

EPIZONE



▶ PROGRAMME  
& ABSTRACTS

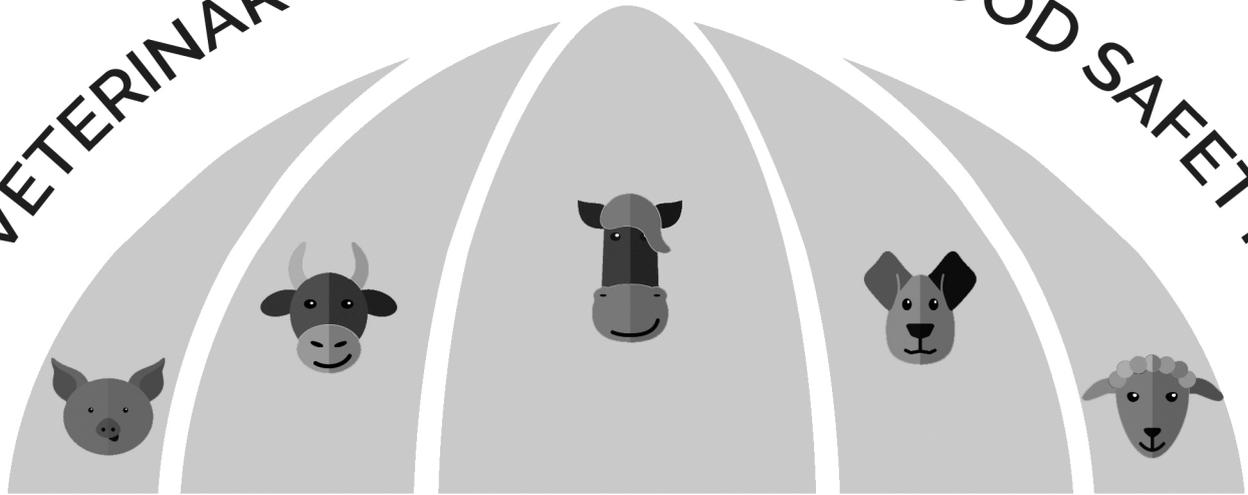
EPIZONE  
10<sup>th</sup> Annual Meeting

GOING  
VIRAL

27-29 September 2016  
MADRID, SPAIN



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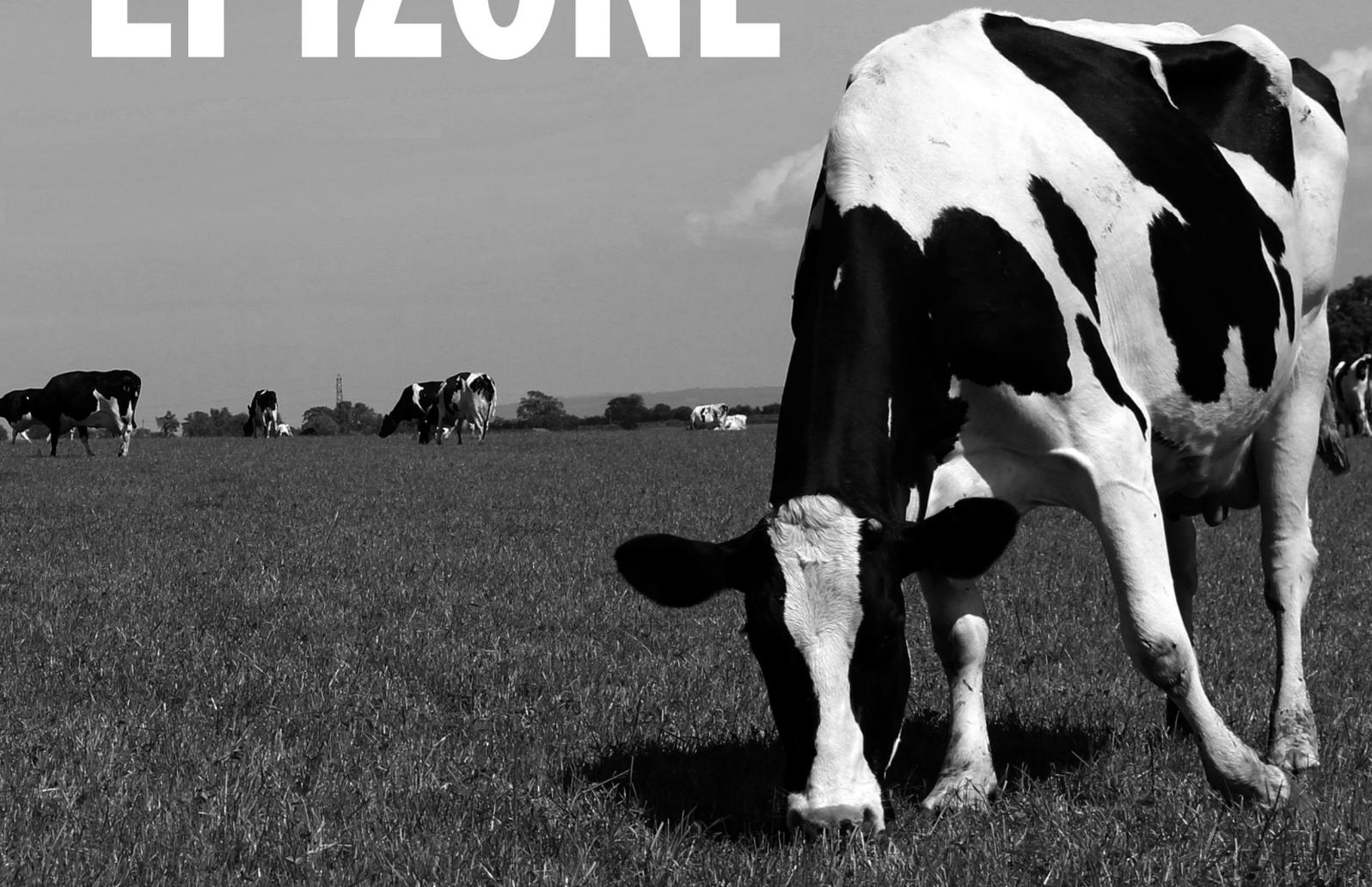
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## Welcome 10<sup>th</sup> Annual Meeting EPIZONE “Going Viral”

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It is a great honour and a privilege for me to give you all our warmest welcome to the Xth Annual Meeting of EPIZONE, the first to be celebrated in Spain. INIA-CISA, as the host institution, has been fully committed on this challenge from the beginning, and we do hope that the final result will be satisfactory for participants, sponsors and organizers. We wish to thank specially to them, as this event could not be possible without any of these essential parts. The financial support from sponsors, the availability and contributions from keynote speakers, the high scientific level of oral and poster presentations from participants and, at the end, curious, active and interested assistants, will contribute to the success of the meeting. From the organizing committee I want also to thank the EPIZONE secretariat and coordinator for their continuous help and implication in the effort. Finally, my gratitude to the people at the local and international scientific and organizing committees that have been working together very hard to yield a balanced, wide-scoped and intense (maybe too intense?) programme. Special thanks to Jovita Fernandez Piñero, who has been in charge of many tasks, and has fulfilled a brilliant labour. And will not forget about the enthusiastic Young Epizone people; thank you for your work and for organizing such a well-designed session.

Under the general title of “Going Viral”, and from a *One Health* perspective, we have outlined three concentric circles defining the main topics:

**Topic I:** Animal Health in a changing World, dealing with global threats for animal health.

**Topic II:** Threats at the European border, paying attention to diseases in the neighbouring areas.

**Topic III:** Current challenges inside Europe, where the main diseases affecting the European countries will be discussed.

As in previous EPIZONE meetings, diagnostics, intervention strategies, epidemiology and surveillance, risk analysis and some other aspects will be approached by recognized experts in specific sessions. Many diseases which are familiar to us will receive attention, from Foot and Mouth Disease to West Nile Virus Disease or the more recent episodes by Lumpy Skin Disease, Pest des Petits Ruminants and some others. African Swine Fever and Bluetongue have been the most “popular” diseases among contributors, this revealing their current relevance.

I hope that the efforts of contributors, sponsors, participants and organizers will provide an opportunity for the “epizootic community” to work together, to plan new initiatives, to interact and to share a good time in Madrid.

Victor Briones

## Acknowledgements

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We are very grateful to the following companies for sponsoring the 10<sup>th</sup> Annual EPIZONE meeting:

- ▶ **Ingenasa** gold sponsor  
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We are also grateful to the following Spanish Organizations for their special support:

**National Institute for the Agricultural  
and food Research and Technology (INIA)**

**Spanish Technology Platform  
for Animal Health (Vet+i)**

## Congress Committees

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### Local scientific and organizing committees (INIA-CISA):

- ▶ Victor Briones, Director, head of both local committees
- ▶ Jovita Fernández-Pinero
- ▶ Maria Luisa Arias
- ▶ Miguel Ángel Jiménez-Clavero
- ▶ Ana de la Torre
- ▶ Fernando Esperon
- ▶ Javier Ortego
- ▶ Noemí Sevilla
- ▶ Alejandro Brun

### International scientific committee:

- ▶ Victor Briones (INIA-CISA, Spain)
- ▶ Stephan Zientara (ANSES, France)
- ▶ Ana Moreno (IZSLER, Italy)
- ▶ Martin Beer (FLI, Germany)
- ▶ Linda Dixon (Pirbright, UK)
- ▶ Anette Botner (DTU, Denmark)
- ▶ Thierry Van der Berg (CODA-CERVA, Belgium)
- ▶ José Manuel Sánchez-Vizcaíno (UCM, Spain)
- ▶ Wim Van der Poel (CVI-WUR, The Netherlands)

### EPIZONE organizing committee:

- ▶ Manon Swanenburg
- ▶ Margriet Vedder
- ▶ Wim Van der Poel



# KEYNOTE SPEAKERS



## José Manuel Sánchez-Vizcaino

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J. M. Sánchez-Vizcaíno is DVM, Ph.D (both from UCM) and Doctor Honoris Causa (UMu). Full Professor of Animal Health at the University Complutense of Madrid and Director of the OIE Reference Laboratory for African Swine Fever and African Horse Sickness.

The Scientific contributions of Professor Sanchez-Vizcaino have notably contributed to the control and eradication of several animal diseases, among them the African Swine Fever, African Horse Sickness and Classical Swine Fever, thanks to the development of new diagnostic methods and fast and sensitive reagents as well as new epidemiologic strategies for the early detection. His actual research areas are: Preventive Medicine and modelling, new diagnostic methods and control strategies for eradication.

He has more than 200 research publications in high international impact journals, international and national awards, and is the author of various chapters in books with international prestige.



## Alexander Morrow

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Dr. Alexander Morrow, BA, MVB, PhD, MRCVS is veterinary surgeon with seventeen years' experience in research working on the pathogenesis and control of *Amblyomma variegatum*-associated dermatophilosis, followed by four years in a research support capacity at Edinburgh University and twelve years in his current position in research programme management with Defra where he is International Evidence Lead Animal Health and Welfare.

He established and coordinated for 10 years the European Collaborative Working Group (CWG) on Animal Health and Welfare research, under the EU Standing Committee on Agriculture Research, and led the associated EU-funded EMIDA ERANET on Emerging and Major Infectious Diseases of Animals and currently leads the STAR-IDAZ global network, Global Strategic Alliances for the Coordination of Research on the Major Infectious Diseases of Animals and Zoonoses, and the associated International Research Consortium, with a higher level of commitment to collaboration, which was launched by the European Commission in January 2016.



## Josué Martínez-de la Puente

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Josué Martínez-de la Puente (Segovia -Spain, 1981) is a biologist working as postdoctoral researcher at the Estación Biológica de Doñana (EBD), an institute from the Spanish Research Council (CSIC). Moreover, he is a researcher in a team included in the CIBEResp network for the study of the transmission of pathogens of public health relevance. His research focuses on the study of the ecological and evolutionary factors determining the dynamic of transmission of vector-borne pathogens affecting wildlife, livestock and humans. During the last years, he has been involved in different EU projects (e.g. EDENEXT and EuroWestNile), integrating a multidisciplinary approach involving virologists, ecologists, entomologists and animal and human health perspectives following the ONE HEALTH framework. He is author of about 60 papers in international scientific journals.



## Aykut Ozkul

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Aykut Ozkul graduated from Ankara University in 1987 with Doctor of Veterinary Medicine title. He has completed his PhD in 1992 in Graduate School of Health, Ankara University. He joined Shafiqul Chowdury's lab School of Veterinary Medicine, Kansas State University in Postdoc position in 1999.

His research group focused on Bovine Herpesvirus type-1, Morbillivirus and some Arbovirus infections. The group primarily interested with molecular epidemiology and vaccinology of given virus infections. He and his research group developed Marker BHV-1 vaccine for local use in 2007 for the very first time. In Morbillivirus researches, the main focus of interest is Peste des Petit Ruminants Virus (PPRV), which is highly contagious and devastating disease of small ruminants in the region. He has also been studying intensively some regional zoonotic arbovirus infections having negative impacts also on human health such as West Nile, Crimean Congo Hemorrhagic Fever infections in departmental BSL-3(+) and ABSL-3(+) facilities for last 6 years.



## Emiliana Brocchi

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Staff Scientist at Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), in Brescia, with 35 years within the organization. Head of Department of Biotechnology and director of the National Reference Centre for Vesicular Viruses, FAO and OIE Reference Laboratories for FMD and SVD.

### Work experience and activities

Development of a multiplicity of monoclonal antibodies against a variety of antigens and animal pathogens, with focus on viruses, with application to diagnosis and research; development of novel diagnostic methods and ready-to-use ELISA kits, for antigen and antibodies detection, based on the use of monoclonal antibodies.

Laboratory diagnosis for FMD, SVD and related pathogens on national basis, international cooperation and diagnostic support to FMD endemic countries.



## Alberto Laddomada

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Alberto Laddomada is graduated in veterinary medicine with post-doctoral studies in microbiology and virology. He worked for about fifteen years as a scientist at the Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy. At that time most of his scientific work was on African swine fever. In 1997 he joined the Animal Health Unit at the European Commission, Brussels, where he dealt with the EU policy and legislation on animal diseases, including classical and African swine fever and avian influenza. He directed the Animal Health Unit from 2007 until 2015. During those years he led the process of adoption of the Commission proposal for the new EU legislation on animal health that then became Regulation (EU) 2016/429 (the “animal health law”). Currently, he is director general of the Istituto Zooprofilattico Sperimentale della Sardegna.



## Marisa Arias

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Since 2005 Marisa Arias is the Technical Director at the Animal Health Research Center (INIA-CISA, SPAIN), Technical Director of the EU Reference Laboratory for African swine fever (ASF), and more recently for the Reference Centre of the Food and Agriculture Organization of the United Nations (FAO) for ASF. Throughout her career, Dr. Arias has been concerned in over seventy two National and EC-funded R&D projects and Agreements for Scientific and Technical cooperation with National and international Institutions and Companies. She has contributed with more than a hundred scientific and technical publications. She has got three national scientific awards for outstanding research, and a merit for the activity research. She has participated often in the capacity of Coordinator and/or Director in over fifty six international Courses on Animal Health organized in Europe, and Central and South America with a special relevance in the last years to ASF.



## Alessio Lorusso

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Alessio Lorusso is a 35-years old Italian DVM with a PhD on coronaviruses of dogs obtained after a two-years training period at the University of Utrecht. Next, he moved to the NADC-USDA of Ames-Iowa for a post doc on the epidemiology and pathogenesis of swine influenza viruses including the pandemic 2009 H1N1. He currently works in the Virology Unit of the OIE Reference Laboratory for Bluetongue of Teramo, Italy. His research activities are not restricted to Bluetongue and related orbiviruses but they also include morbilliviruses of dogs and cats.



## Kris De Clercq

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Doctor in Veterinary Medicine (1981), Faculty of Veterinary Medicine, State University of Gent, Belgium and 'Master of Science in Animal Production' (1983).

Since 1985 working at the CODA-CERVA (Brussels, Belgium), Operational Directorate of Virology. Head of the Unit Vesicular and Exotic Diseases.

Main activities are in foot-and-mouth disease, bluetongue, lumpy skin disease and sheep and goat pox and swine vesicular disease.

Vice-President of the OIE Scientific Commission since 2009.

Head of the OIE Collaborative Centre and the FAO Reference Centre for Vesicular Diseases.

I was coordinator of two EC research projects and a project for the Bill and Melinda gates Foundation.

## Keynotes Lectures

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### Opening lecture:

Prof. JOSÉ MANUEL SÁNCHEZ-VIZCAINO  
(Complutense University, Madrid, Spain)

“One health: a personal view”

### Topic I: Animal health in a changing world

- Keynote lecture 1

Dr. ALEXANDER MORROW (Defra, UK)

“International Research Consortium on Animal Health: Working Together to Address Global Challenges”

- Keynote lecture 2

Dr. JOSUÉ MARTÍNEZ-DE LA PUENTE (Estación Biológica de Doñana-CSIC, Seville, Spain)

“Transmission dynamics of zoonotic arboviruses with avian reservoir”

### Topic II: Threats at the European borders

- Keynote lecture 3

Prof. AYKUT OZKUL (Ankara University, Turkey)

“Current status of PPR, LSD and AKA diseases in Turkey: Risk(s) for European threat”

- Keynote lecture 4

Dr. EMILIANA BROCCHI (IZSLER, Brescia, Italy)

“Foot-and-mouth disease viruses on the move: changing epidemiological patterns”

### Topic III: Current challenges inside Europe

- Keynote lecture 5

Dr. ALBERTO LADDOMADA (IZSSA, Sardinia, Italy)

“Animal disease control: which challenges in the next decades?”

