

EPIZONE

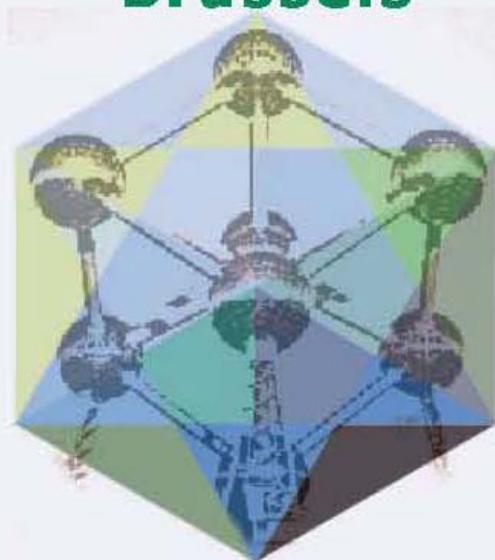
ABSTRACTS

7th Annual Meeting

'Nothing permanent, except change'

1 - 4 October 2013

Brussels



Hosted by



CODA - CERVA

www.epizone-eu.net

The EPIZONE 7th Annual Meeting is hosted by



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7th EPIZONE Annual Meeting "Nothing permanent except change"

The theme of the 7th Annual Meeting endorses the quotation by Heraclitus in antiquity: 'there is nothing permanent except change'. This is particularly true for epizootic viral diseases and not only emphasized by the increased international movement in animals, animal products and people, but also by the possible deliberate introduction of pathogens. In addition, the global climate change has already given concern regarding changes in distribution or severity of many animal diseases and it is clear now that the evolving climatic parameters will further transform the ecology of numerous vectors and pathogens. Moreover, the non-vaccination and eradication policy of the EU is maintained in most circumstances to allow the unrestricted trade of animals and their products worldwide. All these factors make surveillance as the basis for recognition of disease status and subsequent impact on trade. In this context, we must "expect the unexpected" and early detection of potentially epizootic diseases is of the outmost importance.

In addition to the traditional topics, **two specific topics** have been selected:

- Biological control and spatial ecology of epizootics viral diseases with a focus on arboviroses and climatic changes (sessions 1, 5, 6).
- Mitigation strategies: Early warning systems, intervention strategies and emergency vaccination or antivirals in the control of epizootics (sessions 2, 3, 4)

Acknowledgements

We are very grateful to the following companies for sponsoring the 7th Annual EPIZONE meeting:

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- AFSCA-FAVV-FASFC <http://www.afsca.be/home-en/>

In addition to the companies sponsoring the 7th Annual EPIZONE meeting, we also **acknowledge** help of many people in the organization of this meeting including:

- Visit Brussels
- The Federal Agency for the Safety of the Food Chain (FAVV- AFSCA)
- Ana Afonso & Franck Berthe from EFSA
- Luc Niville from SEMICO
- Dominique Fondu, Katia Knapen (CODA-CERVA)
- Wim van der Poel, Manon Swanenburg & Margriet Vedder-Rooties (CVI, The Netherlands)

Local Organizer



CODA - CERVA

Local Organizing and Scientific Committee

Frank Koenen, Chair of the Organizing Committee

Thierry van den Berg, Chair of the Scientific Committee

Brigitte Cay

Kris De Clercq

Bénédicte Lambrecht

Yves Van der Stede

International Scientific Committee

Martin Beer (Germany)

Sandor Belak (Sweden)

Linda Dixon (United Kingdom)

Dirk Pfeiffer (United Kingdom)

José-Manuel Sanchez-Vizcaino (Spain)

Ase Uttenthal (Denmark)

Wim van der Poel (The Netherlands)

Stéphan Zientara (France)

Index

Sponsors	3
Theme	7
Acknowledgements	8
Organization	9
Keynotes Speakers - Biographies	11
Programme	16
EFSA Programme	20
Young Epizone Programme	21
Keynote speakers	22
October 2	
Session 1 – Arboviruses	32
Session 2 – Diagnosis	39
Session 3 – Antivirals & Vaccines	46
Session 4 – Legislation & Intervention Strategies	53
October 3	
Session 5 – Emerging Diseases	61
Session 6 – Epidemiology & Risk Analysis	68
Session 1 continued	75
Session 2 continued	82
Sessions 3 & 5 continued	89
Sessions 4 & 6 continued	96
Posters	103

KEYNOTE SPEAKERS (ALPHABETICAL ORDER)

Short Biography of Martin Beer



Director of the Institute of Diagnostic Virology, Friedrich-Loeffler-Institut

Education (Academic Qualifications)

DMV, Habilitation (venia legend for Microbiology)

Board certified specialist in veterinary Microbiology and Virology.

Employment

1992 – 1995 Doctoral thesis 1995

1995 – 2000 Scientist at the Institute for Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, Munich (Germany)

2000 – present Senior scientist at the Friedrich-Loeffler-Institut, Isle of Riems

2000 – 2004 Head of the National Reference Laboratory for BHV-1 and BVD

2004 – present Director of the Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Isle of Riems

Expertise (summary of research activities)

18-year expertise working with different animal viruses, in particular Pestiviruses (BVDV, BDV and CSFV), animal Herpesviruses (BHV-1), Influenza A viruses and Orbiviruses, with special emphasis on the development of novel vaccines and diagnostics. Recent research projects are focussed on new diagnostic systems (e.g. real-time PCR and microarrays), and the development of marker vaccines as well as marker diagnostics allowing differentiation between vaccinated and infected animals (DIVA-principle). Recent research is also focussed on the pathogenesis of Avian Influenza virus (AIV), Bluetongue virus (BTV) and Schmallenberg virus (SBV).

Short Biography of Kris De Clercq



Doctor in Veterinary Medicine in 1981, Faculty of Veterinary Medicine, State University of Gent, Belgium and 'Master of Science in Animal Production' in 1983. Since 1985 working at the CODA-CERVA. (Veterinary and Agrochemical Research Centre, Brussels-Ukkel, Belgium), Department of Virology. Head of Section Vesicular and Exotic Diseases since 1999. Vice-President of the OIE Scientific Commission since 2009. Head of the OIE Collaborative Centre and the FAO FMD Reference Centre. Since 1995 Member, from 1997 till 2007 Chairman and since 2007 Vice-Chairman of the EuFMD Research Group of the FAO European Commission for the Control of Foot-and-Mouth Disease. Co-ordinator of the EC

research project FMD-ImproCon and FMD-DISCONVAC on the Improvement of FMD Control. Partner in several European and national research projects. Author of 110 peer reviewed scientific papers and 92 international scientific reports on FMD or bluetongue.

Short Biography of Christian Gortazar



Head, SaBio (Sanidad y Biotecnología) research Group, National Wildlife Research Institute IREC (CSIC-Universidad de Castilla – La Mancha). Ronda de Toledo s.n. 13071 Ciudad Real, Spain. Christian.Gortazar@uclm.es

Christian Gortazar (San Sebastián, 1967) got a Degree in Veterinary Sciences at Universidad de Zaragoza, in 1990, and a PhD at the same University in 1997. Since 1999, he is Professor at IREC, a multidisciplinary research institute dealing with conservation and management of wildlife and their habitats (www.uclm.es/IREC). His lecturing on wildlife diseases is part of IRECs MSc and PhD programs, where he has mentored 12 successful international PhD students. He has acted as principal researcher in numerous grants and contracts on wildlife epidemiology and disease control. Research interests include viral, bacterial and parasitic diseases of wildlife, with emphasis on the epidemiology and control of relevant diseases shared with livestock and humans, such as tuberculosis. <http://www.researcherid.com/rid/E-7918-2012>

Short Biography of Guus Koch



Guus Koch is employed as researcher by the Central Veterinary Institute (CVI) part of Wageningen University and Research since 1983. He performed research to the immune system of chicken, pathogenesis and diagnosis of viral infections of poultry notably infectious bronchitis, chicken anemia Marek disease and finally ND and AI. Since 1992, he is head of the Dutch national reference laboratory for Avian Influenza and Newcastle disease. During the high-pathogenicity Avian Influenza epidemic in 2003 in the Netherlands he was member of the CVI crisis team and participated in the National Crisis Team at the former Ministry of agriculture. In 2004 he was stationed at the WHO office in Beijing to advice on outbreak control of HPAI H5N1 in China.

Current Position

Currently he is working senior scientist and head of Dutch NRL for AI and ND at the Department of Virology, CVI WUR – Lelystad, P.O. Box 2003, 8203 AA Lelystad, The Netherlands. E-mail: Guus.Koch@wur.nl

Short Biography of Serge Morand



Serge Morand is interested in evolutionary ecology of host-parasite interactions and population ecology of parasites and pathogens. Field parasitologist, he is concerned at the role of biodiversity as risks and insurance for zoonotic emerging infectious diseases. He is conducting several projects on the impacts of global changes on the links between biodiversity and health in Southeast Asia, using rodent-borne diseases as a model. He is the co-author of several articles and books on these domains. Serge Morand is member of the scientific board of the French Research Foundation for Biodiversity (FRB) and is currently animating the project ATP CIRAD “Emergence and sanitary risks”

Short Biography of Johan Neyts



Johan Neyts is full professor of Virology at the University of Leuven, Belgium. His research is focused on the development of novel antiviral strategies against a number of viruses including picornaviruses (rhinovirus inhibitors for the prevention and treatment of exacerbations of asthma and COPD by these viruses) flaviviruses (mainly dengue) and the hepatitis C virus (HCV). Three (classes of) molecules discovered in his laboratory made it to advanced clinical studies in patients chronically infected with the HCV. His work has been published in more than 280 peer reviewed papers and bookchapters. He is on the editorial board of a number of journals including ‘Antiviral Research’, member of several national and international scientific committees and on the board of the International Society for Antiviral Research. He is the co-founder and Chief Scientific Officer of the spin-out company Okapi Sciences NV, which is developing antivirals for use in veterinary medicine. He teaches medical virology at the school of dentistry and the school of medicine at the University of Leuven. He has been honoured with a number of awards including from the International Society for Antiviral Research, the Royal Belgian Academy of Medicine and the Belgian Fund for Scientific Research.

www.kuleuven.be/regacmt/jn
www.okapi-sciences.com

Short Biography of Francisco Javier Reviriego Gordejo



Dr Francisco Javier REVIRIEGO GORDEJO obtained his PhD (1999) in Veterinary Epidemiology from the Universidad Complutense de Madrid and his degree (1988) in Veterinary Science from the same university. He worked as Official vet for the Autonomous Region of Castilla y Leon where he was responsible for implementing eradication programmes, BSE regional surveillance programme and from 1991 to 1996, he was Head of the Animal Health Laboratory of Avila and before worked as a veterinarian with the Pig Farmers' Association from 1990 to 1991.

He joined the European Commission (DG SANCO) in 2001 as a Legislative Veterinary Officer. In 2005 was Head of Sector Epidemiology and Eradication, and since 2008 is as Head of the Sector Disease Control and Identification.

In addition to his work at DG SANCO, Dr Reviriego Gordejo participated in a number of World Organisation for Animal Health (OIE) ad hoc groups on brucellosis, tuberculosis, bluetongue, epidemiology and modelling. He is founding member of the Spanish Veterinary Laboratory Diagnosticians Association and the Spanish Veterinary Epidemiologists Association. He holds a postgraduate diploma (2000) in Statistics and Design in Health Sciences.

Short Biography of Eeva Tuppurainen



After receiving her veterinary degree from the Faculty of Veterinary Medicine, University of Helsinki, (1986) Eeva Tuppurainen worked for 16 years as a private veterinary surgeon. Between 2002 and 2004 she completed her research-based MSc studies at the Department of Veterinary Tropical Diseases, Veterinary Faculty, University of Pretoria, South Africa on diagnostic methodologies for lumpy skin disease virus (LSDV). Since 2004 she has been working at the World Organization for Animal Health (OIE) Reference Laboratory for lumpy skin disease (LSD), sheep pox (SPP) and goat pox (GTP) at the Pirbright Institute. Since 2006 she has been heading the Capripox Reference Laboratory at Pirbright and in 2007 she

was appointed to OIE international expert for LSD, SPP and GTP. Her special interest is epidemiology, control and eradication of the OIE-listed animal diseases and enhancement of diagnostic capacity in national laboratories in developing countries. Most recently she has been investigating the vector capacity of ixodid ticks for LSDV.

Short Biography of Wim van der Poel



Prof. Dr. Wim H.M. van der Poel, DVM is senior scientist at the Central Veterinary Institute of Wageningen University and Research Centre, The Netherlands. His research activities have focussed on veterinary virology, emerging and zoonotic viruses and food borne viruses. He is also coordinator of the EPIZONE European Research Group, the veterinary research network on epizootic animal diseases.

Previously, from 1996 to 2004, prof Van der Poel has been working on research of viral zoonoses and food borne viruses at the National Institute for Public Health and the Environment in Bilthoven, The Netherlands. At this institute, from 2000-2004, he was also heading the National Reference Laboratory for Microbiological Contamination of Bivalve Molluscs.

Prof. Van der Poel is a member of a number of scientific committees, advisory panels, boards and professional bodies in the field of veterinary virology, and he has been involved in many national and international research projects on viruses in animals and foodstuffs. Throughout his career he has (co)-authored more than 100 scientific papers and reviews in the field of veterinary virology, he has made more than 150 contributions to international conferences, and he has often served as an ad hoc reviewer for scientific journals and granting agencies. In February 2009 he has accepted an honorary visiting chair on Emerging and Zoonotic Viruses at the University of Liverpool, United Kingdom.

Prof. van der Poel graduated in Veterinary Medicine in 1988 and completed his PhD in veterinary virology at the Utrecht University in 1995. He is a registered specialist in veterinary microbiology within the Netherlands Royal Veterinary Association and a registered research worker in medical microbiology within the Netherlands Royal Microbiology Association.

Programme EPIZONE 7th ANNUAL MEETING

Time	Day 1: Tuesday: 01/10	
9.00-17.30 14.00-17.00	Young Epizone, CF Meeting - Small Meetings	Pacheco
16.00 – 18.00	Registration and welcome desks	Pacheco
19.00 - 21.00	Welcome drinks and canapés Belgian Comic Strip Center – Rue des sables, 20 -1000 Brussels (400 m from Pacheco Center)	
Time	Day 2: Wednesday: 02/10: MAIN MEETING	
	AUDITORIUM	ROOM 1
9.00 - 9.20	Introduction: - Mrs S. Laruelle, Minister of Agriculture - Pierre Kerkhofs, General Director CODA-CERVA - Wim van der Poel, coordinator Epizone	
9.20 -10.00	Keynote: <u>Martin Beer (Ge) and Wim van der Poel (NL):</u> Schmallenberg virus	
10.00 - 10.40	Keynote: <u>Kris De Clercq & Johan Neyts (Be):</u> Antiviral therapy for the containment of foot-and-mouth disease virus outbreaks	
10.40 - 11.00	Coffee break	
	Session 1: Arboviruses Chairs: Wim van der Poel & Brigitte Cay	Session 2 : Diagnosis Chairs: Sandra Blome & Frank Koenen
11.00 - 11.15	<u>Kirkland, Peter:</u> Development and application of an <i>in vivo</i> model for studies of vector capacity of Culicoides spp for bluetongue and other arboviruses.	<u>Blaise-Boisseau, Sandra:</u> Development of a multiplex immunoassay for foot-and-mouth disease diagnostic
11.15 - 11.30	<u>Claine, François:</u> Evidences for re-emergence of Schmallenberg virus during summer and autumn 2012 among naïve female lambs and immunized ewes	<u>Pérez, Tereza:</u> One-step immunochromatographic test for the simultaneous detection of antibodies to African and Classical Swine Fever virus in the plasma or serum
11.30 -11.45	<u>Martinelle, Ludovic:</u> Dose dependent challenge of sheep with Schmallenberg virus	<u>Moreno, Ana:</u> PB1-F2 protein and N66S mutation in swine influenza viruses
11.45 -12.00	<u>Vitour, Damien:</u> NS3 of bluetongue virus interferes with the innate antiviral response	<u>Decorte, Inge:</u> Detection of swine influenza virus in oral fluids collected from experimentally infected pigs
12.00 -12.15	<u>van Gennip, René:</u> Non-structural proteins NS3 and NS3a of bluetongue virus are not essential for virus replication	<u>Fowler, Veronica:</u> Evaluation of a simple assay format for the detection of foot and mouth disease virus using reverse transcription loop mediated isothermal amplification
12.15 – 12.30	<u>De Leeuw, Ilse:</u> Bluetongue Virus RNA Detection by Real-Time RT-PCR in Post-Vaccination Samples from Cattle	<u>Aebischer, Andrea:</u> Sample preparation and nucleic acid extraction: the bottleneck in application of pen-side test systems (?)
12.30 - 13.30	Lunch & poster viewing	

13.30 – 14.10	Keynote: <u>Guus Koch (NL)</u> : Phylogenetic analyses as an epidemiological tool to unravel the transmission chain of influenza virus outbreaks.	
14.10 – 14.50	Keynote: <u>Francisco Reviriego - Gordejo (SANCO)</u> : Legislation & Intervention strategies in the EU 2013-2018	
	Session 3 : Antivirals & Vaccines Chairs: Linda Dixon & Kris De Clercq	Session 4: Legislation & Intervention strategies Chairs: Thomas Mettenleiter & Pierre Kerkhofs
15.00 - 15.15	<u>Hamers, Claude</u> : Demonstration of efficacy against challenge of an inactivated Schmallenberg vaccine in cattle	<u>Ruegg, Simon</u> : Assessing the effectiveness of surveillance system components for vector-borne diseases: The example of bluetongue (BT)
15.15 - 15.30	<u>Anderson, Jenna</u> : Development and evaluation of a DIVA subunit vaccine against Bluetongue virus serotype 8 in cattle	<u>Claine, François</u> : Emergence and time course of spreading of Schmallenberg virus in late summer 2011 in a Belgian naïve sheep flock
15.30 - 15.45	<u>Brun, Alejandro</u> : Attenuation of RVFV infection in mice upon inoculation with FMDV synthetic RNAs	<u>Elbers, Armin</u> : Longitudinal monitoring reveals long-lasting virus-neutralizing antibodies against Schmallenberg virus in adult cattle, The Netherlands, 2012-2013
15.45 - 16.00	<u>van Rijn, Piet</u> : Development of reverse genetics for African horsesickness virus	<u>Hoffmann, Bernd</u> : Detection of Schmallenberg virus RNA in bull semen
16.00 - 16.15	<u>Blanco, Esther</u> : 'EPIZONE' Design of novel vaccines based on virus-like particles (VLPs) derived from calicivirus: multimeric presentation of epitopes	<u>Méroc, Estelle</u> : Schmallenberg virus: Results of Belgian cross-sectional serological surveys
16.15 -16.30	<u>Willems, Tom</u> : Alternative foot-and-mouth disease vaccine potency tests based on serology and payload	<u>Deblauwe, Isra</u> : Surveillance of exotic mosquitoes, midges, ticks and their pathogens in Belgium in 2013
16.30 - 18.00	Poster Session with coffee open	NADIR satellite workshop : “Joined forces to optimize animal and public health innovation”.
20.00 - 23.00	Symposium Dinner Chez Léon	
Time	Day 3: Thursday: 03/10: MAIN MEETING	
9.00 - 9.40	Keynote: Christian Gortazar (Spain): Wild boars as viral reservoirs	
9.40 - 10.20	Keynote: Eeva Tuppurainen (UK): Lumpy skin disease, sheeppox and goatpox	
10.20 -11.00	Coffee break and poster viewing	
	Session 5: Emerging diseases Chairs: Stéphane Zientara & Bénédicte Lambrecht	Session 6: Epidemiology & Risk Analysis Chairs: Christine Fourichon & Yves Van der Stede
11.00 - 11.15	<u>Calzolari, Mattia</u> : Arboviruses surveillance by mosquito screening in North-Eastern Italy in 2012	<u>Lebl, Karin</u> : Using interval lagged environmental data to predict the population dynamics of the disease vector Cx. pipiens/restuans
11.15 - 11.30	<u>Dridi, Maha</u> : Evaluation of West Nile Virus (WNV) NS3THR249PRO mutation as a	<u>Saegerman, Claude</u> : A new methodology to assess data availability, accessibility

	virulence marker in a SPF chicken model and in carrion crows (<i>Corvus Corone</i>)	and form for risk analysis
11.30 – 11.45	<u>Balkema-Buschmann, Anne</u> : PCR screening for viral nucleic acid sequences in bat colonies in Germany	<u>Veldhuis, Anouk</u> : Risk factors for introduction and clinical symptoms of Schmallenberg virus in Dutch dairy herds and its impact on productivity
11.45 -12.00	<u>Sarrazin, Steven</u> : Virus transmission and clinical symptoms in calves experimentally infected with a recently discovered hypervirulent BVDV-2c strain	<u>Wernike, Kerstin</u> : Within-herd dynamics of Schmallenberg virus
12.00 - 12.15	<u>Gorna, Kamila</u> : Isolation and molecular characterization of foot-and-mouth disease virus in Benin in 2010	<u>de Koeijer, Aline</u> : Scale and pattern of Q fever transmission through space
12.15 - 12.30	<u>Petrov, Anja</u> : Host response upon infection with moderately virulent Classical swine fever virus strains: impact of race-dependent genetic variations	<u>Georgiev, Milen</u> : Epidemiological determinants are at the core of effective analysis to assess Q fever risk (A review of Q fever in four European countries)
12.30 - 13.30	Lunch & poster viewing	
13.30 – 14.10	Keynote: Serge Morand (Fr): Biodiversity & Epidemiology	
	Session 1 continued Chairs: Martin Beer & Thierry van den Berg	Session 2 continued Chairs: Don King & Steven Van Borm
14.15 -14.30	<u>Coupeau, Damien</u> : Biological and genetic evolution of the hypervariable region in Schmallenberg virus.	<u>Wozniakowski, Grzegorz</u> : Detection of duck enteritis virus (DEV) in ducks, geese, swans and other free-ranging aquatic birds by real-time loop-mediated isothermal amplification
14.30 -14.45	<u>Stockhofe, Norbert</u> : Transplacental infection of Schmallenberg Virus in the first trimester of gestation is highly efficient in sheep	<u>Blaise-Boisseau, Sandra</u> : Development of a single test for exclusion or detection and typing of Foot-and-Mouth disease virus
14.45 – 15.00	<u>Schulz, Claudia</u> : Schmallenberg virus infection in South American camelids	<u>Freimanis, Graham</u> : Investigating the Impact of Nucleic Acid Extraction upon Next-Generation Sequencing of Foot and Mouth Disease Virus (FMDV)
15.00 – 15.15	<u>Moulin, Véronique</u> : Development of a vaccine that protects sheep and cattle against challenge with Schmallenberg virus, a novel Orthobunyavirus recently found in Europe.	<u>Kolbasov, Dennis</u> : Various methods for equine infectious anemia virus detection in experimentally infected animal.
15.15 -15.30	<u>Martinelle, Ludovic</u> : Experimental infection of cattle with Bluetongue virus serotypes circulating in Europe	<u>Fröhlich, Andreas</u> : Adaption of diagnostic methods to different situations of measurement
15.30 -15.45	<u>Feenstra, Femke</u> : RNA elements in open reading frames of the bluetongue virus genome are essential for virus replication	<u>Vrancken, Robert</u> : Antiviral agents to control Classical Swine Fever epidemics
	Coffee break	
	Sessions 3 & 5 continued Chairs: José Manuel Sanchez-Vizcaino & Michele Dottori	Sessions 4 & 6 continued Chairs: Marie-Frédérique Le Potier & Piet van Rijn

16.15 – 16.30	<u>Mulatti, Paolo</u> : Space-time analyses to modulate the West Nile Virus surveillance activities in north-eastern Italy	<u>Sarrazin, Steven</u> : Virulence comparison and quantification of horizontal bovine viral diarrhoea virus transmission following experimental infection in calves
16.30 – 16.45	<u>Sevilla, Noemi</u> : Vaccination with recombinant Adenovirus expressing F or H proteins from the Peste des Petits Ruminants virus can elicit cellular and humoral immune response to the virus.	<u>Ensoy, Chellafe</u> : Exploring Cattle Movements in Belgium
16.45 – 17.00	<u>Castillo-Olivares, Javier</u> : Protective vaccines against African horse sickness virus (AHSV) based on modified Vaccinia Ankara expressing AHSV-VP2	<u>Ferre, Nicola</u> : EPIZONE: Get INSPIREd
17.00 - 17.15	<u>Sevilla, Noemi</u> : Identification of T cell epitopes from non-structural protein NS1 of bluetongue virus serotype 8, capable of cross-reacting with other viral serotypes in mice and sheep.	<u>Claes, Gerwin</u> : An experimental model to analyze the risk of introduction of a duck originated H5 low pathogenic avian influenza virus in poultry through close contact and contaminative transmission
17.15 – 17.30	<u>Smietanka, Krzysztof</u> : Avian influenza virus subtype H9N2 – an emerging threat to poultry in Europe?	<u>Van Loo, Hans</u> : Use of abortion surveillance in cattle to verify the introduction of Schmallenberg Virus in Flanders.
17.30 – 17.45	<u>Kolbasov, Dennis</u> : Experimental reproduction of Epizootic hemorrhagic disease of deers	<u>de Vos, Clazien J.</u> : FEVER: A Framework to assess Emerging VEctor-borne disease Risks for livestock
17.45 – 18.00	Conclusions	
Day 4: Friday: 04/10: EFSA Satellite meeting		

ANIMAL HEALTH AND WELFARE UNIT

AGENDA

EFSA satellite meeting: “Emerging animal diseases in the EU: what have we learned?”

7th Epizone

4 October 2013

9:00-14:30

Pacheco Centre, Boulevard Pacheco 13, 1000 Brussels

Pacheco room

Chair: Simone More

Chair of EFSA Animal Health and Welfare (AHAW) Panel, School of Veterinary Medicine, University College Dublin, IE

Opening: Dr Pierre Naassens, Chief Veterinary Officer, Federal Agency for the Safety of the Food Chain, BE

- 9:00- 9:15 Can we have a fresh look at emerging animal diseases?
Simon More- EFSA AHAW Panel, School of Veterinary Medicine, University College Dublin, IE
- 9:15- 9:45 Revisiting recent unexpected outbreaks in the EU (BTV 8 and SBV)
Wim van der Poel- Central Veterinary Institute, NL
- 9:45- 10:15 Epidemiology and innovative methodologies to support scientific advice on emerging diseases
Dirk Pfeiffer- Royal Veterinary College, UK
- 10:15- 10:45 Hunger for data and information at the onset of emergence
Jose Manuel Sanchez Vizcaino- Universidad Complutense de Madrid, SP
- 10:45-11:15 Risk questions and needs for advice from a risk management viewpoint
Karin Schwabenbauer- Federal Ministry of Food, Agriculture and Consumer Protection, DE
- 11:15-11:30 Coffee break
- 11:30- 13:00 Roundtable discussion
- 13:00-13:15 Conclusions
- 13:30-14:30 Networking lunch



Young Epizone Program 1 st October 2013 @ Pacheco Centre, Room 2	
8:00-8:30	Registration for the YE and EPIZONE 7th annual meeting
8:30-9:30	Icebreaker game
9:30-10:30	Workshop: Working in a project environment, the art of success - introduction
10:30-10:45	Coffee break
10:45-12:15	Workshop: Working in a project environment, the art of success - part 1
12:15-13:15	Lunch
13:15-14:45	Workshop: Working in a project environment, the art of success - part 2
14:45-15:00	Coffee break
15:00-15:45	Workshop: Working in a project environment, the art of success - part 3
15:45-16:00	Break
16:00-17:30	Testimonies from experienced Senior Scientists: Dr. Sandra Blome (Friedrich-Loeffler-Institute, Germany) Dr. Kris De Clercq (Veterinary and Agrochemical Research Centre CODA-CERVA, Belgium) Prof. Dr. Thomas Mettenleiter (Friedrich-Loeffler-Institute, Germany) Prof. Dr. Wim van der Poel (Central Veterinary Institute, The Netherlands)
19:00-21:00	Welcome drinks and canapés EPIZONE 7th annual meeting
20:30-late	Optionally: dinner with YE members in the city of Brussels!

Keynote speakers

KEYNOTE 1 : SCHMALLEMBERG VIRUS

Martin Beer¹ and Wim H. M. van der Poel²

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In November 2011, in Europe a novel orthobunyavirus of the Simbu serogroup, the Schmallenberg-virus (SBV), was discovered using a metagenomic approach. The virus was associated with diarrhea, fever and milk drop in dairy cattle, and malformations of new-born lambs. The outbreak started around the Dutch-German border and resulted in an epidemic affecting cattle, sheep and goat farms all over Europe with acute infections in adult ruminants and malformations in offspring. SBV did not show any zoonotic potential; in a serosurvey humans exposed to infected ruminants were tested for SBV specific antibodies but none of the individuals tested positive. In 2013, SBV spread to countries outside the European Union, including Switzerland, Norway, Russia and bordering countries. In February 2012, the world organisation for animal health (OIE) scientific committee endorsed recommendations for trade, and in March 2012, scientific support studies on Schmallenberg virus were commissioned by the European Commission and the involved EU member states. As part of these studies, and within national research programmes, SBV-specific diagnostic tools were developed, validated and harmonised. Studies e.g. on SBV characterization, SBV pathogenesis, SBV epidemiology and SBV vectors were conducted. SBV was shown to be transmitted rapidly by culicoides biting midges and SBV-specific antibodies were detected in different, mainly ruminant, wildlife species. End of 2012, the detection of SBV-RNA in bovine semen and potential infectivity of such semen was reported for the first time, and subsequently trade measurements were endorsed for bovine genetic products in several countries outside of the European Union. Recently, the first SBV vaccine was licenced in Europe.

In this keynote lecture the most recent results of on-going SBV research activities will be presented and discussed.

KEYNOTE 2: Antiviral therapy for the containment of foot-and-mouth disease virus outbreaks

Kris De Clercq¹ and Johan Neyts^{2, 3}

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Recent European contingency plans envisage emergency vaccination as an animal-friendly control strategy for foot-and-mouth disease (FMD). An antiviral containment strategy for FMD outbreaks may support and complement emergency vaccination in case of an outbreak in Europe and a treat-to-live scenario may spare many animals from being pre-emptively culled. We will discuss the possible use of potent and selective inhibitors of FMD virus-replication as an alternative or complementary measure in outbreak control. Although highly potent and efficient drugs are available in human medicine for the treatment (or prophylaxis) of infections with various herpes viruses, HIV, hepatitis B and C viruses and influenza virus, antiviral drugs are not yet available for the treatment of viral diseases in pets or livestock. An ideal FMDV-antiviral should exert pan-serotype activity, have good (oral) bio-availability and an excellent safety profile, leave no residue, and be stable and inexpensive. Recently, we have made substantial progress towards the development of novel and highly potent molecules that inhibit FMD virus (FMDV) replication *in vitro*. To demonstrate the feasibility of an antiviral approach against FMDV, we will also report on the *in vitro* and *in vivo* antiviral activity of the ribonucleoside analogue 2'-C-methylcytidine, a molecule that was initially developed as a treatment for infections with the hepatitis C virus in man. This molecule was shown to exert *in vitro* antiviral activity against the 7 serotypes of FMD virus and to provide "sterilizing" protection against FMDV infection in (most) severe combined immunodeficient (SCID) mice that had been experimentally infected with the virus. For the preliminary *in vivo* evaluation of antiviral lead molecules, we also refined an infection model in guinea pigs. Intradermal inoculation (with O1 Manisa) resulted within 2 to 4 days post inoculation in generalization of foot-and mouth lesions, a high level of viremia, virus dissemination to internal organs and salivary excretion. Using this model we next explored the effect of 3-oxo-3,4-dihydro-2-pyrazincarboxamide derivative (T-1105) -an inhibitor of influenza replication that has also been reported to inhibit FMDV replication in infected animals. Fourteen/16 T-1105-treated animals were completely protected from generalization of lesions and in 2/16 animals secondary lesions were reduced in comparison to untreated control animals. Our results show the utility of the SCID mouse and guinea pig models for the preliminary evaluation of anti-FMDV drugs and illustrate the potential of small chemical molecules to control FMDV outbreak control.

KEYNOTE 3: Phylogenetic analyses as an epidemiological tool to unravel the transmission chain of influenza virus outbreaks.

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Phylogenetic studies have largely contributed to better understand the emergence, spread and evolution of highly pathogenic avian influenza during epidemics, but sampling of genetic data has never been detailed enough to allow mapping of the spatiotemporal spread of avian influenza viruses during a single epidemic. The rapid evolutionary dynamics of avian influenza viruses suggest that sufficient genetic diversity may be produced during an epidemic in poultry to permit the reconstruction of the inter-flock transmission network, providing important insights for the implementation of efficient control measures

The epidemic of HPAI H7N7 in poultry in the Netherlands in 2003 provided an unique opportunity to study the HPAI transmission dynamics. The epidemic started in high density poultry region in the central part of the Netherlands and gradually spread to the South and further to Belgium and Germany. In addition, virus was detected in 89 diseased people who were handling poultry on infected farms.

We determined virus sequence data from 72% of the infected farms during the 2003 HPAI H7N7 epidemic in the Netherlands. Phylogenetic analyses was used to unravel the pathways of virus transmission between farms and between outbreak areas. The sequence network that was produced can be used to further unravel transmission routes. A few examples will be presented.

KEYNOTE 4: Legislation and Intervention strategies in the EU 2013-2018

Francisco Javier Reviriego Gordejo. EUROPEAN COMMISSION. HEALTH & CONSUMERS DIRECTORATE-GENERAL. Animal Health. Head of Sector Disease Control and identification.

The purpose is to present a European Commission perspective about the next motto from the Health and Consumers Directorate-General of the European Commission after the appealing "prevention is better than cure"¹. The next priorities on animal health, especially for epizootic diseases are also outlined.

The history of the EU veterinary legislation since the times of the so-called EU Trade directives, through the Single Market rules and the Disease Control directives, the preparatory work of for the Animal Health Strategy led to the adoption of the new Animal Health Strategy (2007-2013): "prevention is better than cure" proves that change in the EU veterinary rules is indeed permanent as it is the epidemiological situation.

At present, some milestones of the action plan of the Animal Health strategy are being achieved. As regards categorization and prioritization of diseases a tool for categorization is being fine-tuned though a pilot trial on categorization. The proposal for the Animal Health Regulation and the proposal for the new financing instrument for expenditure relating to animal health have been adopted by the Commission. As regards disease surveillance, the work of the Task Force on animal disease surveillance and the future Animal Disease Information System (ADIS) are new support tools for more performing animal health surveillance.

However, the critical relation between science and policy in the field of animal health, two key players as EPIZONE and SANCO deserve some attention. This relation tends to follow a reactive approach with science production peaking after crises. A more proactive and strategic approach appears to be needed specially for science as policy decision cannot always wait until all relevant scientific questions are answered and solutions in the veterinary field need to be robust.

There are some elements that need to be considered for better intervention in the field of animal health, namely epidemiology and risk communication. Veterinary epidemiology is a precious tool and veterinary epidemiologists are the best placed experts to integrate the "hard sciences" and "social sciences" that are needed to address complex animal health problems that go from outbreak investigation to macroeconomic assessment of animal disease. Risk communication is based on trust and largely relies on relations and confidence established in peace time.

The future of legislation and intervention strategies in the of Animal Health in the EU will build on the opportunities of the new Animal Health Regulation that will set horizontal principles and rules contributing to better overall husbandry, better response to new threats and will provide for less administrative burdens and costs and where involved risks permit so to facilitate movements and trade. Those new rules will provide also for flexibility for disease prevention and control measures proportionate to the risks. The new Animal Health rules will provide further opportunities for flexibility to be adjusted to climate changes and emerging risks, international standards and scientific developments, the variety of sizes and types of establishments, types of animal production and different systems providing equal guarantees (for animal movements, traceability, etc.)

¹ http://ec.europa.eu/food/animal/diseases/strategy/index_en.htm

KEYNOTE 5: Wild boar as viral reservoirs

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Eurasian wild boar (*Sus scrofa*) populations have experienced over the last three decades an unprecedented demographic explosion in their native historic range, from Western Europe to Japan. Feral pigs, which are free-ranging descendants of escaped pigs, or pig x wild boar crosses, are also expanding both geographically and numerically, particularly in the Americas. This comes along with significant increases in pathogen prevalence and distribution, and with a growing risk of dense wild boar (or feral pig) populations being able to maintain the circulation of economically relevant viral agents. In the context of viral diseases, wildlife reservoir hosts are defined as those species able to maintain a given virus in the absence of contact with its domestic host, and able to spill-back the infection to this domestic host. In the case of the pig and the wild boar, this situation is even more complex due to the gradient of existing management situations in both forms: from closed farms to open-air and free-range pig husbandry; and from true wildlife through fenced hunting estates to almost industrial wild boar farms.

Given the relevance of this host group, research is ongoing in a diversity of inter-related fields. This includes (1) wild boar ecology, behavior and management; (2) wild boar – livestock interactions and contact avoidance; (3) epidemiology of diseases shared between wild boar and livestock; and (4) disease control in wildlife reservoirs, among others.

Thanks to the research carried out in the last decade, much more is known on wild boar ecology, behavior and management. Wild boar are adaptable and opportunistic omnivores with a spectacular demographic growth potential. Population increases are exponential in some regions of continental Europe, and expansion still takes place in others (e.g. British Islands and Scandinavia). Survival and population growth is largely limited by (food) resource availability, hunting and diseases, with predators playing only a minor role where present. Wild boar easily move across fences, large rivers and other barriers, and get in close contact with other wild and domestic species.

One specific field where veterinary science overlaps with ecology is the wild boar – livestock interactions and contact avoidance. Tools such as spatial epidemiology, camera trapping and GPS collaring, among others, are facilitating the quantification of direct and indirect contacts between wild boar, other wildlife and livestock. Although wild boar tend to avoid pigs more than expected by random, indirect contacts at waterholes or feeding sites still take place and direct contacts occur if a sow in estrus becomes accessible. Air-borne transmission is also a possibility for certain viral diseases. Innovative research is focusing on the design of innovative tools to avoid such pig – wild boar contacts.

The epidemiology of diseases shared between wild boar and livestock has received much attention. However, some cases such as classical swine fever (CSF) virus or Aujeszky's disease (AD) virus are better understood than others, including quite relevant ones such as foot and mouth disease (FMD) virus and the rising star of wild boar research, African swine fever (ASF) virus. Understanding how and at which rate viral pathogens are transmitted both within wild boar individuals/groups and to in-contact domestic swine as well as identifying factors driving transmission patterns is essential to understand pathogen ecology and design effective preventive and control strategies. Ongoing research is providing new insights on host traits modulating shedding and prevalence of relevant pathogens.

Finally, a particularly promising field is the control of infections shared with wildlife. Setting up a proper disease and population monitoring scheme is the absolute priority before even making the decision of whether or not to intervene. Thereafter, disease control can be achieved by different means, including (1) preventive actions, (2) arthropod vector control, (3) host population control through random or selective culling or through habitat management, and (4) vaccination. The alternative options of zoning or no-action should be considered, too, particularly in view of a cost/benefit assessment. Ideally, tools from several fields should be combined in an integrated control strategy. The success of disease control in wildlife depends on many factors, including the nature of the disease and the characteristics of the pathogen, the availability of suitable diagnostic tools, the characteristics of the wildlife host(s) and vectors, the geographical spread of the problem, the scale of the control effort and the attitudes of the stakeholders.

This presentation will introduce examples based on the APHAEA network (<http://aphaea.eu/>).

KEYNOTE 6: Lumpy skin disease, sheeppox and goatpox

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The *Capripoxvirus* genus within the *Poxviridae* family comprises three members: sheeppox (SPP) virus, goatpox (GTP) virus and lumpy skin disease (LSD) virus. Capripox diseases are notifiable to the OIE due to the substantial economic impact of an outbreak. In outbreaks of the disease morbidity is usually high (SPP/GTP 70-90%, LSD up to 85%). Mortality is relatively low (SPP/GTP and LSD < 10%), although, in young and imported animals it can rise up to 100%. SPP and GTP occur in northern and central Africa, in the Middle East and most of southern Asia. Outbreaks in Turkey have occasionally spread to Greece and Cyprus, whereas LSD is widespread across Africa and endemic in the Middle East. Regardless of the extensive vaccination campaigns carried out in 2012-13 in the Middle East, the spread of LSD has been uncontrollable. The most recent outbreaks of LSD have been reported in Israel, the Palestinian Authority (West Bank), Lebanon, Jordan and Iraq indicating that the disease continues spreading to the north towards Turkey and to the east to Asian countries.

It is widely agreed that the most important mode of transmission of LSDV is likely to be mechanical by a variety of blood-feeding vectors and to a lesser extent via direct contact, semen or via contaminated feed or water. Recently, scientists from the Pirbright Institute and the Department of Veterinary Tropical Diseases, University of Pretoria have been able to demonstrate for the first time a role for hard (ixodid) ticks in the transmission of LSDV. After feeding on infected cattle African brown ear tick (*Rhipicephalus appendiculatus*) males were transferred to feed on naïve animals which became viraemic and seroconverted. Short, low level viraemia (measured by PCR) was also detected in recipient animals through mechanical transmission by African bont tick (*Amblyomma hebraeum*) males. The presence of viral antigen and live virus were demonstrated in the saliva of infected *Amblyomma* and *Rhipicephalus* ticks after feeding on infected cattle either as nymphs or adults. African blue tick (*Rhipicephalus decoloratus*) females were able to pass the virus via their eggs to subsequent larvae. When these larvae were transferred to feed on naïve cattle it became viraemic (PCR) and developed PCR positive skin lesions. In addition, after an oral infection of nymphal or adult *Amblyomma* and *Rhipicephalus* ticks, the presence of the viral antigen was demonstrated using immunohistochemistry in haemocytes and different organs of adult ticks.

KEYNOTE 7: Biodiversity and epidemiology

Serge Morand

The links between biodiversity and the patterns of infectious disease richness and outbreaks are far from being well understood. Biodiversity appears to be a main explanatory factor for the diversity of human infectious diseases, including zoonotic diseases. However, comparative studies suggest that population and wealth (GDP) through the increase of threaten biodiversity may favour epidemics. These results observed at global scale find empirical support at local scale, where most of studies show mostly negative effect of biodiversity reduction on the spread of infectious diseases. Some important questions remain to be explored, concerning either the effects of habitat fragmentation and homogenization or the decrease of genetic diversity in wild life, livestock and poultries on the risks of emergence.

October 2

Session 1: Arboviruses

ORAL: Development and application of an in vivo model for studies of vector capacity of *Culicoides* spp for bluetongue and other arboviruses.

Van der Saag, Matthew¹; Kirkland, Peter D¹

Elizabeth Macarthur Agriculture Institute¹

Key words: bluetongue virus, vector competence, in vivo model

The international profile of arboviruses that are transmitted by biting midges from the *Culicoides* genus has become much more prominent in the last decade with widespread transmission of bluetongue virus (BTV) and more recently the emergence of Schmallenberg virus in Europe. While there is little doubt that most of these viruses are transmitted by *Culicoides* spp, it can be difficult to generate the data required to satisfy criteria for the acceptance of a midge species as a competent vector of a virus. Major elements include proof that the insect of interest takes a blood meal from a particular mammalian host, infection of the insect species with the virus, replication of the virus in the insect and subsequently transmission of the virus back to the animal host. Research to support these criteria can be extremely difficult because there are few midge species that have been colonised and raised under laboratory conditions. Further, transmission experiments typically require ruminants, with associated management, husbandry and ethics considerations. The small size of *Culicoides* spp also makes research with many species in the genus very difficult. Consequently in most circumstances only limited indirect or partial data have been produced to support the role of an individual insect species as a vector. In recent times, this has involved detection of the virus in wild caught insects, use of semi-quantitative 'real time' PCR (qRT-PCR) to demonstrate high levels of nucleic acid consistent with virus replication in the insect, and specifically, high virus levels in the head or salivary glands suggesting a capacity to infect a mammal when taking another blood meal. To address these issues, we have developed an in vivo model using the embryonated chicken egg (ece) and applied it to studies of vector competence of the major Australian vector of bluetongue virus, *Culicoides brevitarsis*, the smallest midge in the genus.

The kinetics of BTV replication and viraemia in inoculated embryos was monitored by qRT-PCR in individual ece by collecting small volumes of blood several times per day. At peak viraemia, wild caught insects (raised as unfed adults, recently emerged from cattle dung) were placed in a small cage attached to the egg shell and allowed to feed on blood vessels of the chorioallantoic membrane, exposed after removing part of the egg shell. Virus levels in the embryo blood were monitored pre and post feeding in each experiment. Engorged midges were held and fed on sucrose for an 8-10 day incubation period. To monitor potential virus replication, a proportion of insects were sampled at different time points and virus loads monitored by qRT-PCR. After the incubation period, pools of surviving midges were allowed to feed on uninfected chicken embryos. After this second feeding period, BTV RNA levels were quantified in individual midges. Virus replication was subsequently monitored in the 'clean' eces to establish whether the midges had transmitted virus.

This model has many advantages, including the capacity to test field caught insects, study 'wild type' virus, has no need for ruminants, can be undertaken at short notice and has the capacity to test large numbers of replicates. Orthobunya and other viruses may also be studied. Results of studies of infection of different populations of *C. brevitarsis* and other species with different serotypes and strains of BTV will be presented.

ORAL: Evidences for re-emergence of Schmallenberg virus during summer and autumn 2012 among naïve female lambs and immunized ewes

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University of Namur¹

Key words: Schmallenberg virus, Re-emergence, Sheep

In late 2011, the sheep flock of the University of Namur (located in the South of Belgium) underwent SBV natural infection. SBV-affected lambs (confirmed RT-qPCR positive) were born during the lambing periods of January and March 2012. In February 2012, 98.8% (417/422) of the adult animals were tested SBV-positive by serum neutralization test (SNT) and no further lamb presenting clinical evidence of in utero SBV infection was born after March 2012. Considering the high SBV seroprevalence in Belgium and within the flock, it was uncertain whether SBV re-emergence would occur during 2012. The objectives of this investigation were (1) to assess by RT-qPCR whether naïve female lambs born between November 2011 and January 2012 would undergo SBV primo-infection, (2) to assess by SNT the level of SBV antibody titers of ewes sampled in February 2012 and February 2013 and (3) to assess whether stillbirths or congenital deformities in lambs born between October 2012 and April 2013 could be associated to SBV infection.

Fifty naïve female lambs born between November 2011 and January 2012 underwent bimonthly blood collections between April and November 2012. Samples were analyzed by SNT and RT-qPCR. 113 ewes whose serum was tested SBV-positive by SNT in February 2012 underwent blood collection in February 2013. Paired samples of 20 ewes were analyzed by SNT. Thorough follow-up of lambings between October 2012 and April 2013 was performed. In case of abortion, stillbirth or presence of congenital deformities in any newborn lamb, RT-qPCR was performed on tissue samples. Results of RT-qPCR were considered positive if Cq value was < 40.0. Results of SNT were expressed as the log₂ transformed dilution that neutralizes 50% of the challenge virus (ED₅₀) and considered positive if log₂ ED₅₀ was > 3.49.

Follow-up of naïve female lambs demonstrated that SBV primo-infection occurred between 25th July and 17th October 2012, with a maximum of positive RT-qPCR results found between 22nd August and 5th September 2012 (Claine et al., 2013). For ewes whose serum was collected twice, median log₂ ED₅₀ (min-max) SBV antibody titers increased from 7.47 (4.98-9.47) in February 2012 to 8.47 (6.48-10.96) in February 2013 ($p < 0.05$). SBV-related abortion (confirmed by RT-qPCR) occurred in two ewe lambs that had undergone SBV primo-infection in autumn 2012. In March and April 2013, two lambs born from previously immunized ewes presented congenital deformities: arthrogryposis associated with sacral spina bifida and left testicular atrophy for one lamb and arthrogryposis associated with cleft palate for the other. Both lambs were tested RT-qPCR positive.

Despite the high SBV seroprevalence after primo-infection in 2011, an important SBV re-emergence occurred during summer and autumn 2012 in this sheep flock located in the South of Belgium. SBV-naïve ewe lambs underwent massive primo-infection and cases of SBV-induced abortions were recorded. In adult ewes, the one log increase of SBV antibody titers recorded between February 2012 and 2013 suggests a booster effect by re-emergence. However, two cases of transplacental transmission of SBV in immunized ewes were recorded and raise questions about the ability of SBV to reinfect previously immunized animals.

Claine F, Coupeau D, Wiggers L, Muylkens B, Kirschvink N. Schmallenberg virus among female lambs, Belgium, 2012. *Emerg Infect Dis* 2013;19:1115-7. <http://dx.doi.org/10.3201/eid1907.121768>

ORAL: Dose dependent challenge of sheep with Schmallerberg virus

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Key words: Schmallerberg sheep dose experimental infection

Schmallerberg virus (SBV) has been identified in Germany in November 2011. It belongs to the family Bunyviridae, genus Orthobunyavirus. The main clinical signs in adult cattle are fever and a significant drop of milk yield for several days, in some cases also diarrhoea and abortions, whereas the affection is considered subclinical in adult small ruminants. The infection is considered as non-contagious, most likely propagated among ruminants by biting midges of the genus *Culicoides*. The most severe impact of the disease on livestock consists in a congenital arthrogryposis/hydranencephaly syndrome affecting lambs, kids and calves in previously SBV free areas. Thus, it is of major concern to be experimentally able to induce in pregnant ruminants a viraemia prone to allow the placental crossing of the virus. In a recent study (Wernike et al., 2012), the use of 1/100 diluted serum to infect calves led to a stronger and more persistent viraemia than pure, 1/10 or 1/1000 diluted serum. We implemented this study to assess the relationship between inoculum dilution and viraemia in sheep.

