals being born infected: performances of surveillance system and diagnostic method.
The risk of BVDV spreading was considered as a function of two elements: permanence of the infected animals in herds and efficacy of infected animal removal. The examined parameters of the permanence risk of the infected animal in the herd were the use of common grazing as well as the application of quarantine in case of animal introduction; whereas animal selling through fairs and markets or slaughtering and direct sale outside the region were the parameters used to determine the efficacy of infected animal removal.
The probability of occurrence of each event was assessed by means of the following descriptive scale: ‘negligible’ when the probability of occurrence of the event is sufficiently low to be ignored, or when possible only in case of exceptional circumstances; ‘low’ when the occurrence of an event is sometimes possible, ‘moderate’ when possible and ‘high’ when clearly possible.
The proposed logical approach has proven to be appropriate for the purpose and useful to evaluate the BVD eradication plan. The model proved to be suitable to individuate the critical points preventing the eradication of the disease and it appears as a useful tool to evaluate the plan capability to achieve the expected goal.
Finally, on the basis of the experimental application of the model, it can be concluded that the probability of introducing BVDV infection in the province of Bolzano was rated as ‘high’, whereas the implemented activities regarding the secondary surveillance of the territory are ‘negligible’.

RA8: An Investigation of the Factors Associated with the Risk of Meat as a Source of Classical Swine Fever Introduction into the UK

Cowan, Lucie[1]; Haines , Felicity[1]; Everett, Helen[1]; Crudgington, Bentley[1]; Drew, Trevor[1]; Crooke , Helen[1]

AHVLA[1]

Key words: Classical swine fever survival of

Introduction:
Classical swine fever virus (CSFV) is a highly infectious disease of pigs, with outbreaks resulting in devastating economic and social consequences. The UK is currently free from CSFV, however in order to minimise the risk of future outbreaks, it is important to identify the likelihood of CSFV introduction to the UK via meats of porcine origin. This will allow for the formation of accurate and updated risk assessments and the definition of effective strategies to mitigate the risk of disease introduction without imposing undue restrictions.
The primary objective aimed to identify the amount of virus necessary to initiate infection if infected material were ingested by swine- the pig oral infectious dose 50% (PID50). Due to a lack of existing data, previous assessments have used 10 TCID50 as an estimated PID50, which is relatively low compared with other viruses. However these estimations were based on pigs infected via the intranasal route. Our second aim is to provide definitive data on the thermal inactivation rate of CSFV in porcine tissues at key temperatures; 68°C represents cooking temperature, 56°C is of relevance for inactivation of virus during composting, 25°C is that of ambient room temperature, and 4°C represents refrigeration temperature.

Materials and Methods:
Six groups of 6, 10-week old pigs were orally inoculated with viral doses of between 101 and 106 TCID50 inside oral baits. Daily clinical scores, rectal and biochip temperatures were obtained from all pigs. Blood and nasal swab samples were taken at two to three day intervals and viraemia and nasal shedding of virus were determined by nucleic acid extraction and qRT-PCR analysis. Development of leucopoenia and thrombocytopenia was measured via FACS analysis. Post mortem examinations determined the presence of pathological lesions. The oral PID50 was calculated using the Spearman-Karber method. To examine how long virus survives in porcine tissue at the key temperatures, muscle, fat and lymph node material were used to generate thermal inactivation curves, and the D values (the time taken for viral load to drop by 1 log) were calculated.

Results:
The oral PID50 was estimated at 105.74 for the highly virulent Brescia. Thermal inactivation results for lymph node at 4°C show a D value of 1.9 weeks. At 68°C, D value for lymph node is 0.47 minutes and for fat is 1.26 minutes. 56°C has a D value of 1.5 minutes for lymph node and 2.45 minutes for fat. These D values are far shorter than those for virus in tissue culture.

Conclusions:
Our study has shown that the PID50 of Brescia is much higher than previously estimated, indicating that the oral route requires a higher infectious dose than other routes of infection.
Assuming a viral titre of 106 TCID50 per gram of infected tissue, thermal inactivation studies have concluded that potentially infected pork products would need to reach a temperature of 68°C for at least 9 minutes in order to completely inactivate CSFV, providing that the centre of the meat reaches this temperature for the full 9 minutes. Moreover, composting of these contaminated materials would need to occur for 18 minutes. UK regulation states that composting must use a minimum temperature of 60°C for at least 2 days, meaning composting regulations in the UK are adequate in providing the necessary conditions to render composted materials CSFV-free.

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EPIZONE 6th Annual Meeting Satellite Symposium on the topic 'Schmallenberg virus'

15th June 2012, Thistle Hotel, Brighton

Schmallenberg virus (SBV) is a novel orthobunyavirus first identified in cattle in Germany in Autumn 2011.

The virus is most likely transmitted by midges (Culicoides spp.), and infections likely occurred in summer and autumn of 2011, but foetuses that were exposed to the virus in the womb were born later. SBV has been detected in several European countries. A risk assessment for public health was issued by the European Centre for Disease Prevention, saying it is unlikely that this new orthobunyavirus can cause disease in humans.

Still a lot of questions remain to be answered. Which vector species is transmitting the disease? Can animals infect each other directly? And of course, where did the virus come from? What can be done to control the virus and can we develop a vaccine? Scientists within Europe need to work together and exchange knowledge to control this new epizootic in Europe.

Schmallenberg virus in Germany, Detection, characterization and experimental infection in cattle

Bernd Hoffmann[1], Matthias Scheuch[1], Dirk Höper[1], Ralf Jungblut[2], Mark Holsteg[3], Horst Schirrmeier[1], Michael Eschbaumer[1], Katja Goller[1], Kerstin Wernike[1], Melina Fischer[1], Angele Breithaupt[1], Thomas C. Mettenleiter[1] Franz Conraths[1] and Martin Beer[1]

[1]Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany; [2] State Veterinary Diagnostic Laboratory (SVUA) Arnsberg, Zur Taubeneiche 10-12, 59821 Arnsberg, Germany; [3]Chamber of Agriculture for North Rhine-Westphalia, Bovine Health Service, Siebengebirgsstraße 200, 53229 Bonn, Germany

In summer and autumn 2011, farmers and veterinarians in North Rhine-Westphalia (Germany), but also in the Netherlands, reported about mild clinical signs in dairy cattle including fever, reduction of milk production and diarrhea. Cattle in several farms showed a similar clinical picture, which disappeared after some days. Most cases in Germany were reported in September and October, and blood and swab samples taken from diseased cattle were tested by real-time RT-PCR for all common bovine viruses like bluetongue virus, epizootic hemorrhagic disease virus, pestiviruses, foot-and-mouth disease virus, bovine ephemeral fever virus or Rift Valley fever virus. However, all samples tested negative and none of the known pathogens could be connected to those cases.

Therefore, a metagenomic approach was chosen to analyze a pool of three selected samples from dairy cattle from a farm near the city of Schmallenberg which had fever and showed a decrease in milk production of more than 30%. The samples were analyzed following a protocol for RNA and DNA preparation and subsequent next generation sequencing in a *Genome Sequencer FLX* instrument (454/Roche) in November 2011. Using a newly developed software routine, all reads were compared to sequence databases, resulting in 7 reads showing a high homology to viruses of the genus *Orthobunyavirus*.

This sequence information was used to develop a specific real-time RT-System which allowed investigation of all collected blood samples from affected cattle farms. Subsequently, the first 5 positive farms could be detected, confirming the presence of “Schmallenberg virus” genome in the blood of several cattle. Subsequently, the virus could be isolated on cell culture and first animal trials in calves were conducted. In addition, the full-length sequence was determined, and it could be shown that the new virus is most related to viruses of the so-called Simbu serogroup.

Since December 2011, more than 1400 cases of SBV-PCR-positive malformed lambs or calves were reported in Germany. Epidemiological data about the distribution of cases, the sero-prevalence in Germany as well as the results from different infection experiments with cattle will be presented and discussed.

OIE experience with the Schmallenberg virus outbreak and the importance of a research network

Dr Elisabeth Erlacher-Vindel, Deputy Head, Scientific and Technical Department, World Organisation for Animal Health,(OIE), 12 Rue de Prony, 75017 Paris, France (e.erlacher-vindel@oie.int).

The World Organisation for Animal Health (OIE) is an intergovernmental organisation with a mandate from its 178 Members to improve animal health worldwide. It is responsible for ensuring transparency of the animal disease situation worldwide, including diseases transmissible to humans, as well as safeguarding the sanitary safety of world trade of animals and animal products and ensuring food safety.

The OIE works with the permanent support of over 265 Reference Laboratories and Collaborating Centres and 11 regional offices worldwide, which play a key role in veterinary scientific research and information.

Collection and publication of veterinary scientific information, notably animal disease prevention and control methods, is one of the main objectives mentioned in the OIE's 5th Strategic Plan.

Following the emergence of Schmallenberg virus in Western Europe, OIE Member Countries asked OIE to provide information and guidance. The OIE decided to convene a meeting of experts at very short notice to review existing knowledge of the new virus and provide information to its Members and stakeholders.

The existing research network made it possible to provide a consolidated expert opinion on Schmallenberg virus very rapidly and to share experiences and available knowledge in a very transparent manner.

Good Veterinary Governance is key to managing emerging diseases, and appropriate decisions have to be taken on a scientific basis. To provide such scientific knowledge, research networks are needed to coordinate scientific projects and provide consolidated expert opinions at an early stage of the event, without unnecessary delay and waste of resources.