- Keynote lecture 6

Dr. MARISA ARIAS (INIA-CISA, Valdeolmos, Spain)

“African swine fever virus: An approach of what we are facing”

- Keynote lecture 7

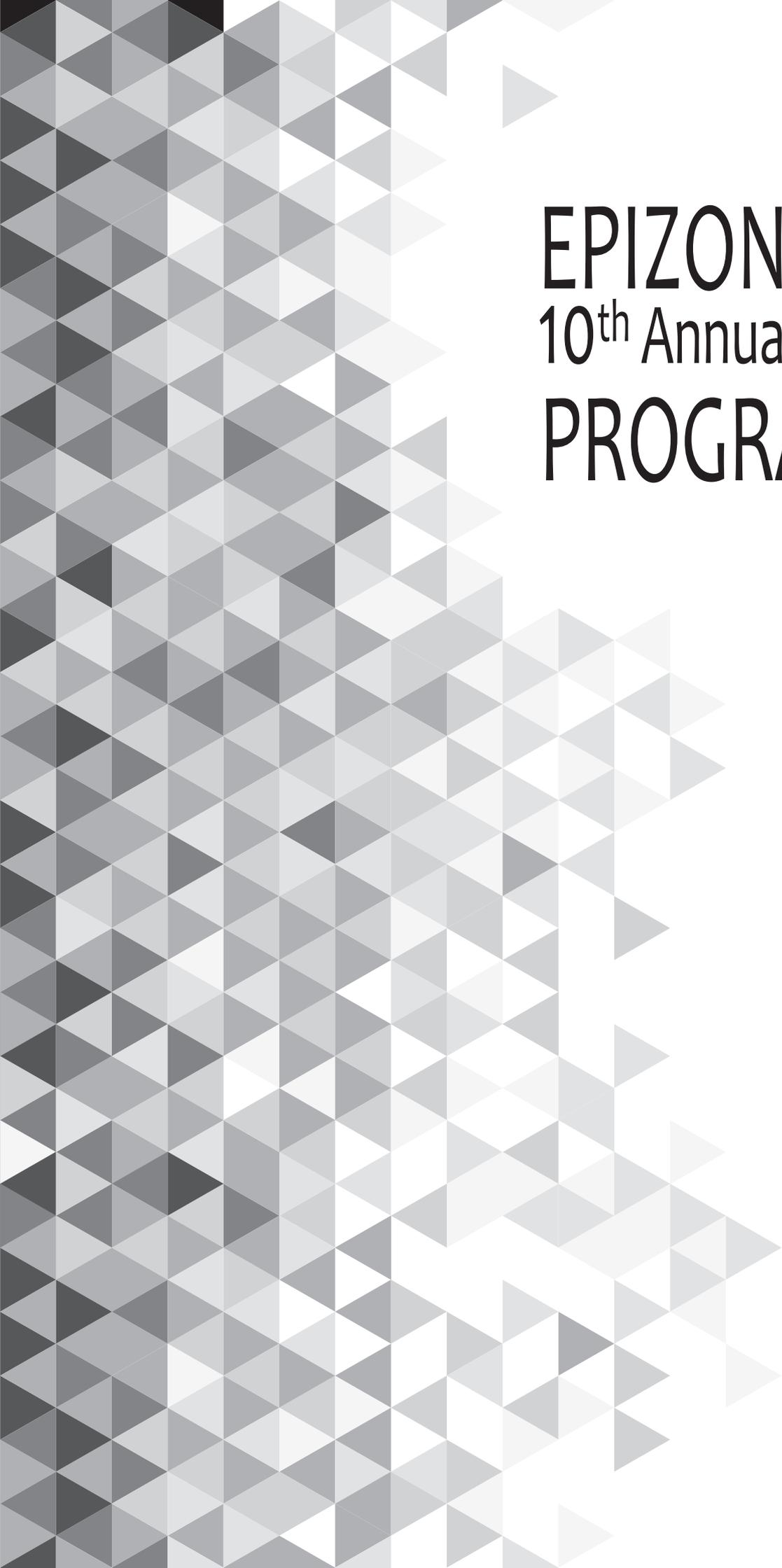
Dr. ALESSIO LORUSSO (IZSAM, Teramo, Italy)

“Bluetongue: a candidate for delisting... ‘yet it moves’”

- Keynote lecture 8

Dr. KRIS DE CLERCQ (CODA-CERVA, Brussels, Belgium)

“Lumpy skin disease: an old disease but a new challenge to Europe”



**EPIZONE**  
10<sup>th</sup> Annual Meeting  
**PROGRAMME**

## Tuesday 27<sup>th</sup> September 2016

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### VENUE NOVOTEL MADRID CENTER

9:00-17:00h	Young epizone room (floor -1)	<b>Young epizone meeting</b> (restricted meeting)
14:00-16:00h	Coordinating forum room (floor -1)	<b>Coordinating forum meeting</b> (restricted meeting)
18:00-20:00h	Floor -1	<b>Registration and poster setting</b>
20:00-22:00h	Welcome area (floor 0)	<b>Spanish welcome cocktail</b>

## Wednesday 28<sup>th</sup> September 2016

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### VENUE NOVOTEL MADRID CENTER

8:00h-onwards REGISTRATION

### PLENARY SESSION (EPIZONE 1 ROOM)

9:00-9:30h	<b>Welcome</b> by <b>WIM VAN DER POEL</b> and <b>VICTOR BRIONES</b> (Carmen Vela, Deputy Minister of R&D, Ministry of Economy and Competitiveness; Manuel Laínez, Director of INIA, to be confirmed)
9:30-10:10h	<b>Opening lecture:</b> Prof. <b>JOSÉ MANUEL SÁNCHEZ-VIZCAINO</b> (Complutense University, Madrid, Spain) “One health: a personal view” (Chairperson: Victor Briones)

## Topic I: Animal health in a changing world

10:10-10:50h	<b>Keynote lecture 1:</b> Dr. <b>ALEXANDER MORROW</b> (DEFRA, UK) “International Research Consortium on Animal Health: Working Together to Address Global Challenges” (Chairperson: Wim Van der Poel)
10:50-11:20h	COFFEE BREAK AND POSTER SESSION
11:20-12:00h	<b>Keynote lecture 2:</b> Dr. <b>JOSUÉ MARTÍNEZ-DE LA PUENTE</b> (Estación Biológica de Doñana-CSIC, Seville, Spain) “Transmission dynamics of zoonotic arboviruses with avian reservoir” (Chairperson: Miguel Angel Jiménez-Clavero)

## Wednesday 28<sup>th</sup> September 2016 (cont) NOVOTEL MADRID CENTER

	EPIZONE 1 ROOM	EPIZONE 2A ROOM
12:00-13:00h	<b>Parallel session I:</b> Diagnostics and Intervention strategies (Chairpersons: Anette Botner and Alejandro Brun)	<b>Parallel session II:</b> Epidemiology&surveillance and Risk assessment (Chairpersons: Emmanuel Albina and Ana de la Torre)
12:00-12:15h	<b>O1.</b> -Screening of tick-borne pathogens in Caribbean ticks using High-throughput qPCR (DOMOTICK Project) (M.Gondard, France)	<b>O9.</b> -Animal Disease Surveillance System in China: the Current Status, Challenges and Opportunities (S.Li, China)
12:15-12:30h	<b>O2.</b> -Multispecie serological assays to detect antibodies specific of Mycobacterium tuberculosis complex in wildlife (A.Ranz, Spain)	<b>O10.</b> -First identification of Mammalian orthoreovirus type 3 in diarrheic pigs in Europe (A.Moreno, Italy)
12:30-12:45h	<b>O3.</b> -Detection of bovine viral diarrhea virus type 2 (BVDV2) causing abortion outbreaks on commercial sheep flocks (L.Elvira, Spain)”	<b>O11.</b> -Virulence of current PEDV-strains in Germany (S.Leidenberger, Germany)
12:45-13:00h	<b>O4.</b> -Efficacy of Suvaxyn CSF Marker (CP7_E2alf) in the presence of pre-existing pestiviral antibodies (S.Blome, Germany)	<b>O12.</b> -Velogenic Newcastle disease virus takes advantage of transmission capabilities to suppress lentogenic virus during coinfection (L.Haijin, France)
13:00-14:00h	<b>LUNCH</b>	
14:00-15:00h	<b>Parallel session I (cont):</b> Diagnostics and Intervention strategies (Chairpersons: Alexander Morrow and Víctor Briones)	<b>Parallel session II (cont):</b> Epidemiology&surveillance and Risk assessment (Chairpersons: Thierry Van den Berg and Fernando Esperón)
14:00-14:15h	<b>O5.</b> -Experimental infection in turkeys with Bagaza virus (R.Villalba, Spain)	<b>O13.</b> -Relationship between landscape characteristics and colony health in Apis mellifera in Tenerife Island (Spain) (S.Barroso, Spain)
14:15-14:30h	<b>O6.</b> -Evaluation of the pathogenicity in grey partridges experimentally infected with Bagaza virus and its potential role as competent host (C.Cano-Gómez, Spain)	<b>O14.</b> -High complexity of West Nile virus lineages and strains detected in the mosquito vector Culex pipiens of northeastern Italy, 2010-2015 (G.Capelli, Italy)
14:30-14:45h	<b>O7.</b> -Divergence in the molecular diagnosis of Porcine Reproductive and Respiratory Syndrome virus (B.Cay, Belgium)	<b>O15.</b> -Concurrent emergence of Usutu and West Nile virus in 2015 in France (S.Lecollinet, France)
14:45-15:00h	<b>O8.</b> -Influence of glycoprotein C on Bovine Herpesvirus-1 virion composition and implications for diagnostics (S. Koethe, Germany)	<b>O16.</b> -Pathogenicity evaluation of twelve West Nile virus strains belonging to four lineages from five continents in a mouse model: discrimination between three pathogenicity categories (E.Perez-Ramirez, Spain)

## EPIZONE 2B ROOM

**Poster presentations on Epidemiology & surveillance and Risk assessment (topics I+II+III)***(Chairpersons: Maria Jenckel and Cristina Cano)*

- 12:00-12:06h P1.** Outbreaks and genetic characterization of porcine epidemic diarrhea (PED) virus in Korea (K.Lee, Korea)
- 12:06-12:12h P2.** Analysing the role of virus populations in chronic infections with classical swine fever virus (M.Jenckel, Germany)
- 12:12-12:18h P3.** Virome characterization of *Culex pipiens* in France through metagenomic analysis (P.Gil, France)
- 12:18-12:24h P4.** Evidence of asymptomatic West Nile virus infections in horses in Central Spain, 2011-2013 (F.Llorente, Spain)
- 12:24-12:30h P5.** Has the evolving cattle transport network in Finland 2009-2015 changed the potential for disease spread? (T.Lyytikäinen, Finland)
- 12:30-12:36h P6.** The potential role of wild pigs in the spread and maintenance of African swine fever at the wildlife-livestock interface in Uganda (K.Stahl, Sweden)
- 12:36-12:42h P7.** A cartographic tool for managing African Swine Fever in Eurasia: mapping wild boar distribution based on the quality of available habitat (J.Bosch, Spain)
- 12:42-12:48h P8.** Evaluation of insect larvae as possible mechanical vectors for transmission of ASFV in wild boar populations (J.H.Forth, Germany)
- 12:48-12:54h P9.** MediLabSecure: Implementing a network of virology and entomology laboratories for a One Health approach of vector-borne and respiratory viruses in the Mediterranean and Black Sea regions (L.Gaayeb, France)
- 12:54-13:00h P10.** Detection and full genome sequencing of Beta-CoV viruses related to Middle East Respiratory Syndrome from two bats in Italy (A.Moreno, Italy)

**13:00-14:00h LUNCH****Poster presentations on Diagnostics and Intervention strategies (topics I+II+III)***(Chairpersons: Saskia Bergervoet and Eva Calvo)*

- 14:00-14:06h P11.** Generation of a recombinant structure of West Nile virus, in a baculovirus system, useful for diagnostic procedures (B.Rebollo, Spain)
- 14:06-14:12h P12.** Infection Dynamics of Respiratory Viruses In Cattle Herds (P.Tuncer, Turkey)
- 14:12-14:18h P13.** Occurrence of Swine Enteric Coronavirus (SeCoV) infection during 2016 within Central Eastern Europe (A.Botner, Denmark)
- 14:18-14:24h P14.** Induction of FMDV- specific T and B cell responses *in vivo* using calicivirus-based virus-like particles (G.Rangel, Spain)
- 14:24-14:30h P15.** Use of baculovirus pseudotyping to characterise the antigenic structure of AHSV-VP2 (M.Aksular, UK)
- 14:30-14:36h P16.** Validation of a new cELISA & qPCR for the diagnosis of African swine fever (L.Comtet, France)
- 14:36-14:42h P17.** Comparative evaluation of the performance of six ELISA tests for the detection of antibodies against African swine fever virus (ASFV) (P. Fernandez-Pacheco, Spain)
- 14:42-14:48h P18.** Generation of African swine fever virus recombinants lacking membrane proteins p22 or pE199L (A.Hubner, Germany)
- 14:48-14:54h P19.** Differential regulation of type I interferon and epidermal growth factor pathways by a Bluetongue virus virulence factor (C.Kundlacz, France)
- 14:54-15:00h P20.** Haematological parameters as a tool to evaluate efficacy of bluetongue virus vaccines in IFNAR(-/-) mice (A.Marín, Spain)

## Wednesday 28<sup>th</sup> September 2016 (cont) NOVOTEL MADRID CENTER

### PLENARY SESSION (EPIZONE 1 ROOM)

#### Topic II: Threats at the European borders

##### 15:00-15:40h Keynote lecture 3:

Prof. AYKUT OZKUL (Ankara University, Turkey):  
 “Current status of PPR, LSD and AKA diseases  
 in Turkey: Risk(s) for European threat”  
 (Chairperson: Jovita Fernández-Pinero)

##### 15:40-16:20h COFFEE BREAK AND POSTER SESSION

##### 16:20-17:00h Keynote lecture 4:

Dr. EMILIANA BROCCHI (IZSLER, Brescia, Italy)  
 “Foot-and-mouth disease viruses on the move:  
 changing epidemiological patterns”  
 (Chairperson: Noemi Sevilla)

#### EPIZONE 1 ROOM

##### 17:00-18:30h Parallel session III:

Diagnostics and Intervention strategies  
 (Chairpersons: Martin Beer and  
 Noemí Sevilla)

17:00-17:15h **O17.**-Concurrent Infection of Bluetongue (BT) and Peste des Petits Ruminants (PPR) in Awassi Sheep in Jordan (N.Hailat, Jordan)

17:15-17:30h **O18.**-Improvement of the protective immune response in swine by co-inoculation of synthetic RNA and an FMDV vaccine. (B.Borrego, Spain)

17:30-17:45h **O19.**-Efficacy and immune responses of DNA and MVA vaccines against Rift Valley fever in sheep (G.Lorenzo, Spain)

17:45-18:00h **O20.**-Replication and shedding of Middle East respiratory syndrome coronavirus (MERS-CoV) in experimentally inoculated pigs and llamas (J.Vergara-Alert, Spain)

18:00-18:15h **O21.**-Structural protein VP2 of African horse sickness virus is not essential for virus replication *in vitro*. (P.van Rijn, Netherlands)

18:15-18:30h **O22.**-The presence of pre-formed antigen in the inoculum of experimental MVA-VP2 vaccines for African horse sickness virus plays an important role in the vaccine immunogenicity. (J.Castillo-Olivares, UK)

#### EPIZONE 2 ROOM

##### Parallel session IV:

Epidemiology&surveillance and Risk assessment  
 (Chairpersons: Wim Van der Poel  
 and Jovita Fernández-Pinero)

**O23.**-Bayesian evolutionary analysis of FMD viruses collected from outbreaks that occurred in Maghreb regions during 2014-2015 (G.Pezzoni, Italy)

**O24.**-Regional evolutionary dynamics of peste des petits ruminants virus in West Africa: influence of livestock trade (A.Bataille, France)

**O25.**-Are camelids and wild boar possible hosts of PPRV? (C.Schulz, Germany)

**O26.**-Rift Valley fever virus and European mosquitoes: vector competence of *Culex pipiens* and *Aedes albopictus*. (A.I.Núñez, Spain)

**O27.**-Outbreak of Epizootic haemorrhagic disease virus in Israel 2015: distribution, duration and infectivity. (N.Golender, Israel)

**O28.**-Animal health risk of legally imported exotic animals into The Netherlands (C.deVos, Netherlands)

20:30-onwards **GALA DINNER AT CASINO GRAN VÍA** (do not forget your original ID/passport!)

Thursday 29<sup>th</sup> September 2016 NOVOTEL MADRID CENTER

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PLENARY SESSION (EPIZONE 1 ROOM)

### Topic III: Current challenges inside Europe

- 9:00-9:40h **Keynote lecture 5:**  
Dr. ALBERTO LADDOMADA  
(IZSSA, Sardinia, Italy)  
*“Animal disease control: which challenges in the next decades?”*  
(Chairpersons: José Manuel Sánchez-Vizcaíno and Marisa Arias)
- 9:40-10:20h **Keynote lecture 6:**  
Dr. MARISA ARIAS  
(INIA-CISA, Valdeolmos, Spain)  
*“African swine fever: An approach of what we are facing”*  
(Chairpersons: José Manuel Sánchez-Vizcaíno and Alberto Laddomada)
- 10:20-11:00h COFFEE BREAK AND POSTER SESSION
- 11:00-11:40h **Keynote lecture 7:**  
Dr. ALESSIO LORUSSO  
(IZSAM, Teramo, Italy)  
*“Bluetongue: a candidate for delisting... ‘yet it moves’”*  
(Chairperson: Javier Ortego)

Thursday 29<sup>th</sup> September 2016 (cont)

NOVOTEL MADRID CENTER

	EPIZONE 1 ROOM	EPIZONE 2 ROOM
11:40-13:10h	<b>Parallel session V:</b> <b>ASF: Diagnostics and Intervention strategies</b> (Chairpersons: <i>Linda Dixon and Carmina Gallardo</i> )	<b>Parallel session VI:</b> <b>BT and other ruminant diseases: Epidemiology&amp;surveillance and Risk assessment</b> (Chairpersons: <i>Ana Moreno and Miguel Angel Jimenez-Clavero</i> )
11:40-11:55h	<b>O29.</b> -Deletion of interferon inhibitory genes from virulent African swine fever virus results in attenuation and induction of protection against challenge (L.Dixon, UK)	<b>O35.</b> -Re-emergence of Bluetongue serotype 8 in France in September 2015 (S.Zientara, France)
11:55-12:10h	<b>O30.</b> -Ornithoros tick salivary, an intrinsic component of vector competence in transmission of African swine fever virus to pigs (M.F.Le Potier, France)	<b>O36.</b> -A Novel Bluetongue Virus in Healthy Goats from Sardinia, Italy (A.Lorusso, Italy)
12:10-12:25h	<b>O31.</b> -Experimental characterization of a recombinant live attenuated African swine fever virus with crossprotective capabilities (F.Rodríguez, Spain)	<b>O37.</b> -Bluetongue: farm risk profile definition by retrospective analysis (F.Loi, Italy)
12:25-12:40h	<b>O32.</b> -African swine fever strain "replaces" attenuated homologous strain after challenge (A.Malogolovkin, Russia)	<b>O38.</b> -Sheep infection studies closely simulating natural transmission suggest vector competence characteristics might influence selection of field reassortment strains of bluetongue virus (K.Darpel, UK)
12:40-12:55h	<b>O33.</b> -Purification and Proteome analysis of African Swine Fever Virus Particles (C.Keßler, Germany)	<b>O39.</b> -Genetically stable infectious schmallenberg virus persists in foetal envelopes of pregnant ewes (N.De Regge, Belgium)
12:55-13:10h	<b>O34.</b> -Role of tetherin restriction factor on African swine fever virus infection (D.Pérez-Nuñez, Spain)	<b>O40.</b> -Cowpox virus-host interactions: Determination of defined virulence factors (A.Franke, Germany)
13:10-14:10h	<b>LUNCH</b>	

14:10-14:50h	<b>Keynote lecture 8:</b>	
	Dr. KRIS DE CLERCQ (CODA-CERVA, Brussels, Belgium) “Lumpy skin disease: an old disease but a new challenge to Europe” (Chairperson: Alejandro Brun)	
	<b>EPIZONE 1 ROOM</b>	<b>EPIZONE 2 ROOM</b>
14:50-16:20h	<b>Parallel session VII:</b>	<b>Parallel session VIII:</b>
	ASF: Epidemiology&surveillance and Risk assessment (Chairpersons: Marie Frederique LePotier and Marisa Arias)	BT and other ruminant diseases: Diagnostics and Intervention strategies (Chairpersons: Stephan Zientara and Javier Ortego)
14:50-15:05h	O41.-Serological survey and geographical analysis of its results in an ASF endemic territory (F.Feliziani, Italy)	O47.-Efficacy of BTVPUR ALSAP 8 in sheep after a single injection (C.Hamers, France)
15:05-15:20h	O42.-Characterization of pig production in Sardinia and its relationship with ASF presence (C.Jurado-Dfiaz, Spain)	O48.-Bluetongue virus infection of bovine monocytes is altered in the presence of Culicoides sonorensis salivary proteins (L.Cooke, UK)
15:20-15:35h	O43.-Tandem repeat sequence in the intergenic region MGF 505 9R/10R is a new marker of the genetic variability among ASF Genotype II viruses (A.Igolkin, Russia)	O49.-Recall T cell responses to Bluetongue virus produce a narrowing of the T cell repertoire (J.M.Rojas, Spain)
15:35-15:50h	O44.-First evidence of an attenuated phenotype of genotype II African swine fever virus in Estonia (L.Zani, Germany)	O50.- First identification and isolation of Camelpox virus in Israel (O.Erster, Israel)
15:50-16:05h	O45.-Experimental pig-to-pig transmission study with a recent European African Swine Fever virus isolate (A.S.Olesen, Denmark)	O51.-DNA Vaccination Regimens Against Schmallenberg Virus Suggest Two Novel Targets For Immunization (H.Boshra, Spain)
16:05-16:20h	O46.-In vivo experimental studies of genotype II African swine fever virus (ASFV) isolates currently circulating in two Estonian counties (C.Gallardo, Spain)	O52.-Development of high resolution melt (HRM) analyses for DIVA and genotyping of Lumpy skin disease virus (LSDV) and Bovine ephemeral fever virus (BEFV) (O.Erster, Israel)
16:20-16:45h	<b>CLOSING SESSION (EPIZONE 1 ROOM)</b>	

## Young Epizone Programme Tuesday 27<sup>th</sup> September 2016

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- 09:00-09:30h**    **Registration for the YoungEpizone members**
- 09:30-10:00h**    **Welcome**
- 10:00-11:00h**    **Icebreaking game**
- 11:00-11:30h**    **Coffee break in the same room**
- 11:30-13:00h**    **Workshop of “Promoting Research” given by **Andres Montero****  
Head of the European Projects Office at the Spanish National Institute  
for Agricultural and Food Research and Technology (INIA)
- 13:00-14:30h**    **Lunch at the Hotel restaurant**
- 14:30-17:00h**    **Presentation of the speakers**
- Prof. Luis Enjuanes**  
*(National Center of Biotechnology)*
- Prof. Esperanza Gomez-Lucia**  
*(Universidad Complutense of Madrid)*
- Dr. Alicia Urniza**  
*(Zoetis Spain)*
- Round table discussion**



**ABSTRACTS**

**TOPIC I**

Animal health in  
a changing world

**PARALLEL SESSION I**

Diagnostics and  
Intervention strategies

**ORAL  
PRESENTATIONS**

## ORAL 1: Screening of tick-borne pathogens in Caribbean ticks using High-throughput qPCR (DOMOTICK Project)

Gondard Mathilde<sup>1,3</sup>, Delannoy Sabine<sup>2</sup>, Devillers Elodie<sup>1</sup>, Valérie Pinarello<sup>3,4</sup>, Liza Al Shikhley<sup>1</sup>, Marc Eloit<sup>5</sup>, Vayssier-Taussat Muriel<sup>1</sup>, Albina Emmanuel<sup>3,4</sup>, Moutailler Sara<sup>1</sup>,

<sup>1</sup>BIPAR JRU, Animal Health Laboratory, ANSES, Maisons-Alfort, France; <sup>2</sup>IdentyPath Platform, Food Safety Laboratory, ANSES, Maisons-Alfort, France; <sup>3</sup>CIRAD, UMR CMAEE, F-97170 Petit-Bourg, Guadeloupe, France, <sup>4</sup>INRA, UMR 1319 MOISA, F-34000 Montpellier, France; <sup>5</sup>Pathogen Discovery Laboratory, Biology of Infection Unit, Inserm U1117, Institut Pasteur, Paris, France.