Twelve "Mourerous" ewes about one year old were used. All the animals were tested seronegative and non viraemic for SBV, bluetongue virus (BTV) and Maedi-Visna virus. Ewes were separated in 4 groups of 3 animals each, and inoculated subcutaneously with 1ml undiluted infectious serum, 1/10, 1/100 and 1/1000 diluted serum depending on their respective group. This undiluted serum was kindly provided by M. Beer, Friedrich Loeffler Institute, Riems, Germany. Animals were daily examined for the detection of clinical signs and temperature rise. Viral RNA was detected in blood, serum and organs using a RTqPCR targeting the S segment. The induction of SBV specific neutralizing antibodies was evaluated by a seroneutralization test, using a SBV isolate from brain tissue of an aborted lamb subsequently passaged four times in BHK21 cells. Ewes were euthanized 10 days post infection (dpi) and necropsied.

None of the animals showed any clinical signs or temperature rise. Viraemia could be detected as soon as 1 dpi in the serum of one ewe infected with undiluted serum and until 9 dpi in another animal of the same group. Mean viraemia duration was overall 6.6 (+/- 1.2) days in the undiluted serum group and 5.2 (+/- 0.8) days in the 1/10 serum group. In the 1/100 only one animal had a detectable viraemia from 3 to 8 dpi. SBV RNA could only be detected at 3 dpi in the serum of one ewe infected with the 1/1000 serum. Maximal viraemia was detected in EDTA blood and serum at 3.9 and 4.3 dpi respectively, and Cq values were on average 1.7 lower in serum. A clear increase in neutralizing antibodies titres could be found, starting at 5 and 6 dpi for the undiluted and 1/10 serum groups respectively. In the 1/100 serum group, only the ewe showing viraemia had a rise in antibodies titres, also at 6 dpi. At necropsy, no particular lesions could be found. In all the animals with a viraemia that lasted more than one day, SBV RNA could be found in spleen, prescapular and mesenteric lymph nodes, the latter showing the lowest Cq values. Viral RNA could also be sporadically found in lungs, tonsil and ovary.

Viraemia earliness, level, and persistence, appeared to be directly dependent on the inoculated dose of SBV. However, in ewes where viral RNA could be detected more than once, no significant difference could be seen in viral detection in organs. Neutralizing antibodies could be detected as soon as 5 dpi. Viraemia length in this study is consistent with recent experimental and field data.

ORAL: NS3 of bluetongue virus interferes with the innate antiviral response

Vitour, Damien¹; Doceul, Virginie¹; Gouzil, Julie¹; Chauveau, Emilie¹; Sailleau, Corinne¹; Bréard, Emmanuel¹; Zientara, Stéphan¹

National Veterinary Research Institute¹

Key words: Bluetongue virus, NS3, type I interferon, inhibition

Bluetongue disease is a major animal health concern transmitted through the bites of Culicoides vectors. Bluetongue virus (BTV), the etiologic agent of the disease, is a dsRNA virus belonging to the genus Orbivirus, into the Reoviridae family. BTV infection triggers the production of type-I interferon (IFN-I) and genomic dsRNA is a strong IFN-I inducer. We recently showed that the RIG-I-like receptor pathway is involved in the innate immune response following BTV infection. Most of viruses have evolved versatile strategies to escape the IFN-I response but until recently nothing was known on the ability of BTV to counteract the innate antiviral response.

In a recent work, we demonstrated that BTV serotype 8 (BTV-8) can dampen the type-I interferon response and that the non structural protein 3 (NS3) is involved in this process. Interestingly, the ability of NS3 to inhibit the IFN- β synthesis seems conserved between different serotypes and strains of BTV. By assessing the activation of multiple promoters through a reporter assay or measuring mRNA expression of several cellular genes, we could find that NS3 specifically modulates the activity of genes that are mostly linked to the IFN-I pathway. In order to precise at which level NS3 interferes with the RLR signaling cascade, we overexpressed MAVS, TBK1, IKK ϵ and IRF3-5D together with increasing amounts of NS3 and assessed the activation of IFN- β promoter in a luciferase reporter assay. We showed that the inhibitory effect of NS3 was reduced upon overexpression of TBK1, IKK ϵ and IRF3-5D, while it was still able to dramatically block MAVS, suggesting that NS3 acts at the level of MAVS or between MAVS and TBK1/IKK ϵ .

In conclusion, we found that NS3 of BTV acts as a powerful antagonist of the innate immune response in non-hematopoietic cells by inhibiting the RLR-dependent signaling pathway. We are now trying to decipher the mode of action of NS3.

ORAL: Non-structural proteins NS3 and NS3a of bluetongue virus are not essential for virus replication

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Central Veterinary Institute of Wageningen UR¹

Key words: orbivirus, bluetongue virus, reverse genetics, NS3,

Bluetongue virus (BTV) is a virus species in the genus Orbivirus of the Reoviridae family. BTV is a nonenveloped insect-borne virus which is released from infected cells by multiple pathways. Unlike other nonenveloped viruses, in addition to cell lysis the newly synthesized BTV particles appear to use a unique budding process. Non-structural protein NS3/NS3a encoded by genome segment 10 (Seg-10) has shown to be involved in the trafficking and egress of bluetongue virus from mammalian and insect cells. Recently, it was shown that NS3/NS3a protein also suppresses antiviral processes by IFN antagonism. Specific domains on NS3/NS3a protein have been recognized, and proposed to be involved in these processes. We have used reverse genetics to study the role of NS3/NS3a in virus replication (van Gennip et al., 2012).

To determine the contribution of different domains, two mutations leading to a frame shift were introduced in Seg-10 resulting in expression of C-terminally truncated NS3/NS3a proteins of 56 and 88 amino acids, respectively. One mutation is located in the first of two late domain motifs. The other is located between the late domain and the first transmembrane region. Initially, both BTV NS3/NS3a mutants showed no CPE, but groups of VP7-immunostained cells were visible. After passaging, both BTV mutants reverted to CPE phenotype comparable to wtBTV, and NS3/NS3a expression returned by repair of the ORF as confirmed by sequence analysis. These results show that, despite remaining extra amino acid residues, there is a strong preference for NS3/NS3a expression. Apparently, both mutant NS3/NS3a proteins are functional with respect to the CPE phenotype.

Then, in order to abolish NS3 and/or NS3a expression, we constructed Seg-10 with one or two mutated start codons (mutAUG1, mutAUG2 and mutAUG1+2), which were subsequently used to generate mutant BTVs with the respective mutations. All mutations of the start codons were AUG(Met)->GCC(Ala) mutations. We were able to rescue all three BTV mutants using reverse genetics, irrespective of the lack of NS3/NS3a expression. All introduced AUG->GCC mutations were genetically stable as confirmed after several virus passages. The lack of expression of NS3, NS3a, or both proteins was confirmed by westernblot analysis. In addition, lack of NS3/NS3a expression by BTV mutant mutAUG1+2 was confirmed by negative immunostaining with NS3/NS3a MAbs, whereas VP7-staining was positive. The growth of BTV mutants mutAUG1 and mutAUG1+2 was attenuated as was shown by a smaller plaque phenotype caused by CPE on BSR cells and growth kinetics on mammalian (BSR) and Culicoides cells (KC). Furthermore, on KC cells mutations in either AUG1 and/or AUG2 lead to reduced release of virus from the cells.

These results clearly show that both NS3 and NS3a are not essential for BTV replication in vitro in both BSR and KC cells. Apparently, NS3 expression is particularly important for virus growth, whereas abolishing of NS3a expression resulted in a less obvious effect on CPE and virus growth on BSR cells. Both NS3 and NS3a seem to be important for virus release from KC cells.

The here presented findings opens possibilities for research on the role of RNA sequences of Seg-10 independent of gene products (Feenstra et al., 2013), on the role of NS3/NS3a proteins in in vivo experiments in both host and insect vector, and on development of BT vaccines without NS3/NS3a expression. Perspective regarding these subjects will be discussed.

ORAL: Bluetongue Virus RNA Detection by Real-Time RT-PCR in Post-Vaccination Samples from Cattle

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Key words: bluetongue; vaccine; RNA detection; postvaccination; disease freedom

Bluetongue virus serotype 8 (BTV-8) was responsible for a large outbreak among European ruminant populations in 2006–2009. In spring 2008, a massive vaccination campaign was undertaken, leading to the progressive disappearance of the virus. During surveillance programmes in Western Europe in 2010–2011, a low but significant number of animals were found weakly positive using BTV-specific real-time RT-PCR, raising questions about a possible low level of virus circulation. An interference of the BTV-8 inactivated vaccine on the result of the realtime RT-PCR was also hypothesized. Several studies specifically addressed the potential association between a recent vaccination and BTV-8 RNA detection in the blood of sheep. Results were contradictory and cattles were not investigated. To enlighten this point, a large study was performed to determine the risks of detection of bluetongue vaccine-associated RNA in the blood and spleen of cattle using real-time RT-PCR. Overall, the results presented clearly demonstrate that vaccine viral RNA can reach the blood circulation in sufficient amounts to be detected by real-time RT-PCR in cattle. This BTV-8 vaccine RNA carriage appears as short lasting.

Session 2: Diagnosis

ORAL: DEVELOPMENT OF A MULTIPLEX IMMUNOASSAY FOR FOOT-AND-MOUTH DISEASE DIAGNOSTIC

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ANSES¹

Key words: FMDV LUMINEX MULTIPLEX SEROLOGICAL DIAGNOSTIC

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals, responsible for important economic losses. FMD virus (FMDV) belongs to the genus Aphthovirus in the Picornaviridae family. Seven immunologically distinct serotypes are described with a high antigenic variability. FMD cannot be differentiated clinically from other vesicular diseases that have similar symptoms such as swine vesicular disease, vesicular stomatitis, and vesicular exanthema. The aim of this project is the development of a multiplex immunoassay for FMD diagnosis by using the Luminex technology. Such a test should allow differential diagnosis of FMD as well as serological discrimination between FMDV infected and vaccinated animals in a single reaction and from a single sample. First stage in the development of this multiplex immunoassay was to produce FMDV antigens compatible with coupling reactions. For this purpose, FMDV proteins were expressed as recombinant 6HisTag viral proteins in *E. coli*, purified and then coupled to Luminex magnetic fluorescent microspheres. Thus, five antigen-coupled bead sets (3D, 3ABC, Vp1 serotype O, A or Asia1) were produced. Simplex immunoassays were then carried out to (1) assess coupling performances (background and specificity) of each bead set using specific monoclonal antibodies or control sera, as well as to (2) set up and optimize the concentration of each reagent. Luminex immunoassays were then performed using bovine sera from uninfected, infected, or vaccinated cattle. Antigen-bead coupling efficiency was monitored in each experiment by anti-His (mAb) reaction. Duplex and triplex tests were then performed using well characterized control bovine serum samples, giving coherent and expected results. A four-plex serological assay was finally developed and evaluated using a standardized bovine serum panel and compared to other usual serological tests.

The development of this bead-based immunoassay gave so far promising results. A Duplex immunoassay was thus developed allowing the simultaneous detection of antibodies against 3D and 3ABC non structural proteins. A four-plex test was also developed allowing detection of anti-3D antibodies and O, A, Asia1 serotyping. Both prototypes were evaluated using a standardized bovine serum panel (36 samples), initially assembled for evaluation of the relative sensitivity of new NSP tests. All the 36 sera were found positive with the Luminex test duplex anti- 3ABC & anti-3D. According to these results, this duplex immunoassay appears as the most sensitive serological NSP method among those compared. Both Luminex detection of anti-3ABC (published results or done at ANSES), give similar results. For the detection of anti-3D and anti-VP1 antibodies (Ab) in a four-plex assay, results of two independant 4plex experiments reveal that all sera are positive for anti-3D Ab. Encouraging serotyping results were also obtained but cross reactivity (A/Asia; SAT2/Asia; A/O) and a loss of sensitivity for detecting anti VP1 antibodies for serotype O were observed. Nevertheless, cross-reactivity is also observed using commercially available Type O Elisa. Future work will include further experimentations to determine sensitivity and improve specificity of the multiplex test. In addition, a five-plex including 3ABC coupled beads as well as multiplex immunoassays including NSP and SP from other vesicular diseases (SVDV, VSV) will be developed.

ORAL: ONE-STEP IMMUNOCHROMATOGRAPHIC TEST FOR THE SIMULTANEOUS DETECTION OF ANTIBODIES TO AFRICAN AND CLASSICAL SWINE FEVER VIRUS IN PLASMA OR SERUM

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INGENASA¹; PRIONICS²; FLI³; CISA-INIA⁴

Key words: African swine fever, Classical swine fever, Rapid tests, diagnostic, Lateral-Flow Devices

African swine fever (ASF) and Classical swine fever (CSF) are two highly infectious diseases of pigs and warthogs. Clinically it is very difficult to distinguish between CSF and ASF infection, and diagnostic tests must always be carried out to confirm infection. The CSF disease occurs in parts of Europe, Asia, America and Africa, meanwhile Europe and its neighbours, except for Sardinia, are free of ASF. However, recently, ASF has appeared in the Caucasus and Russia and both viruses are being detected in these regions. This recent epidemiological situation of both fever viruses has highlighted the need for rapid and reliable tests as an essential tool for disease control.

Due to the long-term persistence of antibodies, the antibody detection techniques are essential in the control of the diseases. To this end, in this work, Ingenasa has developed a rapid and one-step Lateral-Flow Device (LFD) for simultaneous detection and differentiation between ASF and CSF infections in serum.

MATERIALS AND METHODS

The prototype LFD developed is based on a direct immunoassay and it uses three microspheres: blue microspheres which are covalently linked to E2 protein of CSFV, red microspheres which are covalently linked to VP72 protein of ASFV, and green microspheres which are used as test control. For the capture reagent, the two proteins are adsorbed on the nitrocellulose membrane to form two parallel test lines. In addition, the test contains a third line formed by a specific MAb for the control protein.

The antibodies present in the sample react with the latex particles which are coated with specific target proteins. This complex (latex particles/protein/antibodies) flows through the membrane. Depending on the type of antibody present in the sample, different coloured lines will appear. These lines will be used to interpret the test results. The entire test procedure can be completed in 10 minutes.

RESULTS

In order to perform a first validation of the test, 22 and 30 experimental serum samples obtained from different experimental infections of ASF and CSF respectively, were tested and compared with two validated commercial ELISA to ASF and CSF. Moreover 50 serum samples from ASF and CSF from free areas were also tested. The diagnostic sensitivity and specificity values for the developed Lateral-Flow Device were higher than 99% and no cross-reactivity between viruses were observed.

CONCLUSIONS

Even though more samples need to be tested, these preliminary results suggest that the developed duplex LFD provides a reliable method for rapid detection of anti-ASF and anti-CSF antibodies. This one-step assay can be performed rapid and easily without special equipment directly on the field and can provide a useful tool in situations where laboratory support and skilled personnel are limited.

ACKNOWLEDGEMENTS

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ORAL: PB1-F2 protein and N66S mutation in swine influenza viruses

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Key words: PB1-F2 protein, N66S mutation, swine influenza viruses

Recently a novel protein, PB1-F2, encoded by an alternative ORF of the PB1 gene, has been reported. This protein is exclusively found in infected cells, is apparently not incorporated into the virion and contributes to virulence in different ways: promotion of inflammation, up-regulation of viral polymerase activity by its interaction with the PB1 subunit and induction of apoptosis in a cell-type dependent manner. The induction of apoptosis depends on the PB1-F2 localization in the mitochondria, where it decreases the mitochondrial membrane potential. The mitochondrial localization in proteins of 79 and 90 aa and their correlation to the presence of a mitochondrial targeting sequence located in the C-terminal portion were demonstrated. This suggested that proteins below 79 aa may lose their proapoptotic ability. It was also identified that a single residue in PB1-F2 (N66S) affected the severity of the 1918 pandemic and H5N1 influenza virus. Specifically, S at position 66 was associated with high pathogenicity, whereas N at that position resulted in decreased virulence. This study analyzed the characteristics of PB1-F2 of 120 Italian swine influenza viruses (SIVs) divided in 44 H1N1, 28 H1N2, 34 H3N2, 12 2009/pandemic (pdm) H1N1 and two reassortant strains (H3N1 and H1pdmN2). These strains were compared with sequences of SIVs from Europe (EU) and America (US) retrieved in GenBank for a total of 852 sequences. For each subtype, tree databases were created depending on the continent of isolation: H1N1 -121 EU, 151 US; H1N2 - 52 EU, 131 US; H3N2 + H3N1 - 55 EU, 329 US; H1N1pdm + H1pdmN2 - 13 EU; We analyzed the length variation of the protein dividing them in proteins < 79aa and ≥ 79 aa, which are supposed to be functional, and the presence of N66S mutation. Moreover, overall and site-specific dN/dS were estimated using SALC and FEL methods available at the Data-monkey facility. Selection pressure was performed on strains having a complete protein of 90 aa and considered separately European (94 seq) and American (142 seq) SIVs belonging to the three subtypes. The major part of SIVs (80,6%) had a coding reading frame for a functional protein ≥ 79 aa, but the H1N1 SIVs have the highest percentage (44,1%) of non-functional proteins, mainly due to US (58,3%) strains. However the major part of these strains possess genes coding for the C terminus of the protein according with the hypothesis that this part can be expressed independently. The other two subtypes showed functional proteins in 94,5% for H1N2 and 94,3% for H3N2. All the H1N1pdm and H1pdmN2 had a truncated protein of 11aa. Positive selection was then inferred for the entire region of PB1-F2 of US and EU strains, which were subjected to a similar and strong positive selection revealed by the high average dN/dS ratio (4,45 for US and 4,57 for EU). Moreover, site-by-site analysis revealed 28 sites under positive selection for US and 32 sites for EU strains. The presence of N66S mutation has been evidenced in a low percentage of SIVs (2,8%) but with very different frequencies if we analyzed separately the databases. A low frequency (0,2% for US and 2,2% for EU) was observed in all SIVs except the Italian strains, which showed this mutation in 20,7%. Interestingly, this percentage increased to 35,7 if we consider the H1N2 subtype and all the strains showing this change belonged to the Italian reassortant H1N2 strains with a N2 deriving from the recent human H3N2. Further studies should be performed to better investigate the significance of this mutation in the Italian strains.

ORAL: Detection of swine influenza virus in oral fluids collected from experimentally infected pigs

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CODA-CERVA¹

Key words: SIV, Oral fluid, Detection

Introduction

Swine influenza A virus (SIV) is a highly contagious respiratory swine disease with an important economic impact in swine industry. The 2009 pandemic H1N1 virus, which was a reassortant between swine influenza viruses with human and avian influenza viruses, and the possible role of pigs as intermediary hosts or mixing vessel hosts for both avian and human influenza viruses led to an increased surveillance of SIV. However, the most common problem in monitoring SIV remains the short detection period. Since the presence of SIV in oral fluids has only recently been described, it was our objective to evaluate the detection of SIV RNA in oral fluid samples compared to the more commonly used nasal swabs.

Material and Methods

Ten individually housed Belgian Landrace piglets, free of SIV on the basis of screening for the presence of antibodies against SIV by ELISA and routine testing by qRT-PCR, were manually restrained and 0.5 mL of $10^{8.3}$ egg infectious doses (EID₅₀)/mL of a H1N1 strain (sw/Gent/28/10) was administered in each nostril by aerosol inoculation. Six pigs were not inoculated and served as negative control animals. Oral fluid samples were collected at 0, 1, 2, 3, 5, 7, 10, 14 and 21 dpi by means of cotton ropes according to procedures described by Prickett et al. (2008). Nasal swabs were obtained at the same time points and placed in a vial containing 1 mL MEM medium. All samples were immediately chilled on ice, centrifuged at 3000 rpm for 10 minutes and stored as aliquots at -80 °C until use.

SIV RNA from all samples was extracted with the MagMAX Pathogen RNA/DNA kit (Life Technologies) and amplified with the Vetmax Gold SIV detection kit (Life Technologies). All PCRs were run on a LightCycler 480 Real-time PCR system (Roche). In each run, positive and negative controls were tested, along with the samples.

Results

Nasal swabs and oral fluids from all animals collected before the start of the experiment were SIV negative by qRT-PCR. When the nasal swab extraction products were amplified, the H1N1 strain could be detected from 1 till 5 dpi with a detection rate of 100% at 1 to 3 dpi and 20% at 5 dpi. When the oral fluid extraction products were amplified, detectability went from 100% at 1 to 3 dpi; 80% at 5 and 7 dpi; 50% at 10 and 14 dpi to 25 % at 21 dpi. The nasal swab qRT-PCR Ct values ranged from 30.04 to 36.59 whereas the oral fluid Ct values ranged from 26.72 to 37.16. All samples from the negative control animals remained negative throughout the trial.

Conclusion

Our results show that SIV can be detected by qRT-PCR for a longer time period in oral fluids than in nasal swabs. Therefore the collection of oral fluids using ropes could represent a sensitive method for the diagnosis, monitoring and surveillance of SIV in pig populations.

This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (RF 10/6235).

ORAL: Evaluation of a simple assay format for the detection of foot and mouth disease virus using reverse transcription loop mediated isothermal amplification

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Key words: FMDV, LAMP, LFD, Penside Test

Rapid, field based diagnostic assays are extremely useful tools for the control of foot-and-mouth disease (FMD) outbreaks. Current approaches involve either direct detection of FMDV antigen with a lateral flow device (LFD), which has a low analytical sensitivity, or use of portable real-time RT-PCR equipment that has high sensitivity but is expensive.

The objective of this study was to modify a FMDV RT-LAMP assay, to enable detection of dual-labelled products with a LFD and evaluate simple protocols that might be deployed for use in the field. Importantly, this study demonstrated that FMDV RNA could be detected from raw epithelial suspensions, without the need for prior RNA extraction. Furthermore, the RT-LAMP assay could be performed in a water bath without the requirement for a thermal cycler or using a field based isothermal platform (Genie II). The limit of detection was demonstrated to be equivalent to that of a laboratory based real-time RT-PCR assay, and to be 10,000 fold more sensitive than a FMDV-specific LFD currently available for field diagnosis. The FMDV LAMP-LFD was also used to detect FMDV in the air surrounding infected cattle, pigs and sheep after initial collection with portable samplers followed by minimal sample processing, including detection of FMDV from pre-clinically infected pigs.

This study describes an approach that can be used as the basis for a rapid and low cost assay for detection of FMDV. This method has equivalent analytical sensitivity to that of the real-time RT-PCR assay used for routine diagnosis. These results may lead to the development of invaluable tools for controlling FMDV outbreaks and provide “proof of concept” for use of LAMP assays to tackle other challenging diagnosis scenarios encompassing veterinary and human health.

ORAL: Sample preparation and nucleic acid extraction: the bottleneck in application of pen-side test systems (?)

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FLI¹

Key words: pen-side tests, direct RT-qPCR, sample preparation, nucleic acid extraction

Over the past few years, a huge variety of innovative pen-side technologies have been developed for analytical testing directly in the field. Since the majority of these approaches focused on the amplification and detection strategy, sample preparation and nucleic acid extraction remain a bottleneck in the application of pen-side tests.

Under resource-limited settings in the field, direct amplification of nucleic acids from untreated samples would be the most suitable strategy. Therefore, we evaluated the applicability of real-time quantitative RT-qPCR directly from untreated samples using a newly developed kit from Tetracore, Inc. However, since most of the available amplification techniques require extraction and concentration of the target nucleic acid, a rapid magnetic particle based extraction protocol has been developed and evaluated using a KingFisher® Duo system (Thermo). This newly developed protocol was compared to fully automated sample preparation using the EZ1 advanced XL instrument (Qiagen). For the evaluation of the different methods, samples from animals infected with Schmallenberg virus or Bovine viral diarrhoea virus were comparatively analyzed.

First experiences with the direct RT-qPCR kit showed promising results. Further studies are ongoing to identify the performance of this technique with different starting materials like whole-blood, serum, saliva and nasal swabs as well as the applicability for different virus species.

The well-established EZ1 advanced XL instrument allowed the extraction of nucleic acid from all relevant matrices and viruses without limitations. Furthermore, the fully automated system reduced handling steps to a minimum due to the application of prefilled reagents and preprogrammed protocols, which is an advantage for field use by untrained personnel. The shortened KingFisher® Duo extraction protocol required more time for sample preparation, but it allowed completing RNA extraction within 10 minutes without significant loss of sensitivity. This method therefore represents a promising tool for accelerated and simplified sample processing. The suitability of the two extraction techniques for integration in pen-side test systems will be further evaluated using previously developed rapid amplification and detection strategies.

In summary, we will present different strategies for overcoming the bottleneck “nucleic acid release” in molecular pen-side tests. A newly developed rapid nucleic acid extraction protocol using a KingFisher® Duo system will be presented and compared to full-automated extraction using the EZ1 advanced XL instrument. Furthermore, the suitability of the recently introduced direct RT-qPCR kit from Tetracore will be discussed with regard to future integration in molecular pen-side tests.

Session 3: Antivirals & Vaccines

ORAL: DEMONSTRATION OF EFFICACY AGAINST CHALLENGE OF AN INACTIVATED SCHMALLEMBERG VACCINE IN CATTLE

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Key words: Schmallenberg, Vaccine, Efficacy, Cattle, Viraemia

INTRODUCTION

In August 2011, outbreaks of an unknown disease of cattle were reported in both the Netherlands and Germany. From December 2011, abortion and foetal abnormalities, were reported in sheep and cattle in several European countries. A new virus was identified in November 2011 and was associated with both conditions. This agent was named 'Schmallenberg virus' (SBV) after the German town where the virus was first identified. Schmallenberg virus is in the Simbu serogroup of the Orthobunyavirus group. This group of viruses includes many viruses occurring in the Tropics. None had been previously identified in Europe. Although some uncertainty remains on the transmission of SBV, it seems primarily spread by biting insect vectors (midges/mosquitoes).

Herein, we present the results of a vaccination / challenge study demonstrating that administration of an inactivated SBV vaccine in 2 injections, 3 weeks apart, to cattle was able to prevent viraemia.

MATERIAL AND METHODS

Ten weaned calves were randomly allocated to one group of 5 vaccinates and one group of 5 controls. Vaccinates were subcutaneously treated once on day 0 and D21, with 1 mL of an inactivated SBV vaccine (Merial). The other group was left unvaccinated and served as control. Twenty one days after completion of vaccination (ie D42), all calves were challenged with a virulent SBV strain. All calves were then monitored for rectal temperature, clinical signs and viraemia (quantitative RT-PCR) from D43 to D52.

RESULTS

Hyperthermia : an increase of temperature, which temporally corresponded to the period of viraemia was observed in the control group. No increase of temperature was observed in the vaccinated group.

Clinical signs : no significant clinical sign was observed in any of the groups.

Viraemia (qRT-PCR) : All controls were found positive on at least 3 consecutive days. None of the vaccinated animals was ever detected positive.

CONCLUSION

In the present study, vaccination (2 injections, 3 weeks apart) of cattle with the product tested provided full and significant virological protection against a SBV challenge.

ORAL: Development and evaluation of a DIVA subunit vaccine against Bluetongue virus serotype 8 in cattle

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Key words: Bluetongue, DIVA, vaccine, efficacy, cattle

The incursion and circulation of more than nine different serotypes of Bluetongue virus (BTV) in central and northern Europe over the past fifteen years has resulted in decreased animal welfare and production losses. Vaccination is crucial for controlling BTV since conventional biosecurity measures have limited impact on vector-borne diseases. However, as current vaccines have no accepted characteristic allowing for the differentiation of infected from vaccinated animals (DIVA), the use of these products impairs sero-epidemiological surveillance and thus a quick return to BTV-free status as well as serological monitoring of vaccine efficacy in the field. Small ruminants show the most severe clinical signs following BTV infection, but cattle are the virus's main amplifying host and when infected with certain BTV strains, such as BTV-8, they can also display clinical signs. However, protective bovine immune responses against BTV are poorly characterized and there is limited information available about the ability of commercial and experimental vaccines to induce clinical and virological protection against BTV infection in cattle. Our objective was to develop and evaluate the efficacy of a novel DIVA subunit vaccine against BTV-8 in cattle, that later may be developed into a multi-serotype vaccine. We produced recombinant VP2 of BTV-8 and NS1 and NS2 of BTV-2 and formulated the vaccine with the purified proteins and an ISCOM-based adjuvant (SubV). The DIVA characteristic of this vaccine is based on the detection of VP7 antibodies in infected animals, thereby enabling the detection of BTV infection of any serotype.

In the first study, we evaluated the immunogenicity of SubV in comparison with a commercial inactivated vaccine against BTV-8. Cows were subcutaneously immunized twice at a 3-weeks interval with SubV (n=5), the commercial vaccine (n=5), or with placebo (n=5). Humoral and cell-mediated immune responses were monitored before each vaccination as well as at 3 and 6 weeks after the second immunization. Both vaccines induced similar serum neutralizing antibody titers and the specific IgG1 antibody responses detected against VP2, NS1, and NS2 were strongest in cows immunized with SubV. Furthermore, serotype cross-reactive humoral and cell-mediated immunological responses to BTV-8 were detected to NS2 and NS1 of BTV-2, respectively. In the second study, calves were immunized twice at a 3-weeks interval with either SubV (n=6) or placebo (n=6), then challenged with BTV-8 three weeks later in a Biosecurity level 3 facility. Whereas controls developed strong viremia and clinical signs of Bluetongue disease including fever, mucosal congestion, and stiffness, vaccinated calves did not show any of these signs and were strongly protected against BTV infection. Preliminary data indicate that vaccinated animals developed very strong serum neutralizing antibody responses following vaccination. Humoral and cellular immunological analyses are ongoing and clinical, virological, and immunological results of both studies will be presented during the meeting.

Taken together, these data indicate that our novel subunit DIVA vaccine composed of only three BTV proteins is very effective against BTV-8 infection in cattle, and will also enable serological monitoring of any BTV serotype in circulation in vaccinated populations. Furthermore, the serotype cross-reactivity of NS1 and NS2 suggests that this vaccine may be expanded in the future to target multiple BTV serotypes through the addition of purified recombinant VP2 of other serotypes.

ORAL: Attenuation of RVFV infection in mice upon inoculation with FMDV synthetic RNAs

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Key words: Rift Valley Fever, non-coding FMDV RNAs, innate immunity

In this work we have addressed the effect of synthetic, non-infectious, RNA transcripts, mimicking structural domains of the non-coding regions (NCRs) of the foot-and-mouth disease virus (FMDV) genome, on the antiviral, as well as innate and adaptive immune responses against infection by Rift Valley fever virus (RVFV). Groups of 5 mice were inoculated intraperitoneally with 200µg of synthetic RNA resembling the S-regulatory element, the internal ribosome entry site (IRES) and the 3'-NCR of the FMDV genome. The RNA inoculation was performed 24 hours before (-24h), 24 hours after (+24h) or simultaneously (0h) to the challenge with a lethal dose of the virulent RVFV isolate 56/74. Administration of the IRES element provided higher survival rates than administration of S or 3'-NCR elements either at -24h or +24h after challenge. In contrast, when RNA inoculation and viral challenge were performed simultaneously, all mice survived in both IRES and 3'-NCR groups, with an 80% survival in mice receiving the S-fragment RNA. Surviving animals from each group were then re-challenged with a lethal dose of RVFV. Mice that survived this challenge showed neutralizing antibody responses indicative of previous viral replication. All three RNA fragments were able to induce the production of systemic antiviral and proinflammatory cytokines, as early as 4h after inoculation. These data show that triggering of intracellular pathogen sensing pathways is a potential alternative for the prevention or therapeutic treatment of interferon-sensitive viral infections.

ORAL: Development of reverse genetics for African horsesickness virus

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Central Veterinary Institute of Wageningen UR¹; Deltamune²

Key words: African horsesickness, orbivirus, vaccine development, reverse genetics

African horsesickness (AHS) is a notifiable disease of equines. The course of disease is extremely fast and mortality in susceptible horses can be extremely high (>90%). The disease is less severe in African donkeys, zebra's and mules. Horses in European countries can be roughly divided in professional breeding and sport horses, and hobby horses. It can be expected that AHS introduction in Europe will result in a very fast spread of disease over long distances due to the high frequency of (inter)national movements of horses, which will hamper AHS control in an early stage.

Like bluetongue virus (BTV) and Schmallenberg virus, AHS-virus (AHSV) is transmitted by bites of culicoides midges. Recent outbreaks in Europe of culicoides-borne diseases in ruminants have initiated re-evaluation of emergence of AHS in Europe, although it is unknown whether local culicoides species will spread this devastating disease. Evidently, AHS introduction will have a huge economic and socio-emotional impact on the entire European community, irrespective of actual spread of the disease. Therefore, rapid, adequate and acceptable control measures are needed. Control of AHS without vaccination will be difficult or even impossible. Currently, there is no registered AHS vaccine available in Europe. For the last time in the 1980ties, vaccine virus AHSV4LP was successfully and safely used as monovalent vaccine to control an AHSV4 outbreak in Spain and Portugal. This vaccine has been developed by traditional methods in South Africa (Erasmus, 1973). AHSV is one of the most important virus species in the genus Orbivirus of the Reoviridae family. The AHSV serogroup consists of nine serotypes showing little cross protection. Fundamental and applied AHS research on virulence, vector competence, and other viral functions is hampered due to the lack of a reverse genetics system. Alternative methods have been developed to incorporate one or more synthesized segments in the AHSV genome (Matsuo et al., 2010). After our recent success in development of reverse genetics for several BTV serotypes (van Gennip et al., 2012a), we intended to develop a similar system for AHSV.

To develop such a system, the first cell culture adapted vaccine for serotype 4 (here named AHSV4LP) was sequenced by sequencing using 454 technology (Potgieter et al., in prep.). The consensus sequence was used for cDNA synthesis, and subsequently attempts were undertaken to rescue virus from runoff transcripts. Unfortunately, we failed to rescue complete 'synthetic' AHSV4LP (rgAHSV4) with the successful method for BTV. Therefore, a slightly different method was developed which was indeed successful in rescue of rgAHSV4. Subsequently, the exchange of one segment 2 (Seg-2[VP2]) or two segments (Seg-2 and Seg-6[VP5]) of several AHSV serotypes was demonstrated. Further, we were able to introduce mutated genes in rgAHSV4LP. Summarizing, our developed reverse genetics system for AHSV can be used as genetic modification system to generate multi-segment AHSV reassortants and AHSV mutants.

Reverse genetics based on vaccine virus AHSV4LP will be exploited to investigate functions of AHSV proteins. Further, we intend to develop AHS vaccine that will meet criteria like safety, efficacy, onset of duration, duration of immunity and DIVA by genetic modifications. In addition, the same vaccine backbone will be used to exchange serotype defining proteins VP2 and VP5 for vaccine development for other serotypes, as was done for BTV (van Gennip et al., 2012b). An update of the current AHS research will be presented and discussed.

ORAL: 'EPIZONE' Design of novel vaccines based on virus-like particles (VLPs) derived from calicivirus: multimeric presentation of epitopes

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Key words: Vaccine, VLPs, Antibodies, IFNg, Influenza

Virus-like particles (VLPs) have received considerable attention due to their potential application in veterinary vaccines and, in particular, VLPs from rabbit haemorrhagic disease virus (RHDV) have been shown to be good platforms for inducing immune responses against an inserted foreign model cytotoxic epitope (CTL) in mice (2).

Rabbit Hemorrhagic Disease virus (RHDV) is the causative agent of a highly infectious disease of domestic and wild rabbits. RHDV is the prototype strain of the genus Lagovirus within the family Caliciviridae, a group of nonenveloped, icosahedral viruses. Caliciviruses are composed of 180 copies of a single capsid protein.

Our research group has set up a system for the production of large amounts of VLPs derived from RHDV, in insect cells (1, 3). We have identified three independent locations within the gene of the RHDV capsid protein (VP60), where we can insert foreign sequences spanning at least 42 aminoacids in length, without affecting the ability of the resulting chimeric protein to self-assemble into VLPs. Our long term goal is to develop RHDV VLPs as a delivery system for the multimeric presentation of immunogenic epitopes derived from pathogens relevant for animal health.

The aim of the present study was to analyze the potential of chimeric VLPs to induce specific immune responses against foreign epitopes from Feline Calicivirus (FCV) and Influenza virus. To this end we generated chimeric VLPs harbouring such foreign epitopes in different insertion sites. Groups of mice were inoculated with the chimeric VLPs and we analyzed the humoral and cellular responses induced. Furthermore, we assessed the protection conferred by chimeric VLPs harbouring influenza epitopes, against viral challenge. The results obtained indicate that the chimeric RHDV VLPs are able: i) to induce potent antibody responses against foreign B-cell epitopes inserted within VLPs (including neutralizing antibodies) ii) confirm their potential for inducing immune responses against CTL epitopes and iii) the immune response elicited by chimeric VLPs harbouring influenza epitopes confer full protection against a lethal challenge with influenza virus.

In conclusion, RHDV derived-VLPs constitute versatile scaffolds for multimeric antigen display. Moreover, the immunogenic properties of the chimeric RHDV VLPs suggest the potential suitability of these constructions for new vaccine development against animal and human viral infections.

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ORAL: Alternative foot-and-mouth disease vaccine potency tests based on serology and payload

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Key words: vaccine potency, antigen payload, FMDV

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Objective:

This study addresses alternatives to the standard European in vivo vaccine potency test for cattle. Alternative serology-based vaccine potency tests have been published. Now the effect of antigen payload on vaccine potency is investigated.

Materials and methods:

A single batch of the FMD O1 Manisa reference strain was used to produce 5 batches of monovalent double-oil emulsion vaccine with different antigen payloads. Each vaccine batch was tested in triplicate in a standard PD50 test. Cattle serum samples were collected at 21 days post vaccination and antibody titers were determined. The in vivo observations and serological data were analysed and logistic regression models were constructed for assessment of alternative vaccine potency.

Results:

The challenge trials resulted in overall PD50's of 8.8, 4.3, 10.5, 20.1 and 17.7 for the respective antigen payloads of 25%, 50%, 100%, 200% and 400%.

Different logistic regression models were compared for their ability to differentiate between protected and unprotected animals. The model including all parameters (antigen payload, vaccine dose and antibody titer) had a sensitivity and specificity of 76% and 90%, respectively. For the models based solely on antibody titer or antigen payload the sensitivities were 79% and 47% and the specificities were 80% and 81%, respectively.

For models created with the subset of data containing only animals that received the full 2ml vaccine dose the model combining antigen payload and antibody titer performed best (sensitivity: 97%, specificity: 100%), however differences with the model correlating only antibody titer to in vivo protection were small (sensitivity: 93%, specificity: 100%) and the model based solely on antigen payload had a lower sensitivity of 80%.

Discussion:

Vaccine potency can be assessed with alternative tests to the in vivo test. However the confidence of the estimated potency will be influenced by the characteristics of the chosen alternative vaccine potency test.

Session 4: Legislation & Intervention Strategies

ORAL: Assessing the effectiveness of surveillance system components for vector-borne diseases: The example of bluetongue (BT)

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Key words: Surveillance, Bluetongue, Scenario tree modeling

Vector-borne diseases (VBD) are infections transmitted by an arthropod, such as a tick, mosquito or midge. The globalization due to increasing international trade and related movement of animals as well as the ongoing climate change are expected to increase the risk of (re)establishment of several VBD in Europe. Recent outbreaks such as the bluetongue virus serotype 8 (BTV8) in Northwestern Europe, 2006 to 2008, as well as the recent emergence of Schmallenberg virus (SBV) confirm that this risk is real. Effective surveillance systems are essential for the early detection of VBD and to monitor dynamics after an incursion as well as – ultimately – to demonstrate eradication or freedom from infection. The objective of this project was to assess and compare the past and ongoing surveillance systems for BT with the purpose of “demonstration of freedom from infection” for the following countries: Belgium, Switzerland, Netherlands, Germany, Denmark, Sweden and Norway. The project is funded by the EMIDA ERA network. The effectiveness of individual and combined surveillance system components (SSCs) was quantified using scenario tree modeling and simulation. The possible SSCs were: 1) serological survey in cattle, 2) serological survey in sheep, 3) bulk milk serology in dairy cattle, 4) direct pathogen detection in blood samples (antigen detection), 5) vector monitoring (to determine the vector activity period), 6) reporting of clinical signs in cattle and 7) reporting of clinical signs in sheep. To obtain the required input data, a comprehensive questionnaire was developed and completed for each country using telephone interviews. The questionnaire information was used to parameterize the generic scenario tree model to reflect each specific component of each participating country. This generic model was focused on herd level and various risk factors were considered: 1) species (cattle vs. sheep), 2) geographical risk areas (high risk vs. low risk), 3) production type (dairy vs. meat), 4) outdoor access of the animals, and 5) vaccination status of the population. The output of the model was the sensitivity of individual components as well as the combined sensitivity of all components; this sensitivity is the probability of the detection of at least one case during one year.

Results for Belgium and Switzerland will be presented. Belgium’s surveillance was designed to detect 2% BT within-herd prevalence with 95% confidence. The use of serological surveys as well as direct pathogen detection (RT-PCR) reached in initial simulations a component sensitivity of 87%. Switzerland aimed to detect 2% BT within-herd prevalence with 99% confidence. The used serological survey and parallel direct pathogen detection (RP-PCR) reached in initial simulations a component sensitivity of 88%.

The results showed that despite different designs, surveillance systems can achieve comparable detection performance. It highlights that outcome-based surveillance targets provide flexibility in the design of components, taking into account local situation, feasibility and risk factors, as required. In addition to effectiveness, cost is also a key attribute of surveillance. As part of this project, cost-effectiveness will also be assessed.

ORAL: Emergence and time course of spreading of Schmallenberg virus in late summer 2011 in a Belgian naïve sheep flock

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Key words: Schmallenberg virus, Emergence, Sheep, 2011, Belgium

In late summer 2011, Schmallenberg virus (SBV) was circulating in Germany and in the Netherlands and rapidly spread among neighboring countries. During the lambing periods of January and March 2012, signs of transplacental passage of SBV were detected among ewes of the sheep flock of the University of Namur (composed by ~400 ewes and located in the southern part of Belgium). Still- or newborn lambs (n=28) presenting arthrogryposis and scoliosis were observed in January 2012 while abortions of macroscopically normal fetuses (n=8) were observed in March 2012. No SBV cases were detected during lambing periods in October 2011, November 2011 and May 2012. By considering occurrence of SBV-affected lambs and fetuses at different lambing periods as well as results of serum neutralization test (SNT) and RT-qPCR performed on blood samples collected in sentinel sheep during summer and autumn 2011, it was aimed to determine the time point of emergence of SBV as well as the duration of spreading among sheep.

Ewes lambing in January 2012 (n=99) were mated to the rams from 19th August until 5th September 2011, ewes lambing in March 2012 (n=84) from 13th October until 31st October 2011 and ewes lambing in May 2012 (n=80) from 5th January until 23rd January 2012.

During the lambing period of January 2012, 17% (28/163) of produced lambs presented SBV-related deformities. In March 2012, 5% (8/150) of lambs were aborted fetuses. Results of SNT showed that 100% of sentinel sheep (63/63) were seronegative from 10th May until 6th September 2011. Three sentinel sheep were RT-qPCR positive on 6th September 2011. Lambs born in late October 2011 presented anti-SBV colostral antibodies in serum samples collected 36h after colostrum intake. SNT performed on serum samples collected in early February 2012 showed 99% (417/422) of SBV seropositivity.

Results of SNT and RT-qPCR suggest that SBV-infection occurred in early September 2011. By considering the infection time frame compatible with induced deformities in ovine fetuses defined in case of Akabane virus infection (30 to 50 days of pregnancy), ewes giving birth to deformed lambs in January 2012 could have been infected between 18th September and 26th October 2011. Ewes whose lambs were born in late October 2011 probably underwent SBV infection mid-October at latest considering the presence of anti-SBV colostral antibodies in lambs' serum. For ewes lambing in March and May 2012, SBV infection probably occurred before or during early gestation leading to low or no impact on lambings. Accordingly, SBV emergence occurred at begin of September 2011 and ended in late October 2011. In 2012, Claine et al. (2013) demonstrated that the time course of SBV spreading among a sentinel sheep flock (n=50) composed by female lambs born from previously immunized ewes started in mid-July and ended in mid-October. It appears that SBV emergence occurred later in 2011 but lead to more rapid infection of the naïve flock. These results raise questions about the SBV origin and ability to persist from one year to another.

Claine F, Coupeau D, Wiggers L, Muylkens B, Kirschvink N. Schmallenberg virus among female lambs, Belgium, 2012. *Emerg Infect Dis* 2013;19:1115-7.
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ORAL: Longitudinal monitoring reveals long-lasting virus-neutralizing antibodies against Schmallenberg virus in adult cattle, The Netherlands, 2012-2013

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Key words: Schmallenbergvirus, (maternal) antibodies, cattle

Introduction: Schmallenberg virus (SBV), a novel Orthobunyavirus, has swept through the major part of Europe in the period 2011-2013, reaching as far North as Finland, Turkey in the East, and Spain in the South. A vaccine against SBV has been developed and may be a possible preventive instrument against infection. Presently, there is no data available indicating that natural SBV infection results in long-term immunity. Therefore, it is of interest to know how long (protecting) virus-neutralizing antibodies are present in naturally infected animals. Newborn calves acquire passive immunity from their dams by ingestion and absorption of antibodies present in colostrum. Passive immunity can block the production of serum antibodies when vaccine is administered to calves with maternal antibodies. Therefore, it is useful to know how long it takes for maternal antibodies against SBV to disappear in young animals. At a dairy herd, where SBV RNA was detected in a high proportion of *Culicoides* caught in the autumn of 2011, all animals were blood-sampled four times in a period of one year, enabling us to examine the development and the course of neutralizing-antibodies against SBV over time.

Material and Methods: after retrospective detection of SBV RNA in *Culicoides* biting midges, caught at a dairy herd in the autumn of 2011, all ruminants of that herd were blood sampled before the start of a new vector season on 19 April, 2012. The dairy herd consisted of 60 milking cows, and 50 heifers and calves. Blood samples were sent to CVI and tested for antibodies using a virus neutralization test (VNT). Dilutions tested started at 1:4 and ended at 1:512. Titers were expressed on a 2 Logarithm-scale. The whole herd was again blood sampled on 17 September, 9 December, and a last time on 23 April 2013.

Results: At the sampling on 19 April 2012, all adult cows tested positive, only four young calves tested negative. After one year, 98% of the adult cows still tested seropositive, while 50% of the young cows tested positive. Mean₂ Log VNT titer of the adult dairy cows dropped from 8.3 to 6.7 in a period of one year. Assuming that the adult cows became infected around the time that SBV RNA was detected in *Culicoides* on 14 September 2011, this means that at least 19 months after presumed infection cows still had antibodies against SBV. Thirteen calves that were blood sampled \leq 30 days after birth had a mean 2 Log VNT titer of 8 (min: 6.5; max: 9.5). Mean length of time between birth and first appearance of a seronegative status in young calves was 6 months (min: 4 months; max: 8 months). There was a positive relationship between the VNT titer of the dam and the VNT titer of the corresponding calf. Twelve out of a total of 107 cows showed a seroconversion (two or more titer-steps) between two or more sampling occurrences, which can be regarded an indication for circulation of SBV and/or of reinfection in 2012. In our study there is no indication for virus circulation in the winter of 2012-2013. A total of three calves were stillborn during the one-year study period on the farm, but all without the characteristic malformations representative for an SBV infection. This was confirmed by gross pathology at necropsy. Tissue samples of these calves tested negative for SBV by RT-PCR.

ORAL: Detection of Schmallenberg Virus Rna in Bull Semen

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FLI¹

Key words: Schmallenberg virus / real-time RT-PCR / semen / bovine / experimental infection

Purpose: Schmallenberg Virus (SBV), an Orthobunyavirus belonging to the Simbu serogroup, was first discovered in Germany in autumn 2011. In analogy to Akabane virus, SBV-transmission by semen has primarily been considered negligible. However, SBV detection in semen could implicate considerable economic consequences for stock-bull breeders.

Methods: A large number of different manual and automatized extraction procedures were comparatively validated using dilution series of spiked semen, blood samples and minimum essential medium (MEM) as control. Furthermore, SBV-RNA-positive semen samples were used for re-testing. The preferred method was submitted to regional labs in Germany and other laboratories worldwide. In a first ring trial with SBV-positive semen samples the favourite extraction method was further verified.

Using this most suitable extraction procedure, frozen semen collected between May 2012 and November 2012 from 95 seroconverted bulls was analysed for SBV-RNA with real-time RT-PCR (RT-qPCR).

Results: The highest analytical sensitivity for the extraction of SBV-RNA in semen was found for the Trizol® LS Reagent lysis combined with subsequent purification of viral RNA based on magnetic beads. All participating laboratories using the recommend method in the SBV PCR ring trial produced very sensitive results.

At present, 746 semen batches from 95 different SBV-infected bulls were analysed with the recommend extraction method. 29 batches from 11 bulls reacted positively in RT-qPCR (Cq-values: 26–37). At least in one bull, intermittent virus excretion could be observed. In 4 of the 11 bulls, SBV-RNA positive semen was coincidentally detected with first SBV-antibodies.