Schmallenberg virus is a good example that demonstrates the efficiency of an existing research network, and the EPIZONE symposium will further illustrate what can be achieved when "scientific preparedness" is a reality.

Orthobunyaviruses and in particular Simbu serogroup viruses in Europe

Richard M. Elliott, University of St Andrews.

The *Orthobunyavirus* genus within the *Bunyaviridae* family contains more than 170 named viruses that can be conveniently divided into 18 serogroups. The largest of these is the Simbu serogroup with 25 viruses isolated from South America, Africa, Asia and Australia that includes a number of human (Oropouche virus) and animal (e.g. Akabane, Sathuperi, and Shamonda viruses) pathogens. Schmallenberg virus is most closely related to these animal-infecting viruses and thus represents the first occurrence of a Simbu serogroup virus in Europe. The relationships between these different viruses will be discussed. In addition, the establishment of a reverse genetic system for Schmallenberg virus (based on the system first described for the prototype Bunyamwera virus) will be reported. Exploitation of reverse genetics to create recombinant attenuated viruses with vaccine potential will be evaluated.

Schmallenberg virus from policy point of view

Bruschke, Christianne [1]

M of Economic Affairs, Agriculture and Innovation[1]

Key words: Schmallenberg, policy

Schmallenberg Virus (SBV) is an emerging virus detected in November 2011 in Germany. By now the virus has been detected in 8 countries in Europe. There is still much unknown about the virus and infected countries are collecting as many data as possible to get a good insight in the epidemiological situation. Much research is initiated on the pathogenesis of the disease, virus characterization, epidemiology and vaccine development.

An introduction of a new virus in a country or region is always a concern for policy makers. The first question that needs to be answered is whether the virus may also pose a risk for human health. In this case an early risk assessment judged the risk for human health as very low. From a disease control point of view for the national authorities it is furthermore very important to quickly get a good insight in the epidemiological situation. The Friedrich Loeffler Institute that discovered the virus made diagnostic techniques available so all countries had the possibility to test for the virus. To get better insight in the epidemiological situation The Netherlands and some surrounding countries chose to make the disease notifiable and reported positive results to the European Commission and to the OIE. In response to these notifications many third countries have put in place export restrictions which is a big financial burden for exporting countries like The Netherlands, Germany and France. The OIE has made a scientifically based overview of the risks of transmission of the virus by trade of animals or products, based on the currently available knowledge. However one could argue that import restrictions are not proportionate since the economical impact of the disease in the affected countries is not very high.

The introduction of SBV shows that, although the economical impact of a virus is low and it is not a zoonotic disease, there are many policy issues to be dealt with. The policy issues are national, on EU level but also related to trade with third countries.

Experiences with viruses from the Simbu serogroup in Australia and future prospects

P.D. Kirkland, Virology Laboratory, EMAI, Camden, NSW, Australia.

In Australia there are 7 vector borne viruses from the Simbu serogroup, 5 of which infect livestock. There are many aspects of the biology of these viruses that will provide an insight into the epidemiology and pathogenesis of Schmallenberg virus.

The Australian Simbu viruses are spread by Culicoides species, mainly C. brevitarsis. The geographical distribution and seasonal occurrence of a virus is determined by the abundance and activity of the vector. Therefore, virus transmission is usually limited to the summer and autumn months. Unlike bluetongue viruses, the Simbu viruses are transmitted with very high efficiency. Only small midge populations are required to spread a virus in the livestock population. As a consequence there can be a very high annual incidence, often approaching 100%. The mammalian host is partly determined by vector preferences with cattle the main host species but infections occur in small ruminants and equidae.

In a cattle population, a high level of immunity is maintained and disease in locally bred animals is rare. Disease usually occurs following changes to the usual pattern of distribution and activity of the insect. Under climatically favourable conditions, if the insect range expands, and there are pregnant animals present, disease is likely to occur. Under short-term adverse climatic conditions, the midge distribution may contract. When a normal weather pattern returns, and the usual midge distribution resumes, susceptible animals will be infected. Introduction of susceptible stock into an endemic area can result in disease.

The pathogenic Simbu viruses found in Australia are almost affect developing foetus. Only Akabane is a significant pathogen and Aino virus has been infrequently associated with disease. Akabane virus induced disease in sheep in Australia is rare, firstly because few sheep are raised in regions where the main vector is present and secondly because few sheep are pregnant at the time of vector activity. The incidence, severity and range of congenital defects following infection of the bovine foetus is mostly due to the stage of gestation at which infection occurs. Hydranencephaly (HE) is the outcome of infection in the 3rd and 4th months of gestation while arthrogryposis (AG) mostly occurs following infection in the 5th and 6th months. Infection late in gestation can be manifest as encephalitis at birth, with affected calves showing a flaccid paralysis of the limbs. The incidence of defects is greater earlier in gestation but can vary with the strain of virus. An incidence of deformed calves of about 25% is common under field conditions but can be as high as 50%. Experimentally in sheep, the incidence of defects has ranged from 15-85% with different virus strains. The deformities seen in sheep or goats does not show the distinct progression from AG to HE as seen in cattle and affected progeny can show a wide range of abnormalities, including lesions in other organs such as lung and thymus.

Owing to the very high efficiency of transmission of these viruses, it is more likely that an agent such as Schmallenberg will become established in many regions. However, it is possible that the virus will only become endemic in more temperate regions, with incursions into regions that are colder, either due to latitude or at higher altitude, under favourable conditions. Both short and longer term climatic changes may play a role.

Schmallenberg Virus in France

Zientara, Stephan[1]; Lara, Estelle [1]; Breard, Emmanuel [1]; Viarouge, Cyril [1]; Desprat, Alexandra[1]; Vitour, Damien[1]; Adam, Micheline [1]; Chauveau, Emilie[1]; Doceul, Virginie [1]; Marianneau, Philippe[1]; Eleouet, Jean-Francois [2]; Delmas, Bernard [2]; Sailleau, Corinne[1]

Anses[1]; INRA[2]

Key words: Schmallenberg virus, molecular and serological diagnosis

On January 25th, the first case of infection by Schmallenberg virus (SBV) was reported in France. Diagnosis was established from the brain of a newborn lamb showing congenital malformation by using the SBV -specific real time RT-PCR assay developed by FLI which targets segment L of the virus. In March 2012, more than 1000 outbreaks were reported in cattle, sheep and goats.
This communication will describe the measures and actions put in place before and after the report of the first cases in France. The surveillance program will be shortly described.
The diagnostic tools used, developed and validated (molecular and serological assays) will be presented. A network of 57 regional labs has been set up for SBV detection by RT-PCR using commercial kits. The serological methods such as SNT and ELISA (with native and recombinant antigens) have allowed to precise the seroprevalence in some herds. Sequence comparisons between French and German strains will be also related.

Epidemiology of Schmallenberg Virus Infections in Germany

Conraths, Franz[1]; Staubach, Christoph[1]; Sonnenburg, Jana[1]; Fröhlich, Andreas[1]; Kramer, Matthias[1]; Gall, Yvonne[1]; Probst, Carolina[1]; Höreth-Böntgen, Detlef[1]; Teske, Kathrin[1]; Kämer, Doris[1]; Beer, Martin[1]

FLI[1]

Key words: Schmallenberg virus, epidemiology, Germany

After causing mild disease in adult dairy cattle in late summer and autumn 2011, Schmallenberg virus (SBV) infections emerged as a major cause of congenital malformations in sheep, cattle and goats from December 2012 onwards. In Germany, the majority of outbreaks reported so far occurred in sheep holdings, followed by cattle farms and goat holdings. A single case was detected in a European bison. There is serological evidence for SBV infections in roe deer and red deer in an area with a high outbreak density in domestic ruminants. Since mid-February 2012, the number of affected calves increased while the number of reported SBV cases in sheep lambs started to decrease.
The spatial distribution of reported SBV cases within Germany shows considerable variation. While affected farms have been reported from all federal states, the area with highest case density includes North Rhine-Westphalia, Lower Saxony, Hesse and Schleswig-Holstein. There seems to be a West-East- and a North-South-gradient in the density of outbreaks. First epidemiological analyses show that the spatial density of outbreaks in sheep holdings is statistically significantly associated with the population density of sheep. Backward calculations of the likely time of transplacental infection of SBV-infected lambs suffering from congenital malformations of the AHS type suggest that the majority of transplacental infections took place since mid-September 2012. The seasonal peak of the transplacental SBV-infections coincided with the peaks of the BTV-8 infections observed in 2006 and 2007 as well as with the maximum of BTV-8-infected biting midges detected in 2007. These results are in accord with the proposed role of Culicoides spp. in the transmission of SBV.

Role of *Culicoides* biting midges in the transmission of Schmallenberg virus

Simon Carpenter

Culicoides biting midges are among the smallest haematophagous flies commonly found on livestock and have a practically worldwide distribution. In this talk I will outline the lifecycle of *Culicoides* with reference to arbovirus transmission and then discuss their unique biology through comparisons drawn with other blood-feeding Diptera such as mosquitoes and sandflies. These studies will highlight aspects of the epidemiology of *Culicoides*-borne arboviruses that are poorly understood and additionally draw conclusions regarding barriers to virus spread from patterns of bluetongue virus (BTV) movement in southern and northern Europe. I will then specifically examine the reasons why arbovirus transmission appears to be limited to selected species within the genus. This will include a discussion of characterised and inferred barriers to arbovirus dissemination within *Culicoides* of both laboratory and field origin, which are thought to be inheritable and environmental factors modifying such barriers. I will also discuss results of recent studies that have examined the infection and dissemination of Schmallenberg virus in colony lines of *Culicoides* and mosquitoes as models vectors for future research. These results are then compared with those produced for BTV in the laboratory with the same colony lines. Finally, I will consider the future of vector competence research on *Culicoides* and arboviruses worldwide. This will include a discussion of the recently initiated *Culicoides* genome project, based at the Pirbright laboratory, which will enable detailed analyses of genetic components of vector competence and opportunities for worldwide collaboration.