**Keywords:** tick borne pathogens, surveillance, Caribbean, microfluidic real time PCR

Among hematophagous arthropods, ticks transmit the greater variety of pathogens of public health and veterinary importance. Due to socio-economic and environmental factors, such as human practices, increased travel, global market, global warming, and environmental changes, the incidence of tick-borne diseases in both humans and animals is increasing worldwide, leading to a need for extended surveillance tools.

Recently, in Europe a large scale epidemiological study was conducted on 19,474 *Ixodes ricinus* nymphs collected from five European countries using a powerful new high-throughput approach to screen tick-borne pathogens (Michelet et al., 2014). The technology used in this study is a microfluidic high-throughput Taqman realtime PCR (BioMark™ dynamic arrays, Fluidigm Corporation), allowing the simultaneous detection of 25 bacterial, 12 parasitic and 22 viral species across 94 samples of ticks. They successfully determined the prevalence of expected tick-borne pathogens (*Borrelia burgdorferi sensu lato*, *Babesia divergens*, *Tick-Borne Encephalitis virus*, etc.), unexpected (*Borrelia miyamotoi*, *Nairo-like virus*) or rare (*Bartonella henselae*, *Eyach virus*) tick-borne pathogens. This surveillance method represents a major improvement in epidemiological studies, able to facilitate comprehensive testing of tick-borne pathogens in various samples, and which can also be customized for the survey of emerging diseases in different areas of the world.

Caribbean, are a risk area for the (re)-emergence of vector-borne diseases. Population in Caribbean is in expansion, leading to a growing food demand. Maintaining a healthy livestock industry is crucial but often difficult to manage because of tick-borne diseases such as anaplasmosis, ehrlichiosis, or babesiosis. The Caribbean is also a world interface, with numerous air and maritime networks (tourism, and animals trade) leading to a serious risk of dispersion of tick and their tick-borne pathogens in this area. Moreover, few reports are available on tick-borne diseases in the Caribbean and are only focusing on livestock pathogens such as *Ehrlichia ruminantium*, *Babesia (bovis and bigemina)* and *Anaplasma marginale*.

In this context, the DOMOTICK project was designed to apply the high-throughput realtime-PCR technology for a large scale screening of tick-borne pathogens in the Caribbean. Methods included a comprehensive analysis of the literature on tick-borne pathogens, as well as pathogen detection by RNA-sequencing on nucleic acids extracted from ticks collected in Guadeloupe and Martinique to determine which pathogens need to be included in this new high-throughput technology. Preliminary results obtained from NGS analysis suggest that these ticks may harbor more pathogenic microorganisms than the currently known in the Caribbean, such as *Rickettsia* and *Borrelia* species of public health importance. Up to now, 40 bacterial species have been listed, including the genera *Anaplasma*, *Ehrlichia*, *Bartonella*, *Borrelia*, *Rickettsia*, *Mycoplasma*, *Francisella*, *Coxiella*, *Aegyptianella*, *Mycoplasma*; 14 parasites species, belonging to the genera *Babesia*, *Theileria*, *Hepatozoon*, *Leishmania*, *Rangelia vitalii*, *Cytauxzoon felis*; and 25 arboviruses belonging to viral families of *Orthobunyavirus*, *Phlebovirus*, *Nairovirus*, *Asfivirus*, *Thogotovirus*, *Flavivirus*, *Coltivirus* and *Orbivirus*.

The detection tool will be validated with tick samples collected on various vertebrate hosts through the Caribbean islands thanks to the CaribVet network, and to local veterinarians. Results obtained will be used to do an exploratory epidemiological study on the tick-borne pathogens circulating in the Caribbean.

### References:

Michelet L, Delannoy S, Devillers E et al. High-throughput screening of tick-borne pathogens in Europe. *Front. Cell. Infect. Microbiol.* 4, 103 (2014).

## ORAL 2: Multispecies serological assays to detect antibodies specific of *Mycobacterium Tuberculosis* complex in wildlife

Gonzalez I.<sup>1</sup>, Ranz A.<sup>1</sup>, Lopez L.<sup>1</sup>, Ruiz T.<sup>1</sup>, Tapia I.<sup>1</sup>, Fresco A.<sup>1</sup>, Gortazar C.<sup>2</sup>, Casal C.<sup>3</sup>, Navarro J.A.<sup>4</sup>, Venteo A.<sup>1</sup> and Rueda P.<sup>1</sup>

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**Keywords: Tuberculosis, Diagnostics, Wildlife**

For years, the main target in the tuberculosis (TB) control has been cattle; however, other livestock such as goats and pigs, and wildlife (including wild boars, badgers, deer, camelids) can be reservoirs of members of the *Mycobacterium tuberculosis* complex and, therefore, contribute to the maintenance and spread of the disease. However, the diagnosis of TB is still a challenge due to the limitation of *in-vivo* diagnostic tests and the limited sensitivity and specificity of available laboratory tools.

Recent studies showed estimations of 50 million cattle infections with *M. bovis* worldwide at a cost to the agricultural community of 2-3 billion € per year. Under these circumstances, an improved diagnostic method will be very important in the future control of the disease.

The major aim of this study was to develop and validate two multispecies assays based on Double Recognition geometry (ELISA and Lateral Flow assay techniques) to detect specific antibodies of *M. bovis*. A double recognition ELISA (DR-ELISA) based on the recombinant MPB-83 antigen expressed in the baculovirus system, was used to perform serological studies in different animal species (pigs, feral pigs, goats, wildlife...). In parallel, using the same recombinant antigen and the same strategy of double recognition, a new approach was developed based on Lateral-flow devices (LFDs) technology. This represents a well-established technology appropriate to use it in a wide variety of point-of-care (POC) or field-use applications.

To validate these assays, sera from different species were analysed: 170 zoo animals, 616 goats, 61 badgers, 335 deer, 451 alpaca, 351 wildboars, 139 fallow deer, 183 Iberian pigs, 106 Nebrodi black pigs and 181 domestic pigs. Moreover, cross reactivity with *M. avium subsp. paratuberculosis* was determined. For this purpose, sera and plasma of 9 cows and 28 goats positive to paratuberculosis (PTB) and and negative to TB were used.

Results showed that both assays detect specific TB antibodies. None of them showed cross-reaction with PTB antibodies. Depending on the analysed species, sensitivity and specificity of ELISA-DR range between 50-98% and 87-100%, respectively. Concerning to LFA, values range between 53-100% and 60-100% respectively.

As conclusion, the data obtained with both assays show variable values of sensitivity and specificity, depending on the species. Nevertheless, these results give us an idea of their potential as complementary tools to intradermal tuberculin skin test, since the parallel use of both techniques increases the possibility to detect positive animals. Moreover, the pen-side test offers advantages that should be taken into consideration: it is rapid, economic and simple-to use diagnostic tool, since it does not require any kind of instrumentation and the results are interpreted visually.

(FP7-KBBE-2007-1-3-04) Strategies for the eradication of bovine tuberculosis (TB-STEP) and (FP7-KBBE-2013-613799) Integrated solutions for tuberculosis control in animals combining vaccination and multi-species diagnostics (WildTBvac).

## ORAL 3: Detection of bovine viral diarrhoea virus type 2 (BVDV-2) causing abortion outbreaks on commercial sheep flocks

Elvira L.<sup>1</sup>; Fernandez M.<sup>2</sup>, Gutierrez J.<sup>1</sup>, Antón Esnal.<sup>3</sup>, Benavides J.<sup>2</sup>, Pérez V.<sup>2</sup>, de la Torre A.<sup>4</sup>, Álvarez M.<sup>5</sup> and Esperón F.<sup>4</sup>

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**Keywords:** bovine viral diarrhoea virus, sheep, genotyping, abortion outbreaks, natural infections.

### Introduction

Pestivirus genus is a member of Flaviviridae Family, comprising four main virus species: Bovine viral diarrhoea virus (BVDV, including BVDV-1 and BVDV-2), classical swine fever virus (CSFV) and border disease virus (BDV). Although classification refers to the host species they were recovered, numerous investigations have proven that pestiviruses are not highly host-specific and both BVDV and BDV can cross-infect cattle, sheep and goat (Paton, 1995; Pratelli et al., 1999). Previous studies have demonstrated that sheep can be infected with BDV, BVDV-1 and BVDV-2, without complete pathological description (Paton, 1995; Vilcek et al., 1997; Mishra et al., 2007; Braun et al., 2013; Evans et al., 2015). During 2015, severe abortion outbreaks identified as “border disease-like”, based on serology and/or RNA detection by a pan-pestivirus RT-PCR without sequence identification, were diagnosed. We have investigated four of these cases.

### Case description

Four different sheep farms with different location (Toledo, Salamanca, León and Valladolid), aptitude (dairy and meat), and breed (Assaf, Merina and Castellana) were affected. The outbreak consisted on embryo absorption of the fetus, abortions and stillbirths or alive lambs showing nervous symptoms and malformations at lambing.

A complete pathological study was performed including gross pathology, histopathology and immunohistochemical staining of pestiviral antigen. In flock A, an extra label vaccination with Bovilis BVD (MSD AH, 1 ml per animal) was applied to control viral circulation and minimize future problems (Meyer et al., 2012; Poncelet et al. 2007), and next lambing season 270 normal lambing occurred (84% fertility) with only one abortion due to same etiology. The main infectious abortion etiologies were ruled out performing a complete abortion panel: *Brucella spp.*, *Salmonella spp.*, *Campylobacter spp.*, *C. burnetii*, *T. gondii*, *Chlamydomphila spp.*, Schmallenberg and pestivirus. Pestiviral antigen was positive on fetal pool samples from all cases. Molecular detection of pestivirus was performed by a pan-pestivirus RT-PCR which amplifies a 288 bp of the 5' untranslated region (5'-UTR) previously published (Vilcek et al., 1997). Later on, RT-PCR products were sequenced and compared with those from the Genbank. A phylogenetic tree was inferred by the Maximum-Likelihood algorithm with a bootstrap frequency of 1000 replicates. Reference sequences of Border Disease, BVDV-1 as well as all subtypes of BVDV-2 were included.

### Results

Laboratory test for abortifacient agents in samples from flocks A and D, ruled out the main causes of abortion affecting sheep flocks, with the exception of pestivirus infection. All studied lambs from flocks B, C and D showed a variable degree of hydrancephaly. Main gross lesions were thinning of the cortex of both hemispheres, with disappearance of the cerebral convolutions, and cerebellar atrophy. Immunohistochemical labelling of pestivirus antigen (15c5 antibody) showed intracellular labelling of the endothelium and neurons.

Amplification of pestiviral nucleic acids in all samples tested from fetal or lamb organ pools in flocks A, B, C and D, and sequencing of the obtained products were in all cases classified as BVDV-2b.

### Conclusions

As conclusion, BVDV-2 could be underdiagnosed in ewes abortions; surveillance of pestivirus in this farming species should be carried out by molecular techniques with further sequence identification. More studies in order to develop and to test vaccination programs in sheep are also required.

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- 5 Braun et al 2013. Schweizer Archiv für Tierheilkunde 5, 293-298.
- 6 Evans et al. 2015 Small Ruminant Research. 123, 314-319.
- 7 Meyer et al 2012. Journées Nationales GTV, 395-400.
- 8 Poncelet et al. 2007 GTV, Nantes. 699-701

## ORAL 4: Efficacy of Suvaxyn CSF Marker (CP7\_E2alf) in the presence of pre-existing pestiviral antibodies

Dräger, Carolin<sup>1</sup>; Schröder, Charlotte<sup>2</sup>; König, Patricia<sup>1</sup>; Tegtmeyer, Birthe<sup>1</sup>; Beer, Martin<sup>1</sup>; Blome, Sandra<sup>1</sup>

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**Keywords: Classical swine fever, marker vaccine, CP7\_E2alf, efficacy, BVDV antibodies**

Classical swine fever (CSF) is still one of the most important viral diseases of pigs worldwide and outbreaks are notifiable to the OIE. The different control options include (emergency) vaccination, preferably with a vaccine that allows differentiation of infected from vaccinated animals (DIVA principle).

Recently, the chimeric pestivirus “CP7\_E2alf” (Suvaxyn® CSF Marker, Zoetis) was licensed as live attenuated marker vaccine by the European Medicines Agency (EMA). The vaccine virus “CP7\_E2alf” is based on the cytopathogenic Bovine viral diarrhoea virus (BVDV) strain “CP7” expressing the E2 glycoprotein of CSF virus (CSFV) strain “Alfort/187”.

In the context of risk assessments for an emergency vaccination scenario, the question has been raised whether pre-existing anti-pestivirus antibodies, especially against the vaccine backbone BVDV-1, would interfere with “CP7\_E2alf” vaccination and the accompanying DIVA diagnostics.

To answer this question, a vaccination-challenge-trial was conducted with Suvaxyn® CSF Marker and the “gold-standard” of live-modified CSF vaccines C-strain (RIEMSER® Schweinepestvaccine) as comparator. Pre-existing antibodies against BVDV-1 were provoked in a subset of animals through intramuscular inoculation of a recent field isolate from Germany (two injections with an interval of two weeks). Twentyseven days after the first injection, intramuscular vaccination of pre-exposed and naïve animals with either “CP7\_E2alf” or C-strain “Riems” was performed. Seven days later, all vaccinated animals and two additional controls were oro-nasally challenged with highly virulent CSF virus (CSFV) strain Koslov.

It was demonstrated that pre-existing BVDV-1 antibodies do not impact on the efficacy of live attenuated vaccines against CSF. Both C-strain “Riems” and marker vaccine “CP7\_E2alf” were able to confer full protection against highly virulent challenge seven days after vaccination. However, slight interference was seen with serological DIVA diagnostics accompanying the vaccination with CP7\_E2alf. Amended sample preparation and combination of test systems was able to resolve most cases of false positive reactions. However, in such a co-infection scenario, optimization and embedding in a well-defined surveillance strategy is clearly needed.

## ORAL 5: Experimental infection in turkeys with Bagaza virus

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Villalba, Rubén<sup>1</sup>; Varo, José Ignacio<sup>1</sup>; San Miguel, Elena<sup>1</sup>; Ruano M<sup>a</sup> José<sup>1</sup>; Durán, Manuel<sup>1</sup>; Agüero, Montserrat<sup>1</sup>

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**Keywords: Bagaza virus, Flavivirus, turkey**

The geographical distribution of various flaviviruses has increased in recent years in different parts of the world. As most relevant animal health and public health example it is the detection of West Nile Fever virus (WNV) in North America and the Mediterranean basin.

In this context, in September 2010 it was detected and isolated Bagaza (BAGV) virus at the Central Veterinary Laboratory (LCV), National Reference Laboratory for viral zoonosis transmitted by arthropods, associated with an unusual mortality of partridges and pheasants in the south of Spain. At that time in the LCV a specific RT-PCR for detection of BAGV was developed for surveillance purposes.

Experimental infections in both turkeys and hens was planned in the LCV using BAGV isolated in 2010 in order to determinate the relevance of this virus in the animal health field. This work describes and discusses the results of the experimental infection in turkeys.

The first objective of the experimental infection in turkeys was to determinate if BAGV isolated in Spain was able to produce disease in turkeys and to estimate mortality and morbidity caused by this virus in this species, under experimental conditions. The second objective was to evaluate the transmission path by direct contact, using an un-infected group in direct contact with infected animals.

To get the objectives, the presence of clinical signs and also temperature and loss weight was evaluated. Besides it, a laboratory analysis was carried out using both agent detection and serological test. BAGV genome detection by specific RT-PCR was performed in blood, swabs and feather samples along the experiment, and additionally on several organs after the slaughter of the animals. Virus isolation in cell culture was carried out in a few samples to confirm the presence of pathogen producing disease. Seroneutralization test in sera samples was done along the experiment to evaluate the presence of BAGV specific antibodies.

The results obtained confirm that BAGV produces a mild disease in turkeys under these experimental conditions.

## ORAL 6: Evaluation of the pathogenicity in grey partridges experimentally infected with Bagaza virus and its potential role as competent host

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**Keywords:** Bagaza, grey partridge, flavivirus, game birds, pathogenicity.

Bagaza virus (BAGV), synonymous to Israel turkey meningoencephalomyelitis virus (ITV), is a mosquito-borne epornitic virus belonging to the Ntaya serocomplex within the family *Flaviviridae*. BAGV (or ITV) has been found to circulate in sub-Saharan Africa, Israel and India. In 2010, its geographical range extended to Europe when BAGV was first isolated in Spain from sick game birds (red-legged partridges and ring-necked pheasants)<sup>1</sup>. The detection of specific neutralizing antibodies against BAGV in 2011-2012 in game birds from the same area suggested sustained circulation of the virus in Southern Europe in that period<sup>2</sup>.

Experimental infection in red-legged partridges showed that this species is highly susceptible to BAGV infection and disease, and able to spread this virus by direct contact<sup>3</sup>.

The present study aimed at evaluating the pathogenic potential of BAGV in another important European game bird, the grey partridge (*Perdix perdix*) and its possible role as reservoir host.

Five month-old grey partridges were distributed in experimental groups composed of 50% males and 50% females and housed in the biosafety level 3 (BSL-3) animal facilities at INIA-CISA. One group (n=10) was inoculated subcutaneously with an infectious dose of Bagaza virus. Four non-infected partridges were caged in contact with this inoculated group. As control group, 10 animals were sham-inoculated with an equivalent volume of PBS supplemented with 0.2% of albumin and kept in a separated cage. Clinical signs were recorded daily. Body weight, viral load in blood, feathers, oropharyngeal and cloacal swabs (by real-time RT-PCR<sup>2</sup>) and neutralizing antibody titres (by VNT) were monitored for up to 18 days post-infection (dpi). Another group of 12 birds was inoculated with Bagaza virus, housed in a separated cage and euthanized at different dpi to study viral dissemination throughout the body.

All inoculated partridges developed a systemic infection and most were clinically affected, showing weakness, apathy, unresponsiveness, ruffled feathers and ataxia. Also, a significant body weight loss was recorded in the infected with regard to the control group. Six individuals from the infected group evolved favourably while the remaining developed severe symptoms that can be considered irreversible (from partial to complete paralysis and/or a weight lost higher than 30%) and were humanely euthanized. The pathogenicity observed in this species was similar to previous results obtained in red-legged partridges<sup>3</sup>. However, in contrast to what was observed in red-legged partridges, in-contact grey partridges did not acquire the infection during the experiment. Viremia levels in grey partridges were lower and less persistent than those found in red-legged partridges<sup>3</sup>. Infected partridges developed neutralizing antibodies whereas neither contact nor control birds seroconverted.

In conclusion, grey partridges proved to be highly susceptible to BAGV infection but seemed to play a less important role as competent hosts for mosquito-borne and direct contact BAGV transmission than red-legged partridges. On the other hand, it seems highly likely that the observed clinical effects and the poor body condition would have a higher impact in mortality, for instance through increased susceptibility to predation, in natural conditions.

### Acknowledgements:

We thank to the French National Hunting Federation for the financial support (FNC-PSN-PR1-2013) and to Denis Bourasseau and GIBOVENDEE who donate the grey partridges to this study.