Subsequent in vitro and in vivo studies investigating the infectivity of the SBV-positive semen samples are ongoing.

Conclusions: This study provides validated methods for SBV-RNA detection in bull semen. Due to the PCR-positive results despite seroconversion and the potentially intermittent virus excretion, the results of this study might have consequences on trade of semen from bulls in SBV-affected regions.

ORAL: Schmallenberg virus: Results of Belgian cross-sectional serological surveys

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Key words: Schmallenberg virus; Seroprevalence; Belgium; Cattle; Sheep

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A cross-sectional serological study was conducted in the Belgian domestic ovine and caprine domestic population to examine the situation at the end of the first wave of the Schmallenberg virus (SBV) epidemics. One thousand eighty two sheep samples which were collected from 83 flocks all over Belgium between November 2011 and April 2012 were tested with an ELISA kit (ID Screen® Schmallenberg virus Indirect ELISA kit; Montpellier, France) at the Veterinary and Agrochemical Research Centre (CODA-CERVA). The overall between-herd seroprevalence was 98.03% (95%CI: 97.86-98.18) and the within-herd seroprevalence at 84.31% (95%CI: 84.19-84.43). An intra-class correlation coefficient (ICC) of 0.34 was found, indicating that the correlation between two animals within a flock with respect to SBV result was high. A spatial cluster analysis identified a cluster of six farms with significantly lower within-herd seroprevalence in the south of Belgium compared to the rest of the population ($p=0.04$). It was shown that seroprevalence was associated to flock density and that the latter explained the presence of the spatial cluster.

Additionally, 142 goat samples from 8 different flocks were tested for SBV-specific antibodies. The within-herd seroprevalence in goats was estimated at 40.68% (95%CI: 23.57-60.4%).

A total number of 11,635 cattle serum samples originating from 422 herds collected between 2 January and 7 March 2012 were tested using the previously mentioned ELISA kit. Between-herd seroprevalence in cattle was estimated at 99.76% (95%CI: 98.34-99.97), within-herd seroprevalence at 86.3% (95%CI: 84.75-87.71) and the ICC at 0.3. Seroprevalence was shown to be statistically associated to the animal's age ($p<0.0001$): with 64.9% (95%CI: 61.34-68.3) estimated for the 6-12 months of age, 86.79% (95%CI: 84.43-88.85) for the 12-24 months of age and 94.4% (95%CI: 93.14-95.44) for the animals older than 24 months.

We can conclude that, after the first episode, almost every Belgian domestic ruminant has already been infected by SBV. In consequence, if we extrapolate from the other Simbu serogroup viruses, almost all host animals should have developed post infection protective immunity against SBV.

In addition, 7,130 cattle serum samples from 188 herds collected between 1st January and 28 February 2013 were also tested with the ELISA kit. Considering that a herd is positive if at least one sample in the herd was positive, all the herds in the current study were seropositive. Within-herd seroprevalence was estimated at 65.66% (IC95%: 62.28-69.04). Based on the results of the current study, a statistically significant ($p<0.05$) decrease of the level of seroprevalence was observed between the beginning and the end of 2012. This trend was also observed in each of the different age categories. In addition, the seroprevalence level of the different age cohorts did not differ statistically between the two screenings. The results of the current study seem to indicate that the circulation of SBV has slowed down during 2012. This is supported by the fact that few new clinical outbreaks have been reported since summer 2012. The findings also showed that the immunity level of the population stays high after a year, even though, principally because of new-borns, part of the population will continuously remain susceptible.

ORAL: Surveillance of exotic mosquitoes, midges, ticks and their pathogens in Belgium in 2013

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Key words: invasive species, vectors, Culicidae, Culicoides, Ixodidae

Several vector-borne diseases (e.g. Theileriosis, Babesiosis, Bluetongue Virus (BTV), Schmallenberg Virus (SBV), West-Nile Virus (WNV)) are emerging in Belgium or other European countries and some are now endemic. The increase in international transport, the removal of quarantine measures within the EU zone and the growing tourism are responsible for the increasing risk of spread of pathogens and vectors. Climate change also exerts a significant influence on the survival and spread of arthropod vectors (*Dermacentor reticulatus*, *Aedes albopictus*, ..) as well as on the vector competence of these exotic, but also of endemic arthropods. Strengthening surveillance of exotic vectors (mosquitoes, biting midges (*Culicoides*) and ticks) and their pathogens in areas at risk of importation or spread and risk of virus transmission is therefore required. Following the implementation of invasive mosquito surveillance in Belgium in 2012, to evaluate the guidelines of the European Centre for Disease Prevention and Control (ECDC), the surveillance continued in 2013 and also included that of exotic biting midges and ticks, and their most important pathogens.

Objective 1: to detect possible foci of introduction and establishment of invasive mosquito species (IMS) at early stage in Belgium and to screen for pathogens.

- Passive and active sampling from April till November 2013 at platforms of imported used tyres, shelters/greenhouses for imported cutting plants (e.g. Lucky Bamboo), fruits and vegetables, main parking lots along highways at country borders originating from colonised areas, ports and airports.
- Screening of IMS for pathogens (WNV).

Objective 2: to detect possible foci of introduction and establishment of exotic midge species (*Culicoides*, *Ceratopogonidae*) (ECS) at early stage in Belgium and to screen for pathogens.

- Passive sampling from April till November 2013 at main parking lots along highways at country borders originating from colonised areas, shelters for imported fruits and vegetables, ports and airports.
- Screening of ECS for pathogens (BTV, SBV).

Objective 3: to monitor endemic midges (*Culicoides*, *Ceratopogonidae*) and emerging midge-borne diseases.

- Passive sampling of midges from April till November 2013 at a private site in rural area (Betekom).
- Screening of endemic *Culicoides* species for pathogens (BTV, SBV).

Objective 4: to monitor endemic and invasive tick species and emerging tick-borne diseases.

- Active sampling of the invasive tick *Dermacentor reticulatus* from January till June and from September till December 2013 at two colonised sites (De Panne & Moen) and at sites where its presence is suspected (Nature Reserves where cattle or horses are imported from colonised sites).
- Screening of endemic and invasive tick species for pathogens (*Theileria equi*, *Babesia canis*, *Babesia caballi*, *Borrelia*, Tick Borne Encephalitis Virus (TBE)).

The detailed protocol and preliminary results are presented and discussed.

October 3

Session 5: Emerging Diseases

ORAL: Arboviruses surveillance by mosquito screening in North-Eastern Italy in 2012 and 2013

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Key words: Arbovirus; West Nile virus; Usutu virus; mosquitoes; surveillance

West Nile virus (WNV) is an arbovirus circulating between mosquitoes and wild birds, which can affect human beings and horses as dead-end hosts.

Human cases of West Nile neuroinvasive disease are increasingly reported in recent years in Europe, included Italy. Since 2008, to survey the circulation of WNV similar surveillance systems, including mosquito monitoring, were set in Emilia-Romagna (ER), in Veneto (VEN) and Friuli Venezia Giulia (FVG) regions; these three regions cover about 50 thousands km² in the North-East of Italy. Since 2009, both systems recorded the circulation of Usutu virus (USUV), a flavivirus closely related to WNV with a not yet defined pathogenic capacity.

These mosquito-based surveillances include the collection of insects by attractive traps (especially carbon dioxide baited traps) in fixed stations every two weeks from May to October. Mosquitoes are grouped according to species, place and date of sampling, ground in pools (with a maximum of 50 or 200 specimens) and submitted to genus (pan-flavivirus) and species specific (USUV and WNV) molecular analysis.

In 2012 a total of 340,484 mosquitoes were tested in the three regions, 221,722 in ER (1,861 pools sampled in 96 traps) and 118,762 in VEN/FVG (1976 pools in 35 traps). In 2013, to August 8, 439,033 mosquitoes were tested, 160,730 in ER (1458 pools in 88 traps) and 278,303 in VEN/FVG (3719 pools in 54 traps). Composition of mosquito populations sampled were similar in the two surveys, in 2012 season, the most abundant species was *Culex pipiens* (86.1 % in ER, 75.1 % in VEN/FVG) followed by *Aedes (Ochlerotatus) caspius* (10.4 % in ER, 12.8 % in VEN /FVG) and *Aedes vexans* (2.2 % in ER, 1.8 % in VEN/FVG); a relevant number of *Aedes (Stegomyia) albopictus* specimens were also tested (0.7 % in ER, 1.3 % in VEN/FVG).

In 2012 WNV was detected in 11 pools in VEN and in two pools in FVG but was undetected in ER; conversely in 2013, to August 8, WNV was detected in 64 pools in ER and in 24 pools in VEN.

USUV was detected in both plans in the two years of surveillance (128 pools in ER and 86 pools in VEN/FVG). Both viruses were mostly detected in *Cx. pipiens*. In 2012, the overall season minimum infection rate (MIR) for USUV in *Cx. pipiens* was similar in the two surveys (0.41 ‰ in ER and 0.38 ‰ in VEN/FVG). Interestingly USUV was detected also in *Ae. caspius* (three pools) and *Ae. albopictus* (two pools) species, but further data are needed to elucidate the possible vector role of these species.

Utilization of pan-flavivirus PCR also allowed the detection of sequences referable to viruses specific of mosquitoes (Mosquito-only flavivirus), among these *Aedes* flavivirus was detected in 58 pools of *Ae. albopictus* (and in three of *Cx. pipiens*) and *Ochlerotatus* flavivirus was detected in 12 pools of *Ae. caspius* (2012 data). These results confirm the ability of mosquito based surveillances to detect non-target viruses, by using the genus-PCRs.

The detection in 2012 of WNV in VEN/FVG and the inactivity of the virus in ER in the same year raise the question of which factors may allow, or prevent, WNV circulation in neighbouring and similar areas; while the massive circulation of WNV recorded in 2013 in both VEN and ER highlights the importance of identifying drivers that could trigger viral circulation.

Obtained results show a good performance of the two systems in term of sensitivity and precocity in detecting the viral circulation, confirming that, if mosquito trapping effort is intensive, these systems are useful and reliable in terms of assisting the planning of public health policies.

ORAL: EVALUATION OF WEST NILE VIRUS (WNV) NS3THR249PRO MUTATION AS A VIRULENCE MARKER IN A SPF CHICKEN MODEL AND IN CARRION CROWS (CORVUS CORONE)

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Key words: West Nile virus, virulence determinant, Carrion crows, SPF chicken model

West Nile virus (WNV) has become a wide-spread arbovirus in Europe and the Mediterranean Basin countries. This emerging zoonotic disease disseminated 14 years ago in North America where its impact on animal and public health has been considerable. In particular, American crows were highly susceptible to fatal infection with the original American topotype strain NY99 whose virulence was linked to a Thr→Pro mutation located at residue 249 of the viral NS3 helicase. However, the NS3249Pro genotype has arisen recently in the Western Mediterranean region but did not always correlate with an enhanced virulence for birds. This discrepancy brought us to seek to evaluate whether this substitution is responsible for the pathogenicity of the European WNV strain Is98 that had elicited high rates of avian deaths in 1998 in Israel and is characterized by an NS3249Pro genotype. We therefore used two clones derived from the Is98 strain, (1) a wild type (WT) clone characterized by a NS3249Pro genotype and (2) a mutant clone characterized by a NS3249Thr genotype, which we inoculated to wild-caught WNV-sensitive Carrion crows and to a WNV infection avian model, namely, SPF chickens aged one day. Clinical follow-up and viral RNA load monitoring in serum, oral swabs and feathers by an in-house rRT-PCR specific to NS2a viral protein was performed on the experimentally inoculated birds. Data pertaining to these experiments are still under analysis. Nevertheless, preliminary in vitro data suggest that, although both clones replicate as efficiently, the mutant one is less efficient for reinfecting new host cells. WNV epidemiology in the Western Mediterranean region suggests that a proline residue in position 249 of the NS3 protein is not sufficient to enhance virulence for any given WNV strain. Possibly, other residues that differ between strains might also play a role in the observed pathogenicity. Using identical infectious clones that only differ in NS3 residue 249 would allow us to decipher the true weight of the Thr→Pro substitution as a determinant of virulence.

ORAL: PCR screening for viral nucleic acid sequences in bat colonies in Germany

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Key words: bats, virus detection

Bats have been identified as subclinical carriers of zoonotic disease agents worldwide. Recent studies from several work groups also revealed a broad variety of viruses present in European bat species, without having an obvious health implication for the bats. The zoonotic potential of these indigenous viruses seem also to be low, however, it has never been systematically evaluated. In this study we analysed the prevalence of different viruses in almost 1.000 bats belonging to 17 different indigenous German bat species. Bat colonies in Saxony-Anhalt, Bavaria and Mecklenburg-Western Pomerania were sampled by collecting oral swabs, faeces and urine. The samples were PCR screened using conventional nested or semi-nested Pan-assays, in order to detect a broad range of different Astroviruses, Paramyxoviruses, Coronaviruses and Adenoviruses. Amplicons were eventually sequenced and analyzed genetically. Overall virus prevalence rates were between 1 % and 40 %, with variations depending on the virus species, the analysed bat species, and the sampling location. Correlating the virological results with the zoological data for the sampled bat colonies regarding bat species and bat biology will help to elucidate transmission mechanisms for intra- and inter-species infections, and possibly uncover co-evolution events.

ORAL: Virus transmission and clinical symptoms in calves experimentally infected with a recently discovered hypervirulent BVDV-2c strain

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CODA-CERVA¹; FLI²; Ghent University³

Key words: BVDV - horizontal transmission - transiently infected animals - experimental infections

Bovine Viral Diarrhoea Virus (BVDV) is a worldwide spread virus that most commonly infects cattle and can cause considerable economic losses. The virus is characterized by a strong genetic diversity and can be divided into two species: BVDV-1 and BVDV-2. When infecting susceptible animals between the second and fourth month of gestation, BVDV is able to cause persistent infections by infecting the foetus. These immunotolerant persistently infected (PI) animals are important sources of infection as they continuously shed BVDV in large quantities. On the contrary, transiently infected (TI) animals are believed to be less important in the epidemiology of BVDV. However, transient infections with a severe BVDV-2 strain can spread explosively. Only few studies were performed to determine the transmission potential of TI animals.

Recently, a BVDV-2c strain causing severe clinical symptoms and high mortality was described in Germany and the Netherlands. The aim of this study was to reproduce the severe clinical symptoms observed in the field and to determine the transmission potential of animals transiently infected with this hypervirulent BVDV-2c strain following experimental infections in calves.

Ten BVDV-free and BVDV seronegative calves were selected, of which three calves were experimentally infected through intranasal instillation of 5.0 million TCID₅₀ of the BVDV-2c field isolate and housed together with seven contact animals in a single box. Direct contact between all animals was possible during the experiment. Nasal swabs, whole blood and serum samples were collected and clinical characteristics were monitored. Samples were collected daily until 20 days post inoculation (dpi) and from 22 dpi onwards every 2 days. Virus isolation, determination of degree of viraemia, PCR, virus neutralization test and analysis of blood composition were performed on the collected samples. The basic reproduction ratio, a measure of transmission of infection, was calculated using the maximum likelihood estimator.

All three inoculated animals became infected, as whole blood samples became PCR-positive from 2 dpi onwards. A significant raise in body temperature was noticed from 7 dpi to 10 dpi. One animal remained subclinically infected and was PCR-positive until 13 dpi. A second experimentally infected animal suffered from severe diarrhoea and loss of appetite, which started at 8 dpi. This animal recovered 13 dpi, but whole blood samples remained PCR-positive until 16 dpi. The third inoculated animal developed severe diarrhoea 10 dpi, had complete loss of appetite with subsequent significant loss of body weight and died 17 dpi. One of seven contact animals became PCR-positive 11 dpi, indicating the inoculated animals were able to spread the virus. This contact infected animal developed severe diarrhoea with some loss of appetite between 19 and 24 dpi, but recovered afterwards.

Although the transmission study is still on-going, the preliminary results show it was possible to reproduce the severe clinical symptoms observed in the field and the infected animals were able to spread the virus to at least one contact animal. Complete results on virus isolation, degree of viraemia, neutralising antibodies, nasal swabs and basic reproduction ratio will be presented on the EPIZONE congress.

ORAL: Isolation and molecular characterization of foot-and-mouth disease virus in Benin in 2010

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Key words: Foot-and-mouth disease; Virus isolation; West Africa; Benin

Foot-and-Mouth Disease Virus (FMDV) causes a highly contagious trans-boundary disease of cloven-hoofed domestic and wild animals such as cattle, African buffalo, sheep, goats, deer and pigs. FMDV is a member of the Aphovirus genus within the Picornaviridae family. The virus consists of a non-enveloped particle of icosahedral symmetry containing a positive-sense single-stranded RNA genome. There are seven immunologically distinct serotypes, namely O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3. Foot-and-Mouth Disease (FMD) is one of the most economically devastating diseases affecting livestock animals. Several studies have been conducted in the enzootic countries where control measures against this disease have been implemented. However, in West Africa, where constant circulation of this virus is assumed, very few studies on the characterization of circulating strains have been published. This study describes FMDV isolation and characterization from bovine epithelial tissue samples (n=77) collected in 2010 between June and August in three departments (Borgou, Atacora, Alibori) located in the northern part of Benin. Among samples analyzed, 76 were positive by real-time RT-PCR targeting the gene encoding the viral polymerase 3D. The FMDV was isolated from 42 samples. Antigen Capture Elisa (Ag-ELISA) and VP1 coding sequence analysis revealed 33 isolates of serotype O and 9 isolates of serotype A. Serotype O was isolated from samples collected in Borgou (31 isolates) and Atacora (2 isolates). Serotype A was isolated from samples collected in Alibori (5 isolates) and Borgou (4 isolates). Phylogenetic analysis of the VP1 sequence revealed two different groups of type O isolates (>5% of difference), one isolated from Borgou and the other from Atacora. All type A isolates are similar (<5% difference). VP1 sequence comparison with the sequences available in the GenBank database revealed a close relationship of the Benin isolates with strains of topotype O of West Africa (WA) and with strains of African topotype A of genotype VI (<15% difference). Knowledge of the recent strains circulating in this region of West Africa should contribute to better selection of vaccine strains and enable the updating of molecular epidemiology data available for West Africa in general.

ORAL: Host response upon infection with moderately virulent Classical swine fever virus strains: impact of race-dependent genetic variations

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Key words: Classical swine fever, pathogenesis, race-dependent factors

Classical swine fever (CSF) represents one of the most important porcine diseases worldwide and entails tremendous socio-economic consequences. It affects both domestic pigs and wild boar. Depending on host factors and virulence of the CSF virus (CSFV) involved, the clinical course varies from almost inapparent disease to a hemorrhagic fever with high mortality. Over the last decades, moderately virulent CSFV strains predominated in the field showing a strong age dependence of clinical courses. While young animals show a severe multi-systemic disease and succumb to infection within 14 to 21 days, full-grown animals may present only fever and slight depression.

Strains originating from the wild boar population were reported to show differences in virulence and thus clinical course when introduced into the domestic pig population. Factors leading to these differences are completely unknown. Moreover, CSF pathogenesis and host-virus interactions in general are far from being fully understood.

To study race dependent pathogenesis and to define reaction patterns associated with a positive outcome of infection (convalescence), a comparative study was performed using two domestic pig races in comparison to European wild boar. To present one of the most recent field isolates of wild boar origin, infection was carried out with CSFV strain "Roesrath" (EURL data base entry CSF1045). It was originally isolated from a German wild boar piglet in 2009 and is well characterized.

The study design comprised a total of nine German landrace pigs, nine crossbred commercial mast hybrids, and nine European wild boar. Of each race, six animals were infected and three acted as uninfected negative control. Comparative analyses were carried out with regard to clinical, virological and serological aspects as well as blood count parameters. After oronasal virus application clinical signs were assessed daily. Blood samples for laboratory investigations were collected in regular intervals and more frequently in the early phase post infection (after 2, 4, 8, and 24 hours).

Domestic pig races developed fever between days three and five and stayed febrile until euthanasia with the exception of two landrace pigs which became convalescent. In contrast, increases in body temperature were measured just sporadically in wild boar. Furthermore, clinical symptoms appeared to be less severe. Virus was detectable in all animals and to similar amounts apart from antigen detection (ELISA) in wild boar which occurred slightly later and with lower antigen loads.

Similarly to that, serological analyses revealed an early and high production of CSFV-E2-specific antibodies in wild boar but during the course of the disease the German landrace pigs reached equal antibody levels. Seroconversion against glycoprotein Erns was detected in all infected animals, however, highest values were observed in mast hybrids.

In conclusion, race effects on clinical, virological and serological findings were less pronounced than expected and could have been influenced by several host factors such as immune status, origin, concomitant diseases, and age variations. Further studies will target the immune responses (specific and innate) of individual pigs that showed different disease courses to better understand beneficial or detrimental reactions patterns.

Session 6: Epidemiology & Risk Analysis

ORAL: Using interval lagged environmental data to predict the population dynamics of the disease vector *Cx. pipiens/restuans*

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Key words: *Culex pipiens/restuans*, vector, population dynamics

Mosquitoes of the species *Culex pipiens/restuans* are important vectors for a variety of arthropod born viral infections (e.g., West Nile fever, St. Louis encephalitis, Japanese encephalitis, Western equine encephalitis, and Rift Valley fever). To generate a predictive model for the population dynamics of this vector we investigated the associations between 20 years of mosquito capture data and the time lagged environmental quantities daytime length, temperature, precipitation, relative humidity and wind speed.

We averaged time series data from 6 capture sites in Cook County (Illinois, USA) to represent the mean weekly number of mosquito catches on the county level. Using cross-correlation maps (CCMs) we investigated the association between mosquito abundances and environmental quantities (quantified by Spearman's rank correlation coefficient). We incorporated the results obtained from the CCMs into a Poisson regression model to generate the predictive model for the mosquitoes' population dynamics. To optimize the predictive model the time lags obtained from the CCMs we adjusted the lags using a genetic algorithm.

The CCMs showed that mosquito abundances were highly positive correlated with daytime length 4 to 5 weeks prior capture ($r=0.898$) and with temperature during 2 weeks prior capture ($r=0.870$). Negative correlations were found for wind speed averaged over 3 weeks prior capture ($r=-0.621$) and relative humidity averaged 2 to 15 weeks prior capture ($r=-0.561$). Precipitation showed the weakest association with the mosquito capture rates (averaged over 10 weeks prior capture, $r=0.487$).

Cx. pipiens/restuans population dynamics were predicted by integrating the CCM results into a Poisson regression model. This model was applied to simulate the average seasonal cycle of the mosquito abundance. Verification with observations resulted in a correlation (Spearman) of $r=0.937$. Applying the model to the entire 20-years time series resulted also in a suitable fit with $r=0.761$. After the optimization of the time lags with the genetic algorithm the model fit could be further improved. The optimized model resulted in an $r=0.973$ for the average seasonal cycle and an $r=0.800$ for the entire time series.

Our model represents a feasible method to predict the population dynamics of *Cx. pipiens/restuans* mosquitoes. Thus, it could further be used for predicting the occurrence and spread of arboviral diseases transmitted by this vector.

Reference: Lebl, K., K. Brugger, and F. Rubel, 2013: Predicting *Culex pipiens/restuans* population dynamics by interval lagged weather data. *Parasites & Vectors*, 6:129.

ORAL: A new methodology to assess data availability, accessibility and form for risk analysis

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Key words: Data availability, Data accessibility, Data form, Data sources, Venezuelan equine encephalomyelitis (VEE)

Risk assessments are mostly carried out based on available data, which do not reflect all data theoretically required by experts to answer risk questions. The present study aimed at developing methodology to assess data availability, accessibility and form, based on a codification system. This is explained using an exotic viral disease, Venezuelan equine encephalomyelitis (VEE) as case study. The specific data required were organised as a generic exhaustive list of data (types). A direct and an indirect survey allowed elaborating an inventory of data sources. A coding system with reference to data availability, accessibility and form was elaborated. A parallelism was highlighted between data availability and accessibility. Most available data were found in HTML and PDF files, thus not raw tabulated data, which are more suitable for data handling, leading to slower progress in risk assessments that require such data. The form of data plays a key role in the feasibility and rapidness of data management and analysis, through a prompt compilation, combination and aggregation in working databases. Harmonized and standardized data collection systems in animal and public health would provide useful and reliable data and allow assessing data gaps through comparative studies.

ORAL: Risk factors for introduction and clinical symptoms of Schmallenberg virus in Dutch dairy herds and its impact on productivity

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Key words: Schmallenberg virus, dairy cattle, risk factors, impact

Introduction

On November 18th, 2011, the Friedrich Loeffler Institute reported the isolation of a novel orthobunyavirus in German cattle, the Schmallenberg virus (SBV). SBV has also rapidly infected a large fraction of the Dutch ruminant population. This study aimed at quantifying herd-level risk factors for prevalence of SBV and SBV-induced malformations in newborn calves in dairy herds. Also, an attempt was made to estimate within-herd impact of SBV infection on mortality rates, milk production and reproductive performance.

Materials & Methods

A case-control design was used. Seventy-five case herds were selected based on reporting of clinical signs in August/September 2011 and/or compulsory notification of malformations in newborn calves attributed to SBV between December 20, 2011 and July 6, 2012. Seventy-four control herds were selected based on absence of malformations in newborn calves and anomalies in reproductive performance. Up to 70 serum samples were collected per herd and investigated for presence of antibodies against SBV. Risk factors for high within-herd SBV seroprevalence (>50%) and the probability of malformed newborn calves in a herd were quantified using logistic regression analyses. Within-herd impact of SBV (reproductive performance, milk production, mortality) was analyzed descriptively. Farmers were requested to submit any stillborn, aborted or malformed newborn calf. Submitted calves were investigated pathologically and tested for presence of SBV in brain tissue. To gain insight in the level of vertical transmission of SBV, 7 farmers were asked to collect precolostral blood samples from offspring of cows in which the virus has been detected.

Results

Animal-level seroprevalence was 84.4% (95% CI: 70.8-92.3) in case herds and 75.8% (95% CI: 67.5-82.5) in control herds. The results of our study indicated a strong association between grazing and the probability of SBV infection. Herds that were grazed in 2011 had an increased odds (OR 9.9 ; 95%CI: 2.4-41.2) of a high seroprevalence (>50%) compared to herds that were kept indoors in 2011. When grazing was applied in 2011, the odds of malformations in newborn calves tended to be higher compared to herds in which cattle were kept indoors. Incidence of malformations in newborn calves seemed to be associated with clinical expression of the disease in adult cattle, although this effect was influenced by within-herd seroprevalence. The rate of vertical transmission of SBV once a dam gets infected seemed to be low. We calculated that approximately 80% of the cows that were assumed susceptible during the most likely 4-week period of acute infection delivered a vital calf, 5.0-5.8% delivered a non-vital calf, and 4.8-6.7% aborted between day 100-260 post insemination. Based on the detection of SBV in brain tissue of stillborn or malformed calves (11/146), combined with calving data from their herds, we roughly estimated that at least 0.5% of the calves born between February and September 2012 have been infected by SBV. SBV was not detected in precolostral calf serum samples although antibodies were measured in some of them. Maternal antibodies derived by colostrum intake remained present in calf serum samples for approximately 3-4 months of age.

The results of our study indicated a drop in milk production between the end of August 2011 and mid-September (week 35-36). During a 4-week period in which SBV infection was expected to have occurred, the total loss in milk production was estimated at 30 to 51 kilogram per animal. No effect on mortality could be found.

ORAL: Within-herd dynamics of Schmallenberg virus

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Key words: Schmallenberg virus, within-herd dynamics, serology, epidemiology

Almost two years ago, a novel insect-transmitted orthobunyavirus was discovered at the German-Dutch border and named Schmallenberg virus (SBV). Since then, substantial knowledge has been acquired about SBV; however, the information about the initial phase of the disease in 2011 is scarce until now.

In the present study, blood samples from cattle of a farm localised approximately 9 km away from the initial holding in Schmallenberg were taken regularly between May 2011 and January 2012, and tested by an SBV-specific antibody ELISA. Up to the end of September, every tested sample was negative for SBV-specific antibodies suggesting the absence of SBV in that region before autumn 2011. Starting at the end of September, the first cows seroconverted; the within-herd prevalence eventually reached 100% within barely one month. Consequently, SBV has spread rapidly in the tested herd in its first confirmed season in Europe.

Although the “how and where” is not completely understood, SBV survived the winter season 2011/12 and circulated among European livestock again. During the following vector season and winter 2012/2013, a sheep farm not affected before was monitored. Except for dams with 2 or more lambs, all animals are kept outdoors year-round. In January 2013, blood samples were taken from 15 yearlings. All animals were negative in the ELISA; however, 4 sheep tested positive by an SBV-specific real-time RT-PCR. The animals had most likely been infected around the beginning of 2013, in the deep of Central European winter. In the period of presumed infection, the temperatures rose above 5°C for several consecutive days with a maximum of about 9°C, the daily minimum temperatures never fell below 0°C, and there was no snow. Between the end of January and the end of February 2013 further three out of ninety tested sheep seroconverted, in total 13% of the tested samples scored positive in the ELISA. Contrary to the high within-herd prevalence reached after a first infection of a holding with SBV in autumn, the transmission during winter appears to be possible, but with reduced efficacy. The most likely explanation is the reduced activity of the involved insect vectors due to lower outside temperatures.

In conclusion, SBV spread rapidly during the main vector season, but a transmission by haematophagous insects at a much lower level is possible during Central European winter months as well.

ORAL: Scale and pattern of Q fever transmission through space

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Key words: spatial, transmission, Q fever, zoonotic risk,

The recent epidemic of Q fever amongst humans in the Netherlands has triggered many questions regarding transmission and risk of Q fever. Little was known regarding the spatial scale of the transmission and for the possible mechanisms of transmission quantitative information was mostly lacking.

We analysed the available data from the Dutch epidemic, to derive more quantitative insight in the transmission, especially with regard to the human risk. To this end, we quantified the spatial transmission kernels from infected farm to free farm, but also from infected farm to humans.

We found that the transmission between farms behaves as expected, i.e. the kernel suggests that the main transmission between farms follows direct connections, e.g. animal transport, vehicles moving from one farm to another, and people moving between farms. For transmission to humans, a different pattern is found, which suggests random and undirected transmission routes between source and receiver. This suggests that the infection would reach the receiver via a random walk or a Lévy flight pattern, rather than via a direct connection.

From the transmission pattern to humans we conclude that the transmission more likely results from repeated deposition of the infection in the environment with intermittent "flight" phases, where the infection is transported through space e.g. via wind. Transmission to farms on the other hand most likely results from direct contacts and connections between farms.

ORAL: Epidemiological determinants are at the core of effective analysis to assess Q fever risk (A review of Q fever in four European countries)

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Key words: epidemiology, analysis, risk, Q fever

A review on Q fever in four European countries (Bulgaria, France, Germany and the Netherlands) was undertaken by the Animal Health and Welfare Panel of the European Food Safety Authority (EFSA) as part of an evaluation of risks associated with the Q fever outbreak in the Netherlands during 2007 to 2010. The review focused on key epidemiologic features of Q fever in humans and animals in each country, to provide risk managers with an objective information about infection and clinical disease in humans and farmed animals, potential risk factors, control options, and potential reservoirs of infection in nature. The review described the epidemiologic determinants of infection and clinical disease in humans and farmed animals, and patterns of presentation, in time, in space and among population subgroups. A range of susceptible hosts were considered, both human and farm animals, as was the role of some intermediate reservoirs, including ticks, pets, birds and wildlife. The review spanned almost 30 years, encompassing a range of potential ecological or management changes. The biologic pattern of host-pathogen interaction is presented using appropriate epidemiologic measurements, including prevalence for infection and incidence for disease status. Information about risk factors for Q fever are available, however, these are exclusively qualitative. A number of case studies of Q fever outbreaks are presented, highlighting similarities and differences in terms of infection and disease presentation, and putative risk factors. It is clear that the situation is complex, with disease presentation and outbreak occurrence in farmed animals influenced by the husbandry system, and the methods of production and management practices. Control options are presented, and effectiveness assessed where indicated and justified, at least qualitatively. Options for quantitative measurements of efficacy are also presented (e.g. data from case control studies on intensive testing, interventional studies on pathogen survival when manure is treated or on vaccine efficacy). The results are discussed, focusing on implications for future outbreaks and possible variations. Although there is a potential for ecological fallacy, where some of discovered epidemiologic inferences may not be applicable at individual level, overall conclusions at population level may be drawn. There may be limitations in extrapolating these findings to particular events, including involved hosts, geographic locations and time periods. The organized collection of information around key epidemiologic determinants provides a sound evidence base for decision-making, as well as the opportunity to critically evaluate the effectiveness of interventions during outbreak situations.

Session 1 continued

ORAL: Biological and genetic evolution of the hypervariable region in Schmallenberg virus.

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Key words: Schmallenberg virus, hypervariable region, genomic evolution

Schmallenberg virus (SBV) is an emerging arbovirus in Europe. After its identification in 2011, SBV spread rapidly in several European countries. Although its relatively innocuous clinical signs in adult ruminant, SBV causes abortion, stillbirth and congenital malformations in newborn ruminant. Genetic analysis revealed that SBV belongs to the genus Orthobunyavirus within the Bunyaviridae family and is related to the Simbu serogroup viruses. The genome of the genus Orthobunyavirus comprising small (S), medium (M) and large (L) segments encodes six proteins.

The whole genome sequence has been used to analyse the genetic variability of SBV and for the first time within the Bunyaviridae family, a hypervariable region (HVR) has been identified in the N-terminal region of SBV Gc glycoprotein (Coupeau et al., 2013; Fischer et al., 2013). This hypervariable region has been detected in two SBV isolates obtained from malformed lambs born in the same sheep herd in Belgium (Coupeau et al., 2013), and in numerous animals from different herds in Germany (Fischer et al., 2013). Moreover, in vitro growth assays coupled with full length genome sequencing showed an in vitro adaptation of SBV and mutation accumulation inside the hypervariable region in the absence of immune selective pressure (Coupeau et al., 2013).

In this context, we decided to explore the evolution of the hypervariable region during natural infection of SBV. For this, specific time points of SBV infection were investigated (n= number of selected samples): (i) primary SBV infection period observed in ewes during September 2011 (n=3); (ii) the lambing period of January 2012 where numerous malformed lambs were identified (n=16); (iii) the re-emergence period of SBV in the sheep flock during summer and autumn 2012 (n=16) and (iv) the lambing period of January 2013 where few malformed lambs were identified (n=5). In order to compare the extent of the variability in the HVR with a more conserved gene, the S segment was also sequenced in the different samples. Preliminary results show that all HVR sequences were unique in all tested samples. Moreover, phylogenetic analysis shows that the HVR sequence observed during the 2012 re-emergence period seems represent a distinct phylogenetic group.

To conclude, these data are used to better characterise SBV variability at the intra-herd level and virus evolution during the first two years of SBV emergence. Moreover, genomic variability might be associated with the re-emergence of SBV. Indeed, two of the five stillborn lambs observed during the 2013 lambing period are born from ewes that were already infected and immunised in 2011. An overview of SBV genomic evolution is a key step to understand SBV biology and to elaborate vaccine.

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ORAL: Transplacental infection of Schmallenberg Virus in the first trimester of gestation is highly efficient in sheep

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CVI¹; Gezondheidsdienst voor Dieren²

Key words: SBV, transplacental transmission, animal study

In the first year after the recognized introduction of Schmallenberg Virus (SBV) into North-West Europe musculoskeletal malformations and pathological changes of the central nervous system as porencephaly, hydranencephaly and hypoplasia of the cerebellum in new-born lambs and calves were the most intriguing clinical features of this infection (1). Therefore, SBV joins the group of other teratogenic, arthroborne viruses such as Akabane virus. On the basis of epidemiological studies of the recent SBV outbreaks and the comparison with the pathogenesis of Akabane virus, it is assumed that the teratogenic infection takes place in the first trimester; however, the efficiency of transplacental infection generally and in relation to the gestation time point is unknown. Also, information on the transplacental transfer and the virus tropism in the uterus and foetus is lacking. Recent studies on central nervous tissue of naturally infected, new born lambs and calves have described a differential distribution of virus and inflammatory cells, if present in the CNS(2). In this study, the early transplacental infection at 5 and 6 weeks of gestation was examined. 21 SBV sero-negative ewes of primo- or multiparity were inoculated subcutaneously with 1 ml of SBV viraemic calf serum (kind gift of Martin Beer, FLI Riems) at either day 38 (n=10, group 1) or day 45 (n=11), group 2) of gestation. Seven 7 days post inoculation ewes were euthanized and tissue samples were taken from the reproduction tract, especially several placentomes, and from the fetuses (umbilical cord, skull, including CNS and amnion fluid). All samples were investigated by RT-qPCR for the presence of SBV mRNA.

Three days after inoculation of the ewes in all, but one ewe sera were positive in the qPCR (mean PCR ct value: 21,7 (group 1), 20.9 (group 2)) and during necropsy samples were taken from a total of 39 fetuses (n = 20, group 1) and n = 19, group2). No morphological changes were observed at this early time point after infection in any of the fetuses. PCR analyses revealed that placentomes taken at seven days post infection were positive in all viraemic ewes with a mean ct value of 17.1 in group 1 and 18.9 in group 2. In all ewes at least one fetus contained SBV nucleic acid in either umbilical cord or CNS. In 85% of the fetuses of group 1 the umbilical cord was positive for SBV with a mean PCR ct value of 32.2 and 74% of group 2 with a mean PCR ct value of 31.7. Skull tissue (including CNS) was SBV positive in 55% of the fetuses of group 1 and in 74% of group 2 with a mean PCR ct value of 33.0 and 34.1, respectively. In 15% (group 1) and 11% (group2) of the fetal amnion fluids SBV was detected.

From this study it can be concluded, that SBV is able to very efficiently pass the placental barrier and infect the fetus in the first trimester of the gestation.

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ORAL: Schmallenberg virus infection in South American camelids

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FLI¹

Key words: Schmallenberg virus / experimental infection / field study / South American camelids / Germany

Schmallenberg virus (SBV) has been the first Orthobunyvirus of the Simbu serogroup detected in Europe. Culicoides vectors were incriminated for the broad spatial spread of SBV throughout Europe since its discovery in Germany in 2011. Clinical manifestation of the disease in adult ruminants is mild or inapparent, while transplacental transmission of the virus causes congenital manifestations in foetuses and neonates born from dams that were infected the first time in the first third of pregnancy. Serological evidence of a previous SBV-infection demonstrated the susceptibility of South American camelids (SAC) to SBV. However, the pathogenesis of SBV-infection in SAC and their role in the epidemiology of SBV have not yet been investigated.

Therefore, a field study of SBC-infection in SAC in Germany was conducted from September 2012 to autumn 2013. SAC owners and veterinary laboratories were asked to send blood and post-mortem samples and to report whether abnormalities were observed in sampled animals. Serum, plasma and post-mortem samples were analysed by ELISA, serum neutralisation test (SNT) and S-segment-specific real-time RT-PCR (RT-qPCR), respectively. For the validation of serological and virological assays, reference material was collected from three llamas (*Lama glama*) and two alpacas (*Vicugna pacos*) experimentally infected with a SBV-containing serum well characterised by previous experiments. In the five SAC experimentally infected with SBV, no clinical signs were observed during the animal experiment. Similar to experimentally infected ruminants, SBV genome was detected in the first 9 days post-infection (pi), while seroconversion occurred between 9 to 21 days pi. Preliminary data of the field study suggest that about two third of the SAC in Germany were infected with SBV. The proportion of seropositive SAC within herds varied considerably. In all herds, at least one animal was detected seropositive. However, SBV RNA was not detected in any of the samples. Furthermore, clinical signs or congenital malformation were not observed by the SAC owners.

In conclusion, similar to SBV-infected ruminants, SBV genome can be detected in SAC for a short time after SBV-infection and no obvious clinical signs occur in adult animals. However, their role in the epidemiology of SBV has to be further investigated. Final results of the field study will be presented at the meeting.

ORAL: Development of a vaccine that protects sheep and cattle against challenge with Schmallerberg virus, a novel Orthobunyavirus recently found in Europe.

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MSD Animal Health¹

Key words: Schmallerberg, Vaccine, Young and pregnant animals

Decrease in milk production, watery diarrhea and fever were reported in several dairy herds in The Netherlands and Germany in the summer of 2011. Congenital malformations in young animals (lambs and calves) were observed in the following months. The responsible pathogen, a novel Orthobunyavirus, named Schmallerberg virus (SBV), was identified in Western Europe in November 2011. With the support of veterinary experts around the globe, MSD Animal Health has developed in a fast track program a vaccine to be able to protect livestock in Europe against SBV. The efficacy and safety of the vaccine has been tested in young animals (calves and lambs) as well as in pregnant ewes. Different vaccine administration regimes were used. Animals were either vaccinated once or twice and three to four weeks after the last vaccination vaccinates and controls were challenged with a virulent SBV strain. The efficacy of the vaccine was evaluated based on the level of virus neutralizing antibody titers induced after vaccination, the blockage of viraemia and the blockage of vertical transmission to the foetus. SBV neutralizing antibody titers were measured after vaccination. All control animals became viraemic after challenge. Protection (blockage of viraemia and of vertical transmission, as tested by real time RT-PCR) against the challenge was observed in the vaccinated animals. Based on these data good protection against SBV is to be expected in the field and further development of this vaccine is ongoing.

ORAL: EXPERIMENTAL INFECTION OF CATTLE WITH BLUETONGUE VIRUS SEROTYPES CIRCULATING IN EUROPE

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Key words: bluetongue european serotypes experimental infection

Bluetongue virus (BTV) is an Orbivirus (family Reoviridae), transmitted mostly to wild and domestic ruminants by midges of the *Culicoides* genus, and causes bluetongue disease in susceptible species. From 1998 to 2006, Europe had to face an unprecedented emergence of BTV serotypes 1, 2, 4, 8, 9 and 16 (BTV-1, 2, 4, 8, 9, 16), including several countries where the virus has never been detected before. In early 2008, BTV-6 and BTV-11 emerged in the Netherlands and Belgium, respectively, both most likely derived from the misuse of modified live vaccine from South Africa. The presence of competent Palearctic vectors and several serotypes recently described in Europe mainland, with however livestock mostly naïve, trigger the need to evaluate and compare the clinical, virological and serological features of the European BTV serotypes. In this study groups of calves, initially vaccinated against BTV-8 or not, were infected with BTV serotypes lately isolated in Europe, namely BTV-1, 2, 4, 6, 8, 9, and 16. BTV-6 was evaluated through a BTV-8/BTV-6 co-infection. For each single tested serotype, two groups of three male Holstein calves, 6-8 months old, were used: in one group calves were vaccinated against BTV-8 (BTVPUR AISap 8, Merial, Lyon, France), and in the other one they were not. BTV-6/BTV-8 co-infected calves were not vaccinated. Five animals were kept as control.

Each animal was inoculated with 10⁶ TCID₅₀ of the serotype attributed to their group, and co-infected calves received 10⁶ TCID₅₀ of both serotypes. Clinical signs were quantified. Viral RNA was detected, in the blood of the calves and in the organs after necropsy, by serotype specific RTqPCR, using BTV segment 2 as the target. Serological relationship between serotypes was assessed by cross neutralization tests. In vitro growth properties of the different serotypes were compared and expressed in TCID₅₀. Calves were slaughtered 35 days post infection and necropsied.

Most of the infected animals showed very mild clinical signs, however compatible with bluetongue disease, including congestion and crusts on the nostrils and oral mucosa, whichever the considered serotype and the vaccination status. BTV-2, 4 and 6 viral RNA were inconsistently detected and only reached low RNA copies/ml, when compared to BTV-1, 8, 9 and 16. In the co-infected group BTV-8 clearly overtook BTV-6. No significant difference could be found regarding viraemia length in relation to the vaccination status of the animals. However, maximal viraemia of the vaccinated calves ranged from 53 to 91 % of maximal viral RNA copy number/ml of blood of the homologous non-vaccinated animals. In addition, non-vaccinated animals had an overall relative risk of 1.76 (1.02-3.04) of positive viral RNA detection in organs when compared to vaccinated calves. A partial cross reactivity could be shown between BTV-1 and serum from animals infected with BTV-16 and serum of co-infected calves BTV-8/BTV-6. A partial cross-reactivity was also found between BTV-8 versus BTV-1 serum. Growth curve showed no significant differences between serotypes, although BTV-1 had a faster replication within the first 48 hours post-infection.

This experiment allowed to study the potential effect of the BTV-8 vaccination following a challenge with BTV serotypes circulating in Europe (in vivo cross-protection). Furthermore, in vitro cross-reactivity was evaluated between the same BTV serotypes. However the real consequences of these findings in the field under natural circumstances cannot be stated so far.

ORAL: RNA elements in open reading frames of the bluetongue virus genome are essential for virus replication

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Key words: Bluetongue virus, Reverse Genetics, Recombination, Segment 10, RNA function

Several processes involved in replication of segmented double stranded RNA (dsRNA) viruses are still largely unknown. After cell entry, positive sense single stranded RNAs, serving as mRNAs, are synthesized and extruded from core particles. Later after infection, mRNAs are recruited from the cytoplasm and serve as template for synthesis of dsRNA. However, selection of different genome segments, ordering and packaging of dsRNA in the virus particle, and virus assembly are still largely undefined processes and require virus and segment specific RNA-RNA or RNA-protein interactions. RNA sequences involved in these interactions are unknown.

Bluetongue virus (BTV) is a dsRNA virus, causing Bluetongue in ruminants. Bluetongue is arthropod borne, spread by *Culicoides* biting midges, and can course from subclinical to severe haemorrhagic disease. BTV is the prototype of the genus *Orbivirus* in the *Reoviridae* family and consists of at least 26 BTV serotypes of which several are causing economic losses in Europe.

BTV contains 10 dsRNA genome segments (Seg-1-10) encoding seven structural proteins (VP1-7) and at least four non-structural proteins (NS1-4). The proteins encoded by Seg-10 (NS3/NS3a) are non-essential, since BTV with both Seg-10 start codons mutated was propagated in cell lines (van Gennip et al., 2013, in preparation).

This finding led to the unique opportunity to study the role of RNA sequences in virus replication without disturbance of any protein products.

Here, a set of small and large deletions throughout the open reading frame (ORF) of Seg-10 were introduced. BTV deletion mutants were generated by reverse genetics after passaging of transfected cells. Except for the region between both start codons, every region in the ORF could be deleted. A prerequisite for virus propagation was, however, the insertion of RNA sequences originating from different viral genome segments. Only a small deletion at the 3' end of the Seg-10 coding region did not require an insertion. Apparently, RNA sequences from different genome segments were spontaneously inserted during virus rescue by, most likely, a replicative recombination event. Remarkably, all RNA insertions were found in the positive orientation, but were not necessarily in-frame with the ORF of NS3/NS3a. The sequences were inserted in different locations and inversion of the inserts resulted in genetic instability and replacement by other inserts in again the positive orientation. Generation of deletion mutants with synthesized RNA already containing such an insertion was as efficient as the generation of non-mutated BTV. These results show that the insertions are functional and essential for replication of BTV deletion mutants.

We show that RNA sequences within the ORFs of BTV genome segments harbour an important function in BTV replication. The developed system with the protein-lacking genome segment 10 enables research on the role of RNA sequences in RNA replication, virus assembly, RNA recombination and other processes in which RNA-RNA or protein-RNA interactions in the replication of dsRNA viruses are involved. The system is currently also used in the process of developing a new generation of safe DIVA vaccines for Bluetongue.

Session 2 continued

ORAL: Detection of duck enteritis virus (DEV) in ducks, geese, swans and other free-ranging aquatic birds by real-time loop-mediated isothermal amplification

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Key words: duck virus enteritis, detection, real-time loop-mediated isothermal amplification, sequence analysis

Duck enteritis virus (DEV) also known as Anatid herpesvirus 1 causes duck plague (DP) or duck viral enteritis (DVE) in ducks geese and swans. The host range is presumably broader comprising several species of free-ranging aquatic birds. The disease occurs world-wide and is accompanied with high mortality and reduction of egg production among waterfowl thus the epizootic and economical aspects of DEV infection are very important. So far the frequent occurrence of DEV was reported mainly in China while the current epidemiological status in eastern Europe remains unknown. On the other hand, an elaboration of fast and reliable detection methods of molecular biology allows to monitor the occurrence of DEV among aquatic birds. The main goal of our study was to perform the molecular detection of DEV among ducks, Mallards, geese, swans and other free-ranging aquatic birds in Poland. The study was conducted using samples collected between 2006 and 2012 from dead water birds. The presence of DEV UL30 gene encoding DNA-polymerase was detected by elaboration of real-time loop-mediated isothermal amplification (LAMP) that facilitated detection of DEV using real-time PCR system or simple water bath in less than 30 min. The conducted study revealed the occurrence of DEV in 96 out of 132 examined birds (72.7%) predominantly in Mallard ducks (*Anas platyrhynchos*) (26), swans (*Cygnus olor*) (66), and Greylag Geese (*Anser anser*) (2). Interestingly, DEV was also detected in one Grey Heron (*Ardea cinerea*). The conducted sequencing of real-time LAMP reaction mixtures confirmed the identity of DVE. The developed real-time LAMP technique facilitated very fast and firm detection of this virus. These data indicate on the importance of DEV infections in Poland and further study are needed to investigate its exact epizootic and economical impact into production of geese and ducks.