Evidence for Culicoides obsoletus group as vector for Schmallenberg virus in Denmark

Rasmussen, Lasse Dam[1]; Kristensen, Birgit[1]; Kirkeby, Carsten[1]; Rasmussen, Thomas Bruun [1]; Belsham, Graham J[1]; Bodker, René[1]; Botner, Anette[1]

VET-DTU[1]

Key words: Schmallenberg virus RNA, RT-PCR, biting midges, vector,Culicoides spp

Schmallenberg virus (SBV) was first identified in Germany in late 2011 by the Friedrich Loeffler Institute and has now been found in several European countries including Holland, France, Belgium, U.K. and Spain. The disease, which affects sheep, cattle and goats, was first recognized due to transient clinical symptoms including fever, diarrhea and loss of milk production. However, a more significant consequence of infection in pregnant animals is the production of severe congenital malformations in newborn animals, especially lambs. The virus is a member of the Orthobunyavirus genus within the Bunyaviridae family and is closely related to Shamonda and Akabane viruses. These viruses are transmitted by insect vectors (including biting midges (Culicoides sp.) and mosquitoes). To determine whether these insects may act as vectors for SBV, biting midges (Culicoides spp.) caught in October 2011, in the south-west of Denmark (close to the German border), were sorted into pools and tested for the presence of Schmallenberg virus RNA by RT-qPCR. From 18 pools of 5 midges from the C. obsoletus group, 2 pools were both found positive in two separate assays, targeting the L- and S- segments of the SBV RNA. However, 4 pools of C. punctatus s.str were negative. The sequence of 80bp (excluding the primer sequences) from the amplicons (ca. 145bp) was identical to that published for the expected region of the SBV L-segment. The levels of SBV RNA detected in the biting midges were much higher than could be accounted for due to the residue of a blood meal and no ruminant actin mRNA could be detected either. These results strongly suggest that SBV has replicated within specimens of the C. obsoletus group and indicates that these biting midges can act as vectors for this virus. To date (end of March), no cases of disease due to SBV have been detected in sheep, cattle or goats in Denmark.

RT-PCR screening for Schmallenberg virus in Culicoides spp. caught in Belgium in 2011.

De Regge, Nick[1]; Deblauwe, Isra[2]; Vantieghem, Pieter[2]; De Deken, Reginald[2]; Smeets, François[3]; van den Berg, Thierry[1]; Cay, Ann Brigitte[1]

VAR-CODA[1]; Institute of Tropical Medicine[2]; Université de Liège[3]

Key words: SBV, Culicoides, Belgium, RT-PCR, vector

Since Schmallenberg virus (SBV) was first identified by researchers from the FLI (Germany) in November 2011, its presence has in the meanwhile been confirmed in the Netherlands, Belgium, United Kingdom, France, Luxembourg, Italy and Spain. The rapid and large expansion of this virus, together with the knowledge that related viruses belonging to the same Simbu serogroup of Orthobunyaviruses are generally spread by midges and mosquitoes, led to the assumption that also SBV is probably spread by these vectors.
In order to examine the potential role of midges in the spread of SBV, midges caught at several locations in Belgium by UV light traps were analyzed by RT-PCR (protocol for L and S segments detection were kindly provided by FLI, Germany). Till now, midges caught in September and October 2011 were screened after they were morphologically identified at species level and pools consisting of ≤ 20 heads of Culicoides were prepared. Initial experiments confirmed that the RT-PCR detecting the S segment was more sensitive than the PCR detecting the L segment, and was consequently used for further screening. At the time of writing, 214 pools were already screened and 34 pools originating from 8 different locations (Betekom, Berlaar, Eindhout, Verlaine, Boncelles, Sart-Tillman, Nandrin and Bettincourt) were found positive for SBV. These positive pools consisted of heads of parous females of C. obsoletus s.s., C. obsoletus complex, C. dewulfi, C. pulicaris and C. chiopterus. None of the pools containing nulliparous midges tested positive.
These results strongly indicate a role of at least 4 different species of biting midges in the transmission and spread of SBV. This indication is strengthened by the fact that pools consisted exclusively of heads, suggesting that midges act as real amplification vectors and were not simply SBV positive after a blood meal on viraemic animals. More pools of Culicoides will be tested to identify other possible SBV vectors among Culicoides species and Culicoides caught earlier during the vector season will be tested to shed light on the time of introduction of SBV in Belgium.

Comparative analysis to determine the most appropriate tissue for Schmallenberg virus detection by RT-PCR in aborted lambs and calves

De Regge, Nick[1]; Van Loo, Hans[2]; Bertels, Guido[2]; Saulmont, Marc[3]; Vandenbussche, Frank[1]; van den Berg, Thierry[1]; Cay, Ann Brigitte[1]

VAR-CODA[1]; Diergezondheid Vlaanderen[2]; ARSIA[3]

Key words: SBV, diagnosis, RT-PCR

In November 2011, scientists from the Friedrich-Loeffler-Institute (FLI) in Germany identified a new virus in the blood of dairy cows that showed clinical signs such as fever, milk drop and diarrhea. The newly identified virus was named Schmallenberg virus (SBV) and belongs to the Simbu serogroup of the genus orthobunyaviruses. Besides the clinical signs in adult cattle, the virus is transmitted to the fetus when infection occurs during early pregnancy and causes congenital malformations in lambs and calves. Malformations that are often noticed at autopsies are torticollis, arthrogryposis, scoliosis, brachygnathia inferior and brain abnormalities like hydranencephaly and hypoplasia of the cerebrum and/or cerebellum. Since lambs and calves that are suspected to be infected with SBV on the basis of gross lesions need to be confirmed by diagnostic tests, we determined the most suitable tissue for SBV detection by RT-PCR.

Cerebrum, cerebellum, brain stem, spinal cord, thymus, spleen and lymph nodes from suspected lambs were tested by a RT-PCR detecting the L segment of the virus (protocol kindly provided by FLI, Germany) to determine the viral load. As could be expected from the neurological symptoms and lesions, brain material seemed to be the most appropriate matrix to detect SBV by RT-PCR. SBV was detected in the brain stem of all animals that were found positive in at least one tissue tested, followed by cerebellum (83%) and cerebrum (81%), showing that brain stem is the most suitable tissue for SBV detection in aborted lambs. In a considerable amount of lambs that were found positive for SBV in brain samples, SBV could also be detected in lymphoid tissues like spleen (31%), lymph nodes (43%) or thymus (27%), suggestive for a possible lymphotropic tropism of this virus.

For aborted calves, a similar analysis was carried out and the viral load in cerebrum, cerebellum, brain stem, spleen and meconium were also compared but using the more recent RT-PCR detecting the S segment of the virus (protocol also kindly provided by FLI, Germany). Using this more sensitive protocol, the brain stem was found positive in 90% of the calves that were found positive in at least one tissue tested, followed by cerebrum (65%) and cerebellum (48%). Theoretically, a pool consisting of brain stem and cerebrum would lead to a sensitivity of 96 % compared to when all tissues would be tested. Meconium could be found positive in 33% of the cases and spleen only in 5%.

This study shows that SBV can be found in several different organs in aborted lambs and calves infected by the virus. Brain stem however seems to be the preferred matrix for SBV detection by RT-PCR if only one organ has to be considered for testing.

Biological and genetic characterizations of two isolates of Schmallenberg virus (SBV) propagated from naturally infected sheep fetuses

Muylkens, Benoît [1]; Wiggers, Laetitia[1]; Claine, François[1]; Kirschvink, Nathalie[1]

University of Namur[1]

Key words: virus isolation, titration, plaque assay, molecular epidemiology, genetic drift

During the lambing period of January 2012, severe athrogryposis and nervous system defects were observed in 28 lambs delivered by 99 ewes whose breeding period started at mid-August 2011. From RNA samples extracted from brain and blood of the 28 deformed lambs, 10 were tested positive for SBV infection through real-time quantitative reverse transcription PCR (RT-qPCR) developed by the Friedrich Loeffler Institute (Hoffmann et al., 2012), with cycle threshold (Ct) values of 15 to 40. The Ct values obtained on the nervous system of two animals showed high levels of viral RNA copies in these samples respectively tested with Ct of 15.3 and 19.6.

Two viruses were isolated from brain tissues prepared from the two highly positive animals. Pieces of 500 mg of homogenized tissue were suspended in cell culture medium. Serial dilutions (1/2, 1/4 and 1/8) of the tissues suspensions were incubated on Baby Hamster Kidney cells (BHK-21) for virus isolation. The inocula were removed after 3 hours, and replaced with Glasgow Minimum Essential Medium. At the second passage, cell cultures inoculated with SBV-RT-qPCR positive brain tissues showed several plaques compatible with viral cytopathic effect. The amplification of SBV was confirmed by testing in RT-qPCR the culture supernatants collected at two consecutive days. Viral stocks were prepared from passages 3 of the two viral isolates designated SBV-Namur1 (-Na1) and SBV-Na2.