### References

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## ORAL 7: Divergence in the molecular diagnosis of Porcine Reproductive and Respiratory Syndrome virus

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### Introduction:

The Porcine Reproductive and Respiratory Syndrome virus (PRRSv) is a disease endemic on most porcine herds causing significant economic impact in the pig sector. Two genotypes were identified nearly simultaneously in Europe and the USA with antigenic differences that lead to two distinct genotypes: the European type (genotype 1) and the North American type (genotype 2).

### Materials and Methods:

The PRRSv infection status has been followed in Flemish voluntarily participating herds in 2015. Forty two pools of 3 sera samples previously tested positive for PRRSv by means of the vetMAX PRRSV EU/NA real-time PCR assay (LSI) were retested individually (n=126) for PRRSv by 3 different RT-PCR tests including a conventional RT-PCR targeting the ORF5 region; a Sybr green RT-PCR assay targeting the ORF7 region and the commercial VIROTYPE real-time RT-PCR (Qiagen). The ORF5 amplicons were sequenced in order to confirm the present viral genotype. The Sybr green and Virotype PCR assays allow discrimination between PRRSv genotypes on basis of melting temperature (T<sub>m</sub>) or probes specificity respectively.

### Results:

Among the samples only 44% were identified as positive by the 3 molecular assays. Considering the assays individually 84% of samples gave positive result with the conventional PCR, 68% with the Sybr green assay and 65% with the Virotype PCR assay. Sequencing performed on partial ORF 5 amplicons (n=106) indicated the predominance of the genotype 1 (91%). The European type (n=96) was confirmed for 37% of the samples by the Sybr green assay and for 53% by the Virotype PCR assay whereas 13 and 12% of them were identified as genotype 2 by the same tests respectively and for 15 other % the T<sub>m</sub> obtained in Sybr green assay did not allow a distinction between genotypes 1 and 2. In parallel the Sybr green and the Virotype PCR assays have confirmed the North American genotype identification obtained by sequencing for 2 and 3 out of the 4 samples whereas one sample was identified as European strain by both tests. Considering the results of pool testing by the fourth PCR assay (vetMAX, LSI) the accuracy of genotype identification was 83% for the conventional PCR assay; 58% for the Sybrgreen assay and 78% for the Virotype PCR assay.

### Conclusion:

The divergence observed between the different molecular assays is problematic as it demonstrated that none of the tested methods was efficient to ensure a confident detection of virus presence in herds. Moreover it appears that both the melting temperature range from the Sybr green assay and the specificity of the probes could no more be considered as a confident criteria for genotype identification

## ORAL 8: Influence of glycoprotein C on Bovine Herpesvirus-1 virion composition and implications for diagnostics

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**Keywords: Bovine Herpesvirus-1, diagnostics.**

Bovine herpesvirus type 1 (BoHV-1) is a member of the genus *Varicellovirus* within the subfamily *Alphaherpesvirinae* of the family *Herpesviridae*. It is an economically important agent that induces respiratory and genital tract disease in cattle. Disease control is often based on marker vaccination with glycoprotein E (gE) deleted vaccines. The accompanied marker diagnostics to exclude infection with a BoHV-1 field virus is based on the detection of glycoprotein E-specific antibodies. gE, together with gI, forms a functional complex that facilitates direct cell-to-cell spread and is an important virulence determinant acting on neuronal spread *in vivo*.

So far, gE interaction partners other than gI have not been identified for BoHV-1. Therefore, the objective was to screen for viral interaction partners of BoHV-1 gE and to evaluate their impact on gE-ELISA performance.

To this purpose, recombinant BoHV-1 mutants expressing Twin-Strep- or FLAG-tagged gE proteins were generated. gE-containing complexes were purified from the supernatant of infected cells by co-immunoprecipitation and analysed by SDS-PAGE followed by Western blotting.

Western Blot analysis of the co-precipitates confirmed the expected interaction of tagged gE with gI and revealed interactions of BoHV-1 gE with gM and gC but not with viral protein 22 (VP22), gD and gB. To assess whether the interaction of gC with gE has an impact on the gE-antigen-antibody binding properties in a gE-specific ELISA, gC-deleted mutant virus was coated on ELISA plates. Interestingly, the BoHV-1 gE and gE/gI complex-specific antibodies showed enhanced optical density (OD) ELISA values when gC was absent. In contrast, absence of gM had no influence on the gE-antibody binding properties. Preliminary Western blot analyses indicated a relative enrichment of gE in virions of the BoHV-1 gC deletion mutant in comparison to wildtype BoHV-1.

In conclusion, novel interactions of BoHV-1 glycoproteins were identified, and it could be also demonstrated that the use of protein extracts from a gC-deleted mutant improves the signal of a gE-specific ELISA. Further studies will be conducted to elucidate the protein composition of BoHV-1 gC deletion mutant virions in comparison to wildtype BoHV-1 in detail. However, the presented BoHV-1 gC deletion mutant has the potential as a convenient Ag to improve BoHV-1 gE-ELISA diagnostics.



**ABSTRACTS**

**TOPIC I**

Animal health in  
a changing world

**PARALLEL SESSION II**

Epidemiology & surveillance  
and Risk assessment

**ORAL  
PRESENTATIONS**

# ORAL 9: Animal Disease Surveillance System in China: the Current Status, Challenges and Opportunities

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**Keywords: Animal disease, Surveillance, China**

Through the development of China since 1990s, its demand for more, better and safer animal products are growing fast. According to FAO statistics in 2014, China is ranking the world's number one producer of live pigs, poultry, sheep and goats, and the third biggest producer of cattle, which increased 20-30% since the year of 2000. In the meantime, the rapid growth of animal production poses a big pressure to animal disease prevention and control in China. To worsen the situation, the occurrence of a series of public health emergencies closely related to animals, such as SARS and H5N1 HPAI rang the alarm bells and Chinese authority called for a more efficient and efficient surveillance system for animal diseases.

For many years, FAO is involved in China's establishment of its surveillance system through various collaborative projects targeting on specific diseases and long-term capacity building. Great progress has been made and there are still many problems yet to be solved. This presentation will cover the a description of current animal disease surveillance system in China, which will involve relevant policies, systematic structure, key institutions and their mandates, surveillance activities, as well as current challenges and trend of development for the future. It aims to help EU researchers to gain a better understanding of China's animal disease surveillance system, which may lead to more successful collaborations between EU and China in this area.

## ORAL 10: First identification of Mammalian orthoreovirus type 3 in diarrhetic pigs in Europe

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**Keywords: Mammalian Orthoreovirus, Porcine Epidemic Diarrhoea, swine, bats, Italy**

Mammalian orthoreoviruses (MRVs) have long been considered non-pathogenic, although mild respiratory and enteric diseases have occasionally been reported in young animals and children. Several evidences have recently shown that MRVs can cause severe diseases. Cases of neonatal diarrhea and neurological signs in children were associated with both MRV2 and MRV3 in Europe and North America. MRV3 has been recently isolated from piglets with severe diarrhea and respiratory signs in China, Korea and the US, also in association with Porcine Epidemic Diarrhea (PEDV), Transmissible Gastroenteritis (TGEV) and Porcine A-C rotaviruses. In particular, MRV3 was proven to be pathogenic to pigs. In 2015, an important epidemic of PED occurred in Italy caused by S-INDEL strains very closely related to each other and to the US Ohio851 strain. A first attempt to isolate PEDV using VERO cells was conducted collecting 11 swine fecal samples at the beginning of the epidemic, between February and March 2015. Cytopathic effect (CPE) was detected after the first cell passage in one sample. Cell culture supernatant was examined through Electron Microscopy (EM) and RNA was extracted from cell culture and fecal sample and analyzed for MRV using RT-PCRs targeting the L1 and S1 genes. The EM examination of CPE positive cell culture and molecular analyses confirmed the presence of MRV. Full genome sequencing of the isolated virus (MRV3/Sw/It/224660-4/2015) was conducted using an Illumina MiSeq platform. Based on S1 phylogeny, the novel swine MRV strain belonged to the lineage III of the MRV3 and was closely related to human and bat strains and two US porcine MRV3s recently described as associated to PED outbreaks (Figure 1). It shares the highest nucleotide identity with MRV3 SI-MRV01 (98,4%) detected from a child with acute gastroenteritis in Slovenia and with a bat isolate T3/Pipistrellus Kuhl/It/5515-2/2012 (98,2%). The S2 and S3 phylogeny indicated monophyletic groups with US and Chinese pig MRV3 strains and human T1L whereas the S4 revealed a separated group formed by Italian, US and Chinese pig MRV3 strains. The other segments L1, L3, M1, M3, S2, S3 and S4 of the Italian strain were related to US porcine MRV3. Interestingly, the last segments L2 and M2 were closely related to MRVs of bat origin, MRV3 and MRV1 respectively.

We here describe the finding of a MRV3 associated with a PED outbreak in Italy. A similar association was reported in the US during the 2013-2015 PED epidemic, with mortality up to 100% in affected farms. Porcine MRV3s, placed in lineage IV and frequently associated with other enteric viruses, were also described in pigs suffering diarrhea in South Korea. To highlight that Italian and US porcine MRV3 associated to PED outbreaks were characterized by a S1 gene highly related to European bat strains and both fall into lineage III, differently from the Asian MRV3 porcine isolates. The study of potential synergic effects between PEDV and MRV3 is crucial, considering the PED impact on the swine industry. Based on the L2 and S1 genetic distances, it appears that the swine and bat Italian MRV3 are highly correlated. Such evidence arises questions on the epidemiological link between pigs and Kuhl's pipistrelle common in urban environments. The absence of data on the MRVs distribution and genetic characteristics in Europe prevents any hypothesis on the most likely epidemiological links between bats, pigs and humans. The distribution of MRV3 among pigs and bats could probably be widespread in Europe, although it still needs to be further investigated.

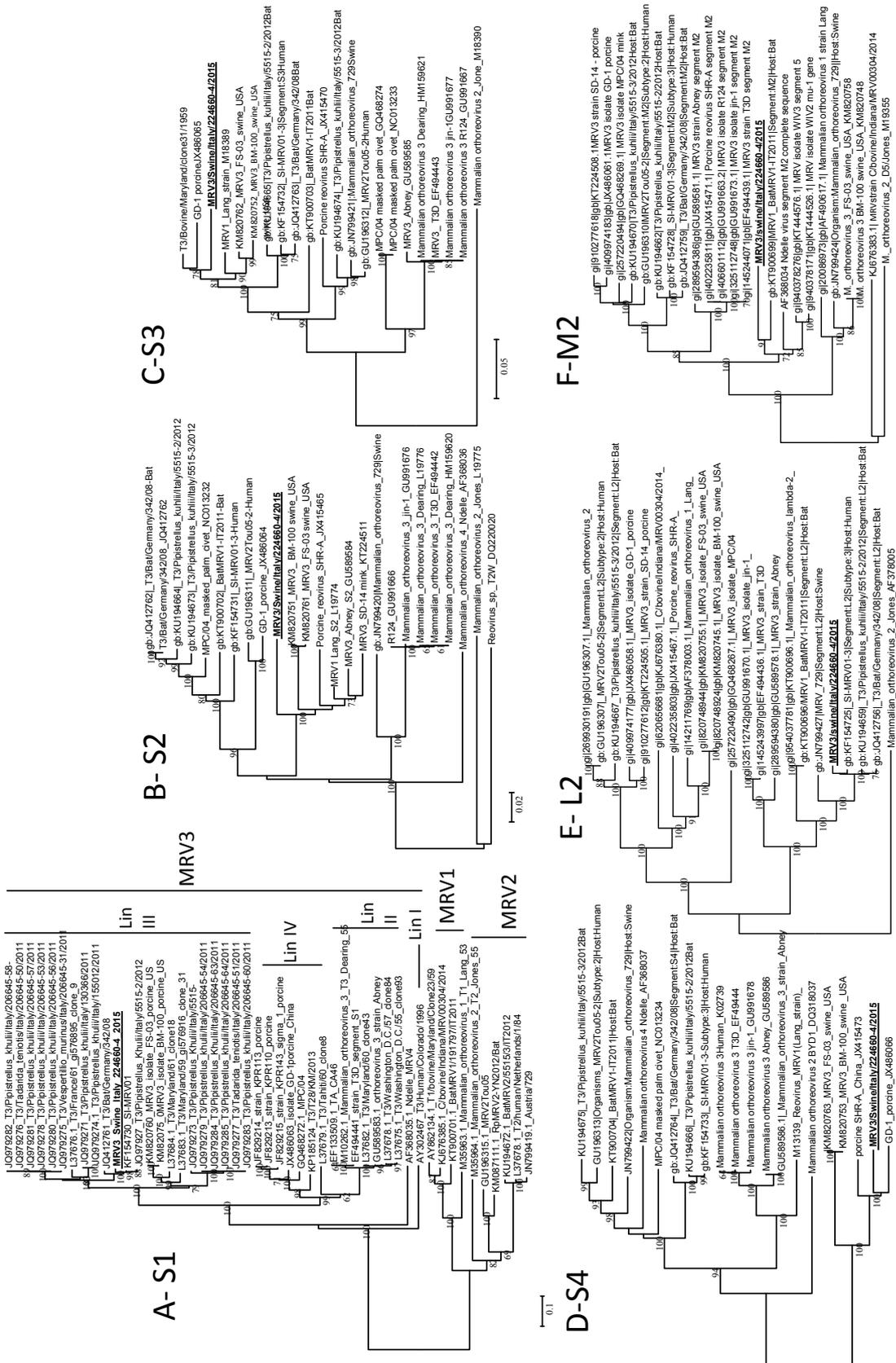


Figure 1 – Phylogenetic analysis of S segments, L2 and M2 of Italian MRV3 strain. Unrooted neighbour-joining trees based on complete nucleotide sequences were constructed with 1000 bootstrap replicates in MEGA 6.0. A) S1 segment; B) S2 segment; C) S3 segment; D) S4 segment; E) L2 segment; F) M2 segment. Bootstrap values higher than 60% was shown. Italian MRV3 strain was underlined and reported in bold face.

## ORAL 11: Virulence of current PEDV-strains in Germany

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**Keywords: Porcine epidemic diarrhea virus (PEDV), virulence, clinical presentation, realtime-RT-PCR**

Porcine epidemic diarrhea (PED) is an acute and highly contagious enteric disease of swine that results in severe enteritis, diarrhea, vomiting, and dehydration. Especially in suckling pigs, mortality can be very high. The causative agent, Porcine epidemic diarrhea virus (PEDV), is an enveloped positive single-stranded RNA virus that belongs to the family *Coronaviridae*, genus *Alphacoronavirus*.

After its first recognition in the 1970s in Europe, the disease caused considerable economic losses, especially in Asia. In May 2013, a highly virulent PEDV variant emerged in the United States (US), with swine farms experiencing explosive epidemics affecting all age classes of animals, with up to 95% mortality in suckling pigs. Since May 2014, several cases of PED were reported from Germany. In most cases, fattening pigs were affected showing high morbidity with almost non-existent mortality. However, some breeding herds also reported high mortality rates of up to 85% in suckling piglets.

For virulence characterization, two groups of sows and their offspring were challenged with current field material from German PED cases (suspension of fecal material and intestines). In group 1 sows were naïve and their offspring was inoculated with the PED preparation at four to six days post farrowing. In group 2 sows were inoculated 21 days prior to farrowing and their offspring was challenged with the above mentioned PED suspension at an age of four to six days. After inoculation all animals were monitored daily for clinical signs and rectal swabs were taken. Viral shedding was detected via an in-house real-time-RT-PCR system.

All piglets of group 1 showed severe diarrhea, vomitus, apathy and anorexia. The naïve sows of group 1 also showed diarrhea, apathy and anorexia, because of being infected by their offspring.

Sows of group 2 showed diarrhea, apathy and anorexia for one day after primary inoculation, while their offspring didn't show any clinical signs indicative for PED. PCR results also suggest that the viral shedding of piglets of group 2 is markedly reduced compared to group 1.

In summary, maternally derived antibodies were able to confer clinical protection but did not completely abolish PEDV shedding. Unprotected piglets showed 100 % morbidity with severity depending on the constitution and milk production of the sow. Lethal cases were mainly observed in weak piglets. In these cases, very early onset of symptoms was observed (< 12 hours post inoculation).

## ORAL 12: Velogenic Newcastle disease virus takes advantage of transmission ability capabilities to control suppress lentogenic virus during coinfection

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**Keywords: Newcastle disease virus, Transmission, Competition**

Since the first Newcastle disease virus (NDV) isolated in 1920s, many other strains have been successfully isolated. Most of these strains are velogenic or at least having a virulent-like fusion protein's cleavage sites. Newcastle disease vaccines were developed several decades ago and since have been used extensively worldwide. This continued vaccination with "old" vaccine strains is thought to drive viral evolution and foster the emergence of virulent strains. Another possibility is the better fitness of virulent strains for transmission and amplification in the targets species that makes lentogenic strains less frequent in the field. In order to test whether the virulent NDV strains take advantages of their transmission capabilities to suppress the nonvirulent strains, reverse genetics was used to generate one velogenic and one lentogenic NDV strains with the same genetic background and with EGFP and RFP markers, respectively. These viruses were used to individually infect or co-infect BHK-21 cells. Both strains could persistently infect the cells and showed similar ability of propagation over 15-16 cell passages. However, when co-infected, the velogenic strain takes control on the lentogenic strain that gradually disappeared until the 10<sup>th</sup> passage. This competition could partially explain why most isolated NDV strains are velogenic.

## ORAL 13: Relationship between landscape characteristics and colony health in *Apis mellifera* in Tenerife Island (Spain)

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**Keywords: Colony losses, Bee viruses, Varroa destructor, Landscape, Biodiversity, Environment**

Colony losses of western honey bee *Apis mellifera* have increased alarmingly in recent years. Viral infections, worldwide introduction of the ectoparasite mite *Varroa destructor*, nutritional deficiencies and environmental conditions are some factors involved in these losses [1]. Despite viruses are usually present in colonies without causing overt symptoms yet can cause colony losses under stressful circumstances. The role and importance of each factor as cause of colony stress is still under study, however the lack of nutritional resources may be one of the main threats considering the characteristics of beekeeping. In this study we examined whether the availability of nutritional resources based on landscape characteristics of apiaries, as botanic biodiversity, may influence honey bee health status, measured as the presence of weak colonies and disease symptoms and also by health indicators. The study was carried out in several apiaries located in natural environments of Tenerife Island (Canary Islands, Spain). Colonies were sampled in late-summer in order to evaluate colony strength and health indicators: presence and load of Varroa mite and deformed wing virus (DWW), black cell virus (BQCV), Israeli acute paralysis virus (IAPV) and sacbrood bee virus (SBV). Colony strength and Varroa load was measured following standard methods from the BeeBook to count for honey bees and mites, whereas viruses were studied using quantitative RT-PCR and colonies were classified attending to their viral load. Colonies were classified as “weak” when they showed symptoms or poor population while the rest of them were categorized as “healthy”, considering the beekeeping management and the season of the year. Landscape of each apiary was studied through a spatial analysis using CORINE Land Cover database and ArcGIS mapping [3]. DWW and BQCV were detected throughout the study, with high prevalence in both cases but medium and low load, respectively. Varroa was also detected in 90% of colonies and IAPV and SBV were not detected in any colonies. DWW load was correlated with Varroa infestation percentage (Spearman correlation test,  $p > 0.05$ ) and both were related to the colony strength (binomial logistic regression,  $p < 0.05$ ). Weak colonies were located in landscapes with less area occupied with wild plants or crops suitable for bee nutrition [4]. This result suggests a crucial role of the landscape characteristics in the survival of colonies. Our results show the importance of evaluating DWW and Varroa in productive apiaries as predictors of colony losses. In addition, we show the need of assessing adequate locations to ensure nutritional resources for honey bees. For this reason, future studies of apiaries maintained in natural environments should take location analysis and into account, in order to improve the availability of resources.

### Acknowledgments:

This work has been partially funded by the project RTA2013-00042-C10 “Holistic evaluation of risk factors in honey bees and wild pollinators: The situation in Spain” (MEyC and INIA), and Ministry of Education, Culture and Sports, which supported SBA with an FPU grant. The authors would like to acknowledge assistance from other members of the SUAT-VISAVET research group and the collaboration of APITEN (Beekeepers’ Association in Tenerife and the veterinary technicians of “Casa de la Miel” (Tenerife).

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## ORAL 14: High complexity of West Nile virus lineages and strains detected in the mosquito vector *Culex pipiens* of northeastern Italy, 2010-2015

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**Keywords: West Nile virus, *Culex pipiens*, north-eastern Italy, WNV lineages, virus strains**

West Nile virus (WNV) represents a serious public health concern for Europe. WNV emerged in northeastern Italy in 2008 and since then it has been constantly detected in humans, animals and the mosquito vector. In order to monitor and control WNV circulation, surveillance programs were implemented both at national and regional level based on serological, entomological and virological monitoring.