ORAL: DEVELOPMENT OF A SINGLE TEST FOR EXCLUSION OR DETECTION AND TYPING OF FOOT AND MOUTH DISEASE VIRUS

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ANSES¹

Key words: FMDV, Diagnosis, typing, multiplex

Foot and mouth disease virus (FMDV) belongs to the Aphtovirus genus in the Picornaviridae family. Seven immunologically distinct serotypes are described worldwide: O, A, C, Asia1, SAT 1, SAT 2 and SAT 3. This virus causes a highly contagious disease of domestic cloven-hoofed animals but can also affect wildlife species. The disease is characterized by the appearance of vesicular lesions on the hooves, the mouth and on the udder of infected females. However, the lesions are very similar to those induced by other vesicular viruses like Vesicular Stomatitis Virus (VSV) and Swine Vesicular Disease Virus (SVDV). FMD is considered to be of socio-economic importance and of significance for international trade and safety by the OIE. Therefore, rapid and accurate detection of all FMDV viruses is essential for immediate implementation of outbreak control measures. Currently, the diagnosis of FMD may require the completion of several tests to detect and type the virus, after reporting of vesicular lesions on host animals. The aim of this project is to develop a new FMDV multiplex molecular diagnostic test for detection or exclusion of vesicular viruses as well as molecular typing of FMDV.

This multiplex detection test is based on the Luminex liquid array technology. Primers were designed to allow amplification of several target genes in a multiplex test as follow:

- (i) FMDV 3D and IRES genes: for the detection of the 7 serotypes strains(pan-FMDV detection set);
- (ii) FMDV VP1 gene: for the molecular typing of each of the 7 FMDV serotypes;
- (iii) SVDV 3D gene: for the detection of all SVDV strains;
- (iv) β -actin gene: as positive internal control.

Each primer set was tested with viral RNA extracted from cell culture, infected or not with FMDV or SVDV to assess their specificity in singleplex. All the primers were specific for their target. Results showed that the 3D pan-FMDV primers detects all the FMDV isolates tested while the IRES pan-FMDV primers detected only some isolates in singleplex. The 3D pan-FMDV test was conserved in the multiplex assay. We used the same approach for the pan-SVDV primers, and these primers were SVDV specific and added to implement the multiplex reaction. Then we developed the test allowing the molecular typing of FMDV strains. Molecular typing of isolates belonging to serotypes A, O, C, SAT3 and Asia 1 was first carried out in singleplex reactions. We were able to detect the five serotypes and each RT-PCR gave a specific signal for their respective strains. The multiplex RT-PCR was performed (combining all the primers pairs giving a 9plex) for both detection and typing of FMDV in one reaction. This test was assessed on cell cultures infected with strains of FMDV and SVDV, and also with field samples.

This test allowed the detection of FMDV and SVDV and in the same reaction, identification of the 5 serotypes O, A, C, SAT3 and Asia 1 in cell culture samples as well as in field samples. However a lower signal was obtained with serotype O strains.

A 9plex Luminex test was thus finally developed allowing detection or exclusion of vesicular viruses as well as molecular typing of FMDV in a single reaction. Using this multiplex detection test, the 3D SVDV and FMDV primers detect respectively all reference strains of SVDV and FMDV tested. Primers targeting VP1 gene, specific for A, O, C, SAT3 and Asia1 serotypes give a specific signal with reference strains of each serotype and also with field samples.

We are currently testing primers allowing molecular typing of SAT1 and SAT2 strains and detection of Vesicular Stomatitis Viruses.

ORAL: Investigating the Impact of Nucleic Acid Extraction upon Next-Generation Sequencing of Foot and Mouth Disease Virus (FMDV)

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PIR¹

Key words: Next generation sequencing, RNA extraction, Foot and Mouth virus

Next generation high-throughput sequencing technologies are powerful tools in the generating of sequence data for use in epidemiological studies and tracing transmission of infections. Foot and Mouth Disease Virus (FMDV) is a highly variable RNA virus with one nucleotide mutation every replication cycle (Klein et al, 2009). The initial quality of the extracted nucleic acid often has a significant impact upon the quality of sequence libraries and reliability of the sequence data generated by these high-throughput techniques (Beerenwinkel et al, 2012).

We performed a full comparison of automated RNA extraction technologies including the Universal Biorobot (Qiagen), MagnaPure (Roche), and Magmax96 (Life technologies). Automated platforms were compared with manual kits, including Trizol (Life technologies) and RNeasy kits (Qiagen) to assess the suitability of such techniques for a highly variable virus such as FMDV. Physical factors of extracted RNA i.e. purity and concentration, were assessed using Bioanalyser and Nanodrop.

Extracted RNA from a fully characterised culture adapted isolate of FMDV UKG was converted to cDNA and used with Nextera XT sample prep kits to construct sequence libraries for sequencing on the illumina Miseq. Final libraries were graded on quality and complexity (using markers such as percentage host derived reads), viral genome coverage and reproducibility. Additionally, the sensitivity of each extraction technique and the qualities of resulting sequence libraries were compared.

After optimisation we prepared cDNA libraries from 30 field isolates selected from the UK 2001 FMDV outbreak archive held at Pirbright. The resulting libraries were sequenced on an Illumina Miseq as per standard protocols hence validating these methodologies. Sequence from isolate libraries supports the use of next-generation sequencing-based strategies in monitoring future outbreaks and tracing transmission of highly variable RNA viruses.

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ORAL: Various methods for equine infectious anemia virus detection in experimentally infected animal.

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Key words: EIAV, experimental infection, PCR, antibodies

Equine infectious anemia (EIA, swamp fever) is a contagious viral disease of equids. It is characterized by intermittent fever, anemia, depression, chronic loss of weight, and cardiovascular system dysfunction.

The causal agent of equine infectious anemia is an RNA-containing virus, a member of the genus Lentivirus of the family Retroviridae.

EIA virus causes a clinical disease and persistent infection.

Currently an agar gel immunodiffusion assay (AGID, Coggins test) is widely accepted and used for EIAV serodetection (Coggins et al., 1972).

Beside the serological assays, sensitive diagnostic tests are also required for EIAV direct detection. Molecular biology techniques allow the EIA virus genome identification both in the disease-affected and recently infected animals in the process of the immune response induction.

PCR is the method most widely used for EIA diagnostics in the period preceding the seroconversion onset. These assays are universal for molecular biology and allowing to set a preliminary diagnosis at the earliest possible time and with high accuracy.

This work is aimed at an experimental reproduction of equine infectious anemia in a susceptible animal and testing of various diagnostic methods developed for this disease.

During the observation period the infected horse had characteristic clinical signs of EIA like fever, loss of weight, and swelling of the legs.

Blood samples from the animal were collected on day 2, 3, 5, 7, 9, and every other day post inoculation. Serum samples were collected on day 7, 14, 21, 28, and 35 post inoculation.

Detection of the virus genome fragments in the material samples was performed using PCR. We used oligonucleotide primers for reverse transcription PCR with an electrophoretic detection described by M.M. Nagarajan and C. Simard, and original oligonucleotide primers and a probe for Real-Time reverse transcription PCR. The primers and the probe for EIAV RNA detection were located within the viral gag gene.

Using the reverse transcription PCR, we detected EIAV viral RNA in blood samples from day 2 up to day 35 post infection (the observation time) and in serum samples from day 2 to day 30 post infection.

In the examination of a sample mucous membrane washout the EIA virus genome was detected. In a washout prepared from the nasal cavity the viral RNA was detected from day 10 up to day 20 post infection, and in a washout from the vagina on days 10 to 25 post infection. In a washout taken from the mouth the EIA virus genome was not detected.

The specific antibodies to the EIA virus were detected using AGID starting from day 30 post infection, while using ELISA from day 21 post infection.

The results of the research showed that the PCR method was a rapid, sensitive and specific diagnostic method for EIA virus genome detection in early periods of the disease.

ORAL: Adaption of diagnostic methods to different situations of measurement

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Key words: imperfect diagnostic methods, cut-off variation, ROC, AUC, sensitivity, specificity

An efficient diagnostic strategy is characterized by its flexibility in terms of its adaptation to specific practical measurement situations. Essentially, it should have the following options for adjusting diagnostic strategies to specific situations of measurement: Pooling, varying cut-offs and combining different diagnostic methods to form a composite diagnostic strategy. Evaluation of the goodness of fit of a diagnostic method is possible in the case of cut-off variation on the basis of sensitivity and specificity in the context of the real or assumed prevalence interval. For this purpose, it is necessary to transform the vector (Se, Sp) of the two-dimensional parameter space into a graded one-dimensional assessment of suitability (ranging from 'unsuitable' to linearly arranged levels of 'suitability') for a given measurement situation. (Partial) ROC analysis is a well-known method for such a transformation. Especially for comparing quality properties of different diagnostic methods, (partial) AUC has a central importance, whose value is the result of the transformation method described above. Another method may consist in calculating minimal misclassification rates. Examples will be used to demonstrate how a cut-off can be generally adapted to a given measurement situation and which prerequisites are necessary for this purpose.

ORAL: Antiviral agents to control Classical Swine Fever epidemics

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Key words: Classical Swine Fever; pestivirus; antiviral; stochastic simulation model; control strategy

Classical Swine Fever (CSF) represents a continuous threat to pig populations that are free of disease without vaccination. When CSF virus is introduced, the minimal control strategy imposed by the EU is often insufficient to mitigate the epidemic. Additional measures such as preemptive culling encounter ethical objections, whereas emergency vaccination leads to prolonged export restrictions. Antiviral agents, however, provide instantaneous protection without inducing an antibody response. The use of antiviral agents to contain CSF epidemics is studied with a model describing within- and between-herd virus transmission. Epidemics are simulated in a densely populated livestock area in The Netherlands, with farms of varying sizes and pig types (finishers, piglets and sows).

Our results show that vaccination and/or antiviral treatment in a 2 km radius around an infected herd is more effective than preemptive culling in a 1 km radius. However, the instantaneous but temporary protection provided by antiviral treatment is slightly less effective than the delayed but long-lasting protection offered by vaccination. Therefore, the most effective control strategy is to vaccinate animals when allowed (finishers and piglets) and to treat with antiviral agents when vaccination is prohibited (sows). As independent control measure, antiviral treatment in a 1 km radius presents an elevated risk of epidemics running out of control. A 2 km control radius largely eliminates this risk.

Sessions 3 & 5 continued

ORAL: Space-time analyses to modulate the West Nile Virus surveillance activities in north-eastern Italy

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IZS-Ve¹

Key words: WNV, Surveillance plan, space-time analysis

West Nile Virus (WNV) reappeared in north-eastern Italy in 2008 and widely expanded in several Italian regions since then, affecting wild birds, equines and humans. Although the number of cases in equine premises decreased in the last two years, likely in response to the vaccination plan implemented in the most affected areas, an increasing number of WNV infections in humans and WNV positive mosquito pools have been identified in 2011-2012. In particular a total of 28 human cases of West Nile Neuroinvasive Disease were identified in 2012 in Italy, of which 25 were detected in Veneto and Friuli Venezia Giulia (FVG) regions; moreover, additional active circulation of WNV was observed in Veneto, in 17 West Nile Fever human cases and in seven blood donors. The situation prompted for a fine-tuned surveillance plan to early detect WNV re-activation in the area. Data from the 2012 WNV surveillance activities on mosquitoes, horses, wild birds and humans in north-eastern Italy were analysed to detect the presence of local clusters of cases, through Bernoulli space-time scan statistics. Logistic regressions were fitted to describe the WNV occurrence in horses, birds and humans within the significant clusters, using the number of mosquitoes and of WNV positive captures collected within 7, 15, 30 or 45 days prior to the observations. Space-time clusters were identified for all of the analysed data-sets. The resulting spatial and temporal windows were largely overlapping, and were located on the border between Veneto and FVG ranging from late July to September. The area encompassed in these clusters was considered as Study Area and further analyses were performed to detect the possible dependence of WNV occurrence in mammals and birds on the viral activity in the vectors in a well-defined time frame. All the logistic regression models showed strong associations between the occurrence of WNV in birds, horses and humans and the circulation of the virus in mosquitoes. In particular, the presence of WNV in wild birds resulted related to the virus circulation in mosquitoes within 7 days prior to the observation, whereas the detection of WNV in horses and humans were significantly associated with the detection of the virus in mosquitoes within 15-30 days. The study allowed to identify a limited area that experienced an excess of risk for WNV circulation in 2012 in all of the considered species. This area should be considered as the starting point for a more fine-tuned and effective surveillance plan, in order to promptly identify any WNV re-activation and/or re-introduction and intercept the outward spread of the disease. Moreover the results provided useful insight on the associations between the WNV circulation in vectors and hosts, suggesting that surveillance in mosquitoes and in birds should be prioritized, although the economical costs for such a surveillance plan could result high and it may be hardly applicable on a large scale.

ORAL: VACCINATION WITH RECOMBINANT ADENOVIRUS EXPRESSING F OR H PROTEINS FROM THE PESTE DES PETITS RUMINANTS VIRUS CAN ELICIT CELLULAR AND HUMORAL IMMUNE RESPONSES TO THE VIRUS

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CISA-INIA¹

Key words: PPRV, vaccine, adenovirus

Peste des petit ruminants (PPR) is an acute, highly contagious viral disease in small ruminants and one of the main constraints in improving small ruminant productivity. The PPRV virion contains two immunogenic integral membrane glycoproteins, the hemagglutinin (H) and the fusion protein (F), to which most of the neutralizing antibodies are directed. Adenoviruses have several attractive features to be good vectors for anti PPRV vaccine: human adenoviruses have been used for several decades as live oral vaccines in US, they can be produced inexpensively with high production, and they induce a strong immune response against vaccine antigens. Thus, we aim to construct and characterize two recombinant adenovirus, expressing the PPRV H and F proteins, and test their immunogenicity in mice.

Second-generation replication-defective human adenovirus type 5 (Ad5) were generated expressing under the EF1 α promoter the H or F genes of PPRV and the red fluorescent protein (RFP) under CMV promoter. Amplified stocks in HEK293A cells, were purified and titrated using standard protocols. Groups of C57BL/6 mice were intramuscularly inoculated with 5x10⁷ ip/mouse (infectious particles) Ad5-F, Ad5-H, Ad5 and PBS, respectively and boosted 3 times. Sera were collected at different times and tested for the presence of PPRV-specific antibodies by ELISA, PPRV-specific neutralizing antibodies in a plaque reduction neutralization assay and T cell specific responses evaluated by ELISPOT and proliferation assays.

The insertion and expression of H and F proteins in the E1A region of the recombinant adenovirus genomes was detected by the co-expression of the RFP in HEK293A, Vero and primary ovine cells. C57BL/6 mice immunized with Ad5-H and Ad5-F elicited a high PPRV-specific IgG response evaluated by ELISA. All vaccinated mice showed PPRV-specific neutralizing antibodies and T cell responses evaluated by different assays.

We demonstrate that recombinant adenoviruses express the biologically active H and F proteins. Furthermore, our results indicate that intramuscular inoculation of mice with Ad5-H and Ad5-F elicited robust anti-PPRV response. Finally, the immune response and protection conferred by the successful strategies developed above will be assayed in sheep, the PPRV natural host.

ORAL: Protective vaccines against African horse sickness virus (AHSV) based on modified Vaccinia Ankara expressing AHSV-VP2

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PIR¹; Zoetis²

Key words: African horse sickness Vaccines Protection MVA-VP2

African horse sickness virus (AHSV) is an arbovirus that causes a lethal systemic disease in Equidae. Control in endemic countries relies on the use of live attenuated vaccines. However, several bio-safety concerns prevent the use of this strategy in non-endemic countries. Furthermore, attenuated vaccines do not discriminate infected from vaccinated animals. For these reasons, several vaccination approaches that have DIVA (differentiating Infected from Vaccinated Animals) capacity have been investigated over the years. We have shown previously that a recombinant modified vaccinia Ankara expressing AHSV-VP2 (MVA-VP2) induced neutralising antibodies in ponies and protection in a mouse model. We have extended these studies and will present recent data demonstrating the protective efficacy of MVA-VP2 against an AHSV challenge in horses. We will also show that a vaccination regime based on MVA-VP2 induced a cross-reactive antibody response that can potentially protect against all AHSV serotypes.

ORAL: Identification of T cell epitopes from non-structural protein NS1 of bluetongue virus serotype 8, capable of cross-reacting with other viral serotypes in mice and sheep.

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CISA-INIA¹

Key words: Bluetongue virus, T cell epitopes, immune response

Bluetongue virus (BTV), an economically important orbivirus of the Reoviridae family, is a non-enveloped, dsRNA virus that causes a haemorrhagic disease mainly in sheep. In order to estimate the importance of T cell responses during BTV infection, it is essential to identify the epitopes targeted by the immune system. We have previously reported the existence of T cell epitopes in mice and sheep from the VP7 core protein of BTV-8. In the present work, we selected potential T cell epitopes for the C57BL/6 mouse strain from the BTV-8 non-structural protein NS1, using H2b-binding predictive algorithms. 3 MHC-class II-binding peptides and 7 MHC-class I binding peptides were selected. Peptide binding assays confirmed all MHC-class I predicted peptides bound MHC-class I molecules. The immunogenicity of these 10 predicted peptides was then determined using splenocytes from BTV-8-inoculated C57BL/6 mice. Three MHC-class I binding peptide consistently elicited peptide-specific IFN- γ production as measured by ELISPOT assay. Cytotoxic T lymphocytes (CTL) were also generated against these 3 peptides in BTV-8 infected mice. Importantly, these CTL were also able to recognise target cells infected with different BTV serotypes. Flow cytometry analysis using intracellular cytokine staining confirmed that CD8⁺ T cells mediated the response to these 3 NS1-derived T cell epitopes. Similarly, one MHC-class II peptides was demonstrated to be a CD4⁺ T cell epitopes using a combination of IFN- γ ELISPOT, proliferation and flow cytometry assays in BTV-8 infected mice. Importantly, two peptides were also consistently immunogenic in sheep infected with BTV-8 using IFN- γ ELISPOT assays. Both of these peptides appear to stimulate CD4⁺ T cells. We are currently investigating the ability of these BTV-specific T cells in sheep to respond to different BTV serotypes. The characterisation of these novel T cell epitopes may also provide an opportunity to develop a DIVA-compliant vaccination approach to BTV.

ORAL: Avian influenza virus subtype H9N2 – an emerging threat to poultry in Europe?

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NVRI¹

Key words: avian influenza, H9N2, turkeys

Avian influenza H9N2 viruses (AIV/H9N2) have been endemic in domestic poultry from Northern Africa and the Middle-East to the Far-East. The viruses cause high economic losses in poultry but their potential impact as zoonotic agents is also of concern. Until recently, the viruses had been sporadically detected in wild birds and poultry in Europe and did not seem to establish themselves in the population of domestic birds (1). However, the most recent reports from Germany suggest that infections with AIV/H9N2 have become endemic, mostly in turkeys, in certain regions of the country (2, 3).

In Poland, the first suspicion of AIV was raised at the end of April 2013 in 5 meat turkey flocks aged 9 weeks (2 flocks) and 13 weeks (3 flocks) located at one farm in the western part of the country. The turkeys were hatched and raised in Germany until the age of 4 weeks, and then moved to Poland and reared until the slaughter age. Clinical signs such as depression, lack of vocalisation, reduced feed and water intake and respiratory symptoms (coughing and sneezing) but no increased mortality were observed. A few weeks later samples from 4.5-16 weeks-old turkeys from another 3 farms in the same region were submitted to the laboratory.

Samples of tracheal and cloacal swabs were tested positive in real time RT-PCR (rRT-PCR)/M (all influenza A type) but rRT-PCR/H5&H7&N1 gave negative results. The conventional RT-PCR for H9 & N2 genes yielded positive results. The isolate from the first case was successfully grown in SPF embryonated eggs and confirmed to belong to H9 subtype in haemagglutination inhibition (HI) test. The virus inoculated intravenously into 10 six-week-old specific antibody negative chickens did not induce morbidity or mortality (intravenous pathogenicity index IVPI = 0.0). Samples of sera from all farms tested positive in both ELISA and HI/H9 test. Preliminary phylogenetic study based on the partial sequencing (approx. 1000 bp) of H9 and N2 genes indicates that there is no link between Polish and Asian outbreaks in poultry but points at wild birds as the primary source of the virus introduction into domestic birds. The frequent and free movement of poultry between EU member states and lack of regulations concerning the control of AIV/H9N2 infections may contribute to the geographical spread of the virus. Further studies are needed to establish the prevalence of infections and to follow molecular markers associated with virus adaptation to poultry and humans.

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ORAL: Experimental reproduction of Epizootic hemorrhagic disease of deers

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Key words: Epizootic hemorrhagic disease of deers, virus, experimental infection, red deer (*Cervus elaphus*), axis deer (*Cervus nippon*)

Outbreaks of epizootic hemorrhagic disease of deers (EHD) are reported annually in North America in the white-tailed deer (*Odocoileus virginianus*), which is mostly affected by peracute form of the disease. Over the last decade virus circulation diagnosed in Eurasia (Turkey, Israel). According to our previous investigations, central, southern and eastern regions of Russia, together with the biggest part of Europe, are secondary risk area for EHDV occurrence. In this territories, the most numerous species, systematically related to white-tailed deer, are red deer (*Cervus elaphus*) and axis deer (*C. nippon*).

The main purpose of our investigations was studying of red and axis deer susceptibility to EHDV.

EHDV serotype 1 (strain New Jersey) was used to infect deer.

On the eighth day, the rise of red deer temperature to 41,10C was observed. On the fourteenth day, mucous membranes of the mouth and anus hyperemia was observed. On 45th day, development of bursitis carpal joints and alopecia on the backs of the animals were noted. Subcutaneous hemorrhage in the back and minor swelling in the dewlap were observed at autopsy. The virus from blood was isolated in cell cultures on 8-20 dpi.

No clinical signs were observed in infected axis deer, as well as in control animal; the temperature was between 38,5-39,00C. Subcutaneous hemorrhages in the back were observed at autopsy. Mucous membrane of the upper lip had small focal reddening. On the diaphragm serous cover and spleen capsule the connective excrescences were observed. On the extremities the swelling of hoof horn and ulcers were revealed. Virus RNA was detected by PCR-RT, developed A. Clavijo et al., from 6 to 23 dpi in the blood samples. Serum antibodies against EHDV were detected by NT from 9 dpi and over a whole period of research (within 23 days).

According to the results of the work we may make the following conclusions:

- experimental infection by EHDV causes acute and subclinical form of the disease in red and axis deer, respectively;
- red and axis deer infected by EHDV may be carriers of the virus;
- real-time PCR based diagnostics is effective for lifetime EHDV detection.

Sessions 4 & 6 continued

ORAL: Virulence comparison and quantification of horizontal bovine viral diarrhoea virus transmission following experimental infection in calves

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Key words: BVDV - transient infections - transmission - experimental infections

Bovine Viral Diarrhoea Virus (BVDV) is a worldwide spread virus that most commonly infects cattle and can cause considerable economic losses. When infecting susceptible animals in early gestation, BVDV is able to cause persistent infections by infecting the foetus. These persistently infected (PI) animals are important sources of infection. On the contrary, transiently infected (TI) animals are believed to be less important in the epidemiology of BVDV. However, transient infections with a severe BVDV-2 strain can spread explosively. Only few studies were performed to determine the transmission potential of TI animals. Nevertheless, understanding the transmission capacity of TI cattle is very useful when establishing BVDV control programmes, especially in cattle-dense regions like Belgium. Therefore the aim of this study was to compare BVDV strain virulence and to estimate basic reproduction ratios for horizontal BVDV transmission by TI cattle following experimental infections using two Belgian virulent BVDV field isolates.

Transmission capacity by TI animals was determined during two trials. In trial 1 and 2 a BVDV-1b and a BVDV-2a strain were used, respectively. In each trial three calves were inoculated with BVDV through intranasal instillation of and housed together with seven contact animals. In case there remained BVDV seronegative animals following the first inoculation of each trial, all seroconverted animals were removed and the experimental design was repeated. To compare virus transmission capacity of TI animals with PI animals, a third trial was initiated. For this a calf persistently infected with a Belgian BVDV-1b strain was commingled with all ten calves of trial 2. During the experiments nasal swabs, whole blood and serum samples were collected and clinical characteristics were monitored. Virus isolation, determination of degree of viraemia, PCR, virus neutralization and analysis of blood composition were performed on the collected samples. The basic reproduction ratio (R₀), a measure of transmission of infection, was calculated using the maximum likelihood estimator.

It was possible to isolate virus and to detect BVDV in nasal swabs from each inoculated animal. The severe clinical symptoms observed in the field with these isolates could not be reproduced during these experiments. Following the first inoculation with BVDV-1b no virus transmission was detected. The experimental infection was repeated by inoculating three animals and following this second inoculation 1 of 4 contact animals was infected. For trial 1 this resulted in a R₀ of 0.25 (95% CI 0.01-1.95). Following the first inoculation with BVDV-2a 1 of 7 contact animals was infected. The experimental infection was repeated by inoculating three animals and none of the three remaining contact animals was infected. For trial 2 this resulted in a R₀ of 0.24 (95% CI 0.01-2.11). In trial 3 all three remaining seronegative calves were infected by the PI animal, which resulted in a R₀ of +∞ (95% CI 0.68-+∞). No blood samples of the seven animals which seroconverted for BVDV-2a in trial 2 were PCR-positive during trial 3.

A very limited horizontal BVDV transmission was observed when TI animals were introduced into a group of susceptible calves, whereas very rapid and efficient transmission was found when a PI animal was introduced. These results suggest TI animals poorly contribute to BVDV spread compared to PI animals. This stresses that the removal of PI animals is essential for BVDV control and eradication, whereas focussing on TI animals should not be necessary.

ORAL: Exploring Cattle Movements in Belgium

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Key words: Cattle movement, Spatio-temporal, Bluetongue

Movement of an animal from one farm to another can lead to the spreading of livestock disease. Therefore, in order to implement an effective control measure against the spreading of the disease, it is important to understand the movement network in a given area. Using the SANITEL data from 2005 to 2009, around 2 million cattle movements in Belgium were traced. Exploratory analysis revealed different spatial structures for the movement of different cattle types: fattening calves are mostly moved to the Antwerp region, while bovines are moved to various parts in Belgium. Based on these differences, movement of bovine would more likely cause a spread of disease to a larger number of areas in Belgium as compared to the fattening calves. Also due to the shorter life expectancy, fattening calves are less likely to spread the disease.

A weighted negative binomial model for the bovine movement revealed hot spot areas of movement; four areas in the Walloon region (Luxembourg, Hainaut, Namur and Liege) are found to be important sources of movement while East and West Flanders are important receivers of movement. This implies that an introduction of a disease to these Walloon regions could result to spreading in the East and West Flanders region. The temporal trend furthermore suggests that intensive surveillance should be performed around spring and autumn. Linking this movement to the spreading of Bluetongue in 2006, results revealed the significant role of cattle movement to the observed pattern of the infection.

ORAL: EPIZONE: Get INSPIREd

Ferre, nicola¹; Rumor, Massimo²; Roccatello, Eduard²; Marangon, Stefano¹

IZS-Ve¹; 3DGIS²

Key words: INSPIRE, conceptual model, data harmonisation

Large amounts of data have been collected and a large number of databases built as part of several EU-funded animal health projects and veterinary activities in general, making a wide variety of spatial data available to the veterinary community. Data layer integration has been hindered mainly by diverse database schemas, geographical models, and spatial distributions. These differences are mostly due to the fact that data acquisition procedures, as information gathering and analysis, are based on a variety of planning criteria. In two recent workshops organized by the European research network EPIZONE (“Data Information Sharing within the Veterinary Scientific Community: Legislative State of the Art and the Role of EPIZONE”; “Data Information Sharing”), it emerged that these data should be interoperable and readily accessible for researchers, policy makers or businesses. The veterinary community is aware that animal health data (including zoonoses data and related spatial information) are expected to contribute to multidisciplinary activities addressing new challenges posed by the global economy. This would enhance the transparency, credibility and efficiency of research and promote the use of data and results for policy-making or business purposes. The need for harmonised veterinary geoinformation is therefore becoming a key topic.

Data harmonisation is also the main objective of the INSPIRE Directive (2007/2/EC), which has entered its implementation phase. The Directive is aimed at providing better, more easily accessible spatial information in Europe for the formulation and implementation of a community policy on the environment. Although the INSPIRE Directive provides a framework and technical specifications for making spatial information created and held by public bodies across Europe interoperable, its relevance to the veterinary community is almost unknown. The aim of this study is therefore to put INSPIRE principles into practice and to present the results of this activity to the EPIZONE community.

The study investigates the feasibility of transposing the “INSPIRE Data Specification on Agricultural and aquaculture facilities” with a view to moving towards georeferenced management of animal holding data. The study would deliver a generic, extendable and interoperable system able to embrace different types of animal holding, from a bee hive to a large poultry farm, and to allow veterinary organizations to adapt their legacy data to the model. The conceptual model is designed to represent animal holding objects at different levels of detail, in order to discern the fundamental patterns, processes and properties of animal holding activities.

The model has been tested according to the provisions of EU regulation no. 1089/2010. Specifically, a schema mapping between the proposed model and the legacy data of a veterinary laboratory has been developed to test which spatial query, quantitative analysis and exploratory data analysis methods could be applied to the veterinary field.

Besides implementation of the conceptual model, some cost-benefit indicators have been collected to quantify the return on the investment. The preliminary results of this activity are also presented.

ORAL: An experimental model to analyze the risk of introduction of a duck originated H5 low pathogenic avian influenza virus in poultry through close contact and contaminative transmission

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Key words: H5 Low Pathogenic Avian Influenza Virus, Interspecific Transmission, Real-time RT-PCR, Close contact transmission, Contaminative transmission

Aquatic wild birds are often carriers of LPAIVs. If H5 and H7 LPAIVs are transmitted to poultry and have the opportunity to circulate, a HPAIV may arise. Contact with aquatic wild birds is one of the most important ways in which these LPAIVs can be introduced into poultry flocks. In this study, the transmissibility of a duck originated H5 LPAIV between ducks and chickens was analyzed in a series of animal experiments, using different transmission routes. Results proved that the outcome of virus intake by chickens exposed to infectious ducks depends on the way the virus is presented. Drinking water contaminated by faeces proved to be the most efficient route by which the virus can be transmitted to chickens. From our results, it appears that measuring the amount of shed viral RNA or virus has only a limited predictive value, whereas data from serology were found to be decisive.

ORAL: Use of abortion surveillance in cattle to verify the introduction of Schmallenberg Virus in Flanders.

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Key words: Schmallenberg virus

In late summer 2011, a non-specific syndrome was reported in cattle in the Netherlands, North-West Germany as well as in the Northern region of Belgium (Flanders). The atypical syndrome was characterized by hyperthermia, milkdrop in dairy cattle and occasionally watery diarrhea. In blood samples of clinically affected animals, the Schmallenberg virus (SBV) was discovered first in Germany in November 2011. So far, it remained unclear how and when exactly this virus arrived in Western-Europe. Since October 2009 a 'protocol abortion' is implemented in the Belgian cattle population. The purpose of this protocol is to encourage veterinarians and farmers to send in sample material (fetus, afterbirth, serum) of cattle that aborted. Beside the mandatory tests to be performed in the mandatory surveillance programme for brucellosis many other diagnostic tests can be performed as well and serve as an incentive for farmers and veterinarians. The aborted cattle population can be considered as a target population in order to follow syndromes or the (re)emergence of diseases.

Hence, the aim of this study was to elucidate when SBV was first introduced in cattle in Flanders using historical sera of cattle that aborted.

Hereto, a total of 975 serum samples of cows that aborted in Flanders between January 2011 and January 2012 were randomly selected (75 samples/month) from the serum bank of the regional laboratory DGZ Vlaanderen. These samples were originally collected (and stored at -80°C) as part of the obligatory abortion surveillance programme of the Federal Agency for the Safety of the Food. The first seropositive samples were found in cows aborting in August 2011. Subsequently, seroprevalence appeared to evolve from 2.86 % (August 2011) to 91.43 % (January 2012) in 5 months' time. Based on these results, it can be concluded that SBV was first introduced in Flanders in late summer 2011. The finding that the seroprevalence evolved quite rapidly suggests a widespread exposure to SBV in 2011.

ORAL: FEVER: A Framework to assess Emerging VEctor-borne disease Risks for livestock

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Key words: vector-borne diseases, livestock, risk assessment, framework, transmission dynamics

Since the incursions of bluetongue virus serotype 8 and Schmallenberg virus in Northwestern Europe, the awareness of emerging vector-borne infections in livestock has increased and risk assessments of such infections have become an important request from policymakers. Risk assessment of exotic vector-borne livestock diseases requires a multidisciplinary approach, taking into account veterinary epidemiology, virology, entomology, ecology, climatology, economy, and – in case of zoonotic infections – human epidemiology.

To prepare for risk assessments of vector-borne diseases in livestock, we developed a Framework to assess Emerging VEctor-borne disease Risks for livestock, FEVER, that can be used for both quick and in-depth risk assessment of exotic vector-borne livestock diseases. The main elements in FEVER are introduction, transmission, establishment, spread, persistence and impact. FEVER uses a stepwise approach, guided by a checklist and/or a questionnaire, thus helping the risk assessor to consider or include all relevant steps into the assessment. Results of risk assessments based on FEVER provide insight into the main elements contributing to the risk which is a prerequisite to identify effective prevention and control measures when preparing for emerging vector-borne diseases. FEVER also helps to identify existing gaps in knowledge and in data that contribute to uncertainty of the risk estimate. Furthermore, FEVER provides consistency in risk assessments of vector-borne diseases enabling comparison between diseases or geographical areas.

FEVER was extensively tested in two international workshops in which the risks of Rift Valley fever (RVF) and Crimean Congo haemorrhagic fever (CCHF) for Western Europe were assessed. Based on these workshops we conclude that FEVER provides a quick and efficient method for a first risk assessment of a vector-borne livestock disease. In a two-day workshop, a lot of information can be obtained in a structured manner from the experts present. Furthermore, such a workshop provides good insight into the main drivers of the risk in a relatively short time span.

The results of the workshops indicated that for both diseases uncertainty about presence and/or competence of the vector in Western Europe is a major knowledge gap. Furthermore, we concluded that FEVER is more easily applied to midge-borne and mosquito-borne infections than to tick-borne infections. Although the main elements of the framework are valid for all three vector species, transmission dynamics of tick-borne infections are rather different from those of midge-borne and mosquito-borne infections. Especially the time scale at which processes occur is much longer for ticks as a result of which tick-borne infections often result in endemicity once introduced.

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POSTERS

ARBOVIRUSES

A1- POSTER: Detection of the Schmallenberg virus in nulliparous *Culicoides obsoletus* complex and *C. punctatus* – the possibility of transovarial virus transmission in the midge population and a new vector

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Key words: Schmallenberg virus, *Culicoides*, transovarial transmission

The epidemiology of the Schmallenberg virus is related closely to its vector ecobiology. Similar to other arthropod-borne viruses of veterinary importance such as BTV and AHS, the occurrence of the disease coincides with the distribution of competent *Culicoides* spp. The mechanism of the emergence of new pathogens, such as SBV, is unclear; however, climate changes and increasing pollution, the globalization of the animal trade and genetic changes both in viruses and in hosts are often mentioned. SBV was shown to overwinter in the area, despite the absence of the competent vector; however, the mechanism has not been clarified. In this study, the possibility of transovarial transmission is postulated on the basis of the evidence of SBV infection in nulliparous females of two *Culicoides* species: *C. obsoletus* complex and *C. punctatus*. Additionally, *C. punctatus* was demonstrated to carry SBV RNA for the first time.

A total of 8,263 female *Culicoides* spp. collected weekly using Onderstepoort UV light traps in 23 different locations scattered over 13 provinces of Poland during the 2011/2012 monitoring program of BTV vector were used in the study. The midges were identified using the taxonomic key of Glukhova (1989) and divided into pools according to the species and parity status. The insect pools were first homogenised in Lysing Matrix D tubes containing 1.4 mm ceramic beads with 1 ml of TRI Reagent and the total RNA was extracted following TRI Reagent manufacturer's instruction. Duplex real-time RT-PCR was performed using primers and probes for simultaneous detection of the viral S segment and culicoid fragment of 18S rRNA. The reactions were performed using AgPath-ID One-Step RT-PCR reagents. The presence of SBV was detected in 44 (10.9%) pools. SBV positive pools were identified in 10 out of 23 locations, spread randomly throughout Poland and without any geographical clustering. Viral RNA was detected only in those pools collected in the last week of August and during the last two months of sampling. None of the midge pools from the previous months was found positive. The majority (82%) of SBV positive pools belonged to *C. obsoletus/scoticus* species. The RNA of SBV was found in 15.6% *C. obsoletus/scoticus* and additionally in 8 out of the 149 (5.4%) *C. punctatus* pools tested. All pools of other midge species were negative. Significant differences in SBV infection rates were observed between midge pools of different parity status. SBV RNA was most frequently identified in gravid midges (36.4%), while in nulliparous, blood-fed and parous midges the percentages were 10.8%, 13.0% and 8.1%, respectively.

Culicoides obsoletus complex proved to be the most important vector of SBV, however *C. punctatus* was postulated another possible vector. Detection of SBV RNA in nulliparous female midges of *C. obsoletus* complex and *C. punctatus* species gave an indirect evidence of the vertical transovarial transmission of the virus which may explain virus overwintering. The timing and the direction of the SBV spread in the midge population confirmed the epizootic situation observed in the ruminants. SBV surveillance in *Culicoides* spp. may be used as an early warning system for the emerging viruses

A2- POSTER: Schmallenberg virus in Culicoides biting Midges in the Netherlands in 2012: a decreased level of circulation compared to 2011

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Key words: Schmallenberg virus, Culicoides, 2012, Netherlands

Introduction: In 2011, a novel Orthobunyavirus of the Simbu serogroup, named Schmallenberg virus (SBV), spread silently across much of northern Europe, infecting ruminant livestock. Several field studies have implicated one or more species of *Culicoides* to act as field vectors for SBV. In the Netherlands, midges of the *Obsoletus* complex and *C. chiopterus* were implicated to act as field vectors for SBV in 2011. We analyzed pools of *Culicoides* - caught in 2012 - for RNA of SBV to test if other *Culicoides* spp. became involved in the spread of SBV and to investigate whether the level of SBV-infected *Culicoides* trapped in 2012 was comparable to those caught in 2011.

Material and Methods: *Culicoides* were trapped almost daily throughout May to October, 2012 at a dairy in the municipality of Ermelo (East Netherlands) and at a location with heifers in the municipality of Speuld (East Netherlands). Captured *Culicoides* were stored in ethanol 70%, and identified morphologically. Midges were divided into 130 species-specific pools, i.e. 50 midges/pool. The number of pools assayed for each species was as follows: *Obsoletus* Complex (42), *C. chiopterus* (21), *C. dewulfi* (26), *C. punctatus* (31), and *C. pulicaris* (10). RNA-extraction was performed as described by De Regge et al. (2012). The RT-PCR cut-off value was set at a cycle threshold (Ct) value of 35. Pools with Ct values >35 were retested and considered positive when confirmed in the retest.

Results and Discussion: Two (1.5%) of the 130 *Culicoides* pools tested SBV-positive by RT-PCR: one pool of midges of the *Obsoletus* complex caught on 14 July 2012 in Ermelo, and one pool of midges of the *Obsoletus* complex caught on 8 August 2012 in Speuld. All 26 pools of *C. dewulfi* tested RT-PCR-negative for SBV, as well as the 21 pools of *C. chiopterus*, the 31 pools of *C. punctatus* and the 10 pools of *C. pulicaris*. This is an indication that no other *Culicoides* spp. became involved in the spread of SBV in 2012 compared to the situation in 2011. The PCR-Ct values of the two positive pools indicated a relatively low viral load. On an individual midge level, assuming one infected midge per positive pool, the proportion of SBV-infected *Culicoides* of the *Obsoletus* complex in 2012 was 0.1% (2/2,100 tested), which was significantly lower compared to the 0.56% SBV-positive midges of the same species in 2011. It is plausible to suggest that the presumed lower level of transmission of SBV in 2012 occurred because only a small fraction of hosts was left susceptible for infection after the massive epidemic in 2011. This may indicate that SBV is being transmitted very efficiently. The speed and spatial dissemination of SBV in Europe has exceeded that demonstrated by Bluetongue virus serotype 8 in the period 2006-2010. SBV-seroprevalence studies executed in ruminant populations so far indicate an amazingly high proportion of animals infected after the first epidemic. In relation to the findings reported in this study it is very hard to predict how the SBV epidemic in Europe will evolve. In countries earlier affected, the remaining susceptible hosts may become infected and in the long run the epidemic could fade-out in these areas. However, while expanding geographically the number of young susceptible animals in the first affected countries at a certain stage might also increase as a result of growth of newborns. In such a scenario whereas the frontline of the epidemic is still moving, infections might reoccur in the primary outbreak regions and it is to be seen where it will end.

A3- POSTER: Transplacental transmission of Bluetongue virus serotypes 8 and 1.

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Key words: Bluetongue, BTV-8, BTV-1, transplacental transmission, sheep

Introduction

Both in the field and in experimental setting, BTV-8/Net2006, the Bluetongue virus (BTV) serotype 8 strain that emerged in Europe in 2006, has shown an unusually high ability to cause foetal infection in pregnant ruminants. In contrast, transplacental transmission of other BTV serotypes, already present in Europe before 2006, had never been demonstrated. To determine whether transplacental transmission is a unique feature of BTV-8, we compared the incidence and pathological consequences of transplacental transmission of BTV-8 to that of another BTV serotype isolated from sheep in Europe: BTV-1.

Materials and Methods

Nine 70-75 days pregnant ewes were infected with either BTV-8 or BTV-1. Four weeks after the infection, the foetuses were collected and examined for pathological changes and BTV immunohistochemistry was performed on tissue sections. Blood and tissue samples from the lambs were tested for the presence of BTV by virus isolation and RT-qPCR. Serotype specific RT-qPCRs were done to confirm the presence of either BTV-8 or BTV-1 in the samples.

Results

BTV-8 RNA and infectious virus could be demonstrated in 12/28 lambs (43%) from 5 ewes (56%). In the BTV-1 group, 6 ewes (67%) transmitted the virus to their lambs, resulting in the infection of 14/17 lambs (82%).

Pathological changes were mainly found in the central nervous system. In the BTV-8 group, lesions consisted of lympho-histiocytic infiltrates, gliosis and slight vacuolation of the neuropil. BTV-1 infection induced a severe necrotizing encephalopathy and severe meningitis, with macroscopic hydranencephaly or porencephaly in 8 out of 14 infected lambs. Immunohistochemistry confirmed the association of BTV with these lesions.

Conclusion

We demonstrated that, in our model, a European isolate of BTV-1 is also capable of infecting lamb foetuses. The incidence of transplacental transmission of BTV-1 was at least as high as that of BTV-8. Moreover, BTV-1 induced severe necrotizing central nervous system lesions, whereas this was not observed in the BTV-8 infected lamb foetuses.

A4- POSTER: Clinical cases of Schmallenberg virus intrauterine infections in Poland

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NVRI¹

Key words: Schmallenberg virus, intrauterine infection, malformations

Schmallenberg virus (SBV) has been associated with subclinical infections or rarely short mild/moderate disease (milk drop, pyrexia, diarrhoea) in adult cattle and late abortions or malformations in newborn cattle, sheep and goats. First cases of SBV intrauterine infections were recognised in cattle and sheep in North-Western Poland between November 2012 and January 2013. More than half of these cases involved newborns which died in the first day of life, whereas the rest cases were stillbirths. The clinical signs of SBV infection in the fetus and newborn were observed in less than 40% of cases. Most common malformations included: scoliosis and kyphosis, arthrogryposis, torticollis and brachygnathia interior. Ten to twenty percent of cases presented hypoplasia of the cerebrum, cerebellum and spinal cord observed at the necropsy. Macroscopic changes of the cranial region included hydranencephaly, porencephaly, hypoplasia to atrophy of hindbrain. Histopathological examination of brain tissue revealed vacuolation and hypoplasia of neuropil and the presence of lymphohistiocytic perivascular infiltration, hypereosinophilic and necrotic neurons and microglial nodules. Focal hypoplasia to atrophy of white matter and mild hypoplasia of the gray matter was observed. The most suitable material for SBV detection in foetuses and newborns proved to be the brain, cerebellum, medulla oblongata and fetal umbilical cord what was correlated to the highest concentrations of viral RNA found in those tissues. However, due to the mostly inapparent SBV infections in adult ruminants, no compulsory reporting of new outbreaks and no monitoring of ruminant SBV infections, the number of SBV clinical cases in the country might be underestimated

A5- POSTER: Arbovirus Monitoring of Mosquitoes in JeJu Island, Republic of Korea in 2012

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Animal and Plant Quarantine Agency¹; Eighth United States Army²; Incheon National University³

Key words: Arbovirus, Mosquito, Polymerase chain reaction

Arbovirus monitoring was conducted in Jeju island to investigate the distribution of mosquitoes and presence of arboviruses in mosquitoes. Jeju Island is the southernmost island in the Republic of Korea, and is therefore regarded to be at a higher risk for the presence of arboviral diseases and provides the ideal environment to study the effects of climate change. For this study, arboviruses which are exotic and/or are of major concern in Korea were selected. This included West Nile virus (WNV), Japanese encephalitis virus (JEV), and Eastern, Western and Venezuelan Equine Encephalitis Viruses (EEE, WEE and VEE). Mosquitoes were collected using mosquito magnet® traps placed around horse barns, international airports and ports in 2012. A total of 11,372 mosquitoes, representing 9 species and 5 genera were captured and the most frequently collected species was *Culex pipiens* (80.09%, n=9,222), followed by *Ochlerotatus togoi* (13.40%, n=1,524), *Aedes albopictus* (4.24%, n=482), *Armigeres subalbatus* (0.86%, n=98), *Culex tritaeniorhynchus* (0.27%, n=31), *Aedes vexans* (0.05%, n=6), *Ochlerotatus koreicus* (0.04%, n=5), *Anopheles sinensis*, and *Culex inatomii* (0.02%, n=2). Of the 11,372 mosquitoes collected, a total of 1,951 mosquitoes were screened for arboviruses by reverse transcription polymerase chain reaction. No WNV, JEV, EEE, WEE and VEE viruses were detected in the samples tested. Despite these results, continuing climate change, affecting the number and distribution of mosquito vectors, will increase the risk of introduction of these arboviruses into Korea. Therefore vector monitoring, performed in conjunction with animal surveillance will have an important role in identifying areas at risk and quickly detecting the introduction of major arboviruses, allowing for the rapid implementation of appropriate preventive and control measures.

A6- POSTER: Genetic variation in the Schmallenberg virus L, M, and S RNA segments present in field-collected Culicoides midges in the Netherlands.

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Animal Sciences Group of WUR¹; CVI²

Key words: Schmallenbergvirus, midges, genetic variation, next generation sequencing.

Biting midges of the genus *Culicoides* have been demonstrated to be responsible for the transmission of Schmallenbergvirus (SBV) among cattle and sheep in the outbreak in Europe which started in late summer 2011. Determination of the species identity of field-collected SBV-positive midges from the Netherlands in August–September 2011 using DNA analysis of the 18S internal transcribed spacer 1 revealed that SBV was harbored in 3 species: *Culicoides scoticus*, *Culicoides chiopterus*, and *Culicoides obsoletus sensu stricto* [1]. To assess genetic variation between SBV present in these midges, five SBV-positive pools of midges (3 *C. scoticus*, 1 *C. obsoletus sensu stricto*, and 1 undeterminable), all scoring an SBV QRT-PCR Ct-value lower than 25 in heads, were used to generate cDNA fragments covering specific regions of the L, M and S genomic RNA segments (~150-300 bp in length). L, M, and S cDNA preparations generated for each pool of midges were tagged with a unique index-sequence as identifier, and all indexed midge pools were mixed and sequenced in a single Illumina MiSeq run. Using this “next generation sequencing” (NGS) approach the genetic variation in SBV between individual midges within a pool and between different pools of midges was determined. Preliminary results revealed that the degree of SBV genetic variation between individual midges, and between different pools, was limited. SBV NGS data obtained from these midges were compared to published SBV sequences originating from cattle and malformed lambs

A7- POSTER: Schmallenberg virus monitoring in Culicoides trapped during 2011 and 2012 in Belgium.

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CODA-CERVA¹; Institute of Tropical Medicine²; Centre wallon de Recherches agronomiques³; Université de Liège⁴

Key words: Schmallenberg virus, Culicoides, rRT-PCR, Belgium

Schmallenberg virus (SBV) was first identified in November 2011 by the FLI (Germany), and has since then been detected in many European countries. To examine the potential role of midges in the rapid expansion of the virus and SBV circulation in Belgium, midges caught with OVI traps from July to October 2011 and 2012 at 4 different regions in Belgium (Antwerp (north-east), Liège (east), Gembloux (centre), Libramont (south)) were screened by rRT-PCR (S segment, protocol FLI) for the presence of SBV.

In 2011, pools consisting of heads (≤ 20 /pool) of parous females morphologically identified upon species level were analyzed. Pools of the *C. obsoletus* complex (*C. obsoletus* s.s), *C. dewulfi* and *C. chiopterus* were found positive indicating that these species might play a role in the transmission and spread of SBV. The first SBV positive midges were found in August 2011 and depending on time and place, a high percentage (up to 30%) of pools were found positive. No positive pools were found in the south of Belgium, correlating with a low seroprevalence rate in sheep and cows at the end of the first vector season (end 2011) in that region.