The biological characterization of the two isolates included (i) viral titration, (ii) plaque assay and (iii) neutralization assays. (i) The viral titers were 2 and 3 log10 lower than the virus titer of the cell adapted SBV-FLI strain. (ii) The plaque assay, adapted from a protocol used for murine norovirus (Mathijs et al., 2010) showed that the plaque sizes and morphologies induced by SBV-Na1 and -Na2 were different from those induced by the SBV-FLI strain. (iii) Virus infectivity of SBV-Na1 and –Na2 was neutralized both by the reference positive serum (provided by the FLI) and by serum obtained from ewes tested positive in SBV serum neutralization assay.
The genetic characterization was based on the PCR amplification and sequencing of the whole S segment and five overlapping fragments encompassing the whole M segment. Sequencing of the S fragment showed three mutations shared by the two isolates, allowing discrimination of the SBV-Na1 and –Na2 viruses from the original German SBV isolate. These mutations were identified before and after the cell culture amplification. Sequencing of the amplicons obtained in the M segment will be completed in the next weeks.

In conclusion, two SBV isolates were obtained from naturally infected sheep fetuses through a direct isolation assay on BHK cells. The preliminary biolological and genetic characterization indicated that the two isolates are different from the original German SBV isolate. The level of genetic drift (both between the two isolates and in comparison with other cattle and sheep SBV isolates) will be further examined through whole genome analysis.

Natural infection of a sheep flock with Schmallenberg virus: clinical, serological and virological features

Kirschvink, Nathalie[1]; Claine, François[1]; Wiggers, Laetitia[1]; Muylkens, Benoit[1]

University of Namur[1]

Key words: sheep, transplacental infection, incidence, PCR, seroneutralisation

The first manifestations of newborn or stillborn lambs that were affected by Schmallenberg virus (SBV) were reported at 23 of December 2011 in the Northern part of Belgium. The sheep flock of the University of Namur was one the first SBV-affected flocks located in the Southern part of Belgium; the first stillbirth of a SBV-affected lamb occurring on 7th January 2012. The present investigation provides information about the clinical manifestations, the serological and virological findings of in utero SBV-infected lambs born in January 2012.
Pregnant ewes (n=99) and their lambs (n= 163) born in January 2012 were investigated. Serum samples were prepared from all ewes. In case of normal lambing and birth of clinically healthy lambs, blood was sampled immediately after birth (T0) as well as 36h after colostrum intake (T36) in randomly selected healthy lambs. In case of SBV-lambing, blood was sampled in all clinically healthy siblings at T0 and T36 and whenever possible, at T0 in lambs showing clinical signs of SBV infection. SBV-affected lambs underwent necropsy within 36h and central nervous system (CNS) tissue was sampled. Virological diagnosis of SBV was performed on CNS tissue by using RTq-PCR and seroneutralisation (SN) for SBV was performed on serum sampled at T0 and T36. RTq-PCR results were considered positive if Ct was lower than 40. Results of SN were expressed as positive or negative.
Among the 99 ewes, 76 gave birth to clinically healthy lambs (n=124) and 23 gave birth to 28 SBV-affected lambs and 11 clinically healthy siblings. Birth of SBV-affected lambs was more frequent in primiparous ewes (9 SBV lambings vs 11 normal lambings) than in multiparous ewes (14 SBV lambings vs 65 normal lambings). Clinical manifestations of SBV-affected lambs included stillborn lambs presenting severe arthrogryposis (n=4), lambs with severe arthrogryposis and dying at birth by asphyxia because of complete muscle paralysis (n=18), and alive lambs showing arthrogryposis of at least one limb (n=6) and that were euthanased shortly after birth. RTq-PCR was positive for 10 SBV-affected lambs. SN revealed positive results in 9/11 SBV-affected lambs at T0. Among clinically healthy siblings of SBV-affected lambs, 6/7 showed SBV-antibodies prior colostrum intake and all were tested positive at T36. Clinically healthy lambs born without SBV-affected siblings (27 lambs tested) did not show SBV antibodies at T0 but were positive at T36, indicating that their ewes had undergone seroconversion. All 99 ewes showed positive results for SN.
This investigation of this sentinel sheep flock revealed that SBV infection had occurred in 100% of the ewes that lambed in January 2012. The incidence of transplacental infection equaled 23% and was higher in primiparous ewes. Transplacental infection led to 17% of lambs showing clinical signs of in utero SBV-infection which could be detected by RTq-PCR in CNS tissue in 37% of the cases. SN assay revealed positive results in 82% of SBV-affected lambs at birth and in 86% of their clinically healthy siblings, whereas gestations leading to exclusively clinically healthy lambs did not suggest the existence of asymptomatical in utero infection by SBV.

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“Schmallenberg” virus from sheep: Molecular characterization of virus present in the brain of a malformed lamb

Hulst, Marcel[1]; Hakze-van der Honing, Renate [2]; Vastenhouw, Stéphanie [2]; Harders, Frank [2]; Hoffmann, Bernd[3]; Beer, Martin[3]; Wellenberg, Gerard [4]; Kortekaas, Jeroen[2]; van der Poel, Wim[2]

Animal Sciences Group of Wageningen UR (ASG) [1]; CVI[2]; FLI[3]; Animal Health Service (GD) [4]

Key words: “Schmallenberg” virus, molecular characterization, brain, malformed lamb

A novel orthobunyavirus, “Schmallenberg virus”, was detected in cattle (BSBV) in the end of the summer in Germany (1), and in late autumn in sheep (OSBV) in the Netherlands. To compare the sequences of the genomic segments (L, M and S) of OSBV present in the brain of a malformed, stillborn lam to that of BSBV originating from blood of cattle, OSBV was sequenced. Using primers derived from the published BSBV sequence (1) overlapping cDNA fragments were amplified directly from total RNA isolated from brain tissue of the affected lamb and analyzed by Sanger sequencing. In addition, 0SBV was isolated and cultured in Vero cells. After grown for 5 passages on these cells virus was concentrated from the culture medium and enriched for negative sense viral RNA segments. A cDNA library prepared from this RNA was sequenced using the MiSeq genome analyzer from Illumina. Full-length genome sequences of the L, M and S segments of native and tissue culture OSBV were assembled and compared to the published sequences of BSBV originating from cattle. The results of these comparisons will be presented.

(1)Bernd Hoffmann, Matthias Scheuch, Dirk Höper, Ralf Jungblut, Mark Holsteg, Horst Schirrmeier, Michael Eschbaumer, Katja V. Goller, Kerstin Wernike, Melina Fischer, Angele Breithaupt, Thomas C. Mettenleiter, and Martin Beer (2012). Novel Orthobunyavirus in Cattle, Europe, 2011. EID Volume 18, Number 3 March 2012.

"Schmallenberg" virus: the EFSA reports on analysis of the epidemiological data and overall assessment of the impact on animal health, animal production and animal welfare together with a characterisation of the pathogen

Afonso A.[1], Willgert K. [1], Richardson J.[1], Cortinas Abrahantes J.[1], Verloo D.[1], Gervelmeyer A. [1], Berthe F.[1]

[1] European Food Safety Authority (EFSA), Parma, Italy

Following a request from the European Commission, the European Food Safety Authority (EFSA) issued a technical report in February 2012 on likely epidemiological scenarios in Europe in relation to the recently detected virus provisionally named "Schmallenberg" virus (SBV) (Simbu serogroup, Bunyaviridae family, genus *Orthobunyavirus*), found in ruminants. The report also included guidance on data to be collected in Member States, with harmonised case definitions and reporting guidelines for a minimum dataset at herd/flock level and an extended dataset at animal level. Two reports on the analysis of epidemiological data collected by affected countries based on that guidance had been issued by the date of abstract submission. The results of further epidemiological data analyses and an overall impact assessment will be presented at the symposium.

As of 16 April 2012, eight Member States (Belgium, France, Germany, Italy, Luxembourg, the Netherlands, Spain and United Kingdom) had confirmed cases of SBV. All affected Member States had reported the number of confirmed herds following viral detection by PCR, virus neutralisation test or serological confirmation and France, Italy, Luxembourg, the Netherlands, Spain and United Kingdom, had also reported the number of suspect herds. Denmark, Ireland, Norway and Switzerland reported suspect herds, none of which were confirmed by laboratory testing. The total number of SBV confirmed herds in Europe as of the 16 April 2012 was 3444. No confirmed acute cases had been reported in adult animals in 2012 to that date.

The data showed a decrease in the number of reports of SBV confirmed herds following a peak in week 9 (February, 27 – March, 4) of 2012. The decrease after week nine was clearly observed in sheep and was most probably linked to the end of the lambing season in affected countries. However, in cattle no clear decrease of suspected or confirmed herds was observed.

The data available up to 16 April 2012 only allowed an impact assessment based upon the comparison between the number of SBV confirmed herds and the total number of herds in each affected country by species. For all affected countries, the number of herds with at least one SBV confirmed animal was low in comparison with the total number of herds. This analysis should be interpreted cautiously since under-reporting or lack of diagnostic confirmation may have affected the ratio.

The data provided up to 16 April 2012 allowed an understanding of the temporal and geographical distribution of the SBV outbreak. As more data becomes available the impact assessment for SBV (in particular within herd and the local impact) could be subject to change.

Evaluation of the zoonotic potential of Schmallenberg virus.