Our aim is to describe the temporal/spatial patterns of lineages/strains of WNV detected by PCR in the mosquito vector *Culex pipiens* in northeastern Italy. This area comprises two regions (Veneto and Friuli Venezia Giulia) divided here in southern part (Rovigo-Verona-Padova provinces), central part (Vicenza-Treviso provinces), costal area (Venice lagoon) and northern part (Udine-Pordenone provinces). From 2010 to 2015, 17,018 mosquito pools, collected during the entomological surveillance in northeastern Italy, were screened for Flaviviruses by an in-house sybr green One-Step Real-Time PCR. Overall, 27 pools of *Cx. pipiens* were positive for WNV-1 and 70 for WNV-2.

In 2010, after only two years from the first report of WNV in Italy, two different strains of WNV-lin1 were circulating in this area, i.e. the original WNV-Italy08 in southern/central areas and the new WNV-1 Livenza strain in the cost. In 2011, WNV-1 Livenza expanded in central/northern areas overcoming WNV-1 Italy08, which apparently disappeared. Meanwhile for the first time a WNV-2 emerged in the north, belonging to the Hungarian clade and sharing high similarity with Hungary-2004 and Greek-2010 isolates. In 2012, WNV-1 Livenza continued to circulate in the cost and the central/northern areas while WNV-2 reached the south. In 2013 the WNV-1 Italy08 re-emerged in the south after three years of silent circulation, slightly different but closely related to the original one; WNV-1 Livenza strain apparently disappeared and WNV-2 spread out in southern and cost areas (55 samples) with six different variants belonging to a new cluster.

In 2014 WNV-2 continued to circulate in southern areas but at the same time a different strain of WNV-2 emerged in the north sharing high similarity with the Russia-2007 and Romania-2013 isolates (Volgograd-2007 strain). In 2015 both the strains of WNV-2 were confirmed to circulate in the same areas of the previous year.

We sequenced the complete genome of WNV-2 Hungary and WNV-2 Volgograd strains in order to determine their phylogenetic relationships with other strains previously isolated in vectors and hosts in Italy and Europe. The WNV-2 Hungary strain showed the highest similarity (99.9%) to the Italian strain detected in a patient from Lombardy Region in 2014 (KP789954), and 0.5% of difference with the Hungary strain (DQ116961), which was isolated in 2004 from a goshawk and considered the ancestor of the WNV-2 Hungarian clade. WNV-2 Volgograd showed a 0.7% of dissimilarity to the Russian strain Volgograd (FJ425721) isolated in 2007. Our sequences differ from each other by 4.1%.

The phylogenetic analyses demonstrated the high complexity of the WNV strains circulating in the area. The findings highlight the fact that new introductions, likely through migratory birds, have occurred in few years (WNV-1 and WNV-2). The patterns of the different WNV strains also indicate the capability of these viruses to become endemic and to rapidly evolve and emerge in different sites. Northeastern Italy is confirmed as a high-risk area for arbovirus introduction, emergence and endemization.

Funding: Veneto and Friuli Venezia Giulia Regions.

## ORAL 15: Concurrent emergence of Usutu and West Nile virus in 2015 in France

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**Keywords: emergence, Usutu virus, West Nile virus, Culex**

Usutu (USUV) and West Nile (WNV) viruses are mosquito-borne *Flaviviruses* amplified in an enzootic cycle involving mainly Passeriformes and Strigiformes birds as reservoir hosts and *Culex* mosquitos as vectors. Although originating from Africa, these viruses have been introduced on several occasions into Europe and have significantly spread during the last decade in Central and Southern Europe, leading to substantial bird fatalities in Central Europe (USUV) or to horse and human neuro-invasive cases (WNV). Even though France had already registered ancient WNV circulation episodes in equids from 1962 to 2006, France had not faced West Nile outbreaks for nearly a decade. Moreover, France had not experienced USUV outbreaks so far.

The French event-based surveillance network SAGIR reported abnormal fatalities of Common blackbirds (*Turdus merula*) in two counties from Eastern France from August 5<sup>th</sup> to October 6<sup>th</sup> 2015. Concomitantly to the first blackbird fatalities, two horses developed meningoencephalitis on August 11<sup>th</sup> and 17<sup>th</sup> in South-Eastern France and referred as WNV-suspect cases to regional veterinary laboratories and ANSES. Flavivirus generic RT-PCR and Sanger sequencing was implemented on bird organs and 65 WNV-suspect horses, as well as a few asymptomatic horses from South-Eastern France, were subjected to WNV serology (competition ELISA, IgM capture ELISA, flavivirus Luminex assay and Virus Neutralization Test).

USUV-infections were confirmed in all the tissues sampled from two Common blackbirds in Haut-Rhin and from one bird sampled on September 23<sup>rd</sup> in Rhône. Furthermore, 49 horses in total were found infected, among which 44 presented clinical signs, namely 41 with meningoencephalitis and 3 with hyperthermia only. Six horses among the 41 with nervous symptoms died from the disease or were euthanized (fatality rate of 14.6%). WNV equine outbreaks were mainly located in the Camargue area, in the vicinity of Arles. 30 kilometres away from horse cases, WNV was evidenced in a febrile patient and in *Culex pipiens* mosquito pools in Nimes. One WNV and three USUV isolates were obtained from dead animals after two to three passages on Vero cells and whole genome sequencing was performed. Interestingly, phylogenetic analysis performed with recent European WNV isolates indicated that the 2015 French WNV strain was genetically close to Western Mediterranean lineage 1 strains, in particular to WNV isolates identified in the Camargue area in 2000 and 2004 (>98% nucleotide identity). This finding and the regular identification of silent WNV circulation events in birds in Camargue suggests WNV endemicity in this area. Phylogenetic analysis of the whole genome of the three USUV isolates demonstrated close genetic relatedness between USUV isolates from Haut-Rhin/France and Germany (99.8% nucleotide identity) and between viral strains from Rhône/France and Spain (99.2% identity). It also showed that French USUV strains from Haut-Rhin and Rhône counties were clearly distinct and arose from at least two independent introduction events.

The concomitant emergence of several WNV and USUV strains in different French regions in August-September 2015 suggests that climatic and environmental conditions in 2015 have promoted *Culex*-borne pathogens amplification. Risk factors of flavivirus emergence will be comprehensively analysed in future studies.

## ORAL 16: Pathogenicity evaluation of twelve West Nile virus strains belonging to four lineages from five continents in a mouse model: discrimination between three pathogenicity categories

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**Keywords:** West Nile virus, Koutango, pathogenicity, mouse model, lineages

Rodent models have been used extensively to study West Nile virus (WNV) infection because they are susceptible dead-end hosts and develop severe neurological symptoms reminiscent of those observed in human WNV neuroinvasive disease. Most of this research has focused on lineage (L) 1 strains, mainly studying the prototype strain NY99, belonging to clade 1a and the Australian Kunjin (KUN) variants belonging to clade 1b. Information about pathogenicity and neuroinvasiveness is lacking for the most recent L1 and L2 African and Euro-Mediterranean strains (some of them involved in recent outbreaks of clinical disease in humans and horses) as well as for newly defined genetic lineages. In this study, 4-week-old female Swiss mice were intraperitoneally inoculated with a collection of 12 WNV isolates, comprising old and recent L1 and L2 strains from Europe, Africa, Australia and North America, the putative L6 strain from Malaysia and the proposed L7 strain Koutango (KOU).

The inoculation of ten-fold dilutions (from 0.1 to 10<sup>5</sup> pfu) of each strain in young Swiss mice allowed the characterization of the assayed isolates in terms of lethal dose 50 (LD<sub>50</sub>), median survival times, replication in neural and extraneural tissues, histopathological lesions in the central nervous system and antibody production. Based on these results, we classified the isolates in three groups: high virulence-HV (including all L1a strains, recent L2 strains and KOU); moderate virulence-MV (B956 strain), and low virulence-LV (KUN and Malaysian isolates). Considering the results from survival curves for each WNV strain and inoculation dose, we determined that the inoculation of a single dose of 1000 pfu would be sufficient to classify WNV strains upon pathotype. This approach would entail an important animal welfare improvement, considerably reducing the number of mice necessary for this type of *in vivo* trials.

We confirmed the enhanced virulence of KOU strain with prominent neuroinvasive and neurovirulent features and a high capacity to cause rapid systemic infection. We also corroborated that differences in pathogenicity among WNV strains do not correlate with the phylogenetic lineage or geographic origin and confirmed that recent European and African WNV strains belonging to both L1a and L2 are highly virulent and do not differ in their pathotype profile compared to the prototype NY99 strain.

### Acknowledgements:

This work was financially supported by the European Commission (EUROWESTNILE-HEALTH-F3-2011-261391)



**ABSTRACTS**

**TOPIC II**

Threats at the  
European borders

**PARALLEL SESSION III**

Diagnostics and  
Intervention strategies

**ORAL  
PRESENTATIONS**

## ORAL 18: Improvement of the protective immune response in swine by co-inoculation of synthetic RNA and an FMDV vaccine

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**Keywords: FMDV, inactivated vaccine, adjuvant, immunomodulation**

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

In previous works we have reported that synthetic non-infectious RNA molecules mimicking structural regions in the FMDV genome, acting as pathogen associated molecular patterns (PAMPs), 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 FMDV, West Nile virus (WNV) and Rift Valley Fever Virus (RVFV). In addition, its co-inoculation with an FMDV conventional vaccine enhanced the immune response elicited after vaccination. On the other hand, these RNAs were able to induce robust antiviral and cytokine responses in porcine PBMCs (peripheral blood mononuclear cells), supporting the potential antiviral and immunomodulatory role of these synthetic molecules in livestock species.

In this work we have analysed the effect of RNA delivery on the immunogenicity and protection against FMDV challenge induced by a conventional type-O FMD vaccine in swine, a natural host farm animal. The co-administration of RNA and a suboptimal dose of FMD vaccine led to higher levels of neutralizing antibodies against homologous and heterologous viral strains, as well as higher anti-FMDV titers at late times post-vaccination. IFN- $\gamma$  specific responses were also higher in PBMCs from pigs inoculated with RNA compared to those from pigs receiving the vaccine alone. When vaccinated pigs were challenged with FMDV, animals showed different levels of protection against disease, with RNA delivery increasing the rate of protection against viral challenge. Our results support the immunomodulatory effect of these RNA molecules in natural host animals and suggest their potential use for FMD vaccines strategies.

## ORAL 19: Efficacy and immune responses of DNA and MVA vaccines against Rift Valley fever in sheep

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**Keywords: Rift Valley fever, DNA vaccine, MVA vaccine, ovine model**

Rift Valley fever virus is transmitted by mosquitoes and causes serious disease in livestock affecting also humans in the form of haemorrhagic fever of fatal consequences. The disease is endemic in Sub-Saharan Africa, although its capacity for wider geographic spread justifies the design of novel control strategies. The aim of this work was to evaluate the immunogenicity and efficacy of DNA and MVA vaccines encoding both viral glycoproteins, a strategy proved successful in laboratory rodents, in an ovine model of RVFV infection. Adult sheep were challenged 12 weeks after the last immunization and both humoral and cell-mediated responses prior and after virus challenge were studied. Strategies based on DNA alone and DNA prime-MVA boost were able to induce rapid and potent *in vitro* neutralizing antibody responses as well as IFN $\gamma$  production after *in vitro* virus specific re-stimulation. In these animals we observed reduced viremia and clinical signs when compared with non-immunized controls. In contrast, sheep vaccinated only with MVA showed increased viremia and fever correlating with the lack of neutralizing antibody responses, despite of inducing cellular responses after the last immunization. However, faster kinetics of neutralizing antibodies and IFN $\gamma$  production after challenge were observed when compared to the control group, indicative of primed immune responses. Interestingly, immune responses were maintained longer in animals vaccinated with the combination of DNA and MVA suggesting that this strategy could provide long lasting immunity. In conclusion, these results suggest that, although these strategies were not fully protective, they were able to mount specific anti-RVFV immune responses, thus warranting further optimization in large animal models of infection.

## ORAL 20: Replication and shedding of Middle East respiratory syndrome coronavirus (MERS-CoV) in experimentally inoculated pigs and llamas

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**Keywords: MERS-CoV, llama, pigs, infection, animal models**

### Introduction:

Emerging and re-emerging pathogens represent a substantial threat to public health, as demonstrated with numerous outbreaks over the past years, including those caused by the Middle East respiratory syndrome coronavirus (MERS-CoV). Dromedary camels (*Camelus dromedarius*) seem to play an important role in zoonotic transmission of MERS-CoV, and the disease is endemic in these animal populations of East Africa and the Middle East. Thus, animal vaccination to prevent virus shedding is urgently required. However, since working with dromedary camels under biocontainment conditions is a difficult task, other animal models could be more suitable for vaccine research. Therefore, we infected llamas and pigs with MERS-CoV.

### Materials and Methods:

Llamas (n=8) and pigs (n=14) were intranasally inoculated with  $10^7$  TCID<sub>50</sub> MERS-CoV/animal. Clinical signs were monitored daily, and nasal and rectal swabs were obtained on days 1, 2, 3, 4, 7, 10, 14 and 24 post-inoculation (PI). Swabs were analyzed for MERS-CoV RNA by RT-PCR. Serum samples were collected weekly from all animals, and tested for IgG antibodies reactive with the spike subunit protein S1 of MERS-CoV by ELISA. Four pigs were euthanized on day 2 PI, and 4 animals of each species were sacrificed on day 4 PI. Remaining animals were euthanized on day 24 PI. Necropsies were performed and respiratory and extra-respiratory tissues were taken for histopathology to describe lesions, for immunohistochemistry (IHC) to detect presence of virus antigen and for RT-PCR to detect presence of viral RNA.

### Results and conclusions:

Both pigs and llamas showed positive RT-PCR results in nasals swabs from days 1 to 10, but only llamas showed moderate nostril mucous secretion. Inflammation and necrosis was mainly seen in the nasal respiratory epithelium of llamas and pigs. Several tissues (mainly from the respiratory tract) were positive by RT-PCR. Antibodies towards the S1 protein were detected by ELISA in all llamas and 4 pigs from 14 days PI onwards. These results prove the susceptibility of llamas and pigs for MERS-CoV infection. Thus, the possibility of MERS-CoV circulation in other geographic areas with populations of camelids and other domestic animals is not negligible.

### Acknowledgments:

ZAPI project (IMI Grant Agreement No. 115760).

## ORAL 21: Structural protein VP2 of African horse sickness virus is not essential for virus replication *in vitro*

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**Keywords:** African horse sickness virus, virus replication, structural protein, orbivirus

Members of the *Reoviridae* family are non-enveloped multi-layered viruses with a double stranded RNA genome consisting of 9-12 genome segments. The *Orbivirus* genus contains many virus species (10 genome segments) such as bluetongue virus (BTV) with at least 27 serotypes, and African horsesickness virus (AHSV) with nine serotypes. African horse sickness (AHS) is a noncontagious disease of equids with a mortality of >90% for domestic horses. This devastating disease is spread by competent species of *Culicoides* biting midges in many African countries. Recent outbreaks of Bluetongue and Schmallenberg (the latter caused by a bunyavirus) in Europe demonstrated that *Culicoides* borne diseases are of serious risk and in particular AHS outbreaks will have a huge socio-economic impact on the community.

Non-structural proteins NS3 and NS4 encoded by Seg-10 and Seg-9, respectively, are not essential for virus replication *in vitro*. Further, *in vitro* reconstitution of BTV doesn't require any of the non-structural proteins, and purified cores particles - without outer capsid proteins VP2 and VP5 - are highly infective. For AHSV, a natural variant of AHSV7 (AHSV-7 tVP2) contains a small in-frame deletion in Seg-2 encoding truncated structural protein VP2 (tVP2), indicating that this region is not essential for virus replication *in vitro* (Manole et al. 2012. *JVirol*). Notably, VP2 of orbiviruses is the determining protein for serotype specific protection and neutralizing antibodies.

We have developed reverse genetics for live-attenuated AHSV for serotype 4 (AHSV4LP) (van de Water et al 2015 *JVirol*), aiming vaccine development according to the novel Disabled Infectious Single Animal (DISA) vaccine for Bluetongue (Feenstra et al. 2014 *JGenVirol*; Feenstra et al 2015 *Vaccine*; Tacken et al., 2015 *Vaccine*).

Here, reverse genetics is used to generate AHSV4 with tVP2 of serotype 7. Similar tVP2 proteins of serotype 4 and 5 are also functional in *in vitro* replication of AHSV. Subsequent studies by expansion of the deletion, out-of-frame deletions, and AUG->GCC mutations in Seg-2 showed that VP2 protein is not required for virus rescue. In order to further confirm dispensability of VP2, in-frame insertion of GFP resulted in fluorescence of infected cells. AHSV without VP2 protein also infects insect cells but virus release is blocked, indicating that virus replication in these cells is disturbed. Westernblot analysis showed that AHSV without VP2 produces core particles after infection. However, VP5 is essential for virus rescue, since virus with a small out-of-frame mutation in Seg-6[VP5] could not be rescued. We conclude that structural outer capsid protein VP2 of AHSV4LP is not essential for *in vitro* virus replication in mammalian cells. AHSV4LP without VP2 protein replicates in mammalian cells, still causing cytopathogenic effect and is released from these cells. It will be very interesting to study AHSV without VP2 protein *in vivo* by experimental infection of horses.

## ORAL 22: The presence of pre-formed antigen in the inoculum of experimental MVA-VP2 vaccines for African horse sickness virus plays an important role in the vaccine immunogenicity

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**Keywords: African horse sickness, Vaccines, MVA, immunogenicity, protection**

African horse sickness (AHS) is a lethal equine disease transmitted by biting midges of the genus *Culicoides* and caused by African horse sickness virus (AHSV). AHS is endemic to sub-Saharan Africa but devastating outbreaks have been recorded periodically outside this region during the last century. The perceived risk of an AHS outbreak occurring in Europe has increased following the frequent epidemics in the continent of bluetongue, a disease of ruminants caused by bluetongue virus, closely related to AHSV.

Live attenuated vaccines for AHS, currently in use in Africa, are considered unsuitable for use in non-endemic countries due bio-safety concerns. The demand to facilitate international travel of horses between Africa and the rest of the world, and the inability of classical AHS vaccines to differentiate vaccinated from infected animals (DIVA), stimulated the development of new vaccines for AHS that could be safe, efficacious and compatible with the DIVA approach.

We have shown in previous studies that recombinant modified Vaccinia Ankara virus (MVA) vaccines encoding the outer capsid protein of AHSV (AHSV-VP2), containing the most relevant virus neutralising antibody (VNAb) epitopes, induced VNAb and protection against AHSV challenge in a mouse model experimental system and also in the target species, the horse. In passive immunisation studies we demonstrated that MVA-VP2 induced immunity and protection is largely associated with VNAb. These vaccines are prepared in chicken embryo fibroblasts and analyses of the inoculum of these experimental MVA vaccines showed that they always contain pre-formed AHSV-VP2.

In this study, we performed a series of experiments with MVA-VP2 vaccines in the mouse experimental system to elucidate the influence in the vaccine immunogenicity of pre-formed AHSV-VP2 present in the inoculum. We performed a comparative analyses of correlates of immunity of mice vaccinated with: a) MVA-VP2 (live); b) MVA-VP2 (live and sucrose gradient purified); c) MVA-VP2 (UV light inactivated); d) MVA-VP2 (UV light inactivated and diluted); e) MVA-VP2 (heat inactivated); f) MVA-VP2 (UV inactivated) + MVA-VP2 (purified); h) MVA-VP2 (heat Inactivated) + MVA-VP2 (purified); and i) wt-MVA (no insert). The results of these experiments showed that levels of protection were maximal using MVA-VP2 (live) vaccine and the protection of all other vaccines correlated strongly with the levels of pre-formed AHSV-VP2 present in the inoculum.

The results and conclusions from these data will be described and discussed in this presentation.



**ABSTRACTS**

**TOPIC II**

Threats at the  
European borders

**PARALLEL SESSION IV**

Epidemiology & surveillance  
and Risk assessment

**ORAL  
PRESENTATIONS**

## ORAL 23: Bayesian evolutionary analysis of FMD viruses collected from outbreaks that occurred in Maghreb regions during 2014-2015

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**Keywords: FMD, Maghreb epidemic, full genome sequencing, phylogenetic evolutionary analysis**

Foot-and-mouth disease virus (FMDV) is a single-stranded positive-sense RNA of the genus *Aphthovirus* (family *Picornaviridae*), which spreads rapidly among cloven-hoofed animals, causing important economic losses. FMDV is classified in seven serotypes and multiple genotypes, topotypes, and lineages, from which new FMD viruses can emerge, challenging diagnostic tools and vaccination control programs.