In 2012, pools of whole parous females (≤ 20 /pool) identified at group level (*Avaritia*, *Culicoides*, *Monoculicoides*) were screened. All pools from July remained negative while positive pools were found at every location in August. In the south (Libramont), more than 50% of the pools from August were SBV positive, indicating virus replication and spread in the (partially) non-protected host population. For Antwerp and Liège, pools from September and October tested negative while those from Gembloux and Libramont remain to be tested. In conclusion, these studies allowed identifying several *Culicoides* species as putative vectors of the recently emerged Schmallenberg virus and confirm the recirculation of the virus in Belgium in 2012.

A8- POSTER: Pathogenesis and transmission of Schmallerberg virus in domestic pigs

Poskin, Antoine¹; Van Campe, Willem¹; Mostin, Laurent¹; Brigitte, Cay¹; De Regge, Nick¹

CODA-CERVA¹

Key words: schmallerberg; pigs; experimental infection; host-range

The Schmallerberg virus (SBV) is a newly emerged virus responsible for a non-specific syndrome including high fever, decrease in milk production and severe diarrhoea in adult cattle. It also causes reproductive problems in cattle, sheep and goat including abortions, stillbirths and malformations. Sequencing of the virus and phylogenetical analysis showed that SBV belongs to the Simbu serogroup of the genus Orthobunyavirus, family Bunyaviridae. Beside the animals mentioned above, SBV has been detected by PCR in bison, deer, moose, alpacas and buffalos and antibodies to SBV were found in fallow deer, roe deer and red deer. The role of pigs in the epidemiology of SBV has not yet been evaluated. This could however be interesting seen their suggested role in the epidemic of the closely related Akabane virus (Huang et al., 2003).

To investigate this issue an experimental infection study was carried out at CODA-CERVA. Four seronegative piglets of 8 weeks old were infected, subcutaneously, with 1ml of SBV infectious serum (FLI) and kept into contact with four seronegative non-infected piglets to examine direct virus transmission. Throughout the experiment blood, swabs and faeces were taken and upon euthanasia at 28 dpi different organs (cerebrum, cerebellum, brain stem, lung, liver, lymph node, kidney and spleen) were collected as well. No clinical impact was observed and all blood samples, swabs, faeces and organs of all pigs tested negative for SBV in PCR. 3 out of 4 infected piglets had seroconverted while the contact piglets remained seronegative.

In conclusion, SBV infection of pigs seems to induce seroconversion but is ineffective in terms of virus replication and transmission indicating that pigs have no obvious role in the SBV epidemiology.

A9- POSTER: Complete Genome Sequence and Phylogenetic Analysis of Bluetongue virus Serotype 16 from Italy

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IZSAM¹; Baseclear BV²

Key words: Bluetongue virus serotype 16, Next Generation Sequencing, Italy

Innovative sequencing techniques or “next-generation” sequencing (NGS), provide high speed and throughput providing a humongous amount of sequences in a single run at reasonably low cost. This study describes the complete genome sequence of a field strain of bluetongue virus serotype 16 (BTV-16) isolated from a cattle in Apulia region (Italy) in 2002. Next Generation Sequencing by Illumina platform delivered the full-length sequences of all 10 segments of the virus. The full genome sequence analysis confirmed that BTV-16 ITL2002 is a reassortant strain having the Seg-5 identical to that of the BTV-2 vaccine strain (RSAvvvv/02) which probably originated from two parental modified live vaccine strains, BTV-16 and BTV-2. Sequence comparisons of Seg-1 to Seg-10 but Seg-5 show that BTV-16 ITL2002 belongs to the major eastern topotypes of BTV. This work further highlights the need for implementation of sequencing techniques and sharing of genomic data.

A10- POSTER: Bluetongue in springboks and wildebeests in the Etosha National Park (Namibia): circulating strains and epidemiology

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IZSAM¹; Ministry of Environment and Tourism, Namibia²

Key words: Bluetongue virus, wildlife, Namibia, epidemiology

Bluetongue (BT) is an important vector-borne disease affecting ruminants worldwide. Little is known about the epidemiology of Bluetongue virus (BTV) in African wildlife. In May 2011, a surveillance program for BTV was organized in the Etosha National Park in Namibia. One hundred and eighty two springboks (*Antidorcas marsupialis*) and fifty blue wildebeests (*Connochaetes taurinus*) were sampled. Blood and serum samples were tested to detect the presence of BTV antibodies and BTV RNA by using competitive ELISA and real-time RT-PCR, respectively. The serum neutralization assay was used to determine the BTV serotype. Bluetongue virus RNA was found in 120 (66%) springboks and 9 (18%) wildebeests, conversely virus isolation was unsuccessful. All wildebeests and 170 (93%) springboks showed BTV antibodies. Twenty five of the 26 recognised BTV serotypes were identified. Interestingly, even though many animals presented antibodies against different serotypes, BTV-1 and BTV-26 were the most frequently detected. It was the first time that the presence of BTV-26 was demonstrated outside Kuwait, the country where it was detected the first time. According to these findings, it appears that BTV widely circulated or is circulating in the Etosha National Park. More studies are warranted in order to establish the role played by wildlife in the epidemiology of BTV and the spread of the newly discovered BTV-26.

A11- POSTER: Pathological manifestations and distribution of viral RNA in malformed newborn calves after natural intrauterine infection by emerging Schmallenberg virus

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University of Liege¹

Key words: Schmallenberg virus newborn calves intrauterine

Schmallenberg virus is the first Orthobunyavirus detected in Europe. It emerged northwest of the continent in the summer 2011 and has since spread rapidly to the point of being detected in the majority of European countries a year later. Ruminants are infected by biting midges (*Culicoides* spp.) which results in a non specific flu-like illness occurring during the summer and autumn, with fever, anorexia, drop in milk production and sometimes diarrhea. Moreover, in a subset of fetuses infected in utero, a congenital malformation/neurological syndrome ensues. In this paper, we report the results of an exhaustive survey of the macroscopic and microscopic morphological alterations detected in a cohort of calves naturally infected in utero and born deformed and we deduce the possible scenario of lesions/disease production. In addition, the distribution of SBV-specific RNA in the different tissues of these congenital cases is reported for the first time, which is essential for diagnostic purposes and for further studies dedicated to the physiopathology of Schmallenberg virus-associated disease.

A12- POSTER: Entomological survey on African Horse Sickness outbreaks in Namibia.

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IZSAM¹; Central Veterinary Laboratory (CVL)²; IZSAM³

Key words: African Horse Sickness, Namibia, Culicoides imicola, Bluetongue

African horse sickness (AHS) is a Culicoides-borne disease affecting equids, endemic in sub-Saharan Africa, including Namibia. However, although the first two species of sub-Saharan Culicoides were described in Namibia, few entomological studies were carried out in this region.

To date entomological studies conducted in Africa have led to the isolation of AHSV from Culicoides imicola in South Africa and Zimbabwe, from Culicoides bolitinos in South Africa and from Culicoides spp. in Kenya and South Africa. In addition, outside the African continent, AHS virus was isolated in 1988 in Spain from C. imicola and from mixed pools of various species, including C. obsoletus and C. pulicaris.

Between 2006 and 2008 a number of AHS outbreaks were observed and described in Namibia, and four of the nine AHSV serotypes (1, 2, 4 and 9) were isolated from horses. In April 2011, an entomological survey was performed in the same area during the outbreaks of AHS. Eight collections were performed at six farms, within a radius of about 400 km around Windhoek, in six district of four Namibian regions: Khomas (Windhoek and Steinhausen), Erongo (Karibib and Omaruru), Otjozondjupa (Okahandja) and Omaheke (Gobabis).

Overall 194,211 Culicoides were collected. Of these Culicoides imicola was largely the most abundant species at all farms (99.4%), whereas Culicoides bolitinos was never found. The Culicoides collected were identified, age-graded and divided in pools ((maximum 100 midges per pool) for AHSV detection.

A total of 18,687 Culicoides divided in 248 pools were tested for AHS by RT-PCR. Of these 227 pools consisted of C. imicola, 13 of C. pycnostictus and 5 of Schultzei complex. Only one pool was analysed of C. nivosus, C. leucostictus and C. tropicalis.

Eighty-one pools tested positive for AHS virus, all consisting of C. imicola collected at Omaruru. In particular, 46 pools consisted of 100 C. imicola and 35 pools of 50 C. imicola. Virus isolation was attempted on 88 pools (n=1,463). Of these, 86 pools consisted of Culicoides imicola, one of Culicoides pycnostictus and one of Schultzei complex.

Three pools, consisting of 100 C. imicola collected at Windhoek farm, were positive for virus isolation, but none tested positive for AHS. One isolate was identified as bluetongue virus (BTV) serotype 1, and the other two as BTV serotype 10.

A13- POSTER: Are bogs reservoirs for emerging disease vectors? Evaluation of Culicoides populations in the Hautes Fagnes Nature Reserve (Belgium)

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Key words: Culicoides, Nature Reserve, Bog, Vector, Culicoides impunctatus

Several species of *Culicoides* (Diptera: Ceratopogonidae) biting midges serve as biological vectors for the bluetongue virus (BTV) and the recently described Schmallenberg virus in northern Europe. Since their recent emergence in this part of the continent, these diseases have caused considerable economic losses to the sheep and cattle industries. Much data is now available that describe the distribution, population dynamics, and feeding habits of these insects. However, little is known regarding the presence of *Culicoides* in unusual habitats such as peaty marshes, nor their potential vector capacity.

This study evaluated *Culicoides* biting midges present in the bogs of a Belgian nature reserve compared to those residing at a nearby cattle farm. *Culicoides* were trapped in 2011 at four different sites (broadleaved and coniferous forested areas, open environments, and at a scientific station) located in the Hautes Fagnes Nature Reserve (Belgium). An additional light trap was operated on a nearby cattle farm. High numbers of biting midges were captured in the marshy area and most of them were *Culicoides impunctatus*, a potential vector of BTV and other pathogens. In addition, fewer numbers of *C. obsoletus*/*C. scoticus* species, *C. chiopterus*, and *C. dewulfi* were observed in the bogs compared to the farm. The wet environment and oligotrophic nature of the soil were probably responsible for these changes in the respective populations. A total of 297,808 *Culicoides* midges belonging to 27 species were identified during this study and 3 of these species (*C. sphagnumensis*, *C. clintoni* and *C. comosiculatus*) were described in Belgium for the first time.

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A14- POSTER: Northward spread of Schmallenberg virus in 2012

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Finnish Food Safety Authority Evira¹

Key words: Schmallenberg virus antibodies, prevalence, distribution, Finland

Introduction

Schmallenberg virus (SBV), the first Orthobunyavirus in the EU region, was discovered in November 2011 by Germans (Hoffman et al. 2012). Real time RT-PCR for virus detection and commercial ELISAs for antibody detection were rapidly developed. In Australia *Culicoides* spp. transmit closely related Akabane viruses much more effectively than bluetongue viruses (Kirkland, personal communication 2012). The same was observed also in the EU. Bluetongue virus never spread to Finland, but the first evidence of arrival of SBV to the country was detection of antibodies in samples taken in routine surveillance at the end of August 2012. Consequently, samples taken for the annual bulk milk survey were also examined for SBV antibodies. It was obvious from the geographical distribution of the antibody-positive holdings, that the latitude does have a substantial effect on the spread. The probability of sustaining the spread decreases along the proportion of infected animals in a herd. The latter can be roughly assessed by determining the level of antibodies in the bulk milk. A further aim of the study was to see how well the latitude coordinate predicts in a linear model the level of the antibodies.

Materials and methods

Samples covering 10% of the dairy herds were collected by dairy industry during February-March 2013. ELISA for detection of SBV antibodies (ID Vet, Grabels, France) in milk samples was used. The geographical distribution of SBV was compared with the average length of the growing season in the country (www.ilmatieteenlaitos.fi/terminen-kasvukausi). The linear model for the latitude coordinate predicting the level of antibodies in bulk tank milk was built with the 'lm' -module of the software from the R Foundation for Statistical Computing (cran.r-project.org, R version 2.15.2).

Results

Overall, 39% of the dairy herds were positive for SBV antibodies. The prevalence approached 100% in the south-west and southern areas of Finland but decreased gradually to sporadic towards the north and north-east areas. No antibodies were detected in Lapland. The latitude coordinate was found to predict the level of antibodies in a significant manner; the p-value of the coefficient was 3.6E-06. However, the coordinate was only a minor factor in explaining the total variability in the antibody levels. In addition, the length of the growing season appeared to correlate positively with the distribution of SBV.

Discussion

The distribution and high prevalence of SBV in south-west and southern parts of the country imply that the three key factors necessary for sustaining the infection existed; i) the density of sensitive hosts was sufficient, ii) competent vectors were present, and iii) temperatures were adequate for the extrinsic incubation period. The observed latitude effect is very probably associated with the mean temperatures. The distribution area appears to coincide with the climatic area where the average day temperature in summer 2012 was at least +14 °C (www.ilmatieteenlaitos.fi/kesa-2012).

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A15- POSTER: Intrauterine Schmallenberg virus infection in a red deer (*Cervus elaphus*) fetus

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University of Liège¹

Key words: Schmallenberg, *Cervus elaphus*, placenta, orthomyxovirus

Schmallenberg virus (SBV), an arthropod-borne orthobunyavirus and fetal neuropathogen in sheep, goat and cattle is epidemic in Europe. Previously, we showed that wild ruminants are also infected by the virus, as evidenced by a very high rate of seroconversion. So far, however, the virus has not been detected in these species, either in healthy, sick or found-dead animals or in their fetuses. In March 2013, a pregnant hind was found dead in the eastern part of Belgium. The animal had an excellent body condition (65 kg). No lesions were identified in the thoracic and abdominal organs. The brain and the skull were intact but clotted blood was present in the spinal canal and in large quantities between the cervical spine and neck muscles. A complete fracture of the third cervical vertebra was demonstrated by radiography, which was assumed to have been fatal. The fetus showed no morphological alteration by visual inspection, whether whole or eviscerated. In particular, the brain and spinal cord were normal, both in terms of volume and shape and that both before and after multiple cross sections. Moreover, the fetus did not show any of the morphological alterations of the neck, trunk or limbs that are suggestive of arthrogryposis. Fetus brain and spinal cord samples were tested for detection of SBV genomic RNA and cellular β -actin transcripts by reverse transcription-quantitative PCR (RT qPCR) using reference protocols. They proved positive for SBV RNA. Then, a fragment of the hind's spleen was tested and it proved SBV-positive too. We concluded that, at the time of her death, (i) the hind was infected with the Schmallenberg virus and (ii) the virus had crossed the placenta, infected the fetus and contaminated its central nervous system. Based on the fetus weight (2 kg), we estimate his age between 4 and 5 months post-coitum. Therefore, assuming that the biology of the virus in wild and domestic ruminants is similar, we can assume that the fetus was already immunologically competent and, accordingly, the fact that no significant damage of the brain and spinal cord was detected comes as no surprise. Thus, like in domestic ruminant species, transplacental infection of the fetus occurs in red deer too. In this context, the evolution of European red deer populations since 2011 should be thoroughly checked to see if contamination of the fetuses in early gestation had an impact on the global population.

A16- POSTER: Schmallenberg and Bluetongue viruses in the Sardinian vector population in 2012

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IZSAM¹; IZS Sardegna²

Key words: Schmallenberg; bluetongue; Sardinia; Culicoides imicola; Culicoides newsteadi

In 2012 Bluetongue (BTV) and Schmallenberg virus (SBV) outbreaks have been reported in Sardinia, Italy. Both viruses emerged in the island at the end of October. Soon after the first notification, an entomological survey was conducted in 15 flocks in which BTV and/or SBV clinical cases were reported. Twenty-nine *Culicoides* collections were performed in seven municipalities of Sardinia including Barisardo, Girasole and Tertenia (Ogliastra province); Mores (Sassari province); Muravera (Cagliari province); Sant'Anna Arresi and San Giovanni Suergiu (Carbonia-Iglesias province). Overall, more than 50,000 *Culicoides* biting midges were collected between October and December. Specifically, *C. imicola* was the species most commonly found (74%) followed by *C. newsteadi* (22%). The remaining 4% of the collected midges included species of the *Obsoletus* complex, *C. punctatus* and *C. pulicaris*. All midges were age-graded, divided in pools of maximum 50 parous females according to species, and tested for BTV and/or SBV by specific real time RT-PCR. Of the 584 pools (23,538 midges) tested, 417 were positive for BTV: among the positive pools, 387 included *C. imicola*, 18 *C. newsteadi*, 11 *Obsoletus* complex and 1 *C. pulicaris*. Of the 727 pools (27,837 midges) tested, two were positive to SBV. These two pools, which included 50 *C. imicola* from the municipality of San Giovanni Suergiu, were also positive for BTV. This survey demonstrated that *C. imicola* could act as a vector of SBV. It could play the vector role for both viruses at the same time. This is also the first time that BTV was detected in midges belonging to the species of *C. newsteadi*.

A17- POSTER: First Detections of Schmallenberg Virus in Northern Ireland

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Agri-Food and Biosciences Institute¹

Key words: Schmallenberg virus, Northern Ireland, RT-PCR

Introduction: Schmallenberg virus (SBV) is an emerging disease affecting ruminants, first detected in Germany in December 2011. Clinical signs in adults include fever, reduced milk yield and diarrhoea. Epidemiological investigations indicate insect vector transmission; vertical transmission in utero has been proven with offspring displaying congenital malformations that include but are not limited to scoliosis, arthrogryphosis, hydrocephalus, torticollis and cerebellar hypoplasia in newborn calves and lambs. Many offspring are stillborn (OIE, 2013). This novel virus is a member of the genus Orthobunyavirus within the family Bunyaviridae. SBV's genome is phylogenetically close to that of Shamonda, Aino and Akabane viruses (Garigliany et al. 2012). This study describes how SBV has been detected in 6 tissue samples from 4 animals in Northern Ireland, using RT-PCR.

Materials and method: Nucleic acids were isolated from tissues of stillborn calves and lambs submitted to the Agri-Food & Biosciences Institute (AFBI) for necropsy. Tissues included brain, spinal cord, lung, small intestine, liver and kidney. Viral RNA was extracted using the RNeasy mini kit (Qiagen, Crawley, UK) in conjunction with a tissue lyser for mechanical disruption of the tissue samples. Extracted RNA was then analysed with the AgPath-ID One Step RT-PCR kit (Ambion, California, US) combined with an SBV S-segment specific TaqMan assay (Bilk et al. 2012), using an ABI 7500 fast Real Time Thermal cycler with the following run conditions: 45°C for 10 minutes; 95°C for 10 minutes; followed by 42 cycles of 95°C for 15 seconds, 56°C for 20 seconds and 72°C for 30 seconds.

Results: To date we have tested 401 tissue samples from 76 animals of which 42 were bovine and 34 were ovine. SBV was detected in the following tissue types; brain, small intestine, liver, lung and kidney however virus was not always detected in every tissue from each positive case. Animals testing positive using this assay were all located within a 23km radius in south eastern Northern Ireland. To date there have been three bovine positive cases and one ovine, all malformed newborns.

Discussion: The tissues available from these positive cases give limited data on the viral distribution. In some cases SBV was only detected in one tissue type. It has been reported that lung, umbilical cord and spinal cord in calves and spinal cord, brain and lung in lambs give positive results more consistently than other tissue types in positive newborns of these species (Bilk et al. 2012). Therefore there is potential scope to improve upon sampling procedures to maximise detection. The small number of positive samples in Northern Ireland could be for several reasons including under-reporting of malformations in ruminants, thus limiting animals available for testing or there is also the possibility that spread has been limited due to the geographical location of Northern Ireland. Recent research has suggested that a marked genetic variability may exist within SBV isolates recovered from stillborn lambs (Coupeau et al. 2013). This is not entirely unexpected in an RNA virus but it does put emphasis on the importance of reporting suspect cases in order to monitor the spread and investigate the evolution of this virus and to inform an appropriate response and future vaccine developments.

DIAGNOSIS

D 1 - POSTER: Detection of African Swine Fever Virus-specific antibodies in pig serum using ASFV P30 and a P30-specific chicken scFv.

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Agricultural Research Intitute¹

Key words: African swine fever, antibodies, P30, scFv

African swine fever virus (ASFV) causes a lethal, highly contagious and economically important disease in domestic pigs. African swine fever (ASF) is difficult to control for several reasons. These include the presence of reservoirs of ASFV in wild pigs and soft tick vectors, the lack of a vaccine and the stability of the virus in pork as well as in the environment. Reliable diagnostic tests and rigid quarantine measures are at present the only tools available for the control of ASF outbreaks.

In this study ASFV P30, a highly antigenic membrane and secreted protein of ASFV, was expressed in a bacterial expression system. Purified inclusion bodies that contained P30 were used as an antigen to select phage displayed antibodies from the Nkuku® library, a large phage displayed antibody based on chicken immunoglobulin genes. Antibody E2 recognised P30 as a phage displayed antibody as well as a soluble fragment (scFv). This antibody could be used in an inhibition ELISA based on P30 antigen to detect the presence of ASFV-specific antibodies in a porcine ASFV reference serum. A small panel of porcine field sera was also tested in this inhibition ELISA and the results indicate that it may provide a viable alternative to other currently used serological assays for ASF.

D 2 - POSTER: Comparison of two Next Generation sequencing platforms for full genome sequencing of Classical Swine Fever Virus

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Key words: Classical Swine Fever Virus, Next Generation Sequencing, platform comparison, Variant analysis

Next Generation Sequencing (NGS) is becoming more adopted into viral research and will be the preferred technology in the years to come. We have recently sequenced several strains of Classical Swine Fever Virus (CSFV) by NGS on both Genome Sequencer FLX (GS FLX) and IonTorrent PGM platforms. In this study, we analyzed NGS data of virus rescued from a CSFV C-strain vaccine strain cDNA clone. The virus analyzed was obtained from a 4th and a 12th passage of rescued virus in SFT cell culture, which had shown a difference in growth kinetics between the passages, and NGS analysis was chosen in order to look for molecular differences. Identical RT-PCR products were run on both GS FLX and an IonTorrent PGM platform for comparison. The NGS data was compared by quality and the percentage mapped reads. Results showed good quality of reads for both platforms and a close to 100% of the reads mapped to the consensus sequence. Additionally, we got an average sequence depth for the genome of 4000 for the IonTorrent PGM and 400 for the FLX platform making the mapping suitable for single nucleotide variant (SNV) detection. The analysis revealed a single non-silent SNV A10665G leading to the amino acid change D3431G in the RNA-dependent RNA polymerase NS5B. This SNV was present at 100% frequency in the 12th passage and only at 55% in the 4th passage, which could explain the difference in growth kinetics between the passages.

D 3 - POSTER: BVR: an Italian Biobanking of Veterinary Resources at IZSLER

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IZSLER¹

Key words: Biobanking; Diagnostic; Animal Diseases

The Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) is a Veterinary Institution of the Ministry of Health. The performed activities include diagnostic services on animal diseases and zoonoses, set up of diagnostic methodologies, control activity on human and animal foodstuffs and applied research in the area of breeding hygiene, in order to improve zootechnic productions and animal welfare.

In this context, it has been developed an Italian Biobank of Veterinary Resources (IBVR), in order to collect biologicals of different types, characterized by well-defined features. In particular, in the infrastructure are stored: cell cultures, bacteria, viruses, parasites, immune sera, monoclonal antibodies, histological samples.

These resources have been identified as reference materials, employed in the routinely activities of IZSLER and at disposal to others.

In order to improve the quality of activities performed by IZSLER it has been developed a harmonized approach on biobanking practices that ensure the availability of biological resources and the cooperation with international laboratories.

The importance of networking of biobanks has been emphasized, in order to promote a global cooperation. This approach will permit to investigate and compare samples and data from different part of the world. Biobank networking is the key area in order to speed up the discovery and the development of new diagnostic tools and therapies.

In particular, this strategy would allow studies with a large number of samples, permitting a major reliability of outcomes and to avoid to repeat experimental studies aimed to obtain reference samples.

D 4 - POSTER: Bayesian evaluation of diagnostic accuracy of two commercial *Leptospira Hardjo* antibody ELISA's in bovine sera.

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Key words: *Leptospira Hardjo* (LH), Bayesian, bovine, diagnostic accuracy

In Belgian cattle, epidemiological information on *L. Hardjo* (LH) seroprevalence is missing, while the diagnosis of leptospirosis remains challenging. A previous gold-standard comparison to an LH-specific microscopic agglutination test (MAT) of two commercial diagnostic ELISA's (Linnodee® and Prionics®), used in Belgium for LH-antibody detection in bovine sera, indicated higher agreement (kappa) for Prionics®. However, though commonly used, MAT is not a perfect gold standard as performance may vary depending on the stage of illness (acute ↔ convalescent), which may compromise its diagnostic characteristics: sensitivity (DSe) and specificity (DSp). Therefore, a no-gold-standard Bayesian evaluation was performed to re-evaluate the diagnostic accuracy of these tests. Two bovine target populations were selected: an abortion population (n=303, prevalence estimated 30%) was tested with all 3 tests and a general population (n=1831, prevalence estimated 1%) with the ELISA's only. The test results were cross-classified with non-interpretable results (NI) alternatively coded as negative, positive or excluded.

A previously published Bayesian model (1,2) was adapted to account for conditional (in)dependence of test results. Prior information was collected to construct several prior-model-data scenarios and a sensitivity analysis led to a final set of scenarios/simulations and data-driven results. The posterior prevalence estimates confirmed what was expected for both populations, thereby validating the models, and for individual tests the credibility intervals of the different simulations overlapped, indicating robustness and insensitivity to model-prior-data scenarios. The posterior estimates of DSe depended on the coding of the NI results and varied between 85-97%, with slight advantage for DSe Linnodee®. The 3 DSe medians were not found to be significantly different and the MAT was indeed found to be imperfect. DSp estimates varied between 80-98% and consistently showed MAT and Prionics® medians as slightly higher compared to Linnodee®, the difference often being statistically significant. The results compared well to the previous gold-standard analysis of the same data.

Advice was given to interpret these results from multiple simulations according to the test's intended purpose. When tests are used to detect exposure to LH in aborting or convalescent cattle, one should ideally select the most sensitive test and interpret NI samples conservatively (as positive) to exclude false negatives. When performing surveillance and controlling for LH-exposure/infection in the general cattle population, one could select for more specificity with a progressive interpretation of NI samples (as negative) to exclude false positives. In the first case, both Linnodee® and Prionics® are considered equally valid choices, in the second case there is a slight advantage for the Prionics® ELISA.

We remark that a larger sample size might still reveal significant differences in DSe, probably in the opposite direction as observed for DSp, with Linnodee® gaining advantage over Prionics®. Given that in certain screening situations (e.g. serial testing) false positives are not a major problem, the test preference may potentially change towards highest DSe. No account was taken of the animal's (unknown) vaccine status and since none of the tests are "DIVA", the seropositive LH-exposed group may include some vaccinated animals. None of the tests were suitable to obtain a causative diagnosis of leptospirosis or to give information on (DSe for) other *Leptospira* serogroups.

D 5 - POSTER: Development of RT-PCR for indication of Aujeszky's disease virus genome

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Key words: Aujeszky's disease virus, glycoprotein D, RT-PCR

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Aujeszky's disease (AD) is an acute viral disease of both domestic and some wild animal species including fur animals and rodents. The disease accounts for great economic losses in the countries with advanced pig industry, affecting primarily sucking and weaned pigs. Early and quick diagnostics of an infectious disease is one of the most important aspects of its effective control and eradication. Therefore, development of an RT-PCR assay aimed at Aujeszky's disease virus genome detection is of vital importance here.

We analyzed nucleotide sequences of various ADV strains available in Gene Bank database, and selected primers and a fluorescent Taq Man probe flanking ADV glycoprotein D gene site for ADV DNA specific amplification.

The primer and the probe specificity levels were evaluated using a panel of DNA samples isolated from culture materials of various Aujeszky's disease virus strains, DNAs of heterologous viruses (namely: CSF and ASF), and also from DNAs isolated from organ samples of intact pigs (including spleen, liver, and lung), and from an intact continuous cell line PSGK.

RT-PCR analytical specificity levels were determined using DNA samples isolated from a series of tenfold dilutions of the virus material.

The DNA isolation from the virus material was carried out using a modified nucleosorbition assay with a sorbent (Boom, 1995). The RT-PCR was performed on a Rotor Gene-6000 cycler (QIAGEN, Germany).

The assay was performed using a standard reagent kit. The results were evaluated through the analyses of fluorescent signal accumulation curves for a FAM channel using the software of the device used.

As a result of the research works carried out we determined that the RT-PCR analytical sensitivity level was 2.0 lg TCID₅₀/cm³. The ADV genome-complementary primers and probe were shown not to interact with the DNAs of heterologous viruses, intact cell culture samples and organs.

Thus, the primers and the probe selected provide a means for ADV genome specific amplification and can be used for the further research works on Aujeszky's disease agent genome detection in field samples.

D 6 - POSTER: Implementation of production of proteins expressed by Baculoviruses in Lepidoptera larvae (*Trichoplusia ni*), preliminary results

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Key words: Baculoviruses, *Trichoplusia ni*, proteins, expression

A large number of proteins of different origin and for different scopes has been successfully expressed in insect cell lines infected with baculovirus where foreign protein genes are placed under the control of a viral promoter. In the last decade an increasing demand for fast and cost-effective production of foreign proteins has been observed. Many are the possible applications of these proteins, from functional analyses to production of antigens for vaccine and diagnostic kits.

In IZSLER the expression of foreign proteins using Sf9 insect cell line infected with recombinant baculoviruses is being active since several years and principally addressed to production of recombinant antigens used in diagnostic kits or in research activities, however this system of production has a potential disadvantage in the cost associated with large-scale tissue culture operations.

Compared to production in cell culture, insect larvae can produce proteins at reduced cost without investment in expensive equipment. The main aim of this study is to establish a laboratory stable colony of larvae *Trichoplusia ni* to be employed as hosts for expression of foreign proteins by infection with recombinant baculoviruses, with the development of standardized artificial diets.

A laboratory colony of *T. ni* was maintained and propagated at the constant temperature of 26°C with relative humidity set at 65% in incubator able to control also the photoperiod (16:8 light/dark). Two different diets were compared in supporting larvae growth.

The Diet 1 was based on Shorey study (1965) and it was composed of soaked beans and brewer's yeast, whereas the Diet 2 was the "Modified form MCMorran Grisdale commercial Diet", acquired from Great Lakes Forestry Centre, Sault Ste. Marie, Ontario. The Diet 1, in respect to the Diet 2, seemed to allow fast development of each stage, shortening of about 28 h the entire development time needed by larvae to reach adult stage. However, in the experiment carried out to test the substrate preference, larvae of the first stage moved towards diet 2 and rejected the diet 1 suddenly after eggs hatching, showing that the alimentary preference of the colony could be important in the establishment of a laboratory stable colony.

For the proteins production experiments fifth-instar larvae were infected individually by injection of 0.1 ml of high titer recombinant baculoviruses stock, expressing the non-structural protein 3 (NS3) of BVDV or the capsid protein (ORF2) of HEV. Infected larvae were kept in incubator at the condition described above and collected after 72h. Larvae were then immediately frozen and kept at -80 °C until processed.

The yield and reactivity of the two proteins were preliminary evaluated with specific Monoclonal Antibodies (MAbs) and positive serum samples in ELISA assays. Both BVDV-NS3 and HEV-ORF2 were produced at significantly higher levels than those obtained by infecting insect cell cultures while maintaining the antigenic properties.

The preliminary results encourage the exploitation of this production system both for internal use and as a service.

Next steps will investigate different inoculation routes (baculovirus injection compared with oral administration with the diet), different payloads and best protein extraction procedures.

D 7 - POSTER: Microarrays As Versatile Multiplex Tools In Veterinary Diagnostics

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Key words: Microarrays, livestock diseases, antibody, antigen, fluorescence, colorimetry

Rapid, easy and reliable diagnostic techniques are needed for the monitoring and the eradication of diseases that afflict domesticated livestock. Microarrays based on the antigen-antibody specific recognition are suitable candidates to fulfil these requirements; their miniaturized format allows for parallel screening and multiplex detection. Hundreds of spotted molecules can be printed on a single modified glass slide, permitting the design of customized microarrays containing the key molecules needed to determine the diseases of choice. In this study, we targeted bovine and porcine diseases, such as BTV, EHDV, PRRSV and CSFV among others. Single modified glass slides with up to 24 identical arrays, capable of performing 24 parallel experiments were used for: (i) antigen or pathogen detection via sandwich assay with antibody microarrays, and (ii) antibody detection in body fluids by indirect assay with antigen microarrays. For comparison, two alternative signal detection methods, fluorescence and colorimetry were used. The fluorescence method required the use of a fluorescence scanner for excitation and subsequent signal detection and quantification, whereas the colorimetric method utilized a common office or film scanner. The microarrays in all cases were able to validate the different pathologies. Even though fluorescence detection was more sensitive than colorimetry, the latter provided robust results and was far more cost effective.. The European Union FP7 Grant No. 289364 (RAPIDIA-FIELD) funds this work.

D 8 - POSTER: A New Diagnostic Tool For Bovine Tuberculosis Control - IDEXX M. Bovis Antibody Test

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Key words: Tuberculosis, ELISA, SICCT, Zoonosis

Introduction

The IDEXX M. bovis antibody test kit is an ELISA designed to detect the presence of antibody to M. bovis in bovine serum and plasma samples. This test could improve bovine tuberculosis (bTB) detection of tuberculosis (TB)-infected animals in TB-infected herds or could be an easy, cost effective surveillance tool in TB-negative regions. The M. bovis ELISA test is validated and certified by the OIE as fit for the purposes defined in the kit insert. Also, USDA license has been obtained in September 2012, and various European countries are looking into registration of the test.

Material and Methods

Characterized serum and plasma samples were obtained from worldwide sources and were used to validate the M. bovis ELISA. Two temporal series were produced from animals exposed to M. bovis and followed over time. Positive samples from 3 different countries were obtained from either culture positive animals (n = 307). Sample sets (n = 100) with varying skin or 5-interferon results were evaluated to demonstrate subsets of positive animals within TB-infected herds (n = 45) and the power of combining tests to increase overall sensitivity. Negative samples (n = 1473) were obtained from 4 different countries with samples originating from negative herds, states or regions. In addition, to understand potential cross-reactivity with other Mycobacteria, samples were obtained from animals exposed to large doses of M. paratuberculosis, M. avium and M. kansasii or from a herd with high Johne's antibody levels.

All samples were evaluated on an M. bovis antibody kit manufactured at production scale, according to the standard kit protocol. Samples with S/P ratios of ; 0.30 were considered positive for M. bovis antibodies.

Results

Data from the M. bovis temporal series revealed that animals can develop antibody titers within weeks of exposure and that an antibody response can be boosted after the application of a skin test. The M. bovis antibody ELISA detected 197/307 samples from culture positive animals resulting in a sensitivity of 64.2% . Using a single test method, between 71.1% and 75.6% of herds would have been detected. Combining tests resulted in an increase in herd sensitivity to between 86.7% (5-Interferon and ELISA) and 97.8% (5-Interferon, SICCT and ELISA). On negative sample sets, the M. bovis ELISA demonstrated a specificity of 98% with no cross reactivity observed on M. paratuberculosis (both experimental and field infected) or M. avium samples. Transient, low level reactivity was observed with animals inoculated with large doses of M. kansasii.

Discussion & Conclusion

The strategic supplemental use of the IDEXX M. bovis antibody test represents a fast, easy, objective and cost effective option for use in bTB programs and can increase overall diagnostic power by detecting subsets of infected animals missed by current methods. This test's high specificity allows for an application of this test in bTB-negative regions.

D 9 - POSTER: Application of Reverse Transcription Real-Time Pcr to Detect the Akabane Virus Rna

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VNIIViM¹

Key words: Akabane virus, cattle, sheep, goats, test - system

The disease Akabane was registered among cattle, sheep and goats in Japan, Korea, Australia, Western Turkey, Kenya, Israel, Cyprus, South Africa, Northern Syria. The Akabane virus is a member of the genus Orthobunyavirus family Bunyaviridae. Established the pathogenicity of the Akabane virus for cattle, sheep and goats. Clinical signs of the disease in cattle include fever, loss of appetite and sometimes diarrhea, drop in milk yield . If an adult ewe or heifer was infected in the early stages of pregnancy, the fetal infection can occur, leading to serious consequences: abortion, birth of premature or dead fetuses and lambs, goats and calves with various malformations. The aim of this work was the development the test - system to detect the Akabane virus RNA by real-time reverse transcription PCR. With order to select primers the nucleotide sequences of Akabane virus were analyzed using the programs "Primer Express" and " Oligo 6.0". For detection of amplification products in the real-time the probe technology Taq-man, containing emitter of fluorescence FAM and quencher BHQ1 was selected.

Analytical sensitivity was determined by the amplification of the virus RNA extracted from consecutive ten-fold serial dilutions of the suspension of brain of infected mice and the infected CV-1 cell culture. The calculated value of analytical sensitivity of RT-PCR in real-time was $1.5 \pm 0.5 \lg \text{MicLD}_{50}/\text{cm}^3$ and $1.0 \pm 0.5 \lg \text{TCID}_{50}/\text{cm}^3$, respectively.

The specificity of the test - system was determined by the examination of RNA of such viruses, as Nairobi sheep disease, Rift valley fever, Ibaraki and bluetongue. The intact samples also were examined. Application of the test-system has allowed to reveal all positive samples. False positive and false negative results were absent.

Thus, the designed test - system is suitable to detect the Akabane virus RNA in the samples of organs and tissues from infected animals and in the infected cell cultures.

D 10 - POSTER: Tick-borne Encephalitis (TBE): development of a serological test for the detection of specific antibodies in animal sera

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IZSLER¹

Key words: Tick-borne Encephalitis, monoclonal antibodies, ELISA

Tick-borne Encephalitis (TBE) is the most important viral tick-borne zoonosis in Europe and many human cases are reported every year. In this study, a competitive ELISA test based on the use of monoclonal antibodies (MAbs) for the detection of specific antibodies for TBEV was developed and then used for a serological survey on the population of wild and domestic ruminants, wild boar and horses in Lombardia and Emilia Romagna.

The viral strain used in the study was the TBEV 103457/2009 (Western European subtype) isolated from the brain of a dog dead with neurological symptoms. Immune sera against TBEV were produced by intramuscularly inoculation of two goats with a viral suspension containing 106TCID₅₀ and blood samples were collected at different times. MAbs were produced in Balb/c mice according to an internally standardized method. Screening and characterization of the hybridomas were carried out by indirect ELISA, indirect immunofluorescence performed in parallel using VERO cells infected with TBEV, West Nile virus (WNV), Usutu virus (USUV), Bagaza virus (BAGV) and non-infected cells, virus neutralization test (VNT) and western blotting (WB). Competitive ELISAs with TBEV positive and negative goat sera were also performed in order to identify MAbs reactive to immunogenic epitopes.

Twenty-one MAbs reactive to TBEV were obtained. Sixteen MAbs were specific for TBEV while five showed cross-reactivity with related flaviviruses. No MAbs showed neutralizing activity and 6 MAbs were positive in WB revealing a band with a molecular weight of 50Kd corresponding to E protein. Four MAbs specific for TBEV (3B10, 4D11, 2G7, 3E12) and 2 MAbs (4D5, 4H1) cross-reactive with all flaviviruses tested were cloned, purified and conjugated with horseradish peroxidase (HRP). Among the TBEV-specific MAbs, based on the ability to inhibit binding of positive sera to virus antigens, 3B10 was finally selected for use in competitive ELISA which was subsequently used as diagnostic test for a serological survey on the population of ungulates in Lombardia and Emilia Romagna. For the competitive ELISA test, 50µl of sera at sequential dilutions starting from 1/5 were incubated for 1h at 37°C in TBEV-coated immunoplates, together with 25µl of HRP-conjugated 3B10 at the pre-determined optimal dilution. After washes, the colorimetric reaction was developed using OPD as substrate, absorbance values at 492 nm were determined and results were expressed as percentage of inhibition. In total, 2714 sheep and goat sera, 350 deer sera, 211 roe deer sera, 777 equine sera and 2519 wild boar sera were collected and examined with the competitive ELISA. A subsequent confirmation of ELISA-reactive sera was performed by VNT. In total, 46 positive sera were identified, 30 of which from wild boar, 13 from horse, 2 from deer and 1 from goat. These results indicate a low prevalence of infection confined to some areas in provinces of Forlì, Ravenna, Modena, Brescia, Bergamo and Parma.

This test, thanks to the principle of the competitive ELISA, can be applied to the analysis of different animal species and can be helpful for describing the epidemiological situation of an area, which may be otherwise difficult to evaluate due to the irregular distribution of TBEV and the low TBEV prevalence in ticks. Indeed, the use of TBEV-specific MAbs in competitive ELISA improves the test-specificity representing a significant aspect for a large-scale screening in areas where there is simultaneous circulation of different flaviviruses.

Acknowledgements:

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D 11 - POSTER: *Mycoplasma hyopneumoniae* prevalence and seasonal effect in Belgian and Dutch pig herds using a tracheo-bronchial swab technique

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Key words: TBS, *M. hyopneumoniae*, season, piglets

Introduction – *Mycoplasma hyopneumoniae* (M.hyo) – one of the main pathogens of the Porcine Respiratory Disease Complex (PRDC) – is still important in modern intensive swine farming in Europe. Recently, a new sampling technique has been developed and validated for use in pigs, namely the tracheo-bronchial swab (TBS) technique [1]. The aim of the present study was to obtain data on distribution of M.hyo infection throughout closed pig herds in Belgium and The Netherlands using the TBS technique during an entire year. Sampling was mainly focused on early diagnosis, since piglets can already be infected during suckling through the sow [2,3,4] and further spread of infection occurs after weaning [5,6].

Materials and Methods – Closed pig farms (n = 176) were randomly selected and in every pig herd, at least 30 piglets were sampled in three age groups. The standard sampling protocol included 20 piglets at 3-5 weeks of age and 10-20 piglets in the 2nd half of the nursery stage (6-11 weeks of age). TBS were collected as described previously [1]. The collected mucus was suspended into 1 mL of buffered saline solution and stored fresh until analysis. Real-time PCR (RT-PCR) analysis was performed according to the standard operating procedure of the laboratory (IVD GmbH, Hannover, Germany) [7] and PCR results were reported as negative or positive for the presence of M.hyo. The detection limit of the RT-PCR test was set at 300 DNA copies of M. hyo per mL of TBS suspension. Several weather data related to the specific sampling period were collected from a central weather station point in the Benelux. Statistical analysis was performed towards seasonal differences and effect of weather characteristics on M. hyo prevalence.

Results – The prevalence of M.hyo in piglets around weaning was highest in Q2 (spring; 9.7%) and lowest during Q3 (summer; 3.8%). In older post-weaning piglets, an increase in M. hyo prevalence could be observed during all seasons, with the highest prevalence in Q4 (autumn; 17.2%) and the lowest prevalence in Q3 (summer; 2.3%). The presence of M.hyo at herd level at 3-5 weeks of age was significantly affected by the precipitation rate ($\beta = -0.026$; $P = 0.03$) during the week preceding sampling. In older post-weaning piglets, the risk for a herd to be M.hyo-positive was significantly affected by season [with the highest risk (OR = 1.91) for M.hyo positivity during autumn and the lowest risk (OR = 9.8) during summer ($\beta = 0.65$; $P = 0.003$)] and the average outdoor temperature ($\beta = 0.02$; $P = 0.007$) during the week preceding sampling.

Discussion – In our study, the individual animal prevalences at 3-5 weeks of age were higher (7.1%) as compared to the study of Villarreal and coworkers [3] using nasal swabs (3.3%). This difference could be explained by the use of the more sensitive TBS technique. Furthermore, in the study of Villarreal et al., 2010 [3], only pig farms with typical clinical signs related to M.hyo were selected, whereas in our study, inclusion criteria did not require specific clinical respiratory problems. These results are in accordance with Segalés et al. (2011) [9], who also observed a seasonal variation in M.hyo prevalence and circulation. In conclusion, although early M.hyo infection may show a seasonal effect, piglets may be infected with M.hyo throughout the entire year.

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D 2 - POSTER: Prevalence of different respiratory pathogens during post-weaning and fattening period in Belgian and Dutch pig herds using a tracheo-bronchial swab technique

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Elanco Animal Health¹

Key words: TBS, PRDC, pigs

Introduction – Besides *Mycoplasma hyopneumoniae* (M.hyo), many other viruses and bacteria can be concurrently present during respiratory problems in pigs, provoking the disease complex known as Porcine Respiratory Disease Complex (PRDC). Diagnosis of infections with these pathogens can be performed using different approaches, including the detection of the pathogen through Polymerase Chain Reaction (PCR) assays. Recently, a new sampling technique [1] has been developed and validated for the detection of M.hyo in pigs using PCR, namely the tracheo-bronchial swab (TBS) technique. With this technique, pathogens present at the level of the trachea-bronchial junction can be recovered and analysed through PCR-analysis. The aim of the present study was to obtain data on the distribution of different pathogens involved in PRDC in closed pig herds in Belgium and the Netherlands using the TBS technique.

Materials and methods – Three hundred and four pig farms were sampled using the TBS technique. In every herd, at least 30 coughing piglets were sampled in at least two age groups (3-5, 6-11 and 12-20 weeks of age). TBS were collected as described previously and analyzed using mPCR and/or dPCR (IVD GmbH, Hannover, Germany). A multiplex (m) [porcine respiratory coronavirus (PRCV), porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine cytomegalovirus (PCMV), porcine circovirus 2 (PCV2)] and duplex (d) [*Actinobacillus pleuropneumoniae* (App), *Haemophilus parasuis* (Hps)] PCR assay were used to detect the different pathogens in the TBS. PCR results were reported as negative or positive for the presence of PRCV, SIV, PCMV, PCV2, App and Hps. For PRRSV, strain type EU/US or both was also reported.

Results – Overall, Hps with virulence factor was present in 78.0, 86.7 and 82.0% of the pigs samples at 3-5, 6-11 and 12-25 weeks of age. In piglets of 3-5 weeks of age, the most prevalent pathogens were SIV (25.3%), PCMV (19.5%), PRRSV-EU (12.7%) and Mhyo (6.4%), whereas in piglets of 6-11 weeks of age, PCMV (25.0%), PRRSV-EU (24.9%), SIV (16.3%) and Mhyo (9.6%) were the most prevalent pathogens. In older pigs (12-25 weeks of age), coughing was mostly provoked by Mhyo (61.3%), PCV-2 (40.3%) and PRRSV-EU (27.3%).

Discussion – The present study clearly shows that different viral pathogens responsible for PRDC may already be present during the post-weaning period. Concerning PRRSV, the most prevalent type was PRRSV-EU, whereas PRRSV-US was far less frequent. It is clear that in several herds, Mhyo is already present in piglets at weaning, further increasing in the second part of the nursery period. These observations are in accordance with Villarreal et al. and Meyns et al. [2,3,4]. Hps was more prevalent than previously assumed. In conclusion, the present study showed that many other respiratory pathogens are present during the post-weaning and fattening period, which may complicate the clinical picture of respiratory disease.

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D 13 - POSTER: Overview on available PCR tests for ASF diagnosis

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CISA-INIA¹

Key words: African swine fever, diagnosis, PCR

A range of fine laboratory techniques is available at present for the routine diagnosis of African swine fever (ASF), allowing the rapid and accurate detection of ASFV-positive and carrier animals. ASFV induces a long-term viraemia in the infected pigs, whereas antibodies come out within the second week post-infection, persisting even for years. Viral DNA detection is the ideal screening virological approach because of the Ag-Ab immunocomplex assembly, which reduces notably the sensitivity of the Ag detection techniques in sub-acute and chronic infections. Although different molecular technologies have been recently applied to develop novel ASFV detection methods (1-4), the PCR is still the preferred molecular technique for the ASF diagnosis. Two OIE recommended methods (5), specifically a conventional (6) and a real-time (7) PCR procedures, are the most widely used at the ASF diagnostic labs. Both reference techniques have been extensively validated over time showing a suitable detection capacity and performance. In the last years, real-time PCR is replacing conventional PCR, and some recently described real-time PCR tests (8-9) are now validated and ready to be used in routine ASF diagnosis. In addition, a real-time PCR (10) kit is commercially available for ASFV (Tetracore Inc., USA) showing a good performance, which is offered in a dried reaction mix format for a simple application. However, reference conventional PCR test is still of great value in the different epidemiological scenarios and should not be totally ruled out. Moreover, some reported gel-based PCR methods have proved useful for specific diagnostic applications, such as the ASFV detection in soft ticks (11) or the simultaneous and differential identification of ASFV and CSFV in a multiplex reaction (12).

A review on the available PCR tests fitting for ASF diagnosis will be presented, and benefits and limitations of the methods will be discussed.

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D 14 - POSTER: AKABANE ANTIBODIES DO NOT CROSS REACT WITH COMMERCIAL NUCLEOPROTEIN-BASED SCHMALLEMBERG VIRUS ELISAs

POURQUIER, Philippe¹; Comtet, Loïc¹

IDvet¹

Key words: SBV, Akabane, ELISA, cross-reactions

Purpose:

Schmallenberg virus (SBV) is a vector-transmitted orthobunyavirus (family Bunyaviridae) which causes foetal congenital malformations and stillbirths in cattle, sheep, and goats. IDvet offers a range of diagnostic tools for the detection of SBV antibodies in multiple species, including indirect and competitive ELISAs.