Reusken, Chantal[1]; van den Wijngaard, Kees[1]; Godeke, Gert-Jan[1]; De Vries, Ankje[1]; van den Kerkhof, Hans[1]; Isken, Leslie[1]; van Pelt, Wilfrid[1]; Koopmans, Marion[1] RIVM[1] Netherlands

Key words: sbv vnt zoonoses

The recent emergence of Schmallenbergvirus (SBV) as a cause of malformations in ruminants has triggered questions of possible risks to human health. In a rapid risk assessment, we concluded that the potential for human infection is low but can not be excluded. Therefore, as a precautionary measure, the Center for Infectious Disease Control in the Netherlands monitors since December 2011 the occurrence of cases of febrile illness within 2 weeks of direct exposure during delivery of ruminants from affected farms. These events triggered a laboratory response to address possible human infections with SBV. The laboratory response included the implementation and validation of a SBV reverse-transcription polymerase chain reaction and virus neutralization test (VNT).
To address the zoonotic potential of SBV, an integrated serological study to assess possible evidence for infections of humans was conducted using the recently developed VNT. These serological studies target populations expected to be at a high risk for exposure, either via the vectorial route or through direct contact by living/working at or in proximity of affected farms. The following serum collections were analysed::
1) sera collected in the period 15 July- 15 October 2011 in municipalities with known affected SBV farms were compared with sera collected in the same period and municipalities in 2010.
2) paired serum samples collected from veterinary students collected in 2006/2008 and 2011;
3) sera collected in March/April 2012 from farmers, workers and residents of affected SBV ruminant farms were compared with sera collected from farmers, workers and residents of ruminant farms in 2009;
4) sera collected from veterinarians who assisted at SBV affected farms since summer 2011 were compared with sera collected from veterinarians assisting at ruminant farms in 2009.

The sampling and testing of farmers, workers (including veterinarians) and residents of SBV affected farms in March/ April 2012 (studies 3 and 4) was part of a larger epidemiological study including questionnaires addressing putative risk factors for SBV exposure/infection. The laboratory results and, if zoonotic transmission occurs, preliminary data on human risk factors will be presented.

Seroprevalence of antibodies to Schmallenberg virus in dairy cattle, winter 2011-2012, The Netherlands

Elbers, Armin[1]; Loeffen, Willie[1]; Quak, Sjaak [1]; de Boer-Luijtze, Els[1]; van der Spek, Arco[2]; Bouwstra, Ruth[1]; Maas, Riks[1]; Spierenburg, Marcel[2]; de Kluijver, Eric[1]; van Schaik, Gerdien[3]; van der Poel, Wim[1]

CVI[1]; NVWA[2]; GD Deventer[3]

Key words: Orthobunyavirus, Schmallenberg virus, seroprevalence, cattle

Introduction
Since the autumn of 2011, infections with Schmallenberg virus (SBV) have been associated with congenital malformations in calves, lambs and goat-kids in at least five European countries. Currently there is limited knowledge specifically related to SBV. In the Netherlands, there is an obligatory reporting of suspect cases (occurrence of malformations of the arthrogryposis hydranencephaly syndrome) followed by confirmatory testing of brain tissue samples by RT-PCR. It is likely that observed suspect cases underestimate the true rate of infection. Therefore there is a definite need for serodiagnostic studies to detect past exposure to SBV in ruminant populations in the affected countries.

Material and Methods
A seroprevalence study was executed to detect past exposure to SBV in dairy cattle in the Netherlands. Furthermore, in order to get some preliminary insight into the within-herd seroprevalence of infected herds (based on PCR test results), in two sheep flocks and two cattle herds a considerable number of animals were blood-sampled.
A stratified random sampling design was set up, with the 12 provinces in the Netherlands as a stratification level. The sampling frame comprised of dairy cattle that were blood sampled in the period November 2011 – January 2012 a) for monitoring testing of antibodies to Bluetongue virus, and b) in the framework of a surveillance investigation in 125 dairy farms to exclude introduction of Brucella and Foot and Mouth Disease. Because we presumed a high intra-class correlation with respect to serological status of animals within herds (based on preliminary test results from a few infected herds), on average two dairy cattle (minimum: 1, maximum: 4) from the same dairy herd were allowed in the sampling list to prevent occurrence of too many cattle from the same herd. This selection procedure resulted in a total of 1,123 samples of dairy cattle from 489 dairy herds to be tested. The mean age of cows tested was 23 months (range: 12 – 79 months). Sera from 1,123 randomly selected dairy cattle were tested for antibodies to SBV using a virus neutralisation test.

Results
SBV-seroprevalence in dairy cattle was 73% (N=1123; 95% confidence interval of seroprevalence (CI): 70 – 75%). SBV-seroprevalence of dairy cattle in the central-eastern part of the Netherlands (N=462; seroprevalence: 83%, 95% CI: 79 – 86%), was significantly higher compared to that in the northern (N=465; seroprevalence: 67%, 95% CI: 63 – 71%) and southern part of the Netherlands (N=196; seroprevalence: 61%, 95% CI: 54 – 68%).
High (70-100%) within-herd seroprevalence was observed in two SBV-infected sheep and dairy herds in which a considerable number of animals was tested.

Implications
We showed a high seroprevalence of antibodies to SBV present in dairy cattle in the Netherlands in the winter of 2011-2012. This indicates widespread exposure to SBV in 2011, and exemplifies the considerable underestimation of the infection rate when one can only rely on observation of clinical suspect cases. Furthermore the SBV-seroprevalence in dairy cattle was significantly higher in the central-eastern part of the Netherlands compared to the northern and southern part of the Netherlands. This could be an indication that introduction of SBV into the Netherlands started somewhere in the eastern part of the Netherlands. Our preliminary results concerning SBV within-herd seroprevalence indicate that by the end of an outbreak season, most of the animals within an affected herd might be infected.

Detection en epidemiological findings of Schmallenberg virus in the Netherlands

van Wuijckhuise, Linda[1]; Veldhuis, Anouk[1]; Carp-van Dijken, Sanne[1]; van Schaik, Gerdien GD[1]

Key words: Schmallenberg virus, impact, risk factors, dairy cattle

The aim of the study is to describe the detection of the Schmallenberg virus (SBV) epidemic in the Netherlands and the epidemiological findings in infected dairy herds. In the Netherlands, a surveillance system is in place for detection of (emerging) infectious diseases at the Animal Health Service. This system consists, amongst others, of a telephone service that provides advice to veterinarians and farmers about animal health related problems that they encounter. The signals that are obtained from the field are discussed weekly in a team of veterinarians, epidemiologists and pathologists. At the end of August 2011, several reports were made – first in the eastern part of the country - of a severe drop in milk production, watery diarrhoea and sometimes fever in dairy cattle. After two weeks, reports were also received from other regions, however reporting ceased in October. Blood and manure samples of clinical cases were then obtained and tested, but no definite causal agent was found. On November 18th, the Friedrich Loeffler Institute (FLI) in Germany reported the isolation of SBV. In the first week of December, an increased number of congenital malformations in new-born lambs throughout the country were reported. Ovine congenital malformations are not uncommon, but usually only a few cases occur per farm. In this case many flocks and many lambs were affected throughout the country. A real-time PCR for SBV was carried out on brain tissue of 54 deformed lambs, on 50 serum samples of dairy cows which had shown clinical signs in August, and on 115 serum samples of healthy cows sampled in November. In 22 lambs and 18 cows the virus was detected. The healthy cows all tested negative. The first clinical cases in calves were reported in December and on January 23rd the first two calves were tested SBV positive. Up to mid-March, about 700 calves, 300 sheep and 30 goats are submitted for necropsy with clinical signs of SBV.
To identify clinical symptoms following SBV infection as well as potential risk factors for introduction of SBV in dairy cattle herds, a case-control study was set up. Data will be collected in 150 dairy herds; 75 confirmed cases of SBV and 75 herds without any signs of SBV. Seventy cows will be sampled per herd and tested in an indirect ELISA. Risk factor and morbidity data will be collected by means of a questionnaire. All stillborn calves and calves that die within 3 days after birth will be necropsied. Calves of 32 dams in infected herds (16 PCR positive / 16 PCR negative) will be tested at birth (pre-colostrum) and in subsequent months with an ELISA and PCR. Census data from sampled herds regarding production, fertility and animal health will be analysed for 2008-2012 using national databases.
Preliminary findings of te impact of SBV on morbidity and mortality rates in adult cattle and calves after primary infection with SBV as well as in calves that have been exposed to SBV during foetal development will be presented. The relation between seroprevalence and the level of clinical symptoms at herd and animal level will be discussed, as well as possible herd- and cow-level risk factors for introduction and within-herd transmission in dairy herds. The study will provide information on the many aspects and impact of a primary SBV infection in dairy herds. Also, the sensitivity of the Dutch surveillance system for SBV and emerging diseases in general is discussed.

Development of a pan-Simbu real-time RT-PCR for the reliable detection of Simbu serogroup viruses

Fischer, Melina[1]; Hoffmann, Bernd[1]; Schirrmeier, Horst[1]; Wernike, Kerstin[1]; Wegelt, Anne[1]; Goller, Katja[1]; Höper, Dirk[1]; Beer, Martin[1]

FLI[1]

Key words: Orthobunyavirus, Simbu serogroup, Schmallenberg virus, real-time RT-PCR

Schmallenberg virus (SBV), a novel Orthobunyavirus from the Simbu serogroup, was first identified in German diary cattle in October 2011 where it caused fever, diarrhea and a decreased milk production. SBV was also detected in sheep and goats where it causes congenital malformations and stillbirths. Concerning this current occasion, we developed a pan-Simbu real-time RT-PCR system for the reliable detection of viruses from the Simbu serogroup and compared it with different diagnostic SBV real time RT-PCR assays. All PCR systems were tested with a panel of different Simbu serogroup viruses as well as several field samples from diseased cattle, sheep and goats originating from all over Germany. The pan-Simbu real-time RT-PCR system was able to detect all tested members of the Simbu serogroup as well as most of the field samples. Furthermore, in silico analyses indicate the capability for the detection of a broad Orthobunyavirus spectrum. For the diagnosis of SBV the SBV-S3 assay turned out to be most suitable with an analytical sensitivity for the SBV-S3 single assay determined as one copy per well and for two duplex assays including an internal amplification control (IC2-RNA, beta-actin) defined as ten copies per well for both duplex systems.