During April 2014 to November 2015 FMDV outbreaks occurred in Maghreb regions, this was the first incursion after 15 years of absence. The phylogenetic analysis on VP1 coding sequences revealed that these viruses belonged to the Ind-2001d lineage within the Middle East-South Asia (ME-SA) topotype. The O/ME-SA/Ind-2001 lineage was initially identified in the Indian subcontinent in 2001 and subsequently became the predominant lineage in the region. Escape of this lineage from the Indian subcontinent has occurred sporadically, causing outbreaks which have been reported in the Arabian peninsula and from 2013 also in Libya, where another type O topotype had been previously circulating.

The objective of the present work was to provide more insights into virus evolution and epidemiological relationships between recent outbreaks in the Maghreb region.

PCR amplicons were obtained from twelve Tunisian viruses, selected from outbreaks of the 2014 Tunisian epidemic wave, and from five Algerian isolates of 2014. The full genome sequences (FGS) were produced by MiSeq instrument (Illumina, USA). The reads were analysed and assembled by SeqMan-NGen 12.0 (Lasergene package; DNASTar, Inc., Madison, WI).

A nearly complete genome sequence of 8076 nucleotides (nt) in length was obtained from each of the isolates, a deletion of 72 nt, not previously reported in other isolates of Ind-2001d lineage, was identified in 5' Untranslated Region. A single large open reading frame (ORF) of 6,999 nt was predicted to encode a polyprotein of 2,333 amino acids.

A Bayesian evolutionary analysis implemented with BEAST software was performed to analyse the phylogenetic relationships between the viral sequences, and to obtain estimates of spatial-temporal origin of the outbreaks. The analysis was carried out separately on the entire ORF and VP1 sequences; the latter enabled us to include more isolates for which FGS was not available yet. According to VP1 phylogeny, a unique introduction of FMD virus occurred in Libya in 2013, from which all the viruses detected in North Africa have evolved. Both ORF and VP1 analyses indicate that all the Maghreb isolates derived from a unique putative common ancestor, that was estimated to be present since beginning of 2014. The presence of a single common ancestor for all the sequences recovered from viruses in the Maghreb provides clear evidence for a single introduction of the virus from Libya into Tunisia. Interestingly, two different introductions in Algeria have likely occurred: in fact, Algerian isolates from 2014 and those from 2015 cluster in different branches, together with different Tunisian isolates. Finally, the 2015 Algerian and Moroccan viruses share a common ancestor and are most closely related. The phylogenetic analysis based on ORF pointed out that Tunisian isolates evolved in three main branches, geographically consistent with animal movements in the country.

## ORAL 24: Regional evolutionary dynamics of peste des petits ruminants virus in West Africa: influence of livestock trade

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**Keywords: PPRV, morbilivirus, phylogenetics, animal movement, transboundary**

Peste des petits ruminants (PPR) is a highly contagious and devastating viral disease of small ruminants. It represents a serious risk for the economy and food security in regions of Africa, Middle East and Asia where the disease is endemic. Integrated knowledge of evolutionary and epidemiological factors underlying PPR virus (PPRV) emergence, persistence and spread are necessary for better guidance of PPR control strategies and their practical implementation. Efforts are especially needed to better understand the regional dynamics of PPRV evolution and endemic transmission.

Here we studied the regional evolutionary dynamics of endemic PPRV in West Africa, focussing on Senegal and neighbouring countries, and assessed the role of livestock trade in explaining the observed viral diversity and phylogenetic patterns. Sheep and goats were sampled in livestock markets and villages across Senegal between 2010 and 2014 and tested for PPRV infection. Other samples were obtained from Mauritania, Mali, and Guinea during the same period. Historical samples (1972-1994) were also collected from the region. In addition, livestock movement data, particularly livestock trade were collected during specific surveys implemented in Mauritania and Senegal.

A total of 55 samples collected from 2010 to 2014 were positive for PPRV. Partial sequencing of the N gene showed that 54 belonged to the PPR virus lineage II (PPRV-II) and one to lineage I. We obtained the sequence of the full N and H genes for all PPRV-II samples, and sequenced the full genome for a subset of recent and historical samples. Phylogenetic analyses showed the presence of at least 4 different, geographically delimited, clades within PPRV-II in West Africa. Samples from Mali were distributed across 3 of these clades, suggesting a central position of the country in regional movement of PPR.

All PPRV-II samples from Senegal were situated within a single clade, but could be separated in distinct clusters. These clusters pointed to virus movement across long distances within Senegal and between Senegal and its neighbours. Transboundary movements involved mainly major sites for commercial animal movement, but also transhumant movement between Senegal and Mauritania. A statistical model of virus genetic distance was fitted with environment and animal movement data to assess if the genetic patterns observed can be predicted by commercial connectivity. We discuss how such landscape resistance analyses based on animal movement can be used to predict PPR transmission pathways and control effort within endemic regions.

## ORAL 25: Are camelids and wild boar possible hosts of PPRV?

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**Keywords: PPRV, transmission, emerging disease, camelid, wild boar**

The worldwide eradication of peste des petits ruminants virus (PPRV; *Morbillivirus*) is planned until 2030. PPRV-vaccination of susceptible species that contribute to PPRV spread is considered an effective tool for PPRV control. In small ruminant populations, up to 100% of PPRV-infected animals show severe clinical signs or succumb the disease. However, the role of most other wild and domestic Artiodactyla species in the epidemiology of PPRV remain unknown, and both cattle and pigs have been considered dead-end hosts for PPRV.

During the last decade, PPRV lineage 4 strains have frequently been detected in Asian and North African countries bordering also Europe. The results of our recent animal trials using a PPRV lineage 4 isolate (Kurdistan/2011) for experimental infection of cattle and pigs confirmed that cattle do not transmit PPRV to goats. In contrast, the transmission experiment with domestic pigs showed that transmission of PPRV to goats and other pigs is possible. Furthermore, recent reports of severe clinical signs and fatalities associated with PPRV-infection in dromedaries have given rise to the question whether camelids might contribute to PPRV spread. Nevertheless, whether wild boar and South American camelids are susceptible to PPRV and might play a role in the epidemiology of PPRV has never been investigated.

Therefore, 6 dromedaries, 6 South American camelids (SAC) and 4 wild boar were intranasally infected with PPRV Kurdistan/2011 in three separate animal trials. After 2 to 3 days post-infection (dpi), 1 to 2 animals of the same or closely related species as well as 2 goats were housed together with the infected animals to study PPRV transmission. Serum, whole-blood, oronasal, conjunctival and fecal swabs were collected in regular intervals and analysed for PPRV, PPRV-RNA and antibodies. Blood and various tissues were collected at post-mortem examination and examined by RT-qPCR, histopathological and immunohistochemical analysis.

Experimentally infected dromedaries and SAC did not show any obvious clinical signs. PPRV-RNA excretion was detected in some of the infected camelids, but none of the contact goats and camelids were infected with PPRV. Seroconversion or a marked rise of antibody levels was found in all SAC and in 4 of 6 dromedaries. In contrast, PPRV-infected wild boar showed a transient rise in rectal body temperature and diarrhoea. PPRV-RNA was detected in swabs and blood samples of all wild boar and preliminary results indicate that similar to domestic pigs also wild boar may transmit PPRV to contact animals. All wild boar seroconverted within 1-2 weeks after infection.

The results of our transmission experiments indicate that cattle and camelids are most likely dead-end hosts for PPRV. Therefore, those species probably don't play a role in PPRV spread and don't have to be vaccinated. In contrast, pigs and wild boar are possible maintenance or spillover hosts that might play a role also in PPRV epidemiology. Further studies are necessary to determine whether pigs, wild boar and other members of the *Suidae* family should be vaccinated against PPRV in affected regions or not, to facilitate PPRV eradication.

## ORAL 26: Rift Valley fever virus and European mosquitoes: vector competence of *Culex pipiens* and *Aedes albopictus*

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**Keyword:** *Culex pipiens*, *Aedes albopictus*, transmission, vector competence, Rift Valley fever virus.

Rift Valley fever (RVF), an emerging mosquito-borne zoonotic disease caused by the Rift Valley fever virus (RVFV). RVFV regularly circulates in endemic areas between wild ruminants and haematophagous mosquitoes. Domestic and wild ruminants, camels and humans are particularly susceptible to RVF. The disease has a grave impact on public health and economy, especially in tropical regions of eastern African countries where numerous outbreaks have occurred since 1931. Favorable environmental conditions and the presence of several potential vector species, like *Culex pipiens* and *Aedes albopictus*, would make possible the introduction and the establishment of this disease also in the European Union countries. In the present study we evaluated the vector competence for RVFV of three different populations of European mosquitoes: *Culex pipiens* form *molestus*, *Culex pipiens* hybrid form and *Aedes albopictus*. Mosquitoes were artificially fed with blood containing virulent RVFV (strain 56/74) in NBS3 conditions. We have used environmental conditions to reproduce the weather during the summer time, in order to estimate accurately the vector competence. After a period of 14 days, infection, disseminated infection and transmission rates and transmission efficiency were analyzed. The virus was able to induce a disseminated infection in *Culex pipiens* hybrid form and in *Aedes albopictus*. Moreover, infectious viral particles were isolated from saliva samples of both species, showing their capacity to transmit RVFV. On the other hand, *Culex pipiens* form *molestus* was not a performing experimental vector for competence studies of RVFV due to low feed rate. The presence of competent populations of *C. pipiens* and *A. albopictus* in Spain indicates that an autochthonous outbreak of RVF may occur in case of virus introduction. Our findings provide helpful information to health authorities in order to set up a future efficient entomological surveillance and vector control programs for RVFV.

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## ORAL 27: Outbreak of Epizootic haemorrhagic disease virus in Israel 2015: distribution, duration and infectivity

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**Keywords: Epizootic haemorrhagic disease virus, abortion, viremia, RNAmia**

In September 2015, after nine years of break, an outbreak of epizootic haemorrhagic disease virus (EHDV) occurred in Israel. The first cases were detected in three dairy cattle farms located near the border with Jordan in the Jordan Valley and in the Dead Sea area. Afterwards, EHD outbreaks spread throughout the country, reaching the Northern area, the Coastal and even the arid desert areas. In total, EHD cases were identified in 88 beef and dairy farms. The main clinical signs observed during the outbreak were reduced milk production, weakness, lameness, excessive salivation, recumbency, fever, light erythema of the nasal and oral mucosae, weight loss and abortion. Respiratory distress, cachexia and death were occasional.

EHDV was identified in whole blood samples from sick animals, as well as in organs from dead animals and aborted fetuses using Real Time reverse transcription polymerase chain reaction (RT-qPCR), whereas sera were tested by EHDV ELISA antibodies. Ten viruses were isolated in embryonated chicken eggs (ECE) and consequently passaged in baby hamster kidney cells (BHK cells).

According to both conventional RT-PCR and RT-qPCR examinations EHDV was found in most tested aborted cattle fetuses during the pick of the current outbreak, which may suggest that EHDV was a major cause of abortion in these animals.

Conventional RT-PCR and consequent sequencing analysis of several partial and two full length VP2 genes were performed from several field samples and all viruses isolated in ECE. It was identified that the causative agent of the disease belongs to EHDV serotype 6. Phylogenetic analysis of the examined sequences showed that VP2 were identical and were closely related to EHDV-6 strains isolated in Algeria and Morocco during the 2006 outbreaks.

Whole blood samples from the same naturally infected animals were used for estimating approximate duration of blood-circulating viral RNA (RNAmia) and viremia. The RNAmia in the infected heifers was at least four months, while high RNA level was observed during first two months following the presumed time of infection, while duration of viremia was probably shorter. Duration of RNAmia was estimated by RT-qPCR tests, when duration of viremia was estimated by virus isolation in ECE and tissue culture cells.

## ORAL 28: Animal health risk of legally imported exotic animals into The Netherlands

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**Keywords: Risk assessment, Exotic animals, Introduction, Impact**

### Introduction

The expanding legal and illegal trade in exotic animals and the limited insight in the numbers and species involved in these trade flows raises concerns, because of its potential contribution to the global dispersion of infectious disease agents. Better insight into the (zoonotic) pathogens that could potentially be introduced by the importation of exotic animals can help to target surveillance efforts on those trade flows that pose the highest risk. The aim of this study was to evaluate the animal health risks associated with the legal importation of exotic mammals, birds, reptiles, and amphibians into The Netherlands.

### Material and methods

An inventory was made of the numbers of exotic animals that arrived in The Netherlands in the period 2013-2014 classified according to taxonomy and geographic origin, the results of which were used to assess the associated animal health risks. These trade figures were linked with information on worldwide occurrence of disease pathogens and the susceptibility of imported species to these pathogens. Then, proxy variables were used to score the *probability* of introduction and subsequent transmission of disease pathogens to livestock animals in The Netherlands (P-score), and the expected *impact* for the livestock sector (I-score).

### Results

In 2013 and 2014, a total of 4,910<sup>5</sup> exotic animals were legally shipped to The Netherlands, 43% of which had The Netherlands as destination. The exotic animals that were imported into The Netherlands originated from 25 different countries worldwide. The majority of imported animals were reptiles (93.8%) and amphibians (5.8%). Birds and mammals were a minority with 0.06% and 0.4%, respectively.

The use of predefined inclusion criteria resulted in a selection of 27 pathogens that were eligible for the risk assessment. For 18 of these pathogens the risk was estimated very low to negligible, because very few or no susceptible animals were imported into The Netherlands from countries where these pathogens were present. The risk of the remaining nine pathogens was assessed using P-I scores. For most pathogens the P-score was relatively high, whereas the I-score was low to moderate for all pathogens. The pathogens with the highest animal health risk of being introduced in The Netherlands via the importation of exotic animals are *Salmonella* (exotic serovars) including *S. arizonae* and *S. pullorum*, and the arboviral pathogens Japanese encephalitis virus (JEV), Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV), and West Nile virus (WNV).

### Conclusion

Based on this study, it was concluded that the animal health risk of introducing exotic pathogens into The Netherlands via the legal import of exotic animals is low. However, amphibians and reptiles might constitute a significant risk for the introduction of *Salmonella* spp. and potentially the arboviral pathogens WEEV, EEEV, JEV, and WNV. These are all zoonotic pathogens that may also have an impact on public health. Currently, no health requirements are imposed on imported reptiles and amphibians except the absence of physical signs of disease. Risk-based testing of imported reptiles and amphibians could contribute to reducing the animal health risk of imported exotic animals.



**ABSTRACTS**

**TOPIC III**

Current challenges  
inside Europe

**PARALLEL SESSION V**

ASF: Diagnostics and  
Intervention strategies

**ORAL  
PRESENTATIONS**

## ORAL 29: Deletion of interferon inhibitory genes from virulent African swine fever virus results in attenuation and induction of protection against challenge

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**Keywords: African swine fever, vaccine, immune evasion, interferon**

African swine fever virus (ASFV) infection can cause an acute haemorrhagic fever in pigs with high mortality. The recent spread of disease in E. Europe has had a high socio-economic impact and threatens the global pig industry. No vaccine is available which limits the options for disease control.

We have investigated the impact of deleting ASFV genes that encode interferon inhibitory proteins on virus pathogenesis and induction of protective immunity. This was predicted as a route for construction of rationally attenuated ASFV vaccine candidates. The genes studied included members of the multigene families (MGF 360 and 505), I329L a homolog of Toll-like receptor 3 and DP148R. The genes were deleted from a virulent genotype I isolate from W. Africa (Benin 97/1) or from an already attenuated genotype I isolate from Portugal (OURT88/3). The OURT88/3 isolate has a large deletion of similar MGF 360 and 505 genes as well as interruptions of other genes compared to virulent isolates. The results showed that deletion of these genes did not affect virus replication in macrophages but had varying effects on the induction of IFN- $\beta$  mRNA and of interferon stimulated genes. Infection with virulent isolates induced very low or undetectable levels of IFN- $\beta$  mRNA in macrophages whereas infection with different deletion mutants induced variable levels of IFN- $\beta$  mRNA. Deletion or interruption of multiple copies of MGF360 and 505 genes and deletion of DP148R resulted in attenuation of the virulent Benin 97/1 isolate and induction of good levels of protection against challenge. In contrast deletion of the DP148R and deletion of I329L from the already attenuated OURT88/3 isolate reduced protection levels and induced signs of chronic disease. The results indicate that deletion of different combinations of IFN inhibitory genes offers a promising route for virus attenuation and induction of protection.

## ORAL 30: Ornithoros tick salivary, an intrinsic component of vector competence in transmission of African swine fever virus to pigs

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**Keywords:** African swine fever, Tick saliva, cells recruitment; macrophages; Langerhans cells

African swine fever (ASF) is a lethal haemorrhagic swine disease with disastrous consequences for pig production. *Ornithodoros* ticks such as *O. erraticus* in Spain and Portugal, and *O. moubata sensu lato* in eastern and southern Africa are able to maintain and transmit the virus, and are competent vectors and reservoirs for ASFV (1). These ticks can maintain ASFV for years and transmit the virus through different routes such as transovarial and/or sexual transmission from tick to tick, as well as horizontal transmission to suids via contaminated saliva or coxal fluid. Vector competence in virus transmission can be related to extrinsic or intrinsic factors. Here we present the intrinsic effect of *O. porcinus* tick salivary gland extract on the African swine fever virus infection in domestic pig. During the early stage of pig infection with ASFV, mononuclear phagocytic cells are the main targets for viral replication (2). Tick saliva has been shown to modulate the host physiological and immunological responses during feeding on skin, thus affecting viral infection.

To better understand the interaction between soft tick, ASFV and pig at the bite location and the possible influence of tick saliva on pig infection by ASFV, salivary gland extract (SGE) of *Ornithodoros porcinus*, co-inoculated or not with ASFV, was used for intradermal auricular inoculation. Our observations focused both on the pig systemic immune response and on pig skin inflammation and cellular modulation (especially LCs and macrophages) at the tick bite location. Unlike previous studies, the assessment of such immune modulations was conducted on the natural host, domestic pigs, and a highly adapted tick-virus association with *O. porcinus* ticks collected from Madagascar and a Madagascan ASF virus strain.

Our results showed that, after the virus triggered the disease, pigs inoculated with virus and SGE presented greater hyperthermia than pigs inoculated with virus alone. The density of Langerhans cells was modulated at the tick bite or inoculation site, either through recruitment by ASFV or inhibition by SGE. Additionally, SGE and virus induced macrophage recruitment each. This effect was enhanced when they were co-inoculated. Finally, the co-inoculation of SGE and virus delayed the early local spread of virus to the first lymph node after the inoculation site. This study has shown that the effect of SGE was powerful enough to be quantified in pig, both on the systemic and local immune response (3).

We believe this model should be developed with infected tick and could improve knowledge of both tick vector competence and tick saliva immunomodulation.

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### Acknowledgments:

This work was partly funded by the European Union Seventh Framework Programme (FP7/2007-2013) under Grant agreement no.311931 – ASFORCE, the General Council of Côtes d'Armor and the Regional Council of Languedoc Roussillon (Chercheur d'avenir project). JB received her PhD grant from CIRAD and ANSES.

## ORAL 31: Experimental characterization of a recombinant live attenuated African swine fever virus with crossprotective capabilities

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**Keywords: African swine fever, vaccine, crossprotection, CD8+ T-cells**

African swine fever (ASF) is a highly infectious disease of obliged declaration to the Animal health world organization (formerly OIE). The complex epidemiological situation of ASF with more than 22 different ASF virus (ASFV) genotypes circulating in Africa, together with the uncontrolled presence of the virus in East Europe suppose a major threat for the world-wide swine industry.

Lack of vaccines available against this disease difficult even more ASF control, today relying in efficient early diagnosis and the culling of infected and in contact pigs. Stamping out policies such as those implemented in the EU, albeit efficient, are not absent of criticisms due to ethical concerns and have demonstrated to be of difficult implementation in less favoured areas of the world including most African countries and more recently, in ASF-affected Eastern European countries. Developing efficient and save vaccines against ASF could definitively contribute to better control ASFV infections in endemic areas and to prevent its entrance/reentrance in ASF-free areas. Therefore, we really believe that investing research efforts on ASF vaccinology is a must.