Given that SBV is closely related to the Akabane virus, and that Akabane virus serology is required for the movement of animals between certain countries, this study examines possible cross-reactions of Akabane virus antibodies with commercial SBV ELISAs.

Methods:

- The following ELISAs were performed as per manufacturer's instructions:
 - o Nucleoprotein (NP)-based SBV ELISAs:
 - ID Screen® Schmallenberg virus Competition Multi-species (cELISA)
 - ID Screen® Schmallenberg virus Indirect Multi-species (iELISA)
 - Commercial NP-based indirect ELISA (Kit A)
 - o ID Screen® Akabane Competition ELISA.
- 2 experimental infection studies were performed using the B8935 and CSIRO Akabane strains. Time-course sera were generated and tested in parallel using the ID Screen® SBV and Akabane ELISAs, and a commercial NP-based SBV iELISA (Kit A)

Results

None of the commercial SBV ELISAs, neither from IDvet nor commercial Kit A, detected sera from animals experimentally-infected with the Akabane virus.

Conclusion

The commercial nucleoprotein-based Schmallenberg ELISAs tested in this study do not cross-react with Akabane antibodies.

D 15 - POSTER: VALIDATION OF A COMPETITIVE ELISA FOR THE DETECTION OF ANTI-INFLUENZA A H7 ANTIBODIES

POURQUIER, Philippe¹; LESCEU, Stéphanie¹

IDvet¹

Key words: Influenza A, H7, ELISA

Purpose:

Influenza A H7 subtypes are associated with highly pathogenic forms of avian Influenza. In April 2013, a new H7N9 strain emerged in China, transmissible from birds to humans, underlining the need for wide scale monitoring of Influenza H7 antibodies.

ELISA testing is an efficient method for the analysis of large numbers of samples, particularly in comparison with Haemagglutination Inhibition (HI).

The ID Screen® Influenza H7 Antibody Competition ELISA is the only commercial ELISA available for the specific detection of H7 antibodies in both domestic and wild avian species. This document summarizes validation data for this assay.

Methods:

ID Screen® Influenza H7 Antibody Competition ELISA: Test performed as per manufacturer's instructions. Microplates are coated with H7 antigen. The conjugate is an anti-H7-peroxidase (HRP) monoclonal antibody.

Results:

Sensitivity: 3 sera from chickens naturally-infected by different H7 strains were tested. All strains were found positive. In addition, 8 H7-positive chicken sera were tested from the FLI Institute reference panel. All sera were found positive.

Specificity:

363 disease-free and specific pathogen free (SPF) sera were tested. Measured specificity = 100% (CI 95%: 98.95% – 100%).

Exclusivity:

- 8, non-H7 Influenza A strains from naturally-infected chickens were tested. All strains were found negative.

- 60, non-H7, AIV-positive chicken sera were tested by the FLI Institute. Most non-H7 sera were found negative.

- 1425 avian field sera, positive for non-H7 Influenza A strains, were tested. 1418 sera were found negative Measured specificity (exclusivity) = 99.51% (CI95%: 98.99% - 99.76%).

- 130 NDV, IBV, or IBD sera were tested. All sera were found negative.

Conclusion:

The ID Screen® Influenza H7 Antibody Competition ELISA demonstrates excellent specificity on disease-free animals, high serotype specificity, and excellent sensitivity.

The test is a rapid and easy-to-use diagnostic tool, well-suited to monitoring outbreaks and epidemiological studies in which massive testing of animals is required.

IDvet welcomes collaborations with laboratories having positive H7 sera in their possession.

D 16 - POSTER: CELL SYSTEM FOR STUDYING VISNA-MAEDI

Baryshnikova, Elena¹; Kislov, Igor¹; Kolbasova, Olga¹

SRI NRIVVaMR¹

Key words: visna-maedi, lung lamb cell culture

CELL SYSTEM FOR STUDYING VISNA-MAEDI

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Purpose

Visna-maedi (VM) - a disease characterized by affects lungs, central nervous system, breast, joints. A result of this work was obtained LL subculture cells. Its morphological characteristics and permissive to virus VM was studied.

Keywords: visna-maedi, lung lamb cell culture.

Introduction

Visna-maedi is a disease characterized by affecting many organs, but in the first place - the lungs in the form of interstitial pneumonia, central nervous system (clinically manifested as slowly progressive encephalomyelitis meningitis and glial type in white matter of the brain and spinal cord of sheep), mammary glands and joints.

The disease is widespread throughout the world.

Visna-maedi is reproduced in cultures of ependymal cells and the choroid plexus of sheep brain in human astrocytes, RS-C-1 and transplantable line of African green monkey cells (Vero), culture cell lung, spleen, abdominal, cardiac, cerebellum, testis, adrenal glands, kidneys sheep, goat synovial membrane (GSM), ovine synovial membrane (OSM).

Materials and Methods

Lung lamb (LL) tissue was collected from a VM virus-seronegative, 3 months old, male goat. Lung cells were cultured in DMEM with 5 % fetal bovine serum at 37°C and 5% carbon dioxide. The solution of 0.02% versene and 0.25% trypsin in a 3:1 are employed.

For LL cell culture we used a method of growing tissue explants After the slaughter of the animal was aseptically collected lungs. Isolated lungs mechanically crushed pieces to the tissue fragments to size of 1-2 mm, then washed three times with DMEM medium with 5% fetal bovine serum and antibiotics. Explants were placed in culture flasks with a culture medium having the same composition and incubated in a CO₂ incubator at 38 ° C. There is change of culture medium twice a week.

The cell culture LL infected with the visna-maedi virus strain M-88 by contacting the virus for 1 h with a monolayer of cells.

Results and discussion.

After 4-5 days of cultivation explants LL cells was noted the intensive growth of cells along the perimeter of adherent tissue fragments.

By 7-8th day confluent monolayer was formed, which was presented to the type of fibroblast-like cells of medium size with a granular cytoplasm and well-defined boundaries. The core is rounded shape with 1-3 nucleoli. When replanting ratio 1:2 monolayer formed by 3-5 days of cultivation and persisted without change of medium on the glass for 8-9 days.

Reproduction VM virus in LL cell culture was accompanied by the formation of syncytia.

Conclusions.

As a result of this work has been obtained subculture LL cells. Morphological characteristics are given. A permissive culture of the cells to the VM virus are showed.

D 17 - POSTER: Validation of the ID Screen Peste des Petits Ruminants Antigen Capture ELISA

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IDVET¹; CIRAD²

Key words: Peste des Petits Ruminants, antigen detection

INTRODUCTION

Peste des Petits Ruminants (PPR) is a contagious disease affecting goats and sheep, primarily in Africa, the Middle East and the Indian subcontinent. It is caused by the Peste des Petits Ruminants Virus (PPRV), a species of the Morbillivirus genus.

The ID Screen® PPR Antigen Capture ELISA is based upon reagents developed at a FAO/OIE PPRV reference laboratory (CIRAD, Montpellier, France). IDvet contributed to the improvement of this test by optimizing test performance and component stability.

This test, which allows for the detection of Peste des Petits Ruminants virus in live animals or for post-mortem diagnosis, may be used with a wide variety of matrices, including oral, nasal or rectal swabs as well as tears, tissue samples, gum debris or PMBC.

This study presents validation data obtained for this test.

METHODS AND RESULTS

The ID Screen® PPR Antigen Capture ELISA was performed as per manufacturer's specifications. Plates are coated with anti PPRV-nucleoprotein (N) capture antibody. The conjugate is an anti-PPRV-N-horseradish peroxidase (HRP) monoclonal antibody.

Comparison with RT-PCR

26 samples from 3 different countries were tested in parallel by RT-PCR and the ID Screen® PPR Antigen Capture ELISA kit. Sample types tested included oral swabs, ocular swabs, mesenteric lymph node homogenate, tears, and nasal swabs.

Test agreement was high for the samples tested.

Comparison with a previously commercialized antigen capture ELISA

7 PPRV strains were tested in parallel on the ID Screen® ELISA and an antigen capture ELISA which was previously available on a commercial basis.

The ID Screen® ELISA showed improved sensitivity on all strains tested, for both strong and weak titres.

Moreover, swab elution efficiency can be greatly improved by using the diluant included in the kit as an elution buffer instead of saline (PBS).

CONCLUSION

The ID Screen® PPR Antigen Capture ELISA demonstrates improved sensitivity on weak and strong positive samples and high correlation with RT-PCR.

It may be used on a variety of sample types, including oral, nasal or rectal swabs as well as tears, tissue samples, gum debris or PMBC.

An easy-to-use test, microplates are supplied coated and in a strip format.

Validation data will be added as it comes available (studies in regions with outbreaks are underway).

D 18 - POSTER: EXPERIMENTAL JOHNIN PPD AND GAMMA-IFN TEST IN *Mycobacterium avium* subsp. *paratuberculosis* INFECTED CATTLE

Mazzone, Piera¹; Vitale, Nicoletta²; Corneli, Sara³; Ricchi, Matteo⁴; Mangili, Pier Mario³; Papa, Paola³; Maresca, Carmen³; Di Paolo, Antonella³; Pezzotti, Giovanni³; Cagiola, Monica³; Arrigoni, Norma⁴

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Key words: MAP Gamma-IFN Johnin

Diagnosis of Paratuberculosis (PTB), due to *Mycobacterium paratuberculosis* (MAP), is usually based on serology and fecal culture. Gamma Interferon (γ -IFN) test, used for ante-mortem diagnosis of bovine Tuberculosis (bTB), due to *M. bovis* (MB), together with Skin Test (ST), may be used also for PTB diagnosis.

γ -IFN test detects cytokine produced by T lymphocytes of infected animals, in response to stimulation with traditional bovine and avian purified protein derivatives, respectively extracted by MB (PPDB) and by *M. avium* (PPDA). Johnin PPD (PPDJ) is obtained from culture of MAP. In this preliminary study, 3 new PPDJs were used in γ -IFN test and compared to classic PPDs, for PTB diagnosis.

PPDJs produced from 2 MAP field strains (A and B) and strain C (ATCC 19698) were tested at two different dilutions (1:5, 1:10). 34 ST bTB negative bovines from 4 bTB officially free herds, with previous PTB clinical cases, were selected and tested by IDVet ELISA, PCR and culture. Evaluation of γ -IFN was performed with BOVIGAM ELISA kit. Samples have been considered positive when Optical Density (OD) value was, at least, twice the OD of the blank. A 2-way analysis of variance (ANOVA), was applied to evaluate "dilution factor" and "strain factor" of PPDJs. Relative specificity of PPDB and PPDJ in bTB diagnosis was calculated using ST as Gold Standard (GS). Relative sensitivity of PPDJ in PTB diagnosis was calculated using as GS positivity in ELISA and/or PCR and/or culture.

26 of 34 animals resulted PTB positive in at least one test. ANOVA showed statistically significant differences between the mean OD of PPDJ for both "dilution factor" and the 1:5 dilution seems to stimulate highest production of γ -IFN for all three PPDJs. For bTB diagnosis, the comparative use of PPDA and PPDJ against PPDB (PPDB OD/PPDA OD; PPDB OD/PPDJA,B,C OD), in 34 bTB negative cattle, yielded a 100% specificity. Finally for PTB diagnosis, PPDJ B, at 1:5 dilution, showed the highest sensitivity 85% while PPDJ A and C, at 1:5 dilution, showed 73%.

Our preliminary results confirm high specificity of PPDA and PPDJs, when used in γ -IFN test for bTB diagnosis. For PTB diagnosis, low sensitivity is influenced by tests used as GS, able to detect advanced stages of infection only. Instead, the use of PPDJ in γ -IFN test seems to be able to reveal earlier stages of infection; in fact, 3 out of 8 subjects previously classified as PTB negatives, but positive to PPDJ γ -IFN-test, became true positive 6 months later to serology and culture. Therefore, in the future will be essential to include more subjects with a long follow up. We also plan to introduce in the stimulation phase a mitogen and a PPDJ produced in the USA (NVSL, Iowa State University Ames), to better evaluate experimental PPDJs.

Our preliminary results confirm high specificity of PPDA and experimental PPDJs, when used in γ -IFN test for bTB diagnosis. For PTB diagnosis, low sensitivity is influenced by tests used as GS, able to detect advanced stages of infection only. Instead, the use of PPDJ in γ -IFN test seems to be able to reveal earlier stages of infection; in fact, 3 out of 8 subjects previously classified as PTB negatives, but positive to PPDJ γ -IFN-test, became true positive 6 months later to serological and culture tests. Therefore, to better evaluate experimental PPDJs, in the future will be essential to include more subjects and make a long follow up on animals involved in the study.

D 19 - POSTER: PRELIMINARY STUDY ON SURVIVAL OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN A BIOGAS PLANT IN ITALY

Mazzone, Piera¹; Corneli, Sara¹; Maresca, Carmen¹; Scoccia, Eleonora¹; Biagetti, Massimo¹; Ciullo, Marcella¹; Pezotti, Giovanni¹; Ricchi, Matteo²; Arrigoni, Norma²

IZSUM¹; IZSLER²

Key words: MAP, Biogas plant

New European laws on renewable energies provide use of animal manure and organic fraction of biomass from agricultural production in biogas plants. After treatment, farmers can use the anaerobic digestion (AD) product as fertilizer. Such action, if on the one side solves energy and environmental problems, on the other creates health-related problems. In fact, in cattle rearing are still present pathogens dangerous for animals and humans, that might resist to AD treatment, with risk of dissemination during the spreading of digestate (DI). Mycobacterium paratuberculosis (MAP), etiological agent of Paratuberculosis (PTB) widespread in manure of PTB affected cattle is characterized by a high resistance in the environment. MAP could be a pathogen potentially resistant to AD treatment, provided in biogas plants. We describe here the preliminary results of an on-going research, focusing on the evaluation of MAP resistance to AD treatments. The aim is to provide health guarantees for future users of final product (DI) and to prevent the diffusion of MAP in environment after DI spreading on agricultural land.

Survey was carried out in a plant for the biogas production, where 29 cattle herds confer slurry and manure. Of the 29 breeding, 24.1% (7 farms) presented in recent years sierological and bacteriological tests positive for PTB. To verify the presence of MAP, we based on samples collected at different points of the system: Pre-tank (PT), where the material directly coming from farms is deposited, Primary fermenter (F1), where takes place the bioactivation of mass, for about 45/50 days, and Secondary fermenter (F2), where the material completes its cycle of metabolism for other 15/30 days. The study has planned to take samples from solid and liquid DI, however, these data are not available yet. A total of 18 samples from PT, 60 from F1 and 60 from F2 were taken. After sampling, direct search of MAP was performed by culture and bio-molecular techniques, according to OIE Manual. Of the 138 samples analyzed, 27 samples resulted positive by bacterial culture, including 12/18 (66,6%) from PT, 14/60 (23%) from F1 and 1/60 (1,7%) from F2. The resulting data show the presence of MAP in the incoming material (PT). MAP showed the capacity to resist to the AD process, but the amount of MAP decreases from PT to the sectors which followed (F1 and F2). The reduction of MAP might be due to 2 hypotheses: the treatment of AD may be able to reduce the amount of MAP; the capacity of F1 and F2 (4000 m³), significantly higher than PT (600 m³), dilutes the charge of MAP, which becomes difficult to detect by bacteriology. Culture for MAP is the best test today available for PTB diagnosis, but detects only 30-40% of infected cattle in a herd, especially animals which shed high amounts of MAP (from 10² to 10⁹ cells/g feces). In our study, in positive samples collected from PT, F1 and F2, we obtained a very low number of colonies with an average of 6, 2 and 1 colonies respectively. These preliminary considerations have led us, to introduce additional techniques for MAP direct detection, such as quantitative Real-time PCR. Therefore, to ensure the safety of final product, further investigations are needed. Moreover to reduce MAP contamination, we have recommended the adoption of Control Programs for PTB in herds that give manure to the biogas plants. In the future the end goal could be to introduce only effluent from farms PTB free.

D 20 - POSTER: qRT-PCR targeting ORF1 a novel alternative for EAV diagnosis

Hans, Aymeric¹; Steinbach, Falko²; Lecouturier, Fanny¹; Koï, Bart³; Juremalm, Mikael⁴; Bouwstra, Ruth³

ANSES¹; AH-VLA²; CVI³; SVA⁴

Key words: Horses, Equine Arteritis Virus, Diagnosis, Epidemiology

Equine Arteritis Virus (EAV) belongs to the arteriviridae family and its genome is a single stranded RNA molecule of 12.7 kb. EAV infects only equids, clinical signs associated to the infection range from fever, legs oedema to abortion of pregnant mares. Following primary infection EAV will usually be eliminated by the host immune response within 30 days, apart in stallions where virus may persist in the reproductive tract, sometimes for years. These stallions shedding EAV in their semen act as a reservoir and are often at the origin of outbreaks. Virus isolation (VI) on cell culture and PCR are the two methods widely used for testing stallion semen against EAV. Main objectives of our project are to harmonize EAV testing in the 4 different collaborating National Reference Laboratories (NRL) and to develop and validate a more sensitive qRT-PCR for EAV diagnosis. First, protocols used in each NRL have been evaluated by organizing a ring trial between the 4 NRLs. Ring trial results analysis, from the 20 samples sent out, shows that each protocol is very specific and overall all 4 NRL obtained satisfactory results with their own protocol. Nevertheless, protocols are not equally transposable to other laboratories. In order to bypass this issue we have decided to develop a new qRT-PCR targeting the open reading frame 1 (ORF1) of EAV genome. ORF1 is encoding the viral non structural proteins as well as the replicase enzyme. Alignment of 40 ORF1 sequences retrieved from GenBank database and from collaborators allowed to design seven primers/probe sets along conserved ORF1 regions. Concentration optimization for each primers/probe sets have been performed as well as the the PCR limit of detection (LOD), which correspond to the minimum amount of copy of the genome detected, and the method LOD, which is the minimum amount of biological materials detectable in a given matrix. PCR LOD is determined using a RNA fragment of the reference strain virus (Bucyrus) in vitro transcribed and method LOD is determined using infectious Bucyrus virus diluted in horse semen. Among the seven primers/probe sets designed two of them show an equal or better sensitivity compare to the reference method developed in 2002 by Balasuriya et al. which targets the ORF7 coding for the viral nucleoprotein. Indeed, PCR LOD results show that primer/probe set # 1 and #6bis obtained 10 and 100 copies of viral genome respectively compare to 100 genome copies for the reference method. Moreover, method LOD for primer set #1 and #6bis are 10 and 30 doses (TCID 50) respectively compare to 20 doses for the reference method. In perspective, we will perform exclusive and inclusive tests to check any no specific amplification of equine viruses such as equine influenza and Equine herpesviruses. In parallel, the protocol developed will be tested by the 4 partners on field samples. In conclusion, we have demonstrated that ORF1 is a good target to detect EAV in semen of shedders stallions and the qRT-PCR developed shows a better sensitivity compare to the reference one published in 2002 that targets ORF7.

D 21 - POSTER: Schmallenberg virus: efficacy of current serological tools

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AH-VLA¹

Key words: SBV, ELISA, NT, diagnosis

Schmallenberg virus (SBV) is a novel Orthobunyavirus first reported from Germany at the end of 2011. The virus incurred into continental Europe some time in summer 2011 and spread across the Netherlands, Germany, Belgium, France and then into the UK. As the transmission occurs predominantly through biting midges, it is likely that SBV arrived in the UK via midges blown across the Channel, similar to the incursion of Bluetongue in the 2007 outbreak. Whilst the virus is closely related to Douglasvirus, Sathuperivirus and Shamondavirus key features in the pathogenesis from Akabanevirus, such as foetal malformations, also apply.

The first malformed foetuses in the UK were demonstrated in January 2012 followed by the occurrence of similar cases across Southern England. Further epidemiological analyses, and taking into account the length of pregnancy, have pointed towards one or more parallel incursions into the UK before October 2011.

In order to determine the extent of the SBV infection, serological tests that deliver an acceptable level of sensitivity and specificity are required. Following up on the first European ring trial we compared the performance of several commercially available ELISA kits from different suppliers with those of our virus neutralisation test which is a virus Plaque Reduction Neutralisation (PRNT) format. NT tests are currently being used as the golden standard reference test.

In summary, the study demonstrates that the second generation ELISAs are more sensitive, but with a variable analytical sensitivity among different suppliers. A particular problem in the comparison of NT and ELISA are the different cut-off points, which will be discussed.

D 22 - POSTER: Molecular double check strategy for the identification and characterization of European Lyssaviruses

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FLI¹; CVI²; DTU Vet³; AH-VLA⁴

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

Lyssaviruses (order Mononegavirales, family Rhabdoviridae), the causative agents of rabies, represent a remarkable public health threat in developing countries. Among human exposures RABV is transmitted predominantly by dog bite; however bat lyssaviruses have also caused human cases. The “gold standard” for post-mortem rabies diagnosis is the fluorescence antibody test (FAT). However, in the case of ante-mortem non-neural sample material (e.g. saliva, cerebral spinal fluid, skin biopsies) or badly decomposed tissues the FAT reaches its limit and the use of molecular methods like reverse transcription PCR (RT-PCR) can be advantageous. In this study we developed a reverse transcription PCR cascade protocol feasible for screening and classification of samples even without any epidemiologic background with emphasis on the most relevant European lyssaviruses.

As a first step two independent pan-lyssavirus assays based on the detection of an intercalating dye are performed in a double check application to increase diagnostic safety. Additionally, two independent internal control systems (endogenous and heterologous) were established. For the second line characterization of the lyssavirus positive samples two independent probe based (TaqMan) species-specific multiplex systems for RABV, EBLV-1, EBLV-2 and BBLV were developed. All assays were successfully validated with a comprehensive panel of 52 lyssavirus positive samples (including RABV, LBV, MOKV, DUVV, EBLV-1 & -2, ABLV, BBLV) as well as negative material from various host species. Furthermore, a synthetic positive control for all assays (intercalating dye and TaqMan assays) was established which enables a quantification of the viral load.

In conclusion the developed pan-lyssavirus real-time RT-PCR assays and the two independent species-specific multiplex real-time RT-PCR systems allow the safe and sensitive screening and detection of all known lyssaviruses in humans and different animals as well as the characterization of the lyssavirus species circulating in the main land of Europe. The presented workflow combines all known advantages of the real-time PCR technology like speed and reduced risk of cross-contamination with improved safety of molecular testing based on double check strategy for the screening as well as the confirmation assays.

D 23 - POSTER: Workflow for the specific detection and characterisation of the Influenza A virus H7N9 (China) by real-time RT-PCR

Hoffmann, Bernd¹; Kalthoff, Donata¹; Harder, Timm¹; Grund, Christian¹; Beer, Martin¹

FLI¹

Key words: Influenza A virus; subtype H7N9; China; real-time RT-PCR

Transspecies transmissions and viral adaptation of influenza A viruses to new host species were responsible for the latest influenza pandemic in 2009. And recently another “animal borne” influenza virus was occasionally introduced into human population: influenza A subtype H7N9. More than 100 humans were infected in China probably after contact to infected poultry. In contrast to influenza A H5N1, which is also an avian influenza virus circulating in Southeast Asia, H7N9 is low pathogenic in its avian host species. Therefore, avian species did not develop clinical signs of illness upon infection with H7N9. Experiments using the ferret model, resembling human influenza virus infection, demonstrated that H7N9 can easily be transmitted via close contact, while airborne transmission between the ferrets was limited. As H7N9 virus is transmitted also between avian species quiet easily, surveillance systems are urgently needed. In addition to the necessity to sensitively track the virus in avian host species, diagnostic approaches to detect the evolutionary scenario of an highly pathogenic virus from the low pathogenic progenitor H7N9 should be set in place. For this purpose subtype (pan-H7 and pan-N9) and strain (Chinese H7N9) specific real-time RT-PCR assays, which target different fragments of the hemagglutinin gene and the neuraminidase gene of the new H7N9 virus were developed and compared to published H7N9 assays. For a complete validation of the pan-assays as well as the strain specific assays 43 H7 and 17 N9 strains were tested and samples from animals experimentally infected with H7N9 were included. The results demonstrated that the new subtype specific pan-H7 and pan-N9 assays detected specifically all tested H7 and N9 strains while all non-H7 and non-N9 strain reacted negatively. Furthermore for the specific detection of the new H7N9 strain from China an exclusively specific H7 assay and an almost specific N9 assay were generated. In contrast the published H7 and N9 assays were not specific for the detection of the new H7N9 virus. This might be a specificity problem for surveillance studies from susceptible animals. In addition a real-time RT-PCR method detecting specifically the mono-basic HA cleavage-site of the new H7N9 virus was developed and validated. Using this method a speedy and easy evaluation and detection of mutations within the mono-basic cleavage-site is possible.

All assays are integrated easily in a general workflow for quick, sensitive and specific identification as well as cleavage-site characterisation of emerging H7N9 virus in humans and animals.

ANTIVIRALS & VACCINES

AV 1 - POSTER: Demonstration of Efficacy against Challenge of an Inactivated Schmallerberg Vaccine in Sheep

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Merial S.A.S.¹

Key words: Schmallerberg, Vaccine, Efficacy, Sheep, Viraemia

INTRODUCTION

In August 2011, outbreaks of an unknown disease of cattle were reported in both the Netherlands and Germany. From December 2011, abortion and foetal abnormalities, were reported in sheep and cattle in several European countries. A new virus was identified in November 2011 and was associated with both conditions. This agent was named 'Schmallerberg virus' (SBV) after the German town where the virus was first identified. Schmallerberg virus is in the Simbu serogroup of the Orthobunyavirus group. This group of viruses includes many viruses occurring in the Tropics. None had been previously identified in Europe. Although some uncertainty remains on the transmission of SBV, it seems primarily spread by biting insect vectors (midges/mosquitoes).

Here, we present the results of a vaccination / challenge study showing that a single administration of an inactivated SBV vaccine was able to prevent viraemia in sheep.

MATERIAL AND METHODS

Eleven weaned lambs were randomly allocated to one group of 5 vaccinates and one group of 6 control sheep. Vaccinates were subcutaneously treated once on day 0, with 1 mL of an inactivated SBV vaccine (Merial). The other group was left unvaccinated and served as control. Twenty one days after vaccination, all sheep were challenged with a virulent SBV strain. All sheep were then monitored for rectal temperature, clinical signs and viraemia (quantitative RT-PCR) from D22 to D31.

RESULTS

Hyperthermia : no hyperthermia was observed in any of the groups.

Clinical signs : no significant clinical sign was observed in any of the groups.

Viraemia (qRT-PCR) : all controls were found positive on 3 consecutive days. None of the vaccinated animals was ever detected positive.

CONCLUSION

In the present study, single vaccination of sheep with the product tested provided full and significant protection against viraemia following a SBV challenge.

AV 2 - POSTER: Implementation Of Diva Genetic Real-Time Rt-Pcr Assay Using A Lapinized Chinese Vaccine Of Classical Swine Fever Virus

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Key words: classical swine fever virus, DIVA genetic real-time RT-PCR assay, lapinized chinese vaccine, virological protection

Classical swine fever is a highly contagious disease. Vaccination of pigs with lapinized Chinese vaccines is still practised in some regions of the world as an efficient measure of control disease. Several disadvantages have been attributed to this type of vaccines such as the replication in lymphoid organs and the antibodies induced upon vaccination that cannot be distinguished from those elicited in natural infections. To address this question, twenty Landrace x Large white cross male pigs, 8 weeks old, were randomized into 3 groups: Group 1 (seven pigs) was vaccinated with 2 mL (100 protective doses 50) of lapinized Chinese vaccine (LABIOFAM S.A., Cuba). In the group 2, seven pigs were left as contact of Group 1. The Group 3 was inoculated with 2 ml of saline solution. Sixteen days later, all animals were challenged with 10⁵ TCID₅₀ of CSFV (Margarita strain). Clinical signs compatible with a classical swine fever infection were recorded. All vaccinated pigs induced a strong interferon gamma response at day 8 after vaccination and reduced significantly the clinical signs in comparison with the control group. At five days post challenge, all vaccinated pigs develop antibodies against E2 glycoprotein. Currently the seroneutralization technique is being conducted. Finally, the use of two techniques of qRT-PCR real time was effective to differentiate the RNA of the vaccine virus from the RNA of the virus used for the challenge. With these techniques, it is feasible to determine the replication of the vaccine virus, as well as the levels of protection in terms of RNA challenge viral load. One analysis that shows a correlation between virological, humoral, cellular immune response and clinical parameters will be presented.

AV 3 - POSTER: In vitro modulation of antibody responses to type I PRRS virus strains

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Key words: PRRS virus, IFN-alpha

The critical issues of PRRS virus (PRRSV) pathogenicity and virus/host interaction are still ill-defined, which actually affects the development of effective disease control strategies based on both clinical and virological protection. In this scenario, the mucosal antibody response of pigs has been substantially neglected in studies on field and experimental infections. Yet, the effective modulation of mucosal antibody responses to PRRSV could be the foundation of novel and effective control strategies. Owing to the above, we investigated the ability of IFN-alpha to modulate antibody production in vitro on the basis of its established properties in other models of antibody response.

The study was carried out in the framework of two experimental infections of PRRS-naïve pigs with EU PRRS virus (PRRSV) strains BS114 / 2000 (attenuated) and BSAL / 2011 (non-attenuated). Serum samples were collected weekly to evaluate the antibody response to the infection. On Post Infection Day (PID) 56, Peripheral Blood Mononuclear Cells (PBMC) and Palatine tonsil cells were cultured in the presence of: 1) PRRSV; 2) PRRSV + 1 U/ml of IFN-alpha; 3) PRRSV + 100 U/ml of IFN-alpha; 4) a cryolysate of MARC-145 cells used for propagation of PRRSV; 5) neither IFN-alpha nor PRRSV (control). Cells were treated with the same virus used for the in vivo infection and incubated at 37 °C in 5% CO₂; supernatants were harvested after 12 days of incubation. The release of PRRSV-specific and non-specific IgA and IgG was measured by isotype-specific ELISAs.

PBMC and tonsil cells of PRRSV-infected pigs did not release PRRSV-specific antibodies in vitro. Tonsil cells obtained from pigs infected with the attenuated strain were significantly stimulated to release IgA immunoglobulins after treatment with PRRSV+IFN-alpha at 1 or 100 U/ml. Interestingly, the cryolysate treatment also caused an increase ($P < 0.05$) of IgA release from PBMC with respect to the control cultures.

The release of immunoglobulins was reduced in cultures of PBMC and tonsil cells obtained from animals infected with the non-attenuated PRRSV strain. In this model, IFN-alpha at 1 and 100 U/ml partly restored IgG release from PBMC only; no effect was showed instead in terms of IgA secretion. Concerning tonsil cells, IFN-alpha at 100 U/ml give rise to an increased production of IgA; no effect was shown in terms of IgG release.

The different properties of the two PRRSV strains could be the result of crucial amino-acid changes in NSP1-beta, previously recognized as an important modulator of Type I IFN responses. In this respect, the attenuated and virulent strains under study showed with respect to the Lelystad reference strain a homology of 99% and 73%, respectively. This could account for the different IFN-alpha responses in vivo after infection and the different outcome of the in vitro IFN-alpha treatments.

AV 4 - POSTER: Recombinant marker vaccine based on DNA and MVA expressing VP2, VP7, and NS1 from BTV-4 partially cross-protects lambs against BTV-8.

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CISA-INIA¹; CIC bioGUNE²

Key words: DNA/rMVA vaccine bluetongue mouse lamb

Bluetongue virus (BTV) belongs to the Orbivirus genus of the Reoviridae family. The development of vector-based vaccines expressing conserved protective antigens results in increased immune activation and could reduce the number of multiserotype vaccinations required. We have now engineered naked DNAs and recombinant modified vaccinia virus Ankara (rMVA) expressing VP2, VP7 and NS1 proteins from BTV-4. IFNAR(-/-) mice inoculated with DNA/rMVA-VP2,-VP7,-NS1 from BTV-4 in an heterologous prime boost vaccination strategy generated significant levels of antibodies specific for VP2, VP7, and NS1, that showed neutralizing activity against BTV-4. In addition, this vaccination regime stimulated specific T cell responses against these three BTV proteins. Vaccination elicited sterile protection against a lethal dose of homologous BTV-4 infection and induced cross-protection against lethal doses of heterologous BTV-8 and BTV-1. In order to evaluate the cross-protection induced by this vaccination strategy in the BTV natural host, lambs were vaccinated with DNA/rMVA-VP2,-VP7,-NS1 from BTV-4 and challenged with BTV-8 two weeks after the second immunization. Preliminary data showed that vaccinated animals presented lower levels of viraemia after challenge than non immunized lambs. In addition, the induction of neutralizing antibodies specific of BTV-8 after the infection with BTV-8 was faster and stronger in vaccinated than in non immunized animals. These data indicate that the immunization with DNA/rMVA-VP2,-VP7,-NS1 from BTV-4 partially protects sheep against an heterologous challenge with BTV-8. The inclusion of the protein NS1 in formulations targeting BTV results therefore, in promising multiserotype recombinant marker vaccines.

AV 5 - POSTER: "EPIZONE" Selection system for rapid and efficient isolation of African swine fever virus recombinants.

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Key words: African swine fever virus, thymidine kinase, recombinants

African swine fever virus (ASFV), the sole member of the family Asfarviridae, genus Asfivirus, causes African swine fever in domestic pigs and wild boars. ASF is a highly contagious hemorrhagic disease with mortality rates up to 100 %. No efficacious vaccine has been obtained yet. The size of the double stranded DNA genome varies between 170 and 190 kbp, and, depending on the virus isolate, contains 150 to 170 open reading frames. ASFV DNA is not infectious per se and therefore isolation of recombinants for molecular analyses of gene functions and generation of novel vaccine candidates requires excessive ASFV helper virus which subsequently needs to be eliminated. Homogeneity of recombinant ASFV stocks requires usually seven to nine consecutive plaque purification rounds which bears the risk of selecting viruses with unwanted mutations that e.g. support replication in cell culture. To reduce significantly the number of plaque purifications, we established a procedure that permits recombinant ASFV isolation after only two rounds of selection. For that, an ASFV-permissive thymidine kinase (TK) -negative cell line (WSL-Bu) was selected and used for the generation of an ASFV mutant in which the viral thymidine kinase (TK) ORF was replaced by the ORF for GFP. With regard to ASFV genome modification, helper virus can be eliminated by choosing an appropriate combination of cells, virus and HAT- or BudR-containing medium.

AV 6 - POSTER: Development of a novel marker vaccine platform for protection against Bluetongue Virus (BTV)

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Key words: bluetongue, vaccine, replicon, DIVA

Bluetongue Virus (BTV) is an economically important member of the genus Orbivirus. Currently, 26 different serotypes of BTV are known. The virus is transmitted by blood-feeding *Culicoides* midges and causes disease in ruminants. In 2006/2007, BTV-8 led to widespread outbreaks in Europe which were eventually controlled employing a conventionally inactivated BTV vaccine. However, this vaccine did not allow the discrimination of infected from vaccinated animals (DIVA) by the commonly used VP7 cELISA.

RNA replicon vectors based on propagation-incompetent recombinant vesicular stomatitis virus (VSV) represent a novel vaccine platform that combines the efficacy of live attenuated vaccines with the safety of inactivated vaccines. Our goal is to generate an RNA replicon vaccine for BTV-8, which is safe, efficacious, adaptable to emerging orbiviruses, and compliant with the DIVA principle.

The VP2, VP5, VP7, VP3 genes encoding for BTV-8 capsid proteins as well as the non-structural proteins NS1 and NS3 were inserted into a VSV vector genome lacking the essential VSV glycoprotein (G) gene. Infectious virus replicon particles (VRP) were produced on a transgenic helper cell line providing the VSV G protein in trans. In vitro expression of antigens was analysed by immunofluorescence using monoclonal and polyclonal antibodies. Sheep were immunized with two different VRP-based vaccine candidates, one comprising the BTV antigens VP2, VP5, VP3, VP7, NS1, and NS3, the other one containing antigens VP3, VP7, NS1, and NS3. Control animals received VRPs containing irrelevant antigen. Virus neutralizing antibodies were induced only when the two antigens VP2 and VP5 were included in the vaccine. These animals were completely protected from challenge infection with homologous BTV-8 challenge and did not show any viremia or clinical signs of disease. Animals immunized with antigens VP3, VP7, NS1, and NS3 developed milder symptoms than control animals but still were viremic.

These results show that the VSV replicon system represents a promising vaccine platform for BTV. In further experiments we will try to exclude VP7 to meet the requirements of a DIVA vaccine.

AV 7 - POSTER: Modern adjuvants do not enhance the efficacy of inactivated African swine fever virus vaccines

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FLI¹

Key words: African swine fever, inactivated vaccine, adjuvants

African swine fever (ASF) is among the most devastating and complex viral diseases of domestic pigs. The disease which is endemic in many countries of Sub-Saharan Africa has recently spread to Trans-Caucasian countries and the Russian Federation where it keeps occurring in domestic pigs and wild boar. There is a clear tendency of spread towards the borders of the European Union and thus contingency plans had to be revisited. Control measures have to rely on sanitary measures as no efficient vaccines exist. Despite intensive research activities, vaccine candidates that have any potential for licensing are lacking. To exclude the possibility that there could be a traditional approach combined with modern technology, a study was undertaken to re-assess inactivated ASFV preparations with state-of-the-art adjuvants.

Traditional inactivated vaccines against ASFV were long shown to completely lack efficacy but since then, considerable improvements have been made regarding adjuvants, especially with action towards cellular immunity, which is vital for the protection against ASFV. In the presented study, ASFV strain "Armenia 2008" was grown on porcine periphery blood monocytes (PBMC) to high titers and inactivated using binary ethyleneimine (BEI). Inactivated preparations were either mixed with Polygen™ (MVP Technologies, Omaha, USA), a co-polymer based adjuvant, known to stimulate humoral and cellular immune responses as well as interferon gamma responses, or Emulsigen®-D (MVP Technologies, Omaha, USA), an oil-in-water emulsion with depot effect that incorporates dimethyldioctadecyl-ammonium bromide (DDA) as T-cell stimulant. For intramuscular immunization of weaner pigs, solutions were prepared with 20 % (v/v) adjuvant. Six weaner pigs received a double immunization with Polygen-adjuvanted virus whereas another 6 weaner pigs received Emulsigen-D-adjuvanted virus. The interval between the immunizations was 3 weeks. Three additional pigs acted as unvaccinated challenge controls. Upon vaccination and challenge infection, rectal body temperatures and clinical scores were assessed daily. Blood collection for serological assays was carried out at days 0, 7, 14, 21, 28, 35, and 42 post vaccination. At day 42 post vaccination, all animals were challenged with ASFV strain "Armenia 2008". Subsequently, serum and EDTA blood samples were collected at days 4, 7, and 10 post challenge infection or at the day of euthanasia for virological and serological assays. Organ samples were collected during necropsy.

Although ASFV-specific antibodies were detectable in all but one vaccinated animal, no protective effect of immunization was observed. All animals developed an acute form of ASF after challenge infection with high fever, reduced feed intake, slight central nervous symptoms, and somnolence. A slightly accelerated course was observed in some vaccinees. All animals succumbed to infection by day 12 post challenge. Virus and genome detection was comparable in all animals. Upon challenge infection, antibodies dropped in immunized animals but were still detectable. No antibodies were found in unvaccinated animals. Concluding, modern adjuvants do not influence the efficacy of inactivated ASFV vaccines. Although ASFV-specific antibodies were present, no protection was observed even after strict homologue challenge. The clinical course in some vaccinees could even indicate an antibody dependent enhancement which could also influence the efficacy of other vaccine approaches.

AV 8 - POSTER: Induction of innate immune responses in porcine PBMCs by RNA transcripts corresponding to non-coding regions of Picornavirus genome

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Key words: adjuvants and antivirals, innate immunity, interferon, swine

Innate immune defenses are essential to control viral infections. Detection of pathogen associated molecular patterns (PAMPs) as non-self by host viral sensors initiates a signalling cascade leading to secretion of type I IFNs, promoting the antiviral state in the host and playing also a role in adaptive immunity.

We have recently described that different highly structured motifs present in the non-coding regions (NCRs) of the FMDV genome, enclosing stable dsRNA regions, are strong inducers of type I IFN, both in vitro and in vivo. When inoculated in mice, these RNAs were able to induce protection against lethal doses of foot-and-mouth disease Virus (FMDV) or West Nile virus (WNV). Besides, when RNA was co-inoculated with a conventional vaccine, the immune response elicited after vaccination was enhanced. These results showed the efficiency in animal models of those RNA regions, likely acting as PAMPs, as synthetic non-infectious antiviral molecules and immune adjuvants.

In this work, we analysed the cytokine responses induced by these RNA transcripts in porcine PBMC (Peripheral blood mononuclear cells) cultures as a first approach to evaluate the potential immunomodulatory role of these molecules in livestock species. Swine is a relevant natural host for many important viral diseases, and many of the viruses causing these diseases are sensitive to IFN. The use of PAMP agonists in farm animals, besides increasing the magnitude of the immune response when used as adjuvants, might reduce the required time lapse between vaccination and protection; this would be particularly useful in emergency vaccination. Our results will provide some insights about the potential of the RNA transcripts as immune antiviral and adjuvant molecules in large animals.

AV 9 - POSTER: UNDERSTANDING THE MECHANISMS OF INTERFERON INDUCTION AND EVASSION BY AFRICAN SWINE FEVER VIRUS AND ITS APPLICATION TO VACCINE DEVELOPMENT.

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PIR¹

Key words: African swine fever virus, vaccines, interferon, immune evasion

African swine fever virus (ASFV) is a large DNA virus that can assume different clinical forms, ranging from an acute haemorrhagic disease to unapparent infections in domestic pigs. The complexity of the immune response has impaired the development of an effective vaccine against ASFV infection. Previous work has established that immunization of pigs with attenuated replication competent ASFV strains can induce good levels of protection against lethal challenge with virulent strains. However, the highly acute nature of the disease caused by the virulent strains in domestic pigs suggests that the porcine innate response is not effective in controlling the early infection until the serological and cellular adaptive immune responses provide immediate protection and, the generation of an immunological memory which is critical for vaccine development. The type I interferon (IFN) response is the first line of innate immune response against viral infection and is also involved in activating the adaptive immune response. Most viruses have mechanisms to evade this pathway. We will present data which investigates the ASFV signals responsible for induction of a type I IFN response in infected macrophages and virus genes involved in suppressing this response.

This information will provide a rational basis for the development of novel vaccine strategies, for example, by deleting genes involved in the evasion of the IFN host response.

AV 10 - POSTER: Bluetongue virus serotype 8 seroprevalence in dairy cattle three and four years after vaccination with an inactivated vaccine in north-eastern Italy

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Key words: BTV-8, vaccination, cattle, Italy

In response to the 2006–2007 European epidemic of bluetongue virus serotype 8 (BTV-8), large-scale vaccination campaigns of susceptible species were launched in most European countries in 2008 using inactivated BTV-8 vaccines. This allowed for safe trade of live animals (Regulation EC 1266/2007) as well as rapid control of the infection. In Italy, vaccination in cattle was performed using Zulvac® 8 Bovis from June–December 2008 and April–November 2009 across the two neighbouring provinces of Verona and Mantua in north-eastern Italy, where BTV-8 cases were recorded. In absence of a serological test able to differentiate infected from vaccinated animals, information on vaccine-elicited antibody dynamic is useful for implementing surveillance activities. For this reason, a cross-sectional serosurvey of BTV-8 neutralizing antibodies in vaccinated dairy cattle three and four years after vaccination was performed in a BTV-free area. In April–May 2012, serum samples from 110 cows (of 12 herds) vaccinated in 2008, and from 270 cows (of 28 herds) vaccinated in both 2008 and 2009, were processed for the presence of BTV-8 neutralizing antibodies using virus neutralization assay. Stratified random sampling according to herd size was carried out and survey design-based sampling weights were used in the analysis to weight the sample back to the population from which it was drawn. Each of the samples was collected and recorded individually. A total of 84 non-vaccinated heifers born from 2010 onwards were also tested for BTV-8 neutralizing antibodies to confirm absence of virus circulation in the sampled herds after vaccination. Overall seroprevalence was 41% (95% confidence interval [CI]: 36–45%). Seroprevalence in animals vaccinated in 2008 only (31%, 95% CI: 23–39%) was significantly lower (χ^2 test, $p = 0.007$) than that recorded in those vaccinated in both 2008 and 2009 (45%, 95% CI: 40–50%). Antibody titres ranged between 10 and 320 (geometric mean [GM] = 22). Log-transformed antibody titres in animals vaccinated in 2008 only (GM = 19) were significantly lower (Student's t test, $p = 0.006$) than those recorded in animals vaccinated in both 2008 and 2009 (GM = 24). Log-transformed antibody titres correlated negatively ($r = -0.15$, $p = 0.003$) with the time interval between the date of last vaccination and that of sampling. Significant age effects were found in seroprevalence (χ^2 test, $p = 0.003$) and log-transformed antibody titres (ANOVA, $p = 0.001$), with seroprevalence being highest in heifers aged 13–36mths at the time of last vaccination (49%, 95% CI: 41–57%) and lowest in calves aged ≤ 12 mths (17%, 95% CI: 7–27%); and antibody titres being highest in heifers (GM = 27) and lowest in adult cows aged ≥ 37 mths (GM = 19). None of the 84 non-vaccinated heifers had detectable BTV-8 neutralizing antibodies. We concluded that despite the significant reduction in seroprevalence and antibody titres from 3 to 4 years post-vaccination and the different age-related responses, vaccine-elicited antibodies can still persist at high levels in a considerable part of the vaccinated population. This implies that for at least 4 years, vaccinated cattle cannot be included in (sero)surveillance programmes. Moreover, the fact that around 1/3 of vaccinated cattle is likely to still be protected 4 years after vaccination provides an explanation of why the control of BTV-8 infection by means of vaccination was so rapid and effective in most European countries despite the reduction in vaccine coverage in 2009–2010.

AV 11 - POSTER: Interference of Newcastle disease and Avian Influenza passive immunity with a recombinant NDV-H5 vaccine efficacy demonstrated in original artificial model of passive immunity transfer in SPF chickens

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Key words: Vaccination, HPAI, rNDV-H5, passive immunity, artificial model

Passive immunity is important early in life, when the immune system is immature and unable to fight off infection. Maternally derived antibodies (MDA) arm young chicks against pathogens previously encountered by the dams. However, MDA can interfere with immune response mounting after vaccinations and reduced their efficacy as observed during Newcastle disease (NDV) and Avian Influenza (AI) vaccination programs. The effect of MDA is usually investigated using the progeny of vaccinated dams which is time consuming, expensive and poorly reproducible. Here, we present a faster, more homogenous, reproducible and cheaper protocol to mimic passive immunity in Specific Pathogen Free (SPF) chicken by injection of a polyserum into the egg yolk at embryonic day (ED) 14, combined with an intraperitoneal injection at day 1. An artificial model reproducing similar levels and duration as a natural passive immunity was optimized for NDV and applied to H5 AI. This artificial model has allowed to evaluate the impact of ND or AI H5N1 MDA on the vaccine efficacy of a recombinant NDV-H5-vectored vaccine (rNDV-H5). Survival, humoral immune responses and viral excretion were evaluated after vaccination and Highly Pathogenic avian Influenza (HPAI) H5N1 AIV challenge of layer chicken. A positive impact on vaccine efficacy was observed in the context of artificial NDV-MDA while the presence of H5-MDA induced a clear negative impact on vaccine induced protection against a HPAI H5N1 challenge.

AV 12 - POSTER: Next Generation Sequencing to investigate in vitro evolution of low pathogenic avian influenza H5N1 virus under strong antiviral (oseltamivir) selection pressure

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Key words: Antivirals, Avian influenza virus, Next Generation Sequencing

Avian influenza virus contains two main surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). HA is responsible for virus binding at cell infection, while NA is responsible for the release of progeny virions. NA inhibitors like oseltamivir (Tamiflu) inhibit neuraminidase activity preventing the release of progeny virus thereby stopping virus replication. Resistance against the antiviral drug oseltamivir has been observed before and was associated with mutations in the NA gene.

This study aimed to investigate in vitro viral evolutionary patterns of a low pathogenic H5N1 subtype virus population under strong antiviral selection pressure. The wild type virus population was first plaque purified to reduce start population diversity. Then the clonal virus was passaged over cell culture under increasing oseltamivir selection pressure. The complete genomes of the wild type virus, the clonal virus and the first 3 passages were sequenced into depth using next generation sequencing.

Although the NA activity dramatically decreased over passages, virus specific RT-qPCR indicated that the virus grew increasingly under increasing antiviral pressure. We were unable to confirm any of previously identified NA mutations associated with NA inhibitor resistance, not even present at a low frequency. Three non-synonymous mutations were fully established in the virus population from the first passage (in PB1 gene: T128S, in HA gene: K205E and in NA gene: N434S). The HA mutation (K205E) increases the negative charge near the receptor binding site of this protein, which is a known compensatory mechanism to reestablish the HA/NA activity balance. This mutation most probably made it possible that our virus population continued growing notwithstanding the presence of oseltamivir and a defective neuraminidase.

In conclusion: in an attempt to set up a controlled in vitro culture experiment to study evolutionary dynamics under strong antiviral selection pressure, we reconfirmed the critical balance of HA and NA activities for efficient replication of influenza A virus.

AV 13 - POSTER: ECONOMIC IMPACT OF USING AN ANTIVIRAL IN THE CONTROL OF A FOOT-AND-MOUTH DISEASE EPIZOOTIC IN SOUTHERN BELGIUM.