Schmallenberg virus outbreak in The Netherlands: Routine diagnostics and test results

Bouwstra, Ruth[1]; van der Poel, Wim[1]; de Kluijver, Eric[1]; Verstraten, Betty[1]; Bongers, Johan[1]

CVI[1]

Key words: Schmallenberg virus, diagnostics, results

At the end of 2011, a new Orthobunya virus named Schmallenberg virus (SBV), was discovered in Germany. Soon thereafter in the Netherlands, the virus was associated with decreased milk production, watery diarrhoea and fever in dairy cows, and subsequently also with congenital malformations in calves, lambs and goat kids. By the 20th of December 2011 in the Netherlands malformations in new-borns of ruminants were made notifiable. After a notification by a farmer or veterinarian, a maximum of five malformed new-borns per farm were necropsied. The diagnosis of Schmallenberg virus disease was based on the pathologic findings and RT-PCR test results of brain tissue of the malformed new-borns. In addition blood samples from mothers of affected new-borns were collected and tested for antibodies against SBV using a virus neutralization test. Between 20th of December and March the 8th, in total 1165 brain tissue samples were tested in the RT-PCR: 577 originated from lambs, 444 from calves and 42 from goat kids. In the VNT 681 blood samples were tested: 329 originated from ewes, 329 from cows and 23 from goats. Results showed that 8% of the tested calf brains, 31% of the tested lamb brains and 12% of the tested goat kid brains were RT-PCR positive. The number of malformed lambs and RT-PCR positive lamb brains decreases over time while the number of malformed calves and RT-PCR positive calf brains increases. In the VNT 95% of the ewes, 93% of the cows and 23% of the goats tested positive. Combining the results of the RT-PCR and the VNT, 20% of all farms tested positive in both the RT-PCR (genetic material of SBV in brain tissue in malformed new-borns) and the VNT (antibodies against SBV in blood from mothers of affected new-borns). In goats the number of seropositives is far lower than in sheep and cattle. Less samples from goats were tested and the estimated seroprevalence is therefore less precise. Furthermore number of RT-PCR positive calves and goat kids is far lower than in sheep. Given that goats, in contrast to cattle and sheep, are often housed indoors, and the SBV is supposedly transmitted by Culicoides vectors, this lower number of test positive results would not be surprising. In addition, the difference in pregnancy length between cows and sheep might explain why it is more difficult to detect genetic material of SBV in brain tissue of calves and, assuming that infection of cows and sheep was around the same period, it might also explain the difference in number of malformations and RT-positives over time. Supposing that all malformations notificated are truly caused by the Schmallenberg virus, on farm level, diagnostic sensitivity of the RT-PCR is much lower in comparison with the VNT. The results reported here are based on testing up to the first week of March 2012. Additional test results which will be available in June will be presented also.

POSTER S1: PRELIMINARY VALIDATION OF THE ID SCREEN® SCHMALLENBERG VIRUS INDIRECT ELISA

Comtet, L[1]; Pourquier, P.[1]; Zientara, S.[2]; Breard, E. [2]; Sailleau , C. [2]; Viarouge, C. [2]; Cay, A.-B.[3]; De Regge, N. [3]

IDvet[1]; Anses[2]; VAR-CODA[3]

Key words: Schmallenberg virus, serology, ELISA

Introduction:
Schmallenberg virus (SBV) is the name given to a vector-transmitted orthobunyavirus related to the Shamonda and Akabane viruses, initially reported in November 2011. The disease has caused foetal congenital malformations and stillbirths in cattle, sheep, and goats. The virus has been detected in Germany, the Netherlands, Belgium, France, Luxembourg, Italy and the United Kingdom.

Serological testing is essential for disease surveillance and epidemiological studies. While antibodies can be detected by virus neutralization and immunofluoresence, these techniques are time-consuming, difficult to implement for large numbers of samples, and do not offer standardized result interpretation.

Available as of March 2012, the ID Screen® Schmallenberg virus Indirect ELISA is the first ELISA developed for SBV diagnosis. The test allows for the detection of SBV antibodies in ruminant serum and plasma. It is a rapid, standardized assay which is automatable and therefore suited to high throughput testing.

This study presents validation data for this test. Data will be added for any second generation tests developed by IDvet between March and June 2012.

Method:
- The ID Screen® Schmallenberg virus Indirect ELISA was performed as per manufacturer's specifications.
- Specificity was studied using panels of sera collected prior to 2010.
- Sensitivity was evaluated on sera tested positive with the virus neutralization test (VNT).
- An experimental infection was performed in April 2012 in order to evaluate the seroconversion response in cattle, sheep and goats.

Results and Discussion:
Preliminary validation studies indicate excellent test specificity and high correlation with other serological techniques, making the the ID Screen® Schmallenberg virus Indirect ELISA an efficient tool for disease surveillance and epidemiological studies.

POSTER S2: DEVELOPMENT OF A REAL TIME RT-PCR DIAGNOSTIC TEST, ADIAVET™ SCHMALLENBERG VIRUS, FOR DETECTION OF THE NEW ORTHOBUNYAVIRUS

Gracieux, Patrice[1]; Versmisse, Yann[1]; Leborgne, Maelle[1]; Blanchard, Beatrice[1]

Adiagene[1]

Key words: Schmallenberg virus, diagnosis, real-time RT-PCR

The Schmallenberg virus (SBV) was isolated for the first time in Germany in 2011 by the FLI from blood of infected cows. The name is based on the geographic origin of the virus (village of the North Rhine-Westphalia). First phylogenic analyses suggest that the novel virus is a Shamonda-like virus within the genus Orthobunyavirus and Simbu serogroup.
Clinical signs of SBV infection in adult ruminants are mainly mild or non-existent. The main clinical signs of SBV are congenital malformations in newborn animals.
The viruses of Simbu serogroup are transmitted by insects (Culicoides midges and mosquitoes). It is likely that SBV is also transmitted by these insects but this has not been confirmed yet.
Viral culture and PCR amplification are the only methods to detect the virus.
Adiagene developed a real time RT-PCR test according to the guidelines of the AFNOR XP U47-600-2 standard for the development and validation of a veterinary PCR kit to detect specifically the Schmallenberg virus.

The real-time ADIAVET™ SCHMALLENBERG VIRUS ready-to-use RT-PCR kit provides a screening assay for the detection of Schmallenberg virus in blood (EDTA whole blood, serum or plasma) and tissue samples of cattle and smaller ruminants. Simultaneous detection of the Schmallenberg virus and an endogenous ruminant gene allows the validation of all the steps (extraction and amplification) of the analysis process for all the samples.
The specificity of the kit was evaluated against 97 organisms preferentially found in the same ecologic niche than SBV and/or close phylogenetically related, No cross-reaction was observed with others organisms tested including Akabane virus, an amplification can be observed with Shamonda virus. This data is in agreement with the phylogenic analysis leading to consider SBV as a Shamonda-like virus.
The specificity of the kit was also evaluated on a panel of 21 qualified SBV RNA solutions provided by European laboratories. Synthetic SBV RNA was produced and quantified to assess the detection limit of the PCR generating a positive result in 95% of cases.

RNA extraction protocols were developed and validated to detect the virus in tissue (brain, spleen), blood and sera sampling from bovine, ovine or caprine. The analysis of the tissue samples needs a grinding step before the RNA purification. Two grinding processes have been validated (Mixer mill (Verder) and Ribolyser (MP Biomedical)). Easy RNA purification protocols have been developed based on silica column or magnetic bead technologies.
The detection limit of the method (RNA extraction and PCR amplification) generating 100% positive results was assessed by spiking SBV-free samples with a titrated viral culture. Each level of spiking was analysed in eight times in two experiments.
The diagnostic sensitivity was evaluated from 32 field samples with known SBV status according to the reference RT-PCR test given by the ANSES Maisons-Alfort (results obtained with FLI methods). The 20/32 SBV positive samples were found positive with ADIAVET™ SCHMALLENBERG VIRUS kit and 12/32 qualified as negative were found negative.

The ADIAVET™ SCHMALLENBERG VIRUS kit allows the specific detection of all Schmallenberg virus tested. The kit showed good performance to detect SBV from field samples. The French Agency for Food, Environment and Occupational Health Safety recommended to veterinary laboratories the use of ADIAVET™ SCHMALLENBERG VIRUS kit.

POSTER S3: DEVELOPMENT OF A SCHMALLENBERG VIRUS ANTIBODY ELISA

Schelp, Christian[1]; Senechal, Yann[1]; Pun, San[1]; Schumacher, Daniel[1]; Gradinaru, Dragos[2]; Egli, Christoph[1]; Troch, Jean-Luc [1]; Leterme, Serge[3]

IDEXX Switzerland AG[1]; IDEXX Montpellier SA[2]; IDEXX Laboratories Westbrook[3]

Key words: Schmallenberg virus, antibody ELISA, Orthobunyavirus

Introduction
Schmallenberg virus was first identified in Germany in late 2011 as a new Orthobunyavirus genetically highly similar to viruses of the Simbu serogroup. The virus infects ruminants such as cattle, sheep and goats. Schmallenberg virus infections have been recently confirmed in several European countries including Germany1, The Netherlands, Belgium, France, United Kingdom, Luxembourg, Italy and Spain. Clinical signs include reduced milk yields, fever, diarrhea, abortions and malformed newborns. Virus detection by real time RT-PCR or virus cultivation is not the method of choice for infection monitoring due to the very short viremic period. Therefore, there is an urgent need for an antibody ELISA to identify infected animals.