In contrast with the recurrent failure of classical inactivated vaccines, live attenuated viruses (LAV) have demonstrated to confer solid protection against ASFV experimental infection. Thus, naturally attenuated ASFV isolates, LAVs obtained by ASFV adaption to grow in tissue culture cells and recombinant live attenuated vaccines, have all demonstrated to confer solid protection against homologous virus challenge but limited protection, if any, have been demonstrated against heterologous ASFV challenges, including against different virus isolates from the same genotype.

Work performed in collaboration between CRESA-IRTA, CBMSO-CSIC and Boehringer Ingelheim Veterinary Research Center (Hannover, Germany), has allowed generating a new ASFV recombinant LAV with crossprotective potential. So far, we have been capable to demonstrate: 1) that deletion of the CD2v (ASFV-Hemagglutinin gene) from the virulent BA71 isolate (BA71ΔCD2), strongly attenuated the virus *in vivo*; 2) that intramuscular inoculation with the recombinant virus (BA71ΔCD2) conferred a dose dependant protection not only against lethal homologous challenge (BA71; Genotype I) but also against heterologous E75 (Genotype I) and Georgiao7 (Genotype II); and 3) that *in vivo* cross protection correlated with the *in vitro* detection of strain-specific CD8+ T-cells in circulation. In spite of these impressive results there is still room for improvement mainly from the biosafety point of view due to rather limited but detectable *in vivo* residual virulence of BA71ΔCD2. The potential use of LAV in the field will be further discussed.

## ORAL 32: African swine fever strain “replaces” attenuated homologous strain after challenge

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**Key words: African swine fever virus, B602L, vaccination, challenge.**

African swine fever is an emerging disease threat for the swine industry worldwide. No effective and safe ASF vaccine is available so far, and progress is hindered by lack of knowledge concerning the extent of ASFV strain diversity and the viral antigens responsible for protection in the pig. Available data from vaccination/challenge experiments in pigs indicate that homologous non-virulent strains may induce protective immunity against ASF.

Here we vaccinated 6 piglets with the attenuated ASFV strain (KK262, genotype I) in order to induce solid immunity. The animals were boosted at day 21 and then challenged with highly virulent homologous strain K49. All animals survived and did not show any clinical signs of ASFV for 28 days post challenge. However, in one animal low level of ASFV DNA was detected at 27 days post challenge. In order to identify the exact ASFV strain we ran B602L gene PCR with following sequencing.

Based on previous results published elsewhere, B602L gene shows extreme divergency even within highly related ASFV isolates. In our case, the ASFV virulent strain (K49) genome was detected in blood samples. The nucleotide sequencing confirmed additional tandem repeat insertion (33 nt) into KK262 genome comparing to K49.

The organ samples (spleen, liver, kidney, lymph nodes) from euthanized animals were analyzed by qPCR and virus isolation using monocyte/macrophages infection. The genome of virulent ASFV was also revealed in spleen samples and lymph nodes of 2 vaccinated/challenged animals. The K49 ASFV strain was also successfully isolated after three continuous passages on swine macrophages.

Therefore, despite of the great protective potential of homologous attenuated ASFV strain the virulent strain still can infect the host and persists for weeks. The attenuated variant might be easily “replaced” by virulent isolate without causing clinical signs of disease that posed a threat for disease transmission

## ORAL 33: Purification and Proteome analysis of African Swine Fever Virus Particles

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**Keywords: ASFV particles, proteome analysis, mass spectrometry**

African swine fever (ASF) is a viral disease that affects members of the *Suidae* family such as bush pigs, warthogs, domestic pigs, and wild boars and is transmitted by direct contact with infected animals or by soft ticks of the *Ornithodoros* genus. Warthogs and bush pigs are generally asymptomatic and are wildlife reservoirs for the virus in Afrika. While ASF had long been endemic in sub-Saharan Africa and in Sardinia, the recent spread into Trans-Caucasian countries has raised concern as ASF severely threatens the European pig industry. In spite of this fact, literature on virus-host interactions that could be used as targets for antiviral strategies is scarce. As the morphogenesis of ASFV is very complex and leads to mature enveloped intracellular virus particles and extracellular virions that carry an additional envelope, the preparation of pure extracellular virions is challenging. To date, highly purified ASF virion preparations have not been analyzed systematically using modern mass spectrometric approaches. The intention of this study is to define the proteome of the ASFV particle and to identify virus-host interactions that potentially play a role in virus morphogenesis.

This work describes the purification of ASFV particles and the characterization of the proteome of mature extracellular ASF virions using shotgun nLC-MALDI-TOF/TOF mass spectrometry. A convenient and efficient workflow for the purification of ASFV particles has been established. ASFV OUR T88/3 was propagated on WSL-HP cells which originate from a natural host (wild boar) using high-density cell culture flasks with 10 layers (HYPERFlask, Corning) to achieve high virus yields. Particles were purified by a combination of differential sedimentation and gradient density ultracentrifugation steps using sucrose and Iodixanol (OptiPrep) as gradient media. Separation was based on particle density as well as on particle dynamics resulting in preparations with high specific infectivity. Use of Iodixanol facilitated the purification of virus, probably due to its low viscosity. Total virus recovery was between 35-70 % after the gradient step. The final virion preparation was characterized by electron microscopy, western blot analysis using virus-specific proteins, and finally by shotgun nLC-MALDI-TOF/TOF mass spectrometry. We present a comprehensive catalog of viral and host-derived structural ASFV proteins.

## ORAL 34: Role of tetherin restriction factor on African swine fever virus infection

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**Keywords: ASFV, virus-host interaction, tetherin**

Tetherin (BST2) is an antiviral protein induced by interferon response. The antiviral activity of tetherin resides mainly in its ability to restrict the release of enveloped viruses. It has been suggested that the sensitivity toward tetherin may be strain specific in viruses such as Influenza. Tetherin is located at the plasma membrane and in intracellular compartments being described to bind to AP complexes, which seems to be responsible of tetherin distribution interchange between cellular membrane/cytosol.

ASFV is a large, enveloped, double stranded DNA virus that infects swine monocytes and macrophages. ASF virulent strains encode CD2v, which has been described by our group to localize together with the molecular adaptor AP-1 during ASFV infection. The repercussion on infectivity of this binding is still unknown, and we investigate whether, first, tetherin may represent an anti-viral activity against ASFV, and second, if it is involved in virulence. We further hypothesize that ASFV could harbour anti-tetherin resistance during virulent strains infection through the control of AP-1 localization possibly based on a CD2v-dependent mechanism.

Our results showed that tetherin expression displayed a strain-dependent behaviour in E-70 vs NHV-infected cells, as demonstrated by qPCR detection of BST2 mRNA. However, both in E70 and NHV-infected COS cells, exogenous HA-BST2h was observed to be down regulated from the cytoplasmic membrane. Preliminary results regarding BST2 localization, AP-1 interaction and the putative antiviral role of tetherin during ASFV infection are presented. These results suggest that BST2 could represent a virulence restriction factor for ASFV, but the mechanisms to the viral control remain still elusive.



**ABSTRACTS**

**TOPIC III**

Current challenges  
inside Europe

**PARALLEL SESSION VI**

BT and other ruminant  
diseases: Epidemiology &  
surveillance and  
Risk assessment

**ORAL  
PRESENTATIONS**

## ORAL 35: Re-emergence of Bluetongue serotype 8 in France in September 2015

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**Keywords: Bluetongue, Re-emergence, Diagnosis, Monitoring**

Bluetongue is an infectious, noncontagious, and arthropod-borne viral disease of domesticated and wild ruminants caused by Bluetongue virus (BTV) (*Orbivirus* genus, *Reoviridae* family). The BTV genome consists of 10 segments of double-stranded RNA. To date, a total of 27 distinct BTV serotypes (BTV-1 to BTV-27) have been identified.

Six years after the introduction of BTV-8 in Europe and after two compulsory vaccination campaigns, France was officially declared “free from BT” in December 2012. Almost 3 years later, the disease made his comeback.

In August 2015, BTV-8 was detected in a sick ram showing clinical signs of BTV infection in central France (department of Allier)(Sailleau et al, 2015). Since its first detection, the virus continued spreading through autumn until the end of December. Sixteen French departments were affected with a total 150 infected herds, of which 95 herds (63.3%) were located in neighboring Allier and Puy-de-Dôme departments (identified as the epicenter of the current BTV-8 outbreak). In May 2016, more than 250 outbreaks were reported.

The BTV-8 strain, designated BTV8-15-01, was isolated from blood after a single passage on BSR cells. Next-generation sequencing was performed on RNA extracts from infected cultures. Sequence analysis of the 10 double-stranded RNA segments (Seg) that constitute the BTV genome showed a close relationship to BTV-8 isolated in France in 2008 (Breard et al, 2016). The sequences of the noncoding regions in Seg-1 to Seg-10 were 100% identical to those of BTV8 FRA2008/28 and FRA2008/29 and only nineteen substitutions were identified in 18,444 coding nucleotides, with a consequence of 4 amino acid changes (1 change in each of Seg-1, 3, 8, and 9). Although the origin of the BTV-8 responsible for the 2006 outbreak has never been defined, the BTV-8 strain circulating in France in 2015 has an almost identical genome to that of the 2008 French isolates. It is likely that the current BTV-8 strain has been circulating, with a low prevalence in the field, possibly by infecting domestic and/or wild ruminants, since it was last detected in France in 2009.

This paper describes the diagnostic tools used for the detection of this BTV strain, the analysis of the virus full-genome sequence, the results of the survey carried out in the field in 2015 and 2016 and the measures of control put in place in France since this virus detection.

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## ORAL 36: A Novel Bluetongue Virus in Healthy Goats from Sardinia, Italy

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**Keywords: Novel bluetongue virus, healthy goats, next generation sequencing, phylogeny**

Bluetongue (BT) is an infectious viral disease of domestic and wild ruminants sustained by the Bluetongue virus (BTV), an *Orbivirus* with a segmented (Seg 1-Seg 10) double-stranded RNA genome. BTV exists so far into 27 distinct serotypes. Since 2000, due to its location in the middle of the Mediterranean basin, Sardinia experienced a series of BTV incursions from countries facing the southern side of the basin thus representing one of the European major BTV invasion corridors. As for the deep economic impact that BTV has on livestock trade from infected areas, a control strategy was implemented in Italy, combining direct and indirect control measures with an intense surveillance plan. During the activities of the plan, we were able to identify in 2015 a putative novel bluetongue virus in two goat farms from Sardinia. The goats were healthy with no evident clinical signs of BT infection. Blood samples were positive by a generic real time RT-PCR (rtBTV) able to detect the genome of all existing BTV serotypes and serum samples were positive for BTV antibodies by cELISA. Serotyping and genotyping were, however, unsuccessful. Therefore, further investigations were carried out including isolation attempts and whole genome sequencing using a combination of sequence-independent/single-primer amplification (SISPA) and next generation sequencing (NGS) straight from two infected blood samples (one for each farm) showing the lowest threshold cycle by rtBTV. Nearly the whole genome sequence (BTV-X ITL2015) was obtained from one blood sample, whereas from the other only partial Seg 3 and Seg 4 sequences were retrieved. Nevertheless, they showed 100% of nucleotide identity with Seg 3 and 4 obtained from the blood sample of the first farm.

Seg 2 of BTV-X ITL2015 shows the highest % of identity (75% nt/77% aa) with BTV-27 from Corsica whereas it is less related with BTV-25 (74% nt/74% aa) and BTV-26 (63% nt/60% aa). VP2 (encoded by Seg 2) of BTV-X ITL2015 belongs to the same nucleotide (K) of BTV-25 and BTV-27 whereas the VP5 (encoded by Seg 6) apparently forms an additional nucleotide (J) as it is more distantly related to the homologous genome segment of BTV-25 (70% nt/76% aa), BTV-26 (73% nt/81% aa) and BTV-27 (70% nt/75% aa). Considering the Seg 2/VP2 identity of BTV-X ITL2015 with those of BTV-25, 26 and 27 and that serum of BTV-X ITL2015 infected goats cannot neutralize any of the existing BTV serotypes, including a chimeric BTV-1 carrying VP2 and VP5 of the uncultivable BTV-25, we propose the existence of a novel BTV serotype which is circulating in goats in Sardinia. Unfortunately all isolation attempts were so far unsuccessful thus hampering proper serological characterization with extant BTV reference serotypes. Viral spread in the farms and further analysis of remaining genome segments are discussed in this study.

## ORAL 37: Bluetongue: farm risk profile definition by retrospective analysis

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**Keywords: Bluetongue, Risk factors, Epidemiology**

At the current state, after 16 years since the first outbreak of Bluetongue (BTV) in Italy, numerous aspects concerning the epidemiology of the disease are more understandable, especially about its spread throughout the territory and the vector dynamics, but there are still many unknown factors that affects its transmission and do not allow us to preventively act to “contain” the impact on the farm at the beginning of a new epidemic season. The changes of this disease and its knowledge during the past decade have involved various aspects of etiology and especially its epidemiology. However, some factors related to the territory and the management remained unchanged during these years. Considering the current difficulties to operate with a mass vaccination using a live attenuated vaccine, the characteristics inherent the farms and the agro-meteorological aspects are the main factors to consider to increment knowledge about the dynamics of the carrier and perform a specific risk analysis for each farm’s type. The aim of thi study is to define a farm risk profile based on the characteristics of susceptible animal breeding that have a role by increasing or reducing the risk of developing BTV, through a logistic analysis model. Animal density, territory characteristics and other intrinsic farm’s parameters were included as explanatory variables to study the correlation with the possible development of focus on larval habitats. This study was carry out according to a retrospective analysis and taking into account the data of all Sardinian farms, related to the years 2012-2015. For all farms included in the model, data about animal density, vaccination, previous BTV outbreak, bovine’s presence, altitude and water mq<sup>2</sup> inside, was recorded considering a 500 m buffer around the farm to define his territory. All variables were useful for the analyses and, in particular, logistic model reveals a protective role of the vaccination on the outbreak (OR = 0.021, 95% CI = 0.013-0.035, p-value <0.0001), and the same effect is represented by the outbreak in the previous year (OR = 0.29, 95% CI = 0.24-0.34, p-value <0.0001). Furthermore strong decrease association with more than 450 metres above s.l. on the outbreak risk (OR = 0.21, 95% CI = 0.18-0.23, p-value <0.0001). The probability of having an outbreak in farms where rivers are present within the territory is 1.44 times higher compared to farms without (OR = 1.44, 95% CI = 1.30-1.60, p-value <0.0001); even the large number of animals (more than 150) increase of 1.53 times (OR = 1.53, 95% CI = 1.42-1.65, p-value <0.0001) the chance of outbreak respect to breeding with less than 150 animals. The risk farm profile as defined by fitted model will enable to provide more specific information about the role of every factor’s effect to the sanitary local units and all those Institutions involved in the BTV fight, useful for the plane of prevention measures in the territory.

## ORAL 38: Sheep infection studies closely simulating natural transmission suggest vector competence characteristics might influence selection of field reassortment strains of bluetongue virus

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**Keywords: Bluetongue virus, reassortment, virulence, vector competence, transmission**

Bluetongue virus (BTV) is transmitted to its ruminant host almost exclusively during blood-feeding of the biologic vector *Culicoides* biting midges and causes a severe haemorrhagic disease, bluetongue, especially in sheep. BTV has a segmented dsRNA genome and simultaneous infection of the same cell by different virus strains can lead to the exchange of genome segments known as reassortment. Hence reassortment strains further complicate BTV strain phenotypic variation within 27 serotypes identified to-date.

It is extremely challenging to experimentally examine the complex interactions between mammalian hosts, insect vectors and transmitted vector-borne viruses and most studies are therefore compromised by needle-inoculation of the virus into the host. We have routinely implemented transmission of BTV to sheep by allowing previously infected *Culicoides* vectors to blood-feed. This more natural infection approach resulted in reliable BTV infection of more than 30 sheep irrespective of BTV strain, even in cases where only 1-3 individual infected *Culicoides* took a blood-meal.

This *Culicoides*-transmitted BTV *in-vivo* infection system was utilised to investigate pathogenesis and transmission characteristics of three parental BTV strains of different serotypes (BTV-1 [MOR2007/01], BTV-8 [NET2006/06] and BTV-4 [MOR2004/02]) and two of their field-isolated natural reassortments, both of the BTV-4 serotype, in sheep. The BTV-4 MOR2009/07 strain is a double-reassortment between BTV-4 (genome segments [Seg] 2,3,6 and 9) and BTV-1 (Seg 1,4,5,7,8 and 10), while BTV-4 MOR2009/10 is a triple reassortment strain between BTV-4 (Seg 2,6 and 9), BTV-1 (Seg 4,5,7 and 10) and BTV-8 (Seg 1,3 and 8).

BTV-1 MOR2007/01 demonstrated highest virulence in sheep with all 4 sheep infected with this strain being euthanized prior to study end. Infection with parental strains BTV8 NET2006/06 and especially BTV4 MOR2004/02 led to less severe clinical disease with clinical outcome of infection varying between individual sheep. Both BTV-4 reassortment strains demonstrated increased virulence in the infected sheep compared to the BTV-4 parental strain.

Blood-feeding of approximately 1200 uninfected *C. sonorensis* per strain on infected sheep at peak viraemia and subsequent incubation resulted in potentially transmissible infections in only a few *Culicoides* individuals for the two parental strains of BTV-1 and BTV-8, both strains exhibiting very low vector competence rates below 1%. On the contrary the BTV-4 parental strain resulted in fully-transmissible infections of blood-fed *C. sonorensis* at average vector competence rates of approximately 10%, despite similar or lower RNAemia levels in sheep. Furthermore high vector competence was also detected in *C. sonorensis* allowed to blood-feed on sheep infected with reassortment strains BTV-4MOR2009/07 or BTV-4 MOR2009/10, the latter reaching average vector competence rates of over 20%. Vector competence rates of all BTV strains remained comparable when membrane feeding *C. sonorensis* on blood collected from the viraemic sheep during peak viraemia. The obtained results suggest that BTV infectiousness for the insect vector might contribute to BTV reassortments being selected and becoming naturally transmitted in the field. Furthermore our studies have demonstrated the reliability of using infected *C. sonorensis* to enable studies of pathogenesis and immune responses to *Culicoides*-borne viruses in the natural host with increased relevance by simulating natural transmission.

## ORAL 39: Genetically stable infectious schmallenberg virus persists in foetal envelopes of pregnant ewes

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Schmallenberg virus (SBV) is a recently emerged virus that induces congenital defects in bovines, ovines and caprines. It was shown before that SBV RNA can persist in maternal, foetal and foetal envelope tissues till the moment of birth when ewes are infected with an SBV infectious serum at day 45 and 60 of gestation. In this study, the infectiousness of this SBV RNA was assessed by virus isolation and its genetic stability during the gestation period was evaluated by sequencing of a 1362bp fragment overlapping the hypervariable region of the M segment. Finally, neutralization tests (VNT) were performed to assess whether the persistent infectious virus could be neutralized by the humoral immune response of the infected ewes.

Five out of 31 SBV qRT-PCR positive organs were found positive in virus isolation. SBV was isolated from the umbilical cord of one ewe inoculated at day 45 of gestation and from three placentomes and one intercotyledonary membrane of four different ewes inoculated at day 60 of gestation. Sequence analysis of the hypervariable region showed that the persisting infectious virus in the placentomes was highly homologous (99.8% (95% CI [99.6%–100%])) to the inoculum and SBV present in serum at the peak of viremia. Furthermore the VNT showed that the infectious SBV present in the foetal envelopes at birth can be neutralized by the humoral immunity present in the infected ewes. An increase in the VNT titre was observed in sera collected at 35 dpi and 90 dpi against the infecting virus, demonstrating an affinity maturation of the SBV specific antibodies during this period.

In conclusion, infectious SBV can persist in foetal envelopes of infected ewes for at least 100 days. This persisting SBV genetically resembles the original inoculum and can be neutralized by the circulating antibodies. These findings are potentially important to explain SBV overwintering.