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Key words: Foot-and-mouth disease, antiviral, control, economic impact

Foot-and-mouth disease virus (FMDV) is a highly contagious pathogen of cloven-hoofed mammals and one of the biggest concerns for veterinary authorities. The control measures to be applied in case of an outbreak vary in function of the disease-free or disease-enzootic status. Vaccination depends on the prior identification of the involved viral serotype and subtype, it confers an immunity limited to 6 months and it requires between 4 to 7 days to trigger the immune response (i.e. immunity-gap). The use of anti-FMDV drugs has been discussed as an alternative or supplementary method to be used in previously FMD-free countries/zones. Such an antiviral treatment could protect against the viral dissemination to fill the gap between vaccination and the rise of a protective immunity. Apart from broad spectrum antiviral agents, such as ribavirin, specific anti-FMDV molecules have been identified in vitro, but none of them has been used in clinical studies involving ruminants or pigs. Next to the anti-FMDV activity, the absence of toxicity and the withdrawal period influencing the food safety, the cost of the treatment would be another important parameter influencing the potential use of an antiviral agent in the control of a FMD outbreak. The aim of this study was to assess the economic impact of using an antiviral in the control of a FMD epizootic in southern Belgium (Walloon Region). This work was based on the results of previous investigations concerning the epidemiological and economic data of a FMD outbreak in Southern Belgium. In the considered scenario, the epizootic was caused by the introduction of an infected cow (during the incubation time) in a beef cattle farm during winter. During the two weeks between the brood cow introduction and the official declaration of the outbreak, animal movements occurred between other beef cattle farms. The economic effects of the epidemic were evaluated taking into account the air-borne transmission of FMDV, the occurrence of animal movements (two scenarios were considered, with a minimum of 2 and a maximum of 17 movements), the presence of bovine and small ruminant farms, as well as pig farms in the protection and surveillance zones around the initial and secondary outbreaks. The wild fauna was not involved in the epidemic. In order to integrate in the above scenario the application of an antiviral agent in the control of the disease, it was assumed that the efficacy of the anti-FMDV drug was proven by reducing viral excretion in infected animals as well as by preventing the infection in animals at risk. Two hypothetical prices were used to introduce in the model the costs related to the administration of the antiviral drug (5€ and 10€ per dose). Furthermore, different strategies of control could be envisaged, such as the administration of the drug to both domestic ruminants and pigs, or depending on the epidemiological role of these species in the FMD transmission and their density in the territory, the administration of the drug to only one of them. Other scenarios could be characterized by the use of the antiviral in the control of the epizootic within the protection and surveillance zones or in only one of them. The costs associated with the use of antivirals in the different proposed scenarios are compared to the costs and socio-economic losses associated with the FMD outbreak and the implementation of control measures.

AV 14 - POSTER: Vaccination against Schmallenberg virus: trivalent Akabane/Aino/Chuzan virus vaccine vs. SBV-vaccine candidates

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Key words: Schmallenberg virus; Vaccine; Orthobunyavirus; Inactivated vaccine

Schmallenberg virus (SBV) is a recently discovered, insect-transmitted, teratogenic orthobunyavirus that infects ruminants. Experiences with viruses closely related to SBV have demonstrated that vaccines are an adequate instrument for disease control. In Japan, for example, an inactivated, trivalent vaccine, containing the orthobunyaviruses Akabane virus (AKAV) and Aino virus (AINOV) and the reovirus Chuzan virus, is available to prevent infectious reproductive disorders in ruminants. Therefore, vaccination will most likely also be an important aspect of disease control for SBV.

In the present study cattle were immunised twice with the inactivated trivalent vaccine against AKAV, AINOV and Chuzan virus. Furthermore, five newly developed, inactivated SBV-vaccine candidates were tested in sheep and cattle, the major target species. The five vaccine formulations differed in cell line used for virus growth, the viral titer before inactivation and the inactivation protocol. Additional sheep and cattle were used as unvaccinated controls. All animals were inoculated with SBV three weeks after the second vaccination. Blood samples were taken weekly and tested by an SBV-specific ELISA and serum neutralization tests for SBV and where required for AKAV and AINOV antibodies. After challenge infection serum samples were obtained daily for 8 days and tested by qRT-PCR based on the SBV S-segment. Autopsy was performed on all animals three weeks after challenge infection, and different lymphoid organs were sampled for qRT-PCR.

In all animals immunised with the trivalent vaccine AKAV- and AINOV-specific neutralizing antibodies were detectable before challenge infection, while control animals remained negative. In both, vaccinated and control animals, SBV- genome was detectable in post challenge serum samples for a minimum of four days, and in the lymphoid organs. In contrast to the trivalent vaccine, four out of five SBV vaccine candidates completely prevented SBV-RNAemia and the fifth reduced it considerably. Anti-SBV antibodies were detected in 21 out of 25 immunised sheep and in 14 out of 16 cattle three weeks after the second immunisation by at least one serological test. The number of animals per group that seroconverted and the strength of the antibody response were dependent on the viral titre prior to inactivation, the production cell line and the applied inactivation protocol.

In conclusion, the Japanese trivalent AKAV/AINOV/Chuzan vaccine did not protect against an experimental SBV infection. In contrast, four of the five newly developed SBV-specific vaccine formulations are promising candidates for disease prevention and could be a valuable tool in SBV control strategies.

AV 15 - POSTER: Characterization and Expression of Immunodominant E Gene of Bovine Virus Diarrhoea Virus (BVDV) Field Isolates from Turkey using Baculovirus Expression System for the Purpose of Preventing of BVDV Infection

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Key words: BVDV, eradication, vaccination, molecular characterization

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Abstract

Bovine virus diarrhoea virus (BVDV) is economically important pathogen for cattle industries. This infection is endemic in some parts of the world and a control program is available that based on a combination of elimination of persistent infected animals and vaccination. Totally 42 BVDV field isolates from cattle in Turkey were characterized using by the sequences of immunodominant envelope (E) gene region of pestiviruses. Selected isolates are among the circulating dominant BVDV field isolates in country. The novel PCR primers were designed to amplify the immunodominant E region of pestiviruses, which is E2. The achieved amplicons from selected local field isolates were transformed to protein using by baculovirus expression system. These proteins could be used in in vivo experiment to produce a recombinant vaccine for a probable national BVDV eradication program that should be included all dominant strains for instance BVDV 1-a, 1-l and 1-d in further studies.

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AV 16 - POSTER: Treatment with anti-rabies VHH derived from llama heavy chain antibodies prevents or delays rabies virus disease and mortality in mice depending on the timing of treatment

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Key words: VHH, nanobody, rabies virus, prophylaxis, protection

VHH are the smallest functional fragments of heavy chain antibodies naturally occurring in Camelids and represent the antigen-binding variable heavy domain. Anti-rabies VHHs with high affinity for the viral glycoprotein G have been selected by phage display. Their simple structure, consisting of a single antigen-binding domain, allows for high solubility, physicochemical stability and high yield production in *E. coli* or yeast. VHH can easily be fused into multimeric constructs with multiple specificities¹⁻³.

Rabies virus is a model neurotropic RNA virus causing an aggressive and lethal infection in the brain of mammals. To study the efficacy of potential antiviral therapies in the brain, we have developed and validated a highly reproducible brain infection model based on intranasal inoculation of virus⁴, which is an excellent model since it leaves the brain mechanically intact. Upon intranasal virus inoculation, viral RNA can be detected already at 1 day post inoculation (DPI) in the olfactory bulbs in part of the mice. At 2 DPI, viral RNA is detectable in the olfactory bulbs of all mice. The virus then spreads quickly to the rest of the brain, resulting in an explosion of the viral load in the brain at 4 to 5 DPI, reaching a plateau from 7 DPI onwards. Symptoms start around 7 DPI and the clinical end point for euthanasia is reached 1-2 days later.

We have used this model to evaluate the protective efficacy of preventive or post exposure treatment with anti-rabies VHH.

In vitro, the monovalent anti-rabies VHH could completely neutralize rabies virus and by combining two VHH with a flexible linker, the neutralizing potency was strongly enhanced to a level equal or superior to that of an antibody⁵.

In mice, preventive administration of low doses of anti-rabies VHH in the nose or the brain 1 day before virus challenge completely prevented disease. Also, simultaneous administration of virus and anti-rabies VHH in the brain, muscle or nose completely protected mice against disease. In contrast, administration of irrelevant VHH invariably yielded lethal disease. Upon intracerebral inoculation of virus and anti-rabies VHH, viral RNA loads remained negligible in the brain and were 106-fold lower than with irrelevant VHH at 7 DPI.

Post exposure treatment was studied by intraperitoneal injection with dimeric anti-rabies VHH or irrelevant VHH at 1 day after intranasal inoculation with the virus. At this early stage of the infection, an important part of the susceptible neurons are still left uninfected, so treatment with compounds that block virus entry and transmission between neurons can still be effective.

VHH were administered intraperitoneally at a dose of 10 mg per mouse (= 47855 neutralizing IU per mouse). In contrast to preventive treatment, administration of VHH at 1 DPI could only prolong median survival by 1 day ($p < 0.05$).

In conclusion, VHH that recognize the surface glycoprotein of rabies virus can completely neutralize virus infectivity in vitro and in vivo when given prior to or together with the virus inoculation. Treatment after the start of the virus infection, when neurons in the olfactory epithelium and olfactory bulbs have already been infected, can delay the median survival time by 1 day. These results are similar to treatment with human rabies immunoglobulins (Imogam®). VHH lack Fc effector functions, but are still able to neutralize rabies virus in vivo. Probably, the protective effect of VHH results from inhibition of virus entry in cells and interruption of the interneuronal virus spread.

AV 17 - POSTER: Early protection against HPAIV H5N1 by single shot application of a live-attenuated influenza variant to mice, chickens and ferrets

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FLI¹

Key words: Avian Influenza, H5N1, vaccine, DIVA, neuraminidase

Highly pathogenic avian influenza (HPAIV) H5N1 sporadically causes severe disease in humans and involves the risk of inducing a pandemic by gaining the ability to transmit from human to human. In naïve poultry (primarily gallinaceous birds) the virus induces devastating disease and commonly used inactivated vaccines are under many conditions not able to fully protect birds from infection.

An optimized vaccination strategy consisting of a one-shot-(oral)-vaccination protocol for birds and mammals – with a fast and strong immune response could probably be able to prevent the spread of the virus.

The vaccine prototype used in this study originated from an HPAI virus A/Cygnus cygnus/Germany/R65/2006 (R65) of subtype H5N1 with a unique deletion of the neuraminidase surface protein. Compared with the intravenous pathogenicity index (IVPI) of the highly virulent ancestor virus (2.97) in 6-week-old chickens, the IVPI of the attenuated neuraminidase-negative mutant was reduced to 0. Furthermore, additional animal trials with chickens could exclude transmission of the mutant.

This study tested the neuraminidase-negative live virus variant as a prototype to induce an early onset of immunity using HPAIV challenge infection after different time points following vaccine application to prove efficacy.

9-week-old ferrets, 6-week-old mice were immunized oronasally and chickens intramuscularly with a single dose of about 104.5 TCID₅₀ per animal 7, 3 and 1 day before lethal challenge infection with HPAIV H5N1. The modified live virus variant protected 100% of chickens, mice and ferrets three days after vaccination against death and severe clinical signs. Additionally, real-time RT PCR analyses of swabs, nasal washing and organ samples proved “sterile immunity” in all animals immunized 7 days before challenge infection. Furthermore, most animals developed high titers of hemagglutinin-specific antibodies after seven days, but no neuraminidase-specific reactivity; a pattern which could be used as the basis of a marker vaccine strategy.

AV 18 - POSTER: A novel silicon nanoparticle PCV2 peptide based vaccine administered intra-nasally to pigs

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Key words: Nanoparticle, Novel, Vaccine, PCV2, intranasal

Introduction: Porcine circovirus type 2 (PCV2) is the main causative agent of postweaning multisystemic wasting syndrome (PMWS) in pigs. PMWS caused a significant economic impact on pork production until the development of vaccines to help control the problem. There are new carrier systems being developed with the potential to advance vaccine technology and delivery routes. This study investigated a novel silicon-based nanoparticle formulation. This unique silicon drug carrier formulation provides a platform system for incorporation as a core technology into innovative and more effective vaccines, moving beyond traditional routes of vaccination, carrier molecules and adjuvants. In this study we detail the development and efficacy of a novel PCV2 nanoparticle vaccine administered intra-nasally using a PCV2 challenge model.

Materials & Methods: Synthetic peptides (Mimotopes) were made, representing the capsid region of PCV2. Lyophilised peptides were prepared in a proprietary bioactive nanoparticle preparation to a final concentration of 250µg/ml. The nanoparticle vaccine was administered to 21 snatch-farrowed colostrum deprived piglets. Piglets were separated into 5 groups: PCV2 challenge only; nanoparticles only; commercial PCV2 vaccine & PCV2 challenge; nanoparticle vaccine & PCV2 challenge and nanoparticle vaccine boost one week after initial vaccination & PCV2 challenge. All nanoparticle recipients were vaccinated with 1mg of vaccine total delivered intra-nasally using a mucosal atomisation device. The experiment ran for 63 days. Initial vaccinations using nanoparticle and commercial PCV2 vaccine formulations were carried out aged 2 weeks; with a nanoparticle boost, the selected pigs were aged 3 weeks. Challenge with PCV2 occurred when pigs were aged 4 weeks. Selected pigs were euthanised and tissues harvested at specified experimental time points. The ability of the commercial PCV2 and nanoparticle vaccines to reduce virus burden in the piglets was assessed by qPCR.

Results: qPCR analysis of selected tissues including mesenteric lymph nodes, spleen, lungs, tonsil and inguinal lymph nodes demonstrated that as the experiment progressed, virus titre reduced across all vaccination groups by an approximate factor of 100. Between Days 40-43 of the experiment, at which selected pigs were post-mortemed, virus titre in nanoparticle vaccinated pigs were comparable to that observed in those vaccinated with the commercial PCV2 vaccine. In lung tissues analysed the titre in selected animals appeared to be approximately 10 fold lower in nanoparticle vaccinated animals than in PCV2 challenged or commercially vaccinated pigs. Those pigs sampled at Days 62-63, upon experiment completion, demonstrated a comparable virus titre in lung tissue in those commercially vaccinated and those receiving nanoparticle preparations. At this stage there was a reduction in virus titre by a factor of 2 in the mesenteric lymph nodes of those pigs that had received a single dose of nanoparticle vaccine in comparison to commercially vaccinated pigs.

Discussion: Using an atomisation device a mist containing PCV2 peptide loaded nanoparticles was delivered into the nasal cavity of pigs allowing for stimulation of an immune response via the mucosal membranes rather than by subcutaneous injection which is the method of delivery for the commercial vaccine used in this study. Using a PCV2 peptide based vaccine and a novel nanoparticle delivery platform technology, it was demonstrated that an effective PCV2 immune response was induced in pigs.

AV 19 - POSTER: "Immunogenicity elicited by dendrimeric peptide constructions against FMDV: influence of epitope multiplicity and conjugation chemistry "

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Key words: "Immunogenicity elicited by dendrimeric peptide constructions against FMDV: influence of epitope multiplicity and conjugation chemistry "

Multimeric presentation, a well-proven way of enhancing peptide immunogenicity, has found substantial application in synthetic vaccine design. Indeed, using such strategy we have reported the improvement of synthetic peptide vaccines against foot-and-mouth disease virus (FMDV), one of the economically most devastating viral disease in animals.

Our previous studies performed in pigs showed that: i) the incorporation of an efficient T-cell epitope (T-3A), highly conserved among FMDV serotypes (Blanco et al., 2001) and ii) the presentation of such epitope joined to four copies of a B-cell epitope (G-H loop, VP1) in a single branched construct, results in a peptide vaccine conferring total protection against FMDV serotype C (Cubillos et al., 2008; 2012).

In order to extend this proof of concept to FMDV serotypes relevant epidemiologically currently, in the present study we have designed new dendrimeric peptides harboring as B-cell epitopes sequences from FMDV serotype O (currently the most widespread serotype). To assess the relevance of multivalency, the FMDV-O dendrimeric peptides were constructed harboring 2 or 4 copies of B-cell epitope, named B2T and B4T. The immunogenicity of such constructions was evaluated in mice model (Swiss strain), showing that multivalency is advantageous over simple juxtaposition but also that bivalent constructions elicit similar or even better immune responses than tetravalent ones. Furthermore, these results were confirmed in swine. Regrettably, the protection against FMDV was not evaluated, due to legal restrictions for FMDV use in vivo in our center, at that time.

In light of these results, we evaluated three approaches to B2T peptides, involving different chemistries for the conjugation of two B epitope peptides to a branching T epitope. Comparison of such B2T with different conjugation chemistries in terms of synthetic efficiency clearly singles out the maleimide-based strategy as most advantageous. Interestingly, this B2T maleimide construction was not only favorable in terms of easy synthesis, but also it was the most immunogenic.

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AV 20 - POSTER: EPIZONE: Effect of vaccination against Salmonella in three pig herds with high infection level

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Key words: Salmonella Typhimurium; vaccination; pig; isolation; ELISA

Currently, the largest number of reported food-borne outbreaks in the EU was caused by Salmonella. Moreover, 56.8% of the confirmed human salmonellosis cases in 2011 could be attributable to pigs. Besides this human health issue, also the economic impact of Salmonella is of concern in pig industry, since several studies have demonstrated a reduced weight gain in subclinical infected pigs. Therefore, control measures in primary production that contribute in reducing Salmonella infections in pigs, and consequently the risk towards human health and pig performance, are of utmost importance. One on-farm intervention method which has been explored previously, is vaccination. However, scarce information is available about Salmonella vaccination in the field for finisher pigs. In addition, only few Salmonella vaccines in pigs are currently commercially available in the EU, and these vaccines are nationally authorized in only four countries (Germany, Poland, Slovakia and Spain).

This study aimed to evaluate the effect and applicability of a double-attenuated Salmonella Typhimurium vaccine (Salmoporc®, IDT, Germany) in one batch of three Belgian Salmonella problem herds (herds with a high level of Salmonella-specific antibodies and with a confirmed bacteriological presence of Salmonella Typhimurium). In each of these herds, 120 piglets were vaccinated at 3 and 24 days of age, while another 120 piglets were left unvaccinated concurrently. After weaning and during fattening, the vaccinated and unvaccinated groups were housed in different, separated pens. Both groups in each herd were monitored till slaughter age, by weighing all pigs individually at 3 days and 27 weeks of age, and by examining serum and individual faecal samples for respectively the presence of Salmonella-specific antibodies (HerdChek Swine Salmonella, IDEXX Laboratories) and Salmonella spp. (ISO 6579 annex D) at 3 days and 10, 16 and 27 weeks of age (n=25-30). At slaughter (29 weeks of age), also ileocaecal lymph nodes (n=70), caecal content (n=35) and carcass swabs (n=10) were collected from each group. Over all herds, the vaccinated animals showed a significant higher (10 weeks of age) and lower (27 weeks of age) S/P ratio in serum, when compared to the unvaccinated ones (P <0.05). In addition, significantly less Salmonella-excreting pigs were present in the vaccinated group at 27 weeks of age (P <0.05). No significant differences over all herds were found between both groups in the mean S/P ratio at 3 days of age, nor in the proportion of Salmonella-excreting pigs at 10 and 16 weeks of age (P >0.05). Furthermore, in each herd a higher daily weight gain was demonstrated in the vaccinated pigs, compared to the control animals (P <0.05). Pertaining to the proportion of Salmonella-positive samples at slaughter, no significant differences between both groups were detected.

This study showed that vaccination against Salmonella Typhimurium might have beneficial effects on daily weight gain and the overall infection pressure in pig herds. However, it was observed that these effects differed substantially between farms. Hence, vaccination is a promising tool in a Salmonella-contaminated environment and in countries where prevalence of Salmonella Typhimurium is high.

LEGISLATION & INTERVENTION STRATEGIES

LIS 1 - POSTER: An economic model for the assessment of cost-benefit effects of mosquito monitoring programs

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Key words: cost-benefit analysis, mosquito monitoring programs, economic model

Economic analyzes for prevention and control of vector-borne diseases are being demanded by the European Union and the science community. However, despite its undoubted importance, such analyzes have hardly been the subject of scientific investigations due to insufficient data.

The aim of this study is to develop an economic model in order to assess cost-benefit effects of mosquito monitoring. To achieve this goal, the model parameters were estimated using mosquito monitoring data in the context of the bluetongue surveillance in Austria from 2007 to 2010. In order to verify the generalization and standardization the model should be applied on the one hand to other mosquito monitoring programs in other countries. On the other hand the model should be applied to monitoring programs of other mosquito species as the *Culicoides obsoletus* spp.

The results of our study should reveal if the economic model can be used in order to calculate cost-benefit effects of mosquito monitoring programs regardless of the country. These results should be utilized to implement more accurate calculations of the financially budget for mosquito monitorings in times of crisis caused by vector-borne diseases.

LIS 2 - POSTER: Schmallenberg virus seroprevalence in ruminants in Poland

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Key words: Schmallenberg virus, seroprevalence, ruminants, Poland

The Schmallenberg virus (SBV) infections were first detected in the ruminants and the insect vector *Culicoides* spp. in Poland in August 2012. Clinical signs of intrauterine infection with SBV including fetal malformations and stillbirths, have been reported in cattle and sheep since autumn 2012. However, because testing and reporting of SBV infections is not obligatory, the epizootic situation of the country was unknown. Therefore, the aim of the study was to estimate the prevalence of SBV infection in the ruminants in Poland between 2010 and 2013.

The study was performed using sera from cattle, small ruminants and wildlife collected during national BTV monitoring program. The sampling was designed in such a way as to enable detection of seroconversion with 95% probability, assuming that the proportion of the seropositive animals in the population at the county level is 20%.

SBV antibodies were detected in 95 out of 1600 ruminants tested (5.9%). Seropositive cattle, sheep and goats accounted for 14.5%, 2.0% and 3.1%, respectively. Wild ruminants including free-ranging bison, red deer and farmed fallow deer were the most affected species with 15.6% having SBV antibodies. No seropositive reactors were found before August 2012. Out of 1204 ruminants tested in 2012, 38 (3.1%) were found SBV seropositive, while in 2013 already 17.9% (57 out of 261) had SBV antibodies. In 2012, seroconversion was found in 7.9%, while from January until May 2013 in 30% of herds tested at least one animal had SBV antibodies. The means of within-herd seroprevalences were 12.1% (range: 11-75%) and 72% (range: 13.6 – 100%) in 2012 and 2013, respectively. The percentage of seropositive animals differed significantly between provinces. Although no statistical correlation between the percentages of seropositive animals and cattle, sheep densities were found, the highest seroprevalence was observed in Podlaskie province (Northeast) which has the highest density of cattle population in the country (42.5 heads/km²).

This retrospective serological study has shown that the Schmallenberg virus has spread across the ruminant population in Poland starting from August 2012. The percentage of SBV seropositive ruminants in 2013 showed significant increase from 2012. Due to the diversified seroprevalence in the herds, the risk of reinfection in the herds infected previously exists.

LIS 3 - POSTER: A surveillance program on canine Leishmaniasis in the public kennels of Emilia-Romagna Region, Northern Italy.

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Key words: Leishmaniasis, Leishmania infantum, dogs, surveillance, kennel

Since 2007 a surveillance plan on vector-borne diseases has been carried out in the Emilia-Romagna Region, Northern Italy. Within this plan a specific program focused on leishmaniasis has been defined. It includes (i) an active surveillance program in public kennels, (ii) a passive surveillance procedure for notification of Leishmaniasis in owned dogs and (iii) an epidemiological investigation after a confirmed human autochthonous cutaneous or visceral leishmaniasis case, along with (iv) the application of diagnostic tests on owned dogs in the area encompassing the patient residence or the probable site of infection. This surveillance program can be seen as a concrete example of the application of the One Health approach, as it requires the collaboration of Public Health Services, Official Veterinary Services, private sector veterinarians and entomologists.

In this paper we report the results of five year activities carried out in public kennels. With the aim of providing health guarantees for dog adoption, a Canine Leishmaniasis (CanL) risk-based surveillance program has been gradually implemented since 2007, including all the kennels of the region two years later. According to this program, specific monitoring activities are performed to verify the presence of sand flies and infected dogs, which are the two main risk factors related to CanL spread. We use the combination of these two risk factors to assign a specific CanL risk-class to each kennel (from 1 to 4, being the class 1 at the highest risk) resulting in different control approaches. Classification of kennels is yearly updated according to surveillance results. Ordinary preventive measures applied in all the kennels are serological testing of all dogs at the moment of admittance to the kennel, clinical surveillance on housed dogs, and individual protections against vector bites as well as therapeutic treatment on infected dogs. The risk-based measures are entomological surveillance in kennels where the vector has not been detected yet, controls on sentinel dogs in kennels housing infected animals and individual anti-vector protections for all dogs housed in class 1 kennels.

From 2007 to 2012 18,806 dogs identified by microchip, housed in 73 public kennels of the whole region, were examined by Indirect Fluorescent Antibody Test, using 1:160 titer as cut-off. On the whole, 528 dogs (2.8%; 95%CI: 2.6%-3.1%) resulted IFAT positive, 43% of which were asymptomatic. Results of serological tests performed on dogs at the admittance to the kennel and annual controls on sentinel dogs were used to estimate CanL risk in the regional territory and to evaluate the efficacy of the preventive measures adopted.

CanL seroprevalence in dogs tested at the admittance in kennel increased significantly from 2010 (1%; 29/2858) to 2012 (2.4%; 69/2841), suggesting a gradual spread of CanL, nevertheless, the number of seroconversions in sentinel dogs did not show a significant variation between 2010 and 2011 (1.2%; 11/896 and 1.6%; 13/825, respectively) and it decreased in 2012 (0.9%; 8/850) indicating the efficacy of the preventive measures applied in kennels.

These data suggest to make an effort to promote the use of individual anti-vector measures (such as pyrethroid impregnated bands or spot-on formulations) also in owned dogs and in private breeding kennels as effective measure to prevent CanL spread.

LIS 4 - POSTER: Risk based surveillance: Bovine Brucellosis in Belgium as a case study.

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CODA-CERVA¹

Key words: Surveillance Bovine Brucellosis

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Belgium was declared officially free of bovine brucellosis in 2003. Following a study carried out in 2009, the surveillance system for brucellosis was revised in Belgium, in accordance with 64/432/CEE and the Royal Decree related (6/12/1978) to bovine brucellosis control and surveillance. The new surveillance system implemented in 2010 was targeted towards reporting and testing of abortions, purchased cattle, followed by the imported cattle. In order to encourage reporting of abortions, an abortion protocol was implemented where each abortion was not only tested for one disease, but for different diseases and free of charge. Also herds that did not report any abortions but that had a high annual birth rate were selected at random during the winter to screen and make sure no brucellosis was present. This new protocol led to the discovery of breakdowns in 2010 and 2012. The aim of the present study was to evaluate the current surveillance program in Belgium, using stochastic simulations. Assumptions regarding diagnostic test sensitivities, design prevalence, were analysed and mitigated in the present exercise using field observations of the 2010 and 2012 breakdowns. Results of the different simulations carried out showed that the overall surveillance system sensitivity across Belgium was optimal to detect a 0.2% prevalence. Surveillance of abortions in the cattle population remained across most of the simulations one of the most efficient surveillance components, followed by testing at purchase.

LIS 5 - POSTER: Serological detection of Schmallenberg virus infections in wild boars, Belgium, 2011-2012

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Key words: Schmallenberg, wild boars, emergence, epidemiology

In the summer/fall of 2011, a nonspecific febrile syndrome characterized by hyperthermia and drop in milk production with occasional reports of watery diarrhea and abortion was reported among dairy cows on farms in northwestern Europe. Further, in November 2011, an enzootic outbreak of malformed neonates emerged in several European countries, with stillbirth and birth at term of lambs, kids and calves with neurological signs or malformations of the head, spine, or limbs. Both syndromes were associated with the presence in the blood (adults) or in the central nervous system (newborns) of a new Shamonda/Sathuperi-like orthobunyavirus, provisionally named Schmallenberg virus (SBV) after the town in Germany where the first positive clinical samples were identified. Defining as precisely as possible the host range of the newcomer is a key point to predict the outcome of the emergence of SBV disease in Europe. In this respect, it must be pointed out that orthobunyaviruses infect more animal species than those in which the foetus is damaged. Recently, serological evidence for SBV infection in wild ruminant species (*Cervus elaphus* and *Capreolus capreolus*) was reported. In the present study, the objective was to seek after serological evidence of SBV infection among wild boars living in a geographical area where exposure to infected insect vectors was high in 2011, as judged from the very high seroprevalence reported among cattle in that region. About 700 animals were sampled during the 2010-2012 hunting seasons. All serum samples collected during the fall of 2010 were seronegative. On the contrary, apparent seroprevalence among wild boars in 2011 was ~27% and started to decline in 2012 (~11%). Acquired immunity against the new virus was thus already very high in the wild boar populations sampled in the fall 2011, suggesting that the new virus had quickly spread throughout the region since its emergence about 250 km northeast in the late summer 2011. The drop in seroprevalence recorded in 2012 suggests that the virus was no more circulating in the region.

LIS 6 - POSTER: Detection of Schmallenberg virus genome in semen of Austrian bulls

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Key words: Schmallenberg virus, semen, RT-qPCR, Austria

Introduction:

Schmallenberg virus (SBV), an orthobunyavirus of the Simbu serogroup, first emerged in 2011 in Germany and The Netherlands and has since spread to most of Europe and possibly beyond. SBV is transmitted by arthropod vectors, but trans-placental transmission also occurs, leading to abortions, stillbirths and birth of malformed and/or weak offspring. Recently, detection of SBV genome in bull semen was reported. Importantly, viraemia and seroconversion was observed in cattle experimentally infected with SBV genome positive semen, raising the question if SBV could also be transmitted by means of artificial insemination (AI). The aims of this study were to validate different protocols for RNA extraction from bull semen and to use the best protocol for retrospective testing of semen samples obtained from seroconverted bulls.

Material & methods:

For validation purposes, culture-grown SBV was spiked in different concentrations into SBV genome negative semen that had been sampled prior to summer 2011. Several protocols for RNA extraction from individual semen charges (straws) were tested; these included silica membrane-based methods that are widely used for RNA extraction from liquid samples, as well as a modified procedure, which employs lysis and phase separation by Trizol LS/chloroform prior to manual or automated cleanup with commercial silica membrane based extraction kits. SBV genome detection was performed by duplex RT-qPCR, targeting both the SBV S segment and beta-actin mRNA. Inhibitory influences possibly exerted by semen were additionally evaluated by RT-qPCR assay targeting synthetic RNA that was spiked into the samples prior to RNA extraction. For the consecutive field study, 164 semen charges were tested so far; these originated from 22 Austrian bulls, which had been sampled from August 2012 to January 2013.

Results:

Trizol LS/chloroform treatment followed by silica membrane based purification enabled the most sensitive detection of SBV genome by RT-qPCR and minimized inhibitory effects from semen. Furthermore, SBV quantification cycle values were in better linear correlation with the amount of SBV spiked into the samples, than with the other RNA extraction protocols tested. Participation in an international SBV RT-PCR proficiency test further confirmed that this protocol was fit for purpose. In the field study, 4.3% (7/164) semen charges obtained from 7 individual bulls tested positive for SBV genome. In individual bulls, SBV genome was thus detected at a single time point only.

Conclusion:

A RNA extraction protocol was established that enables sensitive detection of SBV genome in individual semen charges, ready to use for AI. Using this protocol, SBV genome was detected in field samples from several bulls, confirming reports from other groups. Interestingly, SBV genome detection in semen from Austrian bulls was correlated with rapid increase of SBV seroprevalence among Austrian cattle in summer/autumn 2012.

EMERGING DISEASES

ED 1 - POSTER: Event-based surveillance of equine West Nile fever, Spain

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Key words: West Nile fever, Equine, Virus, Vector-borne disease, Clinical epidemiology

West Nile fever (WNF) is a worldwide viral zoonotic infection caused by a mosquito-borne Flavivirus of the Flaviviridae family. The current conception of WNF epidemiology in Europe combines an enzootic viral circulation in tropical Africa with seasonal introductions of the virus by migratory birds. According to a recent comparative study (Chevalier et al., 2011), the event-based surveillance of horses by equine veterinary specialists appeared to be the most cost-effective system in the European context of WNF. Clinical data issued from an event-based epidemiosurveillance through August 2007 and September 2011 on horses in Spain were statistically compared and used to develop a diagnostic decision tree, both with the aim to improve early clinical detection of WNF in horses. Although clinical signs were variable in WNF horses, several clinical signs were identified to distinguish between WNF versus neurological disease but WNF-negative: photophobia, nasal discharge and paralysis were more associated with WNF. Therefore it can be concluded that clinical examination of neurologically affected horses could potentially provide important clues for early clinical detection of WNF. The study of the clinical pattern of WNF in horses is of paramount importance to enhance awareness and understanding and to optimize surveillance designs for clinical detection of WNF.

ED 2 - POSTER: Evidence of vector competence of *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* ticks for lumpy skin disease virus

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Key words: lumpy skin disease virus, vector competence, transstadial, intrastadial persistence, transmission

Lumpy skin disease is an economically important disease of cattle caused by lumpy skin disease virus (LSDV), a Capripoxvirus and is characterised by fever, enlargement of superficial lymph nodes, lachrymation, salivation and formation of eruptive circumscribed skin nodules. It is endemic in Africa and has also spread to the Middle East.

Transmission of LSDV has been associated with haematophagous insects. Mechanical transmission by *Aedes aegypti* mosquitoes has been reported, while the spread of LSDV from Egypt to Israel in 1989 was related to movement of the stable fly, *Stomoxys calcitrans*. Recent studies have shown the transmission of LSDV by tick vectors.

Vector competence of *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* for LSDV was confirmed by detecting the virus in saliva and in different organs of adult *A. hebraeum* and *R. appendiculatus* following intrastadial and transstadial passage of the virus in the ticks. Tick saliva was tested by virus isolation and real-time PCR, while infection in the tick organs was tested by immunohistochemistry using an immunoperoxidase staining technique. Viral antigen was detected in haemocytes, salivary glands, synganglia, epidermis, fat bodies, and cells of the reproductive tracts of both male and female ticks.

During their life cycle ticks spend long periods off the host. Survival of LSDV in ticks during the moulting process may indicate their potential to serve as over-wintering hosts for the virus. Sexual transmission of LSDV from male to female ticks as well as transovarial passage of LSDV to the next generation of larvae are likely consequences of infection of the tick reproductive systems. Ticks may therefore be both maintenance and reservoir hosts for LSDV.

ED 3 - POSTER: Serological and Entomological surveillance for West Nile Fever in Korea from 2011 to 2012

Lee, J-H¹; Yeh, J-Y²; Park, J-Y¹; Seo, H-J¹; Cho, I-S¹; Kim, M-S¹; Park, EK¹; Kim, S-H¹; Seo, J-E¹; Cho, YS¹

Animal and Plant Quarantine Agency¹; Incheon National University²

Key words: West Nile virus, surveillance, horses, wild birds, mosquitoes

West Nile Fever (WNF) is a mosquito-borne zoonosis caused by West Nile virus (WNV) which belongs to the Flavivirus like Japanese Encephalitis virus (JEV) group. Although no WNF has been reported in Korea, WNF cases in wild birds and humans have been reported in neighboring countries, which demonstrates the possible risk of introduction of WNF into Korea. Therefore, there is a need to conduct WNV surveillance of susceptible animals and entomological studies in Korea. In this study, surveillance was conducted for horses and wild birds from 2011 to 2012, and totals of 2,498 horses and 1,710 wild birds were tested by antibody and/or antigen tests. Antibody testing was conducted by IgM antibody capture ELISA for screening assay and neutralization test for confirmatory assay. Antigen test was conducted by RT-PCR. The horses consisted of racing, riding or breeding horses reared in Korea. Wild bird samples consisted of sera collected from migratory birds and organs collected from dead birds. For examination of possible cross reaction with JEV, neutralization test of JEV was also conducted and determined using a decision algorithm. As a result, no horses or wild birds tested from 2011 to 2012 in Korea were antigen or antibody positive for WNV. Entomological studies were also conducted during the same period, and a total of 24,778 mosquitoes were collected around horse barns, international airports and ports, which were examined for WNV by RT-PCR. The dominant species of mosquitoes in this study were *Culex pipiens*, *Culex tritaeniorhynchus*, and *Aedes vexans* from 2011 to 2012, and no mosquitoes samples were positive for WNV antigen. This study conducted in horses, wild birds and mosquitoes from 2011 to 2012 supports the view that Korea is free from WNV. However, the possibility of introduction of WNV into Korea continues to increase due to climate change and increase in international movement of commodities. Therefore, surveillance and entomological studies will need to be maintained to prevent and quickly identify WNV activity in Korea.

ED 4 - POSTER: Influenza virus in Belgian wild boar population.

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Veterinary Pathology ULg¹; Wildlife Pathology and Diseases ULg²

Key words: influenza, wild boar

Influenza is a seasonal epidemic disease, further responsible for several pandemic outbreaks in human population. Influenza virus uses sialic acids (SA) on target cells as receptor. Human viruses favour α 2-6 linked SA, while avian viruses preferentially recognize α 2-3 linkages. Swine respiratory tract is known to carry both linkages in a more balanced fashion, leading to the hypothesis that pigs are a host of choice for co-infections between avian and human influenza viruses and subsequent genomic reassortments. Such events are thought to be the origin of the emergence of several pandemic strains, including the recent 2009 H1N1 strain. Indoor intensive breeding conditions of pigs in Western Europe render contacts between swine and wild birds highly unlikely. But wild boar often is in close contact with aquatic birds, the reservoir of influenza A viruses. Being so exposed to avian influenza viruses, wild boar seems likely to play the "mixing vessel" role classically assigned to domestic pigs. Influenza seroprevalence in European wild boar populations appears as relatively low in most published studies (below 8 %). Our aim, in the context of increasing wild boar populations in Europe, was to evaluate the apparent seroprevalence for influenza in Belgian wild boars. Foci of high H1 influenza seroprevalence (> 45%) were found in specific hunting territories. The very low concentration of piggeries in the affected regions makes the hypothesis of a recurrent transmission from domestic pigs unlikely and suggests a possible endemic circulation in local wild boar populations. A wild boar H1N2 strain has recently been isolated and phylogenetic studies based on this strain will help to enlighten this point.

ED 5 - POSTER: GENETIC CHARACTERIZATION AND IN VITRO GROWTH KINETICS OF BELGIAN PSEUDORABIES VIRUS ISOLATES ORIGINATING FROM DOMESTIC PIGS AND WILD BOAR

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CODA-CERVA¹

Key words: Aujeszky's disease, wild boar

Aujeszky's disease is an economically important disease in domestic swine caused by the porcine alphaherpesvirus, pseudorabies virus (PRV). As a result of large scale vaccination programs, the disease has been successfully eradicated in domestic pigs in a large part of Europe, including Belgium. Serological studies however show that the virus is still present in the wild boar population. The limited literature on pseudorabies virus circulating in the wild boar population suggests a reduced virulence of these strains in domestic pigs. A recent reintroduction of the virus from the wildlife reservoir into the domestic population has however been reported in France.

To get first insights in the variability and virulence of strains circulating in Belgian wild boar, five PRV strains isolated between 2007 and 2011 originating from wild boar were genetically characterized and in vitro virulence was determined and compared with strains isolated from Belgian domestic swine between 1973 and 1989 and the virulent NIA3 strain.

Genetic characterization was performed by BamHI restriction fragment length polymorphism (RFLP) analysis and phylogenetic analysis based on the sequence of a \pm 800 bp fragment of the glycoprotein C (gC) gene. All wild boar isolates and three of the oldest domestic swine isolates showed a restriction pattern type I, typically found for wild boar strains and older PRV strains. The other domestic pig strains were characterized as type II, which is commonly found in European domestic pigs. Based on phylogenetic analysis, four of the wild boar isolates are identical apart from one nucleotide substitution and belonged to clade B. The other wild boar isolate and all domestic pig isolates belong to clade A.

Furthermore, one step growth curves and sensitivity to interferon alpha and gamma of Belgian PRV isolates were determined to get first insights into the in vitro virulence. One step growth kinetics on both continuous swine testicular (ST) cells and primary porcine cells from skin, lung, kidney and testicular tissues revealed no obvious differences in the in vitro growth capacity between isolates from wild boar and domestic pigs. Also, no differences in sensitivity to interferon alpha and gamma were observed between the different PRV isolates on ST cells.

Our results indicate that intrinsic replication capacity and sensitivity to interferon alpha and gamma of wild boar isolates are not different from domestic pig isolates and that therefore, when reduced in vivo replication is assumed other virulence determinants, such as immune evasion mechanisms, might be hampered. This will be the subject of further studies.

Acknowledgements

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ED 6 - POSTER: MOLECULAR DETERMINANTS OF FOOT-AND-MOUTH DISEASE VIRUS PERSISTENCE

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ANSES¹

Key words: FMDV; persistent infection; MDBK ; QRT-PCR

Foot-and-mouth disease is the most economically important and highly contagious disease of cloven-hoofed animals. Foot-and-mouth disease virus (FMDV), a picornavirus, is able to cause persistent infection in ruminants, besides acute infection. The generation of the carrier state can occur in both initially naïve and vaccinated animals, and can be maintained for many months (up to 2 years in cattle). Such animals represent a potential risk for transmission of virus to susceptible animals. The mechanisms of viral persistence and the determining factors are still unknown. The main objective of this project is to identify the viral and cellular factors involved in the establishment and maintenance of FMDV persistence. This shall be achieved by the identification of changes in host cell gene expression and virus genome associated with virus persistence in an in vitro model. Two major tools are needed for this study, a bovine cell culture model of persistence of FMDV, and the construction of an infectious cDNA clone of FMDV. We managed to get cultured MDBK cells (bovine epithelial cells) that appear to be persistently infected by the FMDV O Mayenne (MDBKfmdvp). These cells were inoculated with a viral clone isolated by plaque purifications of FMDV O/FRA/1/2001 strain. The MDBKfmdvp were maintained in culture and analyzed for the presence of viral RNA by qRT-PCR. Relatively high and more or less constant viral RNA levels are detected during the passages of cells, in cell trypsinates and in the cell culture supernatant. In addition, inoculation experiments of FMDV sensitive cells (IBRS-2, ZZ-R 127, MDBK) with the supernatant of MDBKfmdvp induced lysis of these cells. Further studies will be done to confirm the persistent FMDV infection in these cells and to better characterize them. In the meantime the construction of a full-length infectious cDNA clone of FMDV O/FRA/1/2001 is performed. Once the construction obtained, it will be verified by evaluating infectious properties of rescued virus in comparison with the parental strain virus. Differential expression of essential genes between persistently infected cells, uninfected cells or cells acutely infected, will be studied by DNA microarray approach. Genetic and phenotypic variations of the virus during establishment of persistent infection will also be studied. Viruses isolated from different stages of persistence will be sequenced and compared with the initial inoculum in order to identify mutations potentially linked to the establishment of viral persistence or in its maintenance. "Persistent viruses" will also be used as inoculum on FMDV sensitive cell lines (BHK-21, IBRS-2, ZZ-R 127) to assess their residual virulence. The mutations identified after the sequencing of persistent viruses will be reported by reverse genetics in the sequence of the infectious clone, and their impact on the infectious properties of FMDV will be studied. This work should enable the identification of viral and cellular determinants involved in the establishment and maintenance of viral persistence. The data from this study could be used in the development of improved control strategies to prevent the spread of the disease. The persistent infection cell culture model may have important implications for the study and identification of drugs that may prevent or eliminate FMDV persistence in vivo.

ED 7 - POSTER: The epidemiology of Bovine Ephemeral Fever in Turkey

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Key words: bovine ephemeral fever, cattle, Turkey

Bovine ephemeral fever (BEF) infection was first reported in the mid-nineteenth century when the disease was first observed in East Africa. It has also been reported in Australia and in most countries of Africa, Asia and Japan. In Turkey, BEF infection was first recorded in 1985 with clinical symptoms and macroscopic pathological signs. Then, a lot of outbreaks (in 1996, 1999, 2005, 2008 and 2012) were reported in the southern/south-eastern region where the last outbreaks were observed. The molecular characterisation of BEFVs from 2008 and 2012 outbreaks indicated that the aetiological agents caused these outbreaks were classified into different clusters. This presentation is a summary of BEF virus infection in Turkey, including a historical overview, source of infection, data on the molecular epidemiology, and recommendations to the control of the infection.

ED 8 - POSTER: Determinants of the *Culex pipiens* mosquito dynamics in a West Nile Disease endemic area of Italy

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Key words: WNV, *Culex pipiens*, mosquito population dynamics

Since its reappearance in 2008, West Nile virus (WNV) has expanded in several Italian regions.

In northern Italy the WNV emerged in 2008 in Emilia-Romagna, Lombardy and Veneto regions simultaneously and was repeatedly detected in animals, humans and mosquitoes. Since then, regional entomological monitoring and surveillance programs in animals and humans. WNV has circulated continuously from 2009 to 2012 in the neighbouring regions of Veneto and Friuli Venezia Giulia, while was not detected anymore after 2010 in Emilia-Romagna and Lombardy regions. Recent identifications of multiple viral lineages and strains, together with the yearly recurrence of WNV in the same geographical areas suggest the possibility of both endemisation and repeated introductions via migratory birds. Estimations of the risk of disease spread necessarily require a deeper knowledge of the drivers of the WNV mosquito vectors dynamics. The recent expansion of WNV to previously unaffected areas has been hypothesized to be due also to the increase in the abundance and distribution of potential mosquito vectors, as a consequence of environmental and demographic changes. We analysed the seasonal growth dynamics of *Culex pipiens*, the major WNV vector in Italy. Data on mosquito abundance was obtained on a bi-weekly basis from May to November 2010 – 2012, using CDC-CO2 traps located at 110 sites in Veneto (19), Friuli Venezia Giulia (12) and Emilia Romagna regions (79). A series of different population growth models were fit to field data to evaluate the effect of extrinsic (environmental data: temperature, humidity and precipitation) and/or intrinsic density-dependent factors on *Cx. pipiens* dynamics. Initial comparison of alternative models of density-dependent and independent growth revealed overwhelming support for the density-dependent models, which explained substantially more variation in *Cx. pipiens* population growth rate than any environmental factor on its own. The best overall model for *Cx. pipiens* growth dynamics included both density dependent regulation and independent effects of precipitation, average humidity, and temperature in the two weeks before mosquito capture, indicating the primary importance of environmental conditions during larval development. This evidence of density-dependent population indicates that the per capita growth potential of *Cx. pipiens* populations increases as their numbers fall. Besides potentially posing a challenge to vector populations control campaigns, the results stress the need of incorporating density-dependence for robust prediction of *Cx. pipiens* population expansion and WNV transmission risk.

ED 9 - POSTER: EPIZONE:

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NVRI¹; DTU Vet²

Key words: fish pathogen, hirame rhabdovirus, serological methods, emerging disease

In April 2007, a high mortality (roughly 80–90%) affected a grayling (*Thymallus thymallus* L.) in farm in the south of Poland at a temperature of about 11–13°C. Adult grayling showed clinical signs characteristic of a viral infection, that is, petechial haemorrhages. Several weeks later, in June, significant mortalities were observed in brown trout (*Salmo trutta trutta* L.) in a farm in the same region of Poland, approximately 120 km from the grayling farm. In affected ponds, fish exhibited abnormal swimming behavior (spiraling, lethargy) and darkening of the skin. Stocking material was obtained from earlier infected grayling farm. In both of described cases virus was isolated on cell culture.

In an attempt to identify the viral species, several diagnostics methods were used targeting various fish rhabdoviruses. The presence of VHSV, IHNV and SVCV was tested by ELISA, conventional RT-PCR and a fluorescent antibody test (FAT), but no positive signal was recorded.

Detailed diagnosis started with serological methods, immunisation procedures were followed using New Zealand white rabbits, next neutralisation and immunofluorescence antibody test (IFAT) were conducted.

Neutralisation test results showed that grayling and brown trout virus is similar to the perch rhabdoviruses. The virus isolate used in this test was not neutralised by antisera against SVCV, PFRV, tench rhabdovirus, pike rhabdovirus, MontaRV. In a further study, we applied a 50% PNT test with grayling rhabdovirus isolated in Poland, hirame rhabdovirus against rabbit antisera to homologous viruses.

Cell cultures infected with HIRRV and viruses isolated in Poland showed specific fluorescence in tests with homologous antiserum at titers of 1:100 and 1:200.

Hirame rhabdovirus (HIRRV) is one of the four recognized species within the Novirhabdovirus genus of the family Rhabdoviridae, which also includes Viral hemorrhagic septicemia virus (VHSV) and the type species Infectious hematopoietic virus (IHNV). HIRRV was first isolated during an outbreak among cultured olive flounder (*Paralichthys olivaceus*) and ayu

(*Plecoglossus altivelis altivelis*) in Japan. It was also found in other marine fish in Asia, such as stone flounder (*Kareius bicoloratus*) in China. Experimentally, the virus was shown to be pathogenic for a range of salmonid species in fresh water, including rainbow trout (*Oncorhynchus mykiss*).

The identification of HIRRV in two Polish trout farms can be considered as a emerging disease according to hypothesised relationships between anthropogenic processes and types of disease emergence in freshwater aquatic animals presented by Peeler E.J and Feist S.W. in 2011, but since this outbreaks no cases of HIRRV infection were confirmed in Poland.