Materials & methods
Hundreds of samples originating from clinically affected herds, from virus positive animals and from areas with no Orthobunyavirus presence are being collected. Samples will be further characterized by using an inhouse immunofluorescence assay, PCR and epidemiological and clinical data.
An antibody ELISA will be described. Microtiter plates will be coated with inactivated Schmallenberg-virus antigen. Binding of Schmallenbergvirus antibodies will be visualized by colour change in the wells of the microtiter plate. The diagnostic relevance of the result will be assessed by comparing the optical density (OD) of the samples with the OD of the positive control.

Results
Preliminary data will be presented. Specificity and sensitivity data will be calculated using characterized samples. The data will allow for setting a cut-off to identify Schmallenbergvirus antibody positive and negative samples.

Discussion & conclusions
The data will be discussed and conclusions will be proposed according to obtained antibody test performance.

Acknowledgements
We kindly acknowledge the collaboration with the FLI (Friedrich-Loeffler-Institut), Insel Riems, Germany.
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POSTER S4: First report of Schmallenberg virus in Italy

Ceglie, Letizia[1]; Monaco, Federica[2]; Bonci, Michela[1]; Calistri, Paolo[2]; Bano, Luca[1]; Goffredo, Maria[2]; Belfanti, Ilaria[1]; Pinoni, Chiara[2]; Polci, Andrea[2]; Nardelli, Stefano[1]; Bonfanti, Lebana[1]; Marangon, Stefano[1]; Lelli, Rossella[2]

IZS-Ve[1]; Istituto G. Caporale[2]

Key words: Schmallenberg virus, Italy, goat

In the last months of 2011 and at the beginning of 2012, Schmallenberg virus (SBV) has been reported in ruminants (cattle, sheep, goats and bison) in Germany, the Netherlands, Belgium, United Kingdom, France, Italy and Spain. Preliminary studies on its genome suggest that the virus is a member of the Simbu serogroup belonging to the Bunyaviridae family, genus Orthobunyavirus. Animals infected with SBV show mild clinical signs persisting for approximately a week and characterized by fever, loss of appetite, up to 50% reduction in milk yield and, sometimes, severe diarrhoea. SBV infection is also associated to foetal malformation and stillbirths in domestic ruminants. At the beginning of February, a female goat that died the day after parturition of a healthy kid was submitted for post-mortem examination. The post-mortem revealed the retention of a dystocic foetus showing congenital malformations, namely scoliosis, arthrogryposis and ankylosis of some of the limb joints. Samples of brain tissue and spleen were collected for virus detection. The goat belonged to a small flock with 1 calf and 6 goats located in Northern Italy, in the area of Treviso.
After RNA extraction, samples were submitted to two different one step real-time RT-PCR protocols, developed by the Friedrich Loeffler Institut (FLI), targeting the L1 and S3 genomic fragments, respectively. Brain tested positive to both protocols, whereas spleen tested negative. Spleen and brain samples were sent to the National Reference Centre for Exotic Diseases (ICT- CESME, Teramo) which confirmed the presence of SBV in the brain by qRT-PCR and partial sequencing of the viral genome.
In collaboration with the CESME an epidemiological investigation in the farm was carried out. Epidemiological information on the flock (animal species, number, age and movements) were provided by the farmer. Whole blood and serum samples were collected from all the animals for virological and serological investigation, respectively. All whole blood samples tested negative for SBV, whereas serological positive results were obtained by virus-neutralization (VN) and immunofluorescence (IF) assays performed in Teramo from four goats and the calf.
To date this is the first detection of the new Orthobunyavirus, Schmallenberg virus in Italy. The epidemiological investigations excluded the introduction of SBV in the farm from other EU infected countries. A local virus circulation, therefore, has been confirmed by CESME in the area and 6 Culicoides collections made from September 6th to November 25th2011 in 3 farms around the SBV outbreak were positive by real time RT-PCR for SBV. A re-enforced passive clinical surveillance system has been implemented by Italian Veterinary Authority, focusing on clinical signs in adult animals and abortions, stillbirths and malformed ruminants. Several suspected cases have been submitted to CESME laboratory for confirmation. A clear definition for suspected and confirmed SBV case has been developed in agreement with those suggested by the European Food Safety Authority (EFSA).

POSTER S5: Emergence of Schmallenberg Virus : Development and validation of a PCR detection kit

MAGNEE, Damien[1]; DALY, Stéphane[1]; MOINE, Sandrine[1]; SELLAL, Eric[1]

LSI[1]

Key words: Schmallenberg, RealTime PCR, Outbreak

Between August and December 2011, outbreaks of disease in adult cattle, abortions and births of malformed animals in sheep, cattle and goats were reported in the Netherlands, Germany and Belgium. A new virus was identified as a cause of these problems and was named “Schmallenberg virus” after the place where it was first identified. This virus belongs to the Bunyaviridae family, genus orthobunyaviridae and is closely related to Akabane, Aino and Shamonda viruses. We present here the development of new real-time PCR kits for identification of Schmallenberg virus, by targeting the S segment, and the different validation steps leading to final authorisation for SBV diagnosis by the French National Reference Laboratory (ANSES Maisons-Alfort) and by the Friedrich-Loeffler-Insitut (FLI) in Germany.

Systems for specific detection of Schmallenberg Virus were designed on the basis of the sequence deposited on Genbank. A first system was designed on the L segment. Then, according to the FLI recommendations, we realised a new design, based on the detection of the S segment. The LSI SBVS kit allows the simultaneous detection of SBV target and an endogenous IPC.
Detectability of the both kits was compared with the FLI design using serial dilution of SBV RNAs provided by the Friedrich-Loeffler Institut (Germany). Analytical specificity and sensitivity of L and S segment kit were assessed using several field samples (brains), coming from France and Belgium. Specificity of prototype kits was evaluated on a panel of ruminant pathogens. Both prototype kits were sent to the FLI and the S segment prototype kit was sent to the French NRL for evaluation of their specificity, sensitivity, detectability and repeatability on field samples, in order to receive official validation for diagnosis in France and Europe.

The both systems showed good specificity, with detection of all positive samples and no detection of other ruminant pathogens. These kits have equivalent detectability with the FLI designs, with better sensitivity for the S segment relative to the L segment (for FLI and LSI design).
On 34 field samples (brains coming from stillbirths with malformations), we have better detectability with LSI systems, with 29 positives samples with LSI S segment PCR and 25 positives with FLI PCR.
Characteristics of the both kits obtained at LSI were confirmed at the French NRL and the FLI. Kits showed good sensitivity, specificity and detectability.
The SBV-S segment kit commercialised by LSI received official authorisation for utilisation in the French network for diagnosis of Schmallenberg virus and was also validated by the FLI, for use in European market.

In conclusion, the initial validation of the TaqVet™ SBVS kit at LSI shows good results in sensitivity, specificity and detectability. A panel of 34 field samples show good results, for SBV target and for IPC. All characteristics obtained at LSI were confirmed at the French NRL and at the FLI, giving to this kit an official authorisation for use in SBV diagnosis in France and Europe.

Hoffmann, B. et. al., (2012) Novel Orthobunyavirus in cattle, Europe, 2011 – Center for Disease Control and Prevention.Vol 18, N°3, p1-6.

POSTER S6: Detection of Schmallenberg virus in pools of Obsoletus Complex stored during the Bluetongue Italian surveillance program

Goffredo, Maria[1]; Monaco, Federica[1]; Capelli, Gioia[2]; Polci, Andrea[1]; Quaglia, Michela[1]; Pinoni, Chiara[1]; Montarsi, Fabrizio[2]; Lelli, Rossella[1]; Savini, Giovanni[1]

Instituto "G. Caporale"[1]; IZS-Ve[2]

Key words: Schmallenberg virus, Culicoides, Obsoletus Complex

It is now more than ten years that the Bluetongue (BT) entomological surveillance program is operative in Italy. This activity allowed to store in alcohol 70% Culicoides collections trapped on a weekly basis in farms from all over the country.
Last February Schmallenberg virus (SBV) was detected in a goat foetus and SBV antibodies were found in a cow with stillbirth problems. Both animals were from farms located in the Veneto region.

A SBV retrospective survey was carried out on 87 Culicoides collections caught from six BTV surveillance permanent traps located in the surrounding of the two SBV cases between June and November 2011. The selected sites included Veneto and Friuli Venezia Giulia regions. In particular two were in Treviso province (Istrana and Volpago del Montello), one in Belluno province (Feltre) and three in Pordenone province (Caneva, Montereale Valcellina and Sequals).
Old parous and, when present, engorged females were separately sorted out in pools according to species and tested by qRT-PCR for the presence of SBV.
Of a total of 175 pools examined, 6 were positive to SBV, precisely four collected on September 6th, October 21st, November 3rd and 25th, respectively, from Feltre (Belluno, Veneto), one collected on the 4th of October from Caneva (Pordenone, Friuli), and one collected on November 7th from Istrana (Treviso, Veneto).

All positive pools consisted of species of the Obsoletus Complex. Five of them were composed by parous females (ranging from 5 to 47) and one by a single engorged midge collected from Feltre on September 6th. Culicoides obsoletus sensu strictu was the most abundant species found when nulliparous females of the Obsoletus Complex collected in the selected sites were identified by multiplex PCR.

This study highlights and confirms the benefit of having a good entomological surveillance program in place. It allowed to both monitor the BTV circulation and, retrospectively, get critical information on SBV infection in Italy.
As a result of this retrospective study it was possible (i) to demonstrate that SBV has circulated in at least 3 Italian provinces since early September, (ii) to confirm that species of the Obsoletus Complex play a role in transmitting SBV and (iii) to evidence that C. obsoletus sensu strictu is likely to be the principal vector in the Italian SBV outbreaks.
Studies to determine the exact role of each species of the Obsoletus Complex in transmitting SBV are in progress.

POSTER S7: Preliminary insight into Schmallenberg virus infection impact in sheep flocks

Dominguez, Morgane[1]; Calavas, Didier[1]; Jay, Maryne[2]; Languille, Jerome[3]; Fediaevsky, Alexandre[3]; Zientara, Stephan[1]; Hendrikx, Pascal[1]; Touratier, Anne[2]

Anses[1]; Groupement de défense sanitaire France[2]; Direction générale de l'Alimentation[3]

Key words: Schmallenberg, impact, sheep

Background
Schmallenberg (SBV) virus outbreak emergence is closely monitored in France. Farmers are urged to contact their veterinarian when encountering cases of ruminant neonates or fetuses stillborn, malformed or showing nervous disorders. A brain sample is collected on any suspected newborn cases until a first confirmation of the infection is obtained for the herd. SBV diagnostic is performed in state diagnostic laboratories, using real-time quantitative reverse transcription PCR (RT-qPCR). The first outbreak of SBV infection was confirmed in France, on the 25th January 2012. As of the 30th March 2012, 1,048 SBV positive premises have been identified, 958 sheep, 76 cattle and 14 goat herds.

Method
In the framework of the French National Surveillance Platform for Animal Health, a survey has been implemented in the cattle, sheep and goat SBV positive herds to get an estimate of the apparent attack rate as well as an estimation of the frequency of congenital deformities in newborns. A preliminary investigation is carried out as soon as possible after the first confirmation of the SBV infection. A second investigation will be performed in the same premises at the end of the breeding season to achieve a global impact assessment. Farmers are interviewed using a standardised questionnaire exploring general farm information and information on the females that have given birth and on the newborns. Data is entered into a web-based interface developed for the survey. We present the first results of the preliminary investigation survey in sheep flocks.

Results
As of the 29th March 2012, a preliminary investigation has been carried out in 384 SBV positive sheep flocks, in 38 districts. At the time of the preliminary investigation, a total of 42,219 ewes had already given birth to 68,237 lambs (accounting, on average, for 78% of the ewes in the flocks). Full data characterizing the ewes that had given birth was collected in 362 flocks (accounting for 40 635 ewes). On average in the SBV positive flocks, 34,470 (85%) ewes gave birth normally. Among the 6,165 (15%) ewes that had lambing problems, 4,465 (72%) had of full time birth but at least one of their lambs was deformed, born dead or died within 12 hours after birth and 1,700 (28%) had an abortion. Among the ewes that had lambing problems, 724 (12%) died within 15 days following the delivery.
Full data describing the lambs that were born at the time of the preliminary investigation was collected in 363 flocks accounting for 64 611 lambs. On average in the SBV positive flocks, 54,904 (85%) lambs were healthy; 8,457 (13 %) were born dead or died within 12 hours after birth, 1,250 (2%) showed deformities but were still alive 12 hours after birth (however the viability of these animals was expected to be low in 74% of the flocks).
A total of 6,513 (10%) lambs were deformed. Stiff joints were the most common deformity (observed in 96% of the flocks). Nervous disorders were more uncommon, absent in 63 % of the flocks.

Discussion
Preliminary results of this impact survey provide a first gross estimate of SBV virus infection impact in sheep flocks. The imputability of SBV virus infection in the occurrence of the lambing problems reported in ewes or in the death or deformities reported in lambs is not assessed.
This survey is continuing in sheep, cattle and goat SBV positive herds; the results of the preliminary investigation will be completed by a final assessment at the end of the breeding season.

POSTER S8: Preliminary results of Schmallenberg virus detection in ruminants in Turkey; preparing for the future

Yilmaz, Huseyin[1]; Cizmecigil, Utku[1]; Satir, Esra[2]; Turan, Nuri[1]; Gurel, Aydin[1]; Hoffmann, Bernd[3] van der Poel, W.H.

Veterinary Faculty of Istanbul University[1]; Pendik Veterinary Control Institute[2]; FLI[3]

Key words: Schmallenberg, Virus, Turkey, Cattle, Sheep

The aim of this study was to investigate the presence of Schmallenberg virus in sheep, goats and cattle in Turkey in the same period that the virus has been circulating in Europe. For this, archival samples from sheep, goats and cattle were analysed for the presence of Schmallenberg virus RNA by real-time RT-PCR using spesific probe. Stored cDNA samples and RNA extracted from archival 96 aborted fetuses of sheep (46), goats (11) and cows (38) and samples from the blood of 40 lambs and 20 sheep collected in 2011 from Marmara (EU border), Aegean and Blacksea regions of Turkey were analysed. RNA was also extracted from stored samples. No Schmallenberg virus RNA was detected in any of the samples of sheep and cattle. The investigation is still going on and sequencing will be performed if this virus exists in Turkey.

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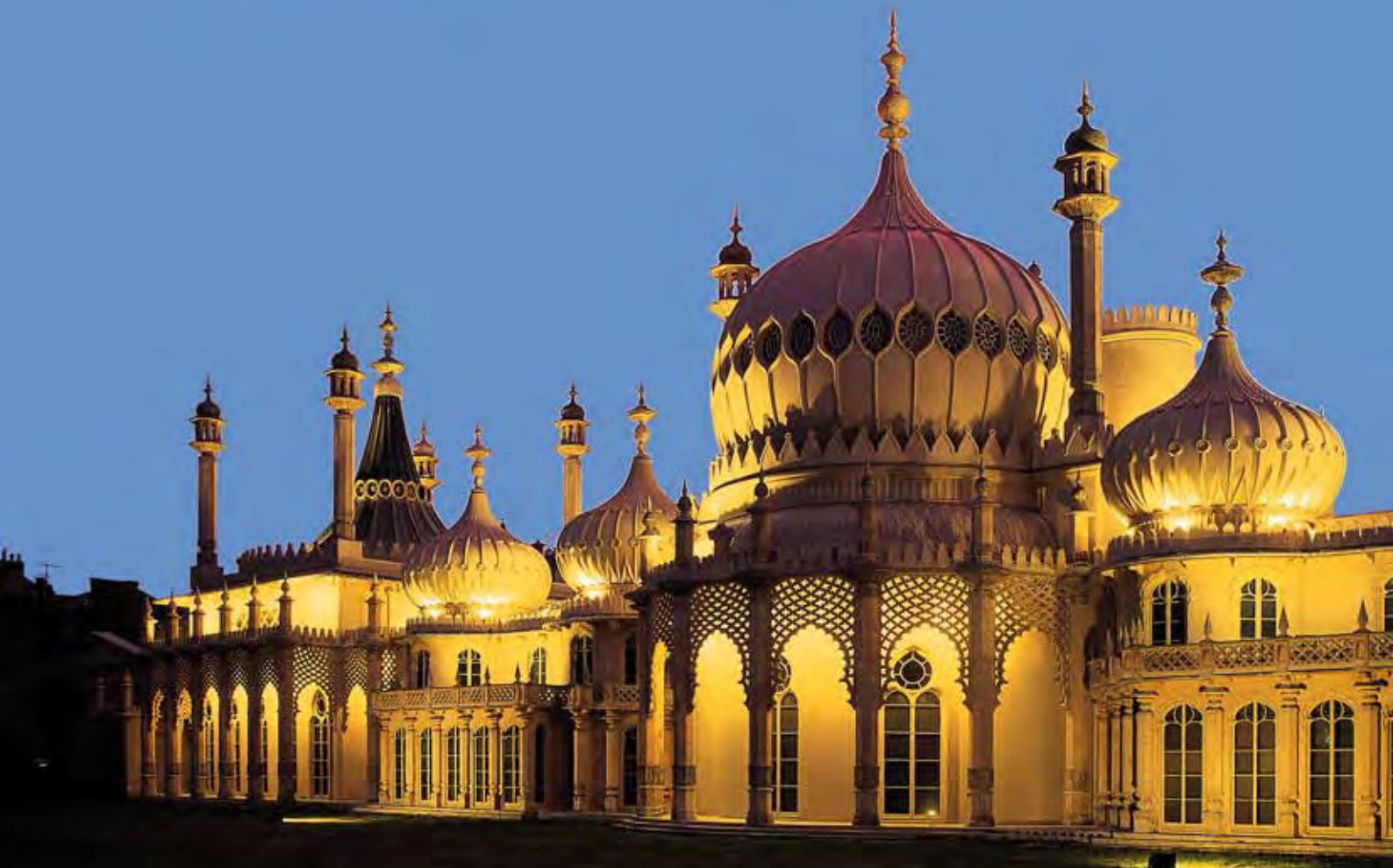
Contact 6th AM and Satellite:

Phone: +44 (0) 1483 232 441
E-mail: epizone.help@iah.ac.uk
www.epizone-eu.net

Contact EPIZONE:

Phone: +31 (0) 320 238 883
E-mail: epizone.cvi@wur.nl
www.epizone-eu.net



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