References

1. Peeler E. J., Feist S. W. (2011): Human intervention in freshwater ecosystems drives disease emergence. *Freshwater Biology*, 56, 705-716.

ED 10 - POSTER: Characterization of a panel of monoclonal antibodies produced against USUTU virus

Lelli, Davide¹; Moreno, Ana¹; Sanz, Antonio²; Stercoli, Lidia¹; Lecollinet, Sylvie³; Pezzoni, Giulia¹; Brocchi, Emiliana¹; Rebollo, Belen²; Sozzi, Enrica¹; Cordioli, Paolo¹

IZSLER¹; INGENASA²; ANSES³

Key words: USUTU virus, monoclonal antibodies

Usutu virus (USUV) is an African mosquito-borne flavivirus of the Japanese encephalitis virus serocomplex which has been detected in dead birds and mosquitoes in several European countries since 2001. In 2009, two human cases with an USUV-related neuroinvasive illness were reported from Italy in areas where a concomitant circulation of the closely related West Nile virus (WNV) was reported. This points the need for diagnostic tools for USUV detection and characterization. Here, we report the characterization of a panel of monoclonal antibodies (MAbs) produced against the USUV strain 200092/2010 isolated from a blackbird in Italy.

To increase the possibility of obtaining MAbs to a variety of epitopes, we employed two different immunization protocols using different antigen preparations. In the first immunization trial, two Balb/c mice were primed subcutaneously with a Betapropiolactone-inactivated and partially purified USUV in complete Freund's adjuvant and boosted intraperitoneally with the same antigen in PBS at intervals of one month. In the second protocol, two mice were primed subcutaneously with the same antigen previously described and boosted intraperitoneally two times at intervals of one month with live virus consisting of a suspension of Vero cells infected with USUV. Three days after the last boost, mice were sacrificed and MAbs were generated according to internal standard procedures. Hybridomas were screened by indirect ELISA performed on USUV-coated immunoplates and by Indirect immunofluorescence (IF) with USUV-infected and non infected Vero cells. MAbs-reactivity was evaluated by IF against the other flaviviruses circulating in Europe including WNV, Tick-borne encephalitis virus and Bagaza virus. MAbs-neutralizing activity was investigated by a virus-neutralization test (VN). Competitive binding assays between MAbs and experimentally positive chicken sera were designed to identify MAbs reactive to immunogenic epitopes. Each MAb was also examined in Western blotting (WB) and indirect ELISA against the following USUV and WNV recombinant antigens produced in E.coli and drosophila cells: WNV envelope protein, USUV and WNV domain III of envelope protein, USUV and WNV non structural protein 1.

A panel of 52 MAbs from four fusion procedures were obtained. Based on their reactivity, all MAbs were first divided in two groups, USUV-specific MAbs (26 MAbs) and flavivirus cross-reactive MAbs (26 MAbs). No MAbs showed VN activity. Twenty-two MAbs resulted positive in WB, 21 of them revealing a band with a molecular weight of 50Kd corresponding to the E protein, and one MAb revealing a band of 10Kd corresponding to the M protein. Within each group of USUV-specific or cross-reactive MAbs, we further identified a sub-group of MAbs which only react in IF against USUV-infected cells while being negative in indirect ELISA against the partially purified USUV (13 MAbs) suggesting a reactivity to non-structural proteins. Based on their reactivity with recombinant antigens, representative MAbs with different patterns of reactivity were selected and cloned. Additional results and complete data will be presented and discussed.

MAbs produced offer a diversified spectrum of reactivity against structural and non structural viral proteins. MAbs showing strong and specific reactivity to USUV antigens may represent essential tools for the development of standardized diagnostic tests for virus and antibody detection.

Acknowledgements:

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ED 11 - POSTER: Tick-borne encephalitis virus (TBEV) – Seroprevalence study for TBEV antibodies in bovine sera in Belgium: a risk-based screening

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Scientific Public Health Institute¹; CODA-CERVA²

Key words: Seroprevalence, seroneutralisation, TBEV

Tick-borne encephalitis (TBE) is a growing public health concern in Europe. An average of about 10000 reported human cases of TBE per year is reported for Europe and Russia. Furthermore, new TBEV foci are emerging or re-emerging in several Western and Central European countries (Donos-Mantke O. et al, 2011; Amato-Gauci A J and Zeller H., 2012). Until now, no autochthonous cases of TBE have been reported in Belgium. However, the risk of TBEV-introduction into Belgium remains high and presently there are suspicions of the presence of contaminated foci in certain regions of Belgium. Until now, two TBEV seroprevalence studies in animals were realised in Belgium. In 2011, serological screening of sentinel dogs detected one dog seropositive for TBEV (Roelandt S. et al, 2011). In 2012, serological screening was conducted on wild sentinel roe deer (*Capreolus capreolus*) and 2 seropositive roe deers were detected (Linden A. et al, 2012). Targeted serological screening of animals such as dogs, cattle or wildlife (e.g. deer, rodents) would contribute in a cost-effective way to continuously survey the potential presence of TBEV in Belgium.

In this study, we selected the three most Eastern provinces of Belgium which are geographically situated closest to known and/or recently emerging TBEV-endemic areas in Germany and France, i.e. the provinces of Luxemburg, Liège and Limburg. Furthermore, the same areas are also known as endemic for Lyme disease (*Borrelia burgdorferi*), another disease transmitted by the same tick as TBEV, i.e. *Ixodes ricinus* (report WIV-ISP, 2011). In total, a sample of 650 bovine sera, from the yearly Belgian Winterscreening serology campaign edition 2010-2011, were tested with a quantitative seroneutralisation assay, designed according to the rabies RFFIT assay. Part of seropositive samples were further tested in a mouse virus-neutralization model. Using a conservative 1/15 cut-off titer, 17 bovines = 2.615% (95%CI: 1.39 – 3.84%) were detected as TBEV-seropositive. Additionally, 6 bovines had a borderline (=suspicious) result ($1/10 < \text{RFFIT titer} < 1/15$), equal to 0.92% (95%CI: 0.19 - 1.66%). Specificity of the RFFIT results was confirmed in the mouse virus-neutralization model. This amounts to a total TBEV-seroprevalence in Belgian cattle between 2.6% - 4.3%. Most of the seropositive animals (n = 21) were localized in Wallonia, three came from Flanders. Bovines with borderline results were often located close to confirmed seropositive animals. The geographical locations roughly coincided with known Belgian hot spots for Lyme disease, where we would also expect possible TBEV circulation in the wild. The animals originated from 10 herds, were autochthonous (no imports), mostly older female beef cattle (> 2 years). The observed seroprevalence in Belgium seems to agree with percentages reported from other countries (Roelandt S. et al, 2010).

In conclusions, we now have strong suspicions that Belgium contains TBEV-endemic areas/foci. Additionally, Juceviciene et al. (2005) comment that seroprevalence and titers in cattle change with the tick season and that during winter the values are at their lowest. Consequently, the real TBEV-seroprevalence in cattle from the targeted areas might be higher than what could be concluded from the “winter” screening samples used in this study.

ED 12 - POSTER: NEXT GENERATION SEQUENCING OF THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS GENOME

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Life Technologies¹

Key words: PRRS virus genome sequencing

Purpose:

Develop a next generation sequencing protocol to sequence the whole PRRS Virus genome to generate sequence information on geographically diverse strains. Using Ion Torrent™ PGM™ sequencing technology, sequencing results can be generated in a short period of time. Using the latest bioinformatics techniques, the sequence information can be compared and contrasted to determine strain similarity based on different ORFs and determine new regions of interest for robust assay development.

Methods

This study uses commercially available kits to isolate and purify PRRSV nucleic acid from animal sourced matrices. Techniques such as ribosomal RNA removal are then used to further enhance PRRSV RNA recovery and to remove endogenous porcine genome sequences. PRRSV genome libraries are then prepared and amplified to capture all portions of the genome. Finally, these libraries are attached to Ion Sphere™ Particles that are loaded onto a sequencing chip and placed on Ion Torrent™ PGM™ for sequence generation. Bioinformaticians then analyze data based on the researchers needs.

Results, Conclusion

This is an ongoing project, initial results are promising. Contigs obtained from de novo assembly of sequence reads span over 98% of the PRRSV genome based on comparison to a reference sequence. Less than 20% of the sequence information generated comes from endogenous porcine origin and that is likely to be reduced as more optimization is performed. We are collaborating with labs in US and in Europe to obtain strains that are currently in circulation. Information generated by this study will increase robustness and effectiveness of future assay designs.

ED 13 - POSTER: Presence of West Nile infection among wild animals in Serbia

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Scientific Veterinary Institute Novi Sad¹; Institute INIA²

Key words: WNV, serology, virus detection, wild boars, roe deer, wild birds, Serbia

West Nile virus (WNV) is a neurovirulent mosquito-transmissible Flavivirus with zoonotic potential, which main hosts are birds, but it also infects other vertebrates, including humans, in which it may cause sporadic disease outbreaks that may result fatal. In Europe, the virus has been present for decades but until the 1990's WNV had caused sporadic outbreaks with rare reports of encephalitis but its epidemiological behaviour changed when it re-emerged with virulence in Romania, Russia and the Mediterranean basin causing dozens of humans and horses deaths. Until 2004, only lineage 1 strains were circulating in Europe, primarily in Mediterranean countries, but since then lineage 2 strains have been isolated in different countries, becoming locally endemic and showing explosive geographic spread throughout central and south-eastern Europe. WNV circulation was examined, for the first time in Serbia, in blood sera collected during 2011 and 2012 from 331 wild boar (*Sus scrofa*), 91 roe deer (*Capreolus capreolus*), and 92 wild resident and migratory birds, belonging to 30 species within 21 families, and the prevalence found were of 15.4%, 17.2%, 23%, and 7.6%, respectively. Preliminary results of viral neutralization in cell culture showed that many of the animals presented neutralizing antibodies that were mainly WNV-specific, as few of them neutralized Usutu virus, the only other flavivirus circulating in Europe. In addition, pools of tissue samples (brain, kidney, lung, spleen and liver) from 82 wild birds belonging to 36 species and 22 families were examined for the presence of WNV-RNA and 8 (9.8%) resulted positives: 3 Northern goshawks (*Accipiter gentilis*), 1 White-tailed eagle, 1 Legged gull (*Larus michahelis*), 1 Hooded crow (*Corvus cornix*), 1 Bearded parrot-bill (*Panarus biamicus*), and 1 Common pheasant. Phylogenetic analysis of partial E region sequences showed the presence of, at least, two WNV lineage 2 Serbian clusters closely related to those responsible of recent outbreaks in Greece, Italy, and Hungary. Full genomic sequence from a goshawk WNV isolate corroborated this data. These results confirm the circulation of WNV in Serbia and remark the risk of infection for humans, mammals and birds so, implementation of a state-of-the art surveillance system for the detection of incursions of WNV into Serbia deems mandatory. Additional epidemiological investigations are currently ongoing.

EPIDEMIOLOGY & RISK ANALYSIS

ERA 1 - POSTER: Characterizing the distribution of BTV-8 and its vectors in Europe by means of Köppen-Geiger climate classification

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University of Veterinary Medicine Vienna¹

Key words: Bluetongue disease, Culicoides, climate classification

The Bluetongue virus (BTV) causes an economically relevant infectious disease in ruminants, such as cattle, sheep and goats. Repeated outbreaks of BTV serotypes 1, 2, 4, 9 and 16 have been reported from the Mediterranean area, in some regions the virus is already endemic. In 2006, the serotype 8 (BTV-8) emerged for the first time in the border area of Germany, Belgium and the Netherlands and spread within a few years over all Central Europe. It is remarkable that Mediterranean countries were not affected by this serotype, whereas even areas north of the 50th latitude were involved. The main vectors are biting midges of the genus *Culicoides*.

The influence of climate on the distribution of this disease has been discussed for years. It is quite well known that the population dynamics of the vectors as well as the extrinsic incubation period depend strongly on the environmental temperature. Here we used climate maps to characterize the distribution of BTV-8 as well as its most important vectors. These maps are based on the Köppen-Geiger climate classification, where each climate class is characterized through a three-letter code. Thus, the Cfb climate (warm temperate, fully humid with warm summer) is dominating in Central Europe. The synopsis of epidemiological and climatological data illustrates the restriction of the BTV-8 outbreak to the Cfb climate. Based on published and own monitoring data we show that the most abundant vector species in Cfb climate belong to the *Culicoides obsoletus* complex (74%). In contrast to Central Europe large parts of the Mediterranean are characterized by Csa climate (warm temperature, dry and hot summer), where *Culicoides imicola* act as the main vector (82%) for sporadic recurring BTV-outbreaks of serotypes 1, 2, 4, 9 and 16.

ERA 2 - POSTER: Genetic and Biological Characteristics of H9N2 Influenza Viruses Isolated from Chickens in China during 2001-2012

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Key words: H9N2, evolution, biological characteristics

H9N2 avian influenza viruses are still circulating in China and responsible for a threat to the poultry industry, even public health. To elucidate the genesis and biology of H9N2 influenza virus, in this study, a total of 20 H9N2 strains were isolated from farms in six provinces in Northern and Eastern China during 2001-2012 in the routine surveillance or some sporadic outbreaks. These H9N2 isolates were purified for three times using specific-pathogen-free (SPF) embryonated chicken eggs before sequenced their complete genomes. On analyzing for the sequences, a conserved motif of all 20 strains is PS(A)RSSR/GLF in HA cleavage site which is a symbol associated with low pathogenicity of influenza virus in chickens. Deduced amino acid sequences show the position 226 amino acid of HA protein in 9 of 20 strains is a Leucine (in H3 numbering), indicating their preference to bind of α -2, 6 Salic Acid receptor. A T300I mutation in three strains (CK/BJ/243/05, CK/HN/323/08 and CK/BJ/243/10) results in loss of a potential glycoprotein site and another P315S mutation in eight strains adds a potential glycoprotein site for which are presumably relevant to the vaccination-mediated selection pressure. There is a deletion between 62-64 amino acids in 18 isolates implying that the short stalk NA might improve neuraminidase enzyme activity and accelerate virus release from infected cells. An S31N mutation in six isolates shows a molecular marker of amantadine resistance which has been reported in the previous studies. NS1 gene of these isolates lacks of the PDZ motif in comparison to that of QA/HK/G1/97, which is related to the virus escape and antiviral effect. In phylogenetic tree analysis, most segments of these 20 strains share common ancestors from CK/BJ/1/94, CK/SH/F/98 and QA/HK/G1/97 isolates which have formed into 3 independent lineages in China. Among them, HA, NA and NS genes of all strains are directly derived from CK/BJ/1/94; M genes of 7 strains are divided into QA/HK/G1/97 lineage. Especially, we interestingly found that the PA genes in two strains (CK/HN/321/08 and CK/HN/323/08) bearing a full-length NA are clustered into CK/Korea/323/96 branch, and the PB2 genes of two recent isolates (CK/SD/513/11 and CK/GS/419/12) do not belong to any reference lineages and are from unknown lineages. Thus, we found seven new genotypes in 20 strains based on the previous genotyping approach. To investigate the replication ability in mice, we selected six isolates from different genotypes for infection experiment. The results showed that three viruses of six H9N2 are able to replicate in mice lung without adaptation and result in weight loss to some extent. Taken together, our findings demonstrate that the rapid continued evolution of H9N2 viruses currently circulating in China occurred via the point mutation and/or reassortment, and emphasize the importance to strengthen the continued influenza virus surveillance in chicken flocks as well.

ERA 3 - POSTER: Genetic diversity of Newcastle disease virus in Pakistan: A countrywide perspective

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Key words: Lineages, Newcastle disease virus, Pakistan, Phylogenetic analysis, Poultry

Newcastle disease (ND) is one of the most deadly diseases of poultry around the globe. The disease is endemic in Pakistan and recurrent outbreaks are being reported regularly in wild captive, rural and commercial poultry flocks. Though, efforts have been made to characterize the causative agent in some parts of the country, the genetic nature of strains circulating throughout Pakistan is currently lacking. To ascertain the genetics of NDV, 452 blood samples were collected from 113 flocks, originating from all the provinces of the country, showing high mortality (30–80%). The samples represented domesticated poultry (broiler, layer and rural) as well as wild captive birds (pigeons, turkeys, pheasants and peacock). Samples were screened with real-time PCR for both matrix and fusion genes and positive samples were subjected to amplification of full fusion gene, subsequent sequencing and phylogenetic analysis. The deduced amino acid sequence of the fusion protein cleavage site indicated the presence of motif (112RK/RQRR↓F117) typical for velogenic strains of NDV. Phylogenetic analysis of hypervariable region of the fusion gene indicated that all the isolates belong to lineage 5 of NDV except isolates collected from Khyber Pakhtoon Khawa (KPK) province. A higher resolution of the phylogenetic analysis of lineage 5 showed the distribution of Pakistani NDV strains to 5b. The isolates from KPK belonged to lineage 4c; the first report of such lineage from this province. Taken together, data indicated the prevalence of multiple lineages of NDV in different poultry population including wild captive birds. Such understanding is crucial to underpin the nature of circulating strains of NDV, their potential for interspecies transmission and subsequent disease control strategies.

ERA 4 - POSTER: Culture-Independent analysis of respiratory microbiota of birds in the free range system

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Key words: Culture-independent analysis, Respiratory microbiota, Birds, 454-Pyrosequencing, Taxonomic diversity

Knowing the microbial diversity in the respiratory system of birds is important for (i) monitoring health conditions of birds, (ii) surveillance of endemic infections in a particular geographical region and their subsequent zoonotic potential, (iii) improvement in molecular diagnostics and disease control, and (iv) substantial information about isolation and characterization of previously uncultured microorganisms. Using limited prior knowledge of organisms and their culturing needs, a little is known of the bacterial community present in the respiratory system of birds. Here, we describe the first culture-independent analysis of the respiratory microbiota of birds raised in the free-range system. During respiratory disease outbreak in the Punjab province, with clinical symptoms different than previously known for viral and bacterial infection, equal numbers of tracheo-bronchoalveolar lavage were collected from clinically healthy (n=6) and diseased birds (n=6) well before administration of antibiotics. All samples were subjected for 16S rRNA-gene-based (V1–V5, ~ 900bp) 454-pyrosequencing with subsequent quality taxonomic assignment using MOTHUR-BLASTn-MEGAN software pipeline. Summarization of almost all of the reads to the corresponding bacterial domain and its subsequent descendants represented rigorous quality control through filtering of the chimeric sequences and PCR mismatches when assigning taxonomic units. The quality sequences retrieved from clinically healthy (n=72,726) and diseased birds (n=72,967) showed high diversity corresponding to 8 phyla, 55 families, 59 genera and 24 bacterial species. Rarefaction plot suggested that nearly all of the bacterial diversity has been captured from each group of birds. Compared to healthy birds, Shannon-Weaver diversity index and rarefaction plot suggested less taxonomic diversity and richness in diseased birds (1.32 vs 1.29). However, as indicated by Simpson-Reciprocal diversity index at the genera node, abundance of similar taxonomic node was more frequent in diseased than healthy birds (3.27 vs 2.68). Of the bacterial communities identified in clinically healthy birds at phyla node, Proteobacteria (33,532, 86.42%) was found in greater abundance followed by Firmicutes (1,932, 4.97%), Actinobacteria (1,433, 3.69%), Bacteroidetes (1,137, 2.93%), Tenericutes (335, 0.86%), Fusobacteria (316, 0.81%), Cyanobacteria (106, 0.27%), and Chlamydiae/Verrucomicrobia (6, 0.015%). Likewise, in diseased birds, Proteobacteria was the most abundant (41,185, 76.29%) followed by Tenericutes (9,098, 16.85%), Firmicutes (1,859, 3.44%), Actinobacteria (1,285, 2.4%), Bacteroidetes (514, 0.95%), Fusobacteria (22, 0.04%), Acidobacteria (9, 0.02%), and Cyanobacteria (9, 0.02%). Further analysis toward lower taxonomic nodes identified reads corresponding to 51 families, 48 genera, and 15 species in clinically healthy birds, whereas 43 families, 46 genera, and 19 species were identified in the diseased birds. Importantly, among those microorganisms, some were assigned to genera but not to species taxonomic level. Identified bacterial species such as *Enterococcus cecorum*, *Kushneria* Sp. Z35, *Bacteroides acidofaciens* and *Acinetobacter* Sp. N15, all being associated with various diseases in humans and other mammals, help us monitor their zoonotic nature and transmission. Further molecular and clinico-epidemiological studies for the organisms identified at genera node or above with subsequent 16S rRNA-gene sequencing and phylogenetic analysis will allow us better understand the role of bacterial species identified in sick and healthy birds, and determine its significance to public health.

ERA 5 - POSTER: Assessment of African Swine Fever status in Swaziland, southern Africa

Boshoff, Carin¹; Bastos, Armanda²; Dube, Mzwandile³; Heath, Livio⁴; Dube, Mzwandile³

Tshwane University of Technology¹; University of Pretoria²; Ministry of Agriculture³; Onderstepoort Veterinary Institute⁴

Key words: African swine fever, Swaziland, Ornithodoros ticks, PCR

African Swine fever is a devastating haemorrhagic disease of domestic pigs, caused by African Swine fever virus, the only known DNA arbovirus and sole member of Asfarviridae family. Argasid ticks of the genus *Ornithodoros*, which are widely distributed throughout southern Africa, play a primary role in maintenance and spread of the virus, within the endemic sylvatic cycle. Currently no vaccines are available and disease prevention relies on regulated control zones and strict bio-security measurements. The demarcation of control zones in South Africa is based on surveys conducted in the 1980's during which the distribution of ASF-positive *Ornithodoros* ticks was determined. The north-eastern part of South Africa, including parts of the Limpopo, North West, Kwa-Zulu Natal and Mpumalanga provinces fall within the ASF control zone. The ASF status of Swaziland, which is situated between Kwa-Zulu Natal and Mpumalanga provinces, is currently unknown. A recent study making use of a molecular approach revealed that the virus may have disappeared from Mkuze Game Park in Kwa-Zulu Natal province. This wildlife reserve which is still incorporated within the control area is bordered by Swaziland in the north, which in turn is bordered by Mozambique in the east and Mpumalanga in the North. Thus the country of Swaziland, which is surrounded by regions in which ASF presence has been recorded, can potentially serve as an uncontrolled reservoir of infection or is an area under constant threat of incursion. To distinguish between these two possibilities, the ASF status of Swaziland was assessed in this study by sampling *Ornithodoros porcinus* ticks from seven Swaziland Nature reserves. The ticks were individually tested with the aid of a highly sensitive, tick-specific, duplex PCR. Amplification of the p72 gene target confirms ASF virus genome presence, whilst 16S tick host gene amplification serves as an internal control for genomic DNA integrity. To date, all ticks from Swaziland screened using this molecular approach were negative for the presence of ASF virus. Whilst these results suggest that Swaziland appears to be free of the disease, the presence of the soft tick vector and the sylvatic cycle hosts, together with the country's proximity to the ASF-positive areas of South Africa and historically positive areas of Mozambique, indicate that regular monitoring of the ASF status of Swaziland is of regional interest.

ERA 6 - POSTER: Peste des petits ruminants virus in Pakistan

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Key words: Peste des petits ruminants virus, Pakistan, Small ruminants, DNA sequencing, Genetic characterization

Peste des petits ruminants is a highly contagious disease of domestic and wild small ruminants and camels. Despite of continuous use of live attenuated vaccine, the disease is repeatedly being reported in small ruminants of Pakistan. Very limited information is available on the genetic diversity of the peste des petits ruminants virus (PPRV) in Pakistani small ruminants. This study was performed to provide information on the molecular characteristics, genetic diversity and the phylogenetic relationship of PPRV strains causing continuous outbreaks in Punjab, Pakistan. Thirty-five samples from 6 distinct outbreaks, suspected for PPR based on clinical picture and history, were confirmed to be PPRV positive using real-time PCR against the nucleoprotein (N) gene of the virus. Sequence analysis of the N gene of PPRV indicated that representative isolates were monophyletic and has relationship with previously characterized Pakistani strains of PPRV. However, these PPRV strains were significantly distinct from previously characterized PPRV strains from Pakistan. The results indicate that there are at least two different genetic variants of PPRV circulating in Pakistani small ruminants and PPR has established as an endemic infection in the country. These findings are essential in expanding the data on the genetics of local PPRV strains.

ERA 7 - POSTER: Assessment of African Swine Fever status in Swaziland, southern Africa

Boshoff, Carin¹; Bastos, Armanda²; Dube, Mzwandile³; Heath, Livio⁴; Dube, Mzwandile³

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Key words: African Swine Fever, Ornithodoros ticks, Swaziland, PCR

African Swine fever is a devastating haemorrhagic disease of domestic pigs, caused by African Swine fever virus, the only known DNA arbovirus and sole member of Asfarviridae family. Argasid ticks of the genus *Ornithodoros*, which are widely distributed throughout southern Africa, play a primary role in maintenance and spread of the virus, within the endemic sylvatic cycle. Currently no vaccines are available and disease prevention relies on regulated control zones and strict bio-security measurements. The demarcation of control zones in South Africa is based on surveys conducted in the 1980's during which the distribution of ASF-positive *Ornithodoros* ticks was determined. The north-eastern part of South Africa, including parts of the Limpopo, North West, Kwa-Zulu Natal and Mpumalanga provinces fall within the ASF control zone. The ASF status of Swaziland, which is situated between Kwa-Zulu Natal and Mpumalanga provinces, is currently unknown. A recent study making use of a molecular approach revealed that the virus may have disappeared from Mkuze Game Park in Kwa-Zulu Natal province. This wildlife reserve which is still incorporated within the control area is bordered by Swaziland in the north, which in turn is bordered by Mozambique in the east and Mpumalanga in the North. Thus the country of Swaziland, which is surrounded by regions in which ASF presence has been recorded, can potentially serve as an uncontrolled reservoir of infection or is an area under constant threat of incursion. To distinguish between these two possibilities, the ASF status of Swaziland was assessed in this study by sampling *Ornithodoros porcinus* ticks from seven Swaziland Nature reserves. The ticks were individually tested with the aid of a highly sensitive, tick-specific, duplex PCR. Amplification of the p72 gene target confirms ASF virus genome presence, whilst 16S tick host gene amplification serves as an internal control for genomic DNA integrity. To date, all ticks from Swaziland screened using this molecular approach were negative for the presence of ASF virus. Whilst these results suggest that Swaziland appears to be free of the disease, the presence of the soft tick vector and the sylvatic cycle hosts, together with the country's proximity to the ASF-positive areas of South Africa and historically positive areas of Mozambique, indicate that regular monitoring of the ASF status of Swaziland is of regional interest.

ERA 8 - POSTER: Bacterial diversity of field-caught mosquitoes from different regions of Belgium and potential impact on virus transmission.

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Univesity of Liege¹

Key words: Mosquito, Endosymbiotic bacteria, Disease Transmission, Belgium

Several disease vectors presented a resistance to various pesticides currently used. Endosymbiotic bacteria was an alternative solution found because of their probably interactive effects with their host. In this study, six species of Culicidae belonging to three genera (*Culex pipiens* s.l., *Culex torrentium*, *Culex hortensis*, *Anopheles clavigere*, *Anopheles maculipennis* s.l., *Culiseta annulata*) mosquito field from eight sites of Belgium were used for the screening of the presence of six genera endosymbiotic bacteria (*Wolbachia* sp, *Comamonas* sp, *Delftia* sp, *Pseudomonas* sp, *Acinetobacter* sp and *Asaia* sp). In order, these endobacteries were chosen because of their probably role in mosquitoes and in the last extension, their use as a target approach "bio-control" mosquitoes later. The study was conducted by the method of normal PCR (Polymerase Chain Reaction) with appropriate primers using Taq polymerase. A total of 144 samples of larvae and 66 adult specimens of all species were tested. For the mosquito in Belgium, two endosymbiotic bacteria presented a most specificity: *Wolbachia* and *Acinetobacter*. Our results allowed us to confirm the absence of *Wolbachia* in *An. clavigere*, *An. maculipennis* s.l and *Cx. torrentium*. *Acinetobacter* was found in every species. Current advances in understanding the mosquito-microbiota relationships may have a great impact in a better understanding of some traits of mosquito biology and in the development of innovative mosquito-borne disease-control strategies aimed to reduce mosquito vectorial capacity and/or inhibiting pathogen transmission.

ERA 9 - POSTER: Geographic distribution of tick infesting wild fauna and potential vector of tick-borne diseases in Northern Italy

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IZSLER¹

Key words: tick, TBD, wild animals, Italy

Emilia Romagna and Lombardia regions are located in the northern part of Italy. Various tick-borne diseases (TBD) occur in this area, where Lyme disease, transmitted by *Ixodes ricinus*, is frequently diagnosed in humans. This work is an attempt to gather suitable data for surveillance on Ixodid fauna of wildlife hunter-killed animals and for risk assessment on tick-borne diseases in our regions. Diagnosis of TBDs increased over the last years and attention of public health services focused on emerging vector borne diseases and wild fauna transmitted diseases. In this study, Ixodid ticks were collected from wild animals after being hunter-killed or found dead and submitted to IZSLER laboratories for necropsy during 2008-2012, from two Italian region, Lombardia and Emilia-Romagna. Ticks were removed and identified following Manilla taxonomic keys and mapped using an open source software (QuantumGis) at municipality level.

A total of 6675 tick exemplars were collected from 268 municipalities in five years of surveillance. We sampled a total of 13 different mammals species: the most sampled animals were hunted game animals like roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*) and hare (*Lepus europaeus*). Mean tick intensity (mean number of ticks per host) was determined in each host species: the greater number of ticks per host was on hedgehog (t.i.=15,7) and hare (t.i.=10,6). Tick infestation differ between host species ($p<0.05$, χ^2): wild boar was found infested by eight tick species, followed by fox (infested by seven species). *D. marginatus* is significantly associated ($p<0.05$, χ^2) to wild boar and is rarely observed on other mammals, while *I. ricinus* was collected on all mammal species considered but with different abundance; in roe deer and hare about 90% of ticks collected were *I. ricinus*.

We found tick species that are vectors of many TBD: *I. ricinus* represent the main tick species (N=3939; 59%) and it's well distributed in all northern Italy. Other vectors are *R. sanguineus* (n=891; 13%), *I. hexagonus* (n=669; 10%), *R. turanicus* (n=596; 9%), *D. marginatus* (n=439; 7%) and *H. marginatum* (n=37; 0,6%).

Historical data on presence/abundance of disease vectors are essential to understand TBD epidemiology and to gather data for diseases transmission. Our surveillance system allows to map geographic distribution of ticks species at municipality level and provide a good base to detect and respond to vector-borne diseases related health threats.

ERA 10 - POSTER: Risk Assessment of Rift Valley Fever in Western Europe.

Fischer, Egil¹; De Vos, Clazien¹; De Koeijer, Aline¹

CVI¹

Key words: Vector-borne, livestock, risk, introduction, spread

Since the epidemic of bluetongue (BTV-8) in Europe, the awareness of emerging vector-borne infections increased, and risk assessments of such infections became an important request from policymakers

Rift Valley Fever (RVF) is a viral vector-borne zoonotic infection with a wide host range that is transmitted by several mosquito species. RVF causes high mortality among young ruminants and can cause abortions. In humans the disease can lead to disabilities and death, although most infections will only cause mild disease or will go unnoticed. Until the beginning of this century the disease was confined to Africa, but the introduction of RVF into the Arabian peninsula has increased the fear of introduction into Europe.

A full risk assessment of an infectious disease is a challenge in itself, since combining the probability of introduction with the impact of an exponentially growing epidemic is not straightforward. The complexities of vector-borne infections, such as RVF, further enhance the challenge of such a risk assessment.

Therefore, we applied a recently developed framework (1) that guides the user stepwise through the risk assessment of a vector-borne infection to assess the risk of RVF for Europe. A two-day workshop was held with international experts in which all relevant aspects of this infection were considered. Two aspects of the risk assessment, the risk of introduction and the transmission potential (2), have been studied in more detail for the situation in the Netherlands to obtain a more quantitative assessment of the risk.

Results indicate that an epidemic of RVF following an introduction may occur in Western Europe if local mosquito species are able to transmit RVF. Such an outbreak may affect a very large number of animals before detection, while the epidemic amongst humans is likely to remain limited. The probability of a RVF epidemic depends highly on the specific region in which and time at which the virus is introduced. Both the results of the expert workshop and the quantitative assessments show that the main uncertainties in the risk assessment of RVF for Europe follow from unknowns about the local vectors, such as abundance and the interaction with local hosts.

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ERA 11 - POSTER: Diagnosis and Investigation of an Outbreak of Porcine Reproductive and Respiratory Syndrome (PRRS) in Ireland during spring 2013

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Key words: Diagnosis, RT-PCR, PRRSV, Ireland

Introduction: Porcine Reproductive and Respiratory Syndrome (PRRS) also known as 'blue-ear pig disease', is an economically important disease of production pigs worldwide. PRRS virus (PRRSV) is a member of the Arteriviridae family. Clinical signs include late abortions, high neonatal mortality rates, low fertility and problems associated with weight loss and hypothermia. PRRSV was first discovered in the 1980s in The Netherlands and simultaneously in the mid-Western United States. The genomes of these two prototypical strains diverge by ~40%. In recent years small divergent changes have occurred in both strains but currently European and N. American are currently the only two discovered genotypes. In March 2013, a commercial company in the Republic of Ireland producing boar semen for artificial insemination raised concerns over potential infection with PRRSV. Subsequently, farms throughout both the Republic of Ireland and Northern Ireland using this semen for insemination contacted the Agri-Food & Biosciences Institute (AFBI) regarding concerns over infected stock. This study details the emergency response by AFBI, during the initial period of this outbreak.

Materials and method: In total, 840 sera from gilts/sows and 87 unused semen batches were received for PRRSV screening from 2 local boar studs and 17 farms representing a number of farms from both the Republic of Ireland and Northern Ireland. Separate semen and sera samples were pooled, where practicable, reducing the actual number of samples tested to 340. The total nucleic acids were extracted using the Roche Magna Pure 96 extraction system (Roche, West Sussex, UK). Amplification was performed using a duplex TaqMan® based RT-PCR capable of detecting both European and North American strains. Thermal-cycling was performed using an Applied Biosystems (California, US) 7500 Fast PCR system and an Ambion AgPath-ID One-Step RT PCR kit (California, US) using the following conditions: at 45°C for 10 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 45 seconds (Kleiboeker et al. 2005).

Results: Analysis of RT-PCR results indicated that 8.6% of pooled serum samples and 1.2% of pooled semen samples tested positive for the presence of European PRRSV nucleic acids. No North American PRRSV nucleic acids were detected in any of the samples tested.

Discussion: Given the structure of the Irish pig industry and the use of artificial insemination throughout the island, the results provided by AFBI to this PRRSV outbreak demonstrates the impact of infection at a boar stud. The possibility remains that infection rates could be greater across the island of Ireland than the ~10% suggested here. This kind of outbreak in such a local market could have a hugely damaging impact to the Irish pork producing economy. Further research is being conducted that will detail fully investigate the sequence underpinning these European strains, permitting phylogenetic analysis. This outbreak underlines the need for rapid diagnostic response to stem a potential outbreak, thus limiting the impact on an economy reliant on maintaining the high quality output and the reputation of its agri-food industry.

ERA 12 - POSTER: Epidemiological modeling for the assessment of bovine tuberculosis surveillance in Emilia-Romagna Italy

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Università di Parma¹; IZSLER²; University of California Santa Cruz, CA, USA³; Stanford University⁴

Key words: Epidemiological model, Network, Tuberculosis, Dairy Cattle

The modeling approach to study infectious diseases has become increasingly popular in last decades. Models offer the opportunity to test the interaction between epidemics and surveillance or control measures to contrast them. Consequently, they can be useful tools to assess the performance of health policies in advance on their adoption or in condition of freedom from disease when no actual performance assessment is possible.

In this work we study the impact of the introduction of a pathogen in a disease-free region as a consequence of livestock movement. The case of study is represented by the possible introduction of bovine tuberculosis (bTB) in the dairy cattle system of Emilia-Romagna Region, Italy. Currently the Region is bTB-free and surveillance for this disease is based on three pillars: systematic intradermal tuberculin testing of cattle over 24-months of age, with three-year turnaround time, intradermal tuberculin testing on moved cattle and post-mortem inspection of slaughtered animals. The dairy farming system of the Region is constituted by closed housing of cattle with no grazing, therefore the only direct contacts among animals of different farms are through movement of cattle among farms, nearly exclusively for restocking.

The goal of this study is to assess the effectiveness of current and alternative surveillance strategies in detecting the infection in the dairy farming system. To this purpose, we built an epidemiological model of both the within-farm and the between-farm bTB dynamics. Within-farm dynamics is described by a Susceptible-Exposed-Infected model, while between-farm dynamics is described by a network model, in which nodes represent farms and links are constituted by possible cattle movements between farms.

Preliminary results suggest that each pillar of the present surveillance has pros and cons. Intradermal tests on moved cattle and post-mortem inspection of slaughtered animals perform better in detecting outbreaks involving more than one farm (major outbreaks), while they perform worse for single-farm outbreaks. On the contrary, routine tests are less effective for major outbreaks and more effective for single-farm ones. Surveillance strategies alternative to the present ones have been explored by the model.

ERA 13 - POSTER: Spatio-temporal quantitative risk assessment of introducing African Horse Sickness into Western Europe

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INRA¹; ANSES²; CIRAD³; CVI⁴

Key words: risk assessment, culicoides, arbovirus, african horse sickness

African horse sickness (AHS) is a highly fatal viral vector-borne disease, which is transmitted between equine hosts by *Culicoides* midges. Given the estimated rate of 90% lethality in horses, the introduction of AHS virus (AHSV) in Europe would have dramatic consequences for the horse industry. The last outbreak in Europe was in Spain in 1987 following the importation of infectious Namibian zebras. The European import regulations have become stricter since the 1980s, so the risk of introduction of AHSV may also have changed since. However, the recent introductions in Northern Europe of bluetongue virus serotype 8 (BTV-8) in 2006 and Schmallenberg virus in 2011, two infections that are also transmitted by *Culicoides*, highlight the interest of an updated risk assessment of AHSV introduction in Europe. This is especially relevant for France that will organize the next World Equestrian Games (Deauville 2014). The objective of this study was to quantify the spatio-temporal risk of introduction of AHSV in Western Europe by assessing three important pathways of introduction. The experience gained during the last BTV and Schmallenberg outbreaks is helpful in evaluating some of these pathways.

To analyze the introduction risk of AHSV from various pathways a quantitative risk assessment was conducted based on a recently developed framework for risk assessment of emerging vector-borne livestock diseases(i). Model calculations were performed in Microsoft Office Excel 2010 and @Risk 6.1. The model incorporates three potentially important routes of introduction of the virus: (1) legal importation of an infectious equine, (2) introduction of an infectious vector through wind spread and (3) introduction of an infectious vector through transports of bovines. Different categories of AHS risk regions have been identified based on historical AHS occurrence, the presence of the main vector *C. imicola* and whether regions are a part of the European Union. The model uses monthly time steps. The spatial scale is a split of countries based on the administrative areas combined with climatic data and the distribution of equine and bovine populations. The main input data into the model are *Culicoides* abundance, average monthly temperature, the number of bovines and equines imported into each region considered, and the winds coming from North Africa.

Stochastic simulations allow calculation of a spatio-temporal risk pattern for introduction and helps in identifying periods and areas with increased risk. The different pathways considered are compared and their relative importance is assessed. Preliminary results for France show that their relative importance depends on the areas and months considered. These results are based on past data as a proof of principle but can be updated at any moment to calculate the current risk of introduction. This last point could allow health authorities to reduce the risk by implementing targeted surveillance activities and protective measures.

(i) C. de Vos, J. Bremmer, M. Hoek, E. Fischer, A. de Koeijer, Risk Assessment Framework for Emerging Vector-Borne Livestock Diseases. Report 11-CVI0168. Available at: <http://edepot.wur.nl/198115>

ERA 14 - POSTER: Evidence of a recombination event in Swine Vesicular Disease Viruses circulating in Italy

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Key words: Phylogenetic analysis, Swine Vesicular Disease Virus, NGS, recombination

Swine Vesicular Disease Virus (SVDV) is a positive sense 7400 bp long RNA virus belonging to family Picornaviridae, genus Enterovirus. During the last decade in Italy SVDV has been controlled by a surveillance and eradication program, also based on a virological surveillance in faecal samples. Circulating strains were monitored by molecular epidemiology studies based on the nucleotide sequence of a portion of highly conserved 3D gene coding for the RNA polymerase RNA-dependent enzyme. From 2004, phylogenetic analysis evidenced the simultaneous circulation in Italy of two SVDV genomic sub-lineages: one comprises viruses typically evolved in Italy since 1992 (Italian sub-lineage) and the other including isolates strictly related to viruses first detected in Portugal in 2003/2004 as well as some viruses from Italy isolated since 2004 (Portuguese sub-lineage). This epidemiological situation was confirmed by phylogenetic studies based on the coding region of the structural protein VP1 showing a 100% of concordance with the 3D gene pattern of clustering. From 2010 the 5'UTR region fragment of 26 SVDV strains was sequenced by Sanger method within the framework of Real-time PCR assay validation studies. The phylogenetic analysis, using Neighbor-joining method and based on the three genomic regions mentioned above, showed that seven out of 26 strains cluster within the Italian sub-lineage according to the 3D gene fragment, but they are classified as Portuguese sub-lineage considering the 5'UTR fragment and the VP1 region. These results suggested a possible recombination between the Italian and Portuguese sub-lineage genomes, presumably occurred during co-infection events. To gain insights regarding relative contribution of recombination to the generation of SVDV genetic diversity, we performed Next Generation Sequencing (NGS) analysis on nine isolates grown in cell-cultures (including both Italian and Portuguese isolates and four presumed recombinant SVDV strains). The RNA was extracted from the infected cell culture supernatants by using QIAmp Viral RNA kit (Qiagen). In order to amplify almost all SVDV genome, a One step PCR generating long overlapping fragments (3327 bp and 5010 bp) was carried out using a proofreading enzyme mixture (Platinum Taq Hi-Fidelity, Invitrogen). The NGS was performed using MiSeq system (Illumina), the sequencing library were prepared by standard Illumina Nextera protocol. The nearly full-length genome sequences were achieved by multiple alignments using SeqMan NGen (DNASTAR software package). To further investigate the potential recombination events both Recco (Recombinant Analysis using cost optimization) and the Recombinant identification program (RIP) based on pairwise sequence comparison were used. One significant recombination event was evidenced with both programs and a P-value of 0.000999 was obtained by Recco method: in fact all the recombinants presented the 5'UTR and P1 codifying region of the Portuguese sub-lineage and the remaining genome (codifying for the non-structural proteins) of the Italian one. These results indicate that recombination occurs between SVDV isolates contributing to SVDV genetic novelty. The NGS platform will be further exploited to investigate on the spatio-temporal epidemiology of SVDV, to study its genome evolution in nature and contribution of recombination to SVDV genetic diversity.

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ERA 15 - POSTER: Next Generation Sequencing and Phylogenetic Analysis of the UK 2001 Foot and Mouth Disease Virus outbreak

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Key words: Next generation sequencing, Foot and Mouth disease virus, UK, 2001

In 2001, the United Kingdom livestock industry was devastated by an epidemic of Foot and Mouth Disease Virus (FMDV) caused by Pan Asia O strain of the virus. With the rapid spread of the disease throughout the United Kingdom a total of 2,030 farms were declared as 'infected premises' (IP's), with many attributed to 'local spread'. Previous studies have looked at viruses from the outbreak at consensus level between farms using traditional Sanger sequencing techniques (Cottam et al, 2008; Konig et al, 2009). As part of the EPI-Seq research consortium we will use high-throughput sequencing technologies to determine the evolution of the virus to a high resolution, between all IP's identified in the outbreak. Samples of infected tissue, taken at original IP's were stored at the Pirbright Institute (PI), where they constitute a unique specimen repository for examining the microevolution of FMDV during a major outbreak of significant economic and sociological consequence. Here we report on a project in which we plan to investigate samples from 1500 confirmed IP's held within the repository using Illumina next generation sequencing to trace virus transmission between IP's.

Total RNA will be extracted from archived samples and converted to cDNA using gene specific primers, prior to the construction of sequencing libraries using the Nextera XT technology (Illumina). Sequencing libraries will be validated by Bioanalyser and sequencing will be performed on an Illumina Miseq using standard sequencing protocols. We will use this data to investigate transmission between different IPs using both phylogenetics and Bayesian analysis techniques correlating with spatio-temporal and geographic data collected during the outbreak. We will also focus on particular regions of interest during the outbreak (i.e. Cumbria and Devon), in order to confirm local spread patterns and transmission between IPs.

The project will determine the microevolution of the virus and trace transmission between IPs using phylogenetic analysis to confirm 'local spread'. The project will examine the evolution of a highly variable RNA pathogen of particular veterinary interest during an outbreak of significant size to a high resolution. This information will provide useful insights into FMDV evolution and strategies for monitoring future outbreaks.

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ERA 16 - POSTER: Impact of landscape component for CSFV management and confinement in wild boar: the French example

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Key words: Classical swine fever; vaccination; wild boar; barriers; dispersal

During the French CSFV vaccination campaign of wild boar in the Vosges du Nord area in 2006-2010, some seropositive animals (242 on 11,441 analyzed) were detected in the surrounding monitoring area. This area was separated of the vaccinated area by a physical barrier constituted by the A31 motorway and the Sarre River. The aim of this study was to analyze the spatio-temporal and individual risk factors associated with seropositivity in the monitoring area using generalized linear models. Seroprevalence remained under 5% in the three sub-areas of the monitoring area (defined according to the distance to the barrier) with no increase along the five years period of study. The most important effect was the proximity to the barrier: most of the seropositive animals were present in the vicinity to the barrier. Age (oldness) and year period (spring and summer) were also shown to be important for their distribution. Within the closest area to the barrier, the area of forest of each municipality appeared to be the main factor explaining the occurrence of seropositive animals, followed by the distance to the river (decrease of distance), the presence of forestry continuity on both side of the barrier, and the distance to the motorway (decrease of distance). Results of the model associated with the absence of viral detection suggested that seropositive animals corresponded to dispersed wild boar coming from the vaccinated area. This survey confirmed the effectiveness of physical barriers delimiting an area naturally infected by CSFV and managed by oral mass vaccination. It explored the spatio-temporal behaviour of seroprevalence in a surveillance area, describing dispersal of animals on large distances, which may be helpful to understand wild boar ecology.

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ERA 17 - POSTER: HOW TO FACE THE RISK OF A NEW BTV – OUTBREAK

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Key words: Bluetongue outbreak risk

Bluetongue is a vector-borne viral infectious disease of domestic and wild ruminants, especially sheep, non-contagious for human. The disease is been transmitted by Culicoides (biting midges).

At present, 26 serotypes of the virus are known in different parts of the world and the following types are currently identified in Europe : 1, 2, 4, 6, 8, 9, 11, 16, 25 and now even 14 in Poland.

Due to the long viraemia (up to 9 weeks) RT-PCR is been used for diagnostic clarification following first clinical signs. The TaqVet™ Bluetongue Virus NS3 - All genotypes - designed as a PAN test covering all 26 serotypes, allows the simultaneous detection of the gene coding for NS3, common to all the types of Bluetongue virus, and of the sequence of an endogenous internal control (IPC - Internal Positive Control). It does not cross-react with EHDV.

The current EU strategy concerning bluetongue is based to continue on the three facts: surveillance, movement restrictions and vaccination.

For surveillance to use the LSIVet™ Ruminant Bluetongue Advanced II - Serum kit with a recombinant VP7 protein of the Bluetongue virus, serotype 8. This protein is highly preserved and specific of the 26 known serotypes of the Bluetongue virus.

ERA 18 – POSTER: Estimating transmission rate of African swine fever in domestic pigs through experimental studies

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African swine fever (ASF) is a deadly virus disease of domestic pigs with severe socio-economic impact. Several characteristics of ASF virus (ASFV) make its control and eradication difficult, including the absence of available vaccines, the potential long survival in infected material and contaminated animal products, and the potential existence of a wild boar/tick reservoir. Information on transmission rate parameters is essential to develop effective surveillance, control and eradication programmes. Three transmission experiments were conducted in The Pirbright Institute high containment facilities using the highly virulent strain, Georgia 2007/1. The ASF transmission process between experimentally inoculated and contact domestic Large White pigs based on viraemia and clinical data was reconstructed in a stochastic Susceptible-Infected-Removed (SEIR) model. Three different experimental designs were used to allow for direct and/or indirect transmission between infected and non-infected animals to occur. Pigs were observed and scored for clinical signs and samples collected from blood, oral, nasal and rectal swabs for measurement of ASFV. The pigs were euthanized at a moderate severity end-point. For each experiment, the transmission rate parameters, β_w and β_b , were estimated using a maximum likelihood method based on a binomial distribution of the number C of animals being infected in a given pen during a given time step. Such parameters are defined as the number of new infections per unit of time (usually per day), caused by one typical infectious animal in a fully susceptible population within and between-pen, respectively. A first approximation of the within-pen transmission rates (β_w) based on the viraemia data and the clinical data has been estimated and discussed. These transmission rate parameters for ASF will make a contribution towards advancing the understanding of the epidemiology of ASF and inform the design of control strategies and preventive measures.

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