## ORAL 40: Cowpox virus-host interactions: Determination of defined virulence factors

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**Keywords: Cowpox virus, Virulence, Pathogenicity**

The pathogenic potential of different cowpox virus (CPXV) strains is rather variable. The knowledge about virulence factors of CPXV field strains is crucial to generate risk assessments for circulating viruses in voles (= reservoir host) and viruses infecting accidental hosts such as rats, cats or humans. Therefore, the objective of our study was the identification and confirmation of defined CPXV virulence markers. Two representative CPXV isolates, one isolate from a rat and one cell culture-adapted virus, which have strikingly different pathogenic potential, were compared. *In vivo* experiments demonstrated that animals infected with the rat-derived CPXV developed severe disease with mortality rates of up to 100 %. The genome of this highly virulent CPXV specifies additional open reading frames (ORF) when compared to the laboratory strain. Specifically, additional sequences were identified in the *ati*, *p4c*, *NMDAr*, *7tGP*, *D7L* and *CrnE* genes. Phenotypic correlates of these genotypic differences were investigated *in vitro* and *in vivo* by the generation and testing of chimeric viruses as well as single gene mutants. While the *in vitro* growth analyses revealed no differences between the generated CPXV strains, the morbidity and mortality rates in Wistar rats as animal model were quite different. These first data suggest that *NMDAr*, *D7L* and *CrnE* might be important virulence factors of CPXV and we concluded that genotypic differences are indeed related to different pathotypes.



**ABSTRACTS**

**TOPIC III**

Current challenges  
inside Europe

**PARALLEL SESSION VII**

ASF: Epidemiology  
& surveillance and  
Risk assessment

**ORAL  
PRESENTATIONS**

## ORAL 41: Serological survey and geographical analysis of its results in an ASF endemic territory

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**Keywords: African swine fever, serological tests, geographical information systems**

African swine fever (ASF) was introduced in Sardinia in late 1978 and remains still endemic. Despite the application of severe measures the outcomes were unsatisfactory and indeed, beginning in 2011, a significant epidemic wave of the disease was observed. One of more important instrument provided by the annual eradication program is the serological monitoring of domestic and wild pig population. Each year the eradication program impose the serological and clinical check of pig herds as well as a large sample of shoot wild boars during the hunting season; moreover in case of ASF outbreak the plan provide the serological surveillance of pig herds situated in the surveillance and protection zone. Currently the eradication plan indicates the ELISA test for the screening whereas the positive samples should be confirmed by Immunoblotting test.

In this contest a lot of data are available sometimes difficult to interpret; in this work we try to investigate the epidemiological means of the serological survey even using geographic tools.

For this study all the serological samples carried out by the Istituto Zooprofilattico Sperimentale of Sardinia in 2015 were considered; specifically seropositive samples (to the screening test and/or confirmatory test) were evaluated and geographical information systems (GIS) was used to map and investigate the positive (almost one samples seropositive) farms.

The spatial analysis was conducted through the creation of dot, concentration maps and hotspot areas. The mapping and the geographical calculation were done using the freeware software QGIS 2.4.0-ChugiaK.

A total of 89,150 sera from 11,292 farms were tested, of these 11,006 were georeferenced and 152 were positive.

Dot and distribution maps, using absolute numerical value (representing seropositive farms), have been highlighted 6 hotspot areas that are located in the area known as the ASF historical endemic area. In fact in this area most of positives farms are localized.

The detection of serological positive animals is an important indicator of viral circulation. In the ASF endemic area of Sardinia the serological survey remains a strategic instrument and a deep evaluation of data is fundamental to understand the epidemiological dynamic of infection. This is valid for wild boar population as well as for the domestic one, because gives the evidence of illegal animal movements and hide infection outbreaks. Often the finding of serological positive is the only track of viral spread (if no viremia is detected), but anyway it is relevant to take in mind these important data and give them the correct epidemiological mean. The application of geographical tools in this field is very useful to individuate the high risk areas and to evaluate the spread of infection in the target population.

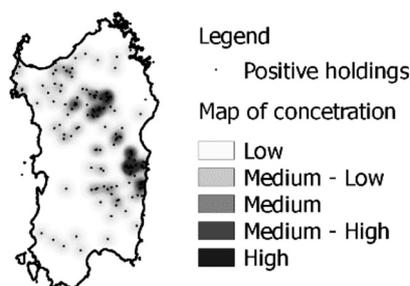


Figure 1: Concentration map of farms positive for ASF diagnostic tests.

## ORAL 42: Characterization of pig production in Sardinia and its relationship with ASF presence

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**Keywords: pig production, swine trade, Sardinia**

Agriculture is an important sector in Sardinia that generates together with fishing and silviculture more than 900 million € on average per year. Livestock production is mainly focused on sheep and dairy, while pigs remain as secondary activity. Pig husbandry in Sardinia has been affected for decades by African swine fever (ASF), a high consequential notifiable disease, present since 1978. ASF presence involves restriction of exports, which could have limited growth and progress of pig production. However, very few data is available about pig production system on the island, how it works and the potential relationship with ASF. Considering that new regulations are being established to eradicate ASF, promoting farm professionalization, rising biosecurity levels and increasing veterinary control on pig farms, we considered essential to characterize the current swine system, identify trade and husbandry patterns in order to be able to check efficacy and implementation of these new measures.

In order to elucidate that, a retrospective study was performed to study the characteristics of pig production and pig trade channels on the island. For that purpose, data on pig farms and pig movements between 2010-2015 was analysed, pig farms were categorised according to current regulations, and pig movements/trade patterns were analysed by using time series and social network analysis (SNA). In addition, productivity levels of swine farms were estimated considering farm typology, characteristics of the local production and the number of productive years of the farm.

Results showed that more than 90% Sardinian farms are breeding farms, of which around 70% had less than 5 animals registered in the official census. Semi-extensive farms were mainly located in Northern provinces whereas intensive (closed) farms were more common in Central and Southern regions. Data processing showed that around 60% studied farms had declared any movements throughout their productive life. Significant seasonality in self-consumption and domiciliary slaughters was identified in winter, especially on small and medium farms. In contrast, movements to slaughterhouses registered two maxima over each year within summer and winter, whereas movements between farms were randomly reported along study period. SNA showed a local network with almost always unidirectional movements between neighbouring farms, involving a supplier farm and a recipient farm. Likewise, most farms usually sent animals to nearby slaughterhouses. Regarding productivity rates, more than 90% premises with registered movements reported lower productivity rates than expected.

Sardinia pig production is mainly based on non-professionalised small-scale producers established to satisfy self-consumption. Marked seasonality on pig slaughtering and self-consumption was identified in winter potentially due to increase demand for pig meat and pork products during Christmas. Based on productivity estimation, many pig movements may go un-reported on Sardinia. However, positive trends in reporting movements was observed since 2012 which may reflect an improvement in compliance with regulations. Discordance between official reported data and estimated productivity reflects potential illegal trade on the island that may be bypassing veterinary controls. All above constitute potential risks that could favour ASF spread and persistence on the island. Moreover, results get sight into time periods when control on farms should be implemented in order to avoid entrance and spread of infectious diseases through animal movements.



## ORAL 44: First evidence of an attenuated phenotype of genotype II African swine fever virus in Estonia

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**Keywords: African swine fever virus, attenuation, pathogenesis, characterization**

African swine fever (ASF) is a severe, multi-systemic disease of pigs that is caused by the eponymous double-stranded DNA virus (ASFV) of the genus *Asfivirus* within the *Asfarviridae* family. In 2014, ASF was re-introduced into the European Union, affecting domestic pigs and wild boar in the Baltic States and Poland. Up to now, new cases have been reported especially from wild boar every week. However, the geographic region remains rather stable. This epidemiological behaviour was rather unexpected based on the experimental findings that the virus strains involved showed exceptionally high virulence. It was anticipated that the virus would either spread rapidly or die out due to self-limitation. Factors leading to the observed long-term outbreak are far from being understood. One explanation could be virus attenuation. In fact, wild boar hunted in Northern Estonia were tested ASFV-positive without showing obvious clinical or pathological signs. To explore the reason for this phenomenon, a re-isolated Estonian virus was biologically tested in different experiments.

In a first experiment, ten sub-adult wild boar were inoculated. Here, the virus showed high virulence, and nine out of ten animals succumbed to infection showing typical lesions. Subsequently, a virus re-isolated from the recovered animal was utilized in two trials with different pig breeds. Trial A comprised 12 minipigs, trial B five domestic fattening pigs. In both trials, oronasal inoculation was carried out using a blood suspension containing at least  $10^{4.5}$  hemadsorbing units. Upon infection, clinical signs were recorded daily. Furthermore, blood and swab samples were taken at regular intervals. Necropsy was carried out on all animals and organ samples were collected. Blood, swab and organ samples were subjected to PCR analyses and virus isolation, serum samples were analyzed using commercial ELISA kits and indirect immunoperoxidase test.

In trial A, all animals developed fever and unspecific clinical signs within the first week post inoculation. However, nine out of twelve minipigs survived the acute phase and were slaughtered in good health status at 36 days post inoculation (DPI). One animal was found dead after blood sampling and two others were euthanized due to severe respiratory distress. These deaths were not clearly linked to the disease course. Necropsy revealed that two of the inoculated minipig sows were pregnant. Organ pools of the fetuses were tested by PCR and were found negative. Antibodies were detected in all convalescent animals by the end of the trial.

Animals of trial B displayed a similar disease course with all animals surviving till the end of the trial at 36 DPI. Apart from a severe fibrinous pericarditis in one of the animals, necropsy did not reveal any signs indicative for ASF or any other disease. While clinical signs were completely absent from 19 DPI, high ASFV genome loads were still detectable by PCR till the end of the trial.

Summarizing, an apparently attenuated virus strain was re-isolated from the initial trial. Animals of different pig breeds, i.e. minipigs and domestic fattening hybrids, showed similar disease courses that were, in terms of virus detection, still comparable to the highly virulent ancestors. However, clinical signs and mortality were drastically reduced. Under field conditions, these unspecific clinical signs could easily go unnoticed and thus complicate disease control tremendously. For the wild boar situation in Estonia, circulation of attenuated strains is likely and should be further investigated.

## ORAL 45: Experimental pig-to-pig transmission study with a recent European African Swine Fever virus isolate

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**Keywords: African swine fever virus, Polish isolate, transmission**

### Introduction

African swine fever (ASF) is a severe viral haemorrhagic disease of domestic pigs and wild boar - usually causing high mortality. The disease is caused by African swine fever virus (ASFV), a large DNA virus that is the sole member of the genus *Asfivirus* within the family *Asfarviridae*.

In 2007, ASFV was introduced into Georgia and subsequently into other Transcaucasian Countries, the Russian Federation, Ukraine and Belarus, where it has caused outbreaks in wild boar and domestic pigs. In early 2014, it spread to Eastern Europe (Poland and the Baltic countries), where outbreaks in both wild boar and domestic pigs have been notified.

There is a non-negligible risk of ASFV introduction to Western Europe including Denmark via returning trucks, movement of people and other human-induced activities. Currently, there is limited knowledge of clinical and pathological characteristics, modes-of-transmission and transmission parameters of the new European ASFV strains. Knowledge that is vital in the improvement and evaluation of our current ASFV contingency plans.

### Materials and methods

To gain knowledge of the above listed parameters, an *in vivo* infection study was conducted using a recent European ASFV isolate (from Poland, February 2015) in 8-9 weeks old pigs. Four pigs were inoculated intra-nasally with the virus and 12 susceptible contact pigs were exposed to the inoculated pigs, either directly (direct contact within- and between pens) or by air. Six pigs were kept as negative controls. After euthanasia of the four inoculated pigs and the 12 contact pigs, four control pigs were moved to the infected stable unit in order to investigate the possibility of indirect transmission via a contaminated environment.

Clinical scores, including rectal temperature, were recorded from the pigs each day. Serum, EDTA, oral, nasal and rectal swabs were collected from the pigs, while manure and air samples were obtained from the environment. Samples have been tested for the presence of ASFV by quantitative real-time polymerase chain reaction (qPCR) and by virus isolation in cell cultures (VI). Serum samples were tested for the presence of ASFV antibodies by competitive enzyme-linked immunosorbent assay (ELISA) and by immunoperoxidase monolayer assay (IPMA).

### Results

In this study it was shown that intranasal inoculation, direct contact, air contact and indirect contact (through a contaminated environment) resulted in development of acute disease characterized by high fever and depression. Other symptoms, including hyperemia, bleedings and convulsions were only seen in some pigs. The pathological findings varied considerably between pigs. However, in most pigs, haemorrhagic lymph nodes, hydroabdomen and coagulation deficiencies were observed. Blood, swab samples, manure and air samples were found to be ASFV positive in PCR, while VI results are currently awaited. Antibodies were not detected in serum samples.

Data from the experiment is currently being incorporated into an existing within-herd ASF spread model and will be used to model the spread of ASFV within and between Danish pig herds.

## ORAL 46: *In vivo* experimental studies of genotype II African swine fever virus (ASFV) isolates currently circulating in two Estonian counties

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African swine fever (ASF) is one of the most important re-emerging diseases of swine. It was introduced in 2007 into Georgia in the Trans Caucasus and rapidly spread to neighbouring countries including the Russian Federation. Since then, ASF continued to further expand on North and West to countries neighbouring the European Union (EU) including Ukraine and Belarus. In 2014 ASF entered the EU in Poland, Lithuania, Latvia and Estonia most likely through incursions of infected wild boar from Eastern Europe neighbouring countries, as Belarus. During 2015 and 2016 have had a dramatic increase in numbers of cases in wild boar in the EU with a continuing dispersal particularly in Lithuania, Latvia and Estonia. Experimental infections of pigs and wild boar with ASF virus (ASFV) isolates from the earlier phases of the epidemic in the Eastern Europe have indicated that these are highly virulent and induce acute form of ASF which result in the death of almost 100% of pigs before an antibody response is developed. However, since 2015, in certain areas of the affected Eastern EU countries, there has been an increasing evidence of seropositive wild boar with high antibody titers and low virus presence. This pattern of infection is related to animals surviving for more weeks than expected, and even some of them may be recovering from the disease. It could be due to acquired immunity after the primary infection, and/or the presence of related viruses of reduced virulence that could emerge after the circulation of the ASF virus within the wild boar population. In order to obtain a precise description of the clinical, virological and pathological features induced in domestic pigs infected with the currently circulating ASFV isolates in the EU, an animal trial has been conducted at the EU Reference Laboratory (EURL) for ASF. Two groups of six domestic pigs were housed in separate compartments and two pigs in each group were inoculated by the intramuscular route with 10 HAD<sub>50</sub>/ml of two different ASFV Estonian isolates; i) in group I animals were inoculated with the Es15/WB-Valga6 ASFV isolated from a seropositive wild boar in Valga region in 2015, and ii) in group II the domestic pigs were inoculated with the Es15/WB-Tartu14 ASFV isolate, representative of the new ASFV genetic variant (GII-CVR-2) which is co-circulating in Tartu region since July 2015 within the wild boar population. In both, at the start of the experiment, four out of the six pigs were housed together with the inoculated pigs as in-contact animals, to better resemble the possible transmission routes in field.

An acute fatal disease was developed in one out of the two inoculated animals, in group I that died at 11 days post inoculation (dpi). The second inoculated pig showed, however, a delayed course of the disease, resembling the same as that seen in two out of the four in-contact animals, which died or were slaughtered from 23 to 25 days post exposure (dpe). Two naturally infected “in contact” animals survived the infection which was characterized by the presence of clinical signs similar to that observed in the dead animals although with less intensity. Both inoculated and in contact pigs seroconverted at the second week after the infection and blood remained PCR-positive after a month they were infected.

In group II, the two inoculated animals developed an acute form of ASF and died or were slaughtered at 7 and 11 dpi. One out of the four in-contact pigs showed severe disease and was slaughtered at 14 dpe. The three remaining naturally infected “in contact” animals survived to the infection showing unspecific clinical signs related to the chronic form of ASF which were characterized by recurrent transient fever, joint swelling and respiratory distress with spasmodic coughing. Weak and transient viremia was detected at the second week post contact in these three in-contact animals and become seropositive at 14 dpe yielded very high values (>10<sup>5</sup>) until the end of the experiment.

This information is very valuable for more effective control -eradication programs of ASF in the affected areas where genotype II ASFV viruses are circulating.

*This work has been funded by the INIA AT2015 -002 project, in collaboration with the Estonian National Reference Laboratory and the European Union Reference Laboratory for ASF (EURL-ASF).*



**ABSTRACTS**

**TOPIC III**

Current challenges  
inside Europe

**PARALLEL SESSION VIII**

BT and other ruminant  
diseases: Diagnostics and  
Intervention strategies

**ORAL  
PRESENTATIONS**

## ORAL 47: Efficacy of BTVPUR ALSAP 8 in sheep after a single injection

Galleau, Stéphanie; Hamers, Claude; Bibard, Amandine; Blanchet, Michel; Besancon, Laure; Goutebroze, Sylvain

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**Keywords: Bluetongue, BTV-8, Vaccination, Duration of Immunity, Viraemia**

### Introduction

BTVPUR ALSAP 8 is a vaccine centrally registered in the EU since 2009 to immunize cattle and sheep against the bluetongue virus serotype 8. During the development of this vaccine, it was demonstrated that, in both sheep and cattle, 2 injections at a 3-4 weeks interval are able to protect all animals for 1 year, not only against clinical signs (disease protection) but also to prevent viraemia (epidemiological protection).

Therefore, for both species, the SPC indicates a primary vaccination including 2 injections (3-4 weeks apart) for active immunisation of sheep and cattle to prevent viraemia and to reduce clinical signs caused by bluetongue virus serotype 8, with onset and duration of immunity of 3 weeks and 1 year after the primary vaccination course respectively.

During this development, the efficacy of the vaccine was also tested after a single injection and protection was assessed by BTV-8 virulent challenges performed after 6 months or after 12 months. We report hereafter the results of these single shot experiments.

### Material and Methods

In this experiment, 29 weaned, BTV naive lambs were randomly allocated to a control group (G<sub>0</sub>) of 15 animals and a vaccinated group (G<sub>1</sub>) of 14 animals. On Day 0 (D<sub>0</sub>), all animals of the G<sub>1</sub> group were administered a 1 mL subcutaneous injection of a BTVPUR Alsap 8 vaccine, formulated at low antigen dose. Controls remained untreated. All lambs were maintained in insect-proof facilities until their challenge and in high containment thereafter. On D<sub>196</sub>, 7 vaccinates and 7 controls were challenged with a virulent BTV-8. The remaining 7 vaccinates and 8 controls were challenged on D<sub>365</sub> with the same virulent BTV-8 isolate. During the immunization phase, animals were serologically monitored at key time points. During the challenge phases, rectal temperatures, clinical signs and viraemia were monitored for 14 days.

### Results

**Six months challenge:** all controls developed hyperthermia, typical clinical signs of Bluetongue and high viraemia titres. Conversely, none of the vaccinates ever presented such sign or was found viraemic at any time point. Full protection of all vaccinates was demonstrated.

**Twelve months challenge:** all controls developed hyperthermia, typical clinical signs of Bluetongue and high viraemia titres. In the vaccinated group 6 out of the 7 vaccinates never presented such sign nor was found viraemic at any time point. The 7th vaccinate presented frank hyperthermia, typical signs of Bluetongue and moderate viraemia titres. The reason for this animal not being fully protected remain unclear but the consequence is that prevention of viraemia was not demonstrated in every single animal after a 1 shot administration.

### Conclusion

These results demonstrate that a single injection of BTVPUR ALSAP 8 in sheep provides full clinical and virological protection for 6 months and a very significant protection for 1 year.

In some types of production models, such a vaccination schedule could be of economical, epidemiological and practical interest for protection during the vectorial season.

## ORAL 48: Bluetongue virus infection of bovine monocytes is altered in the presence of *Culicoides sonorensis* salivary proteins

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**Keywords: Bluetongue virus, *Culicoides*, vector-pathogen-host interaction, monocytes, cytokines**

*Culicoides* biting midges are the biological vector for numerous arthropod-borne viruses (arboviruses) of livestock such as Bluetongue virus (BTV) and Schmallenberg virus. Arboviruses are transmitted to their vertebrate hosts during blood-feeding of their arthropod vector and are deposited into the skin alongside the pharmacologically active saliva of the vector. The saliva of haematophagous arthropods contains a mix of proteins which facilitate successful blood meal acquisition by counteracting blood coagulation and local immune responses. Importantly, many arboviruses such as BTV, West Nile virus and Rift Valley fever virus display increased viral infectivity and/or virulence when inoculated into the vertebrate host alongside their arthropod vectors saliva. The most likely explanation for this phenomenon is immune-modulatory effects of arthropod saliva indirectly benefiting the co-inoculated virus.

The mechanisms of how *Culicoides* salivary proteins modify infectivity and virulence of *Culicoides*-borne viruses, such as BTV, are largely unexplored. We have previously shown that proteases within *Culicoides* saliva can directly modify the BTV particle by cleaving the outermost protein VP2, resulting in increased infectivity for *Culicoides* derived cells, however the effect on infectivity for ruminant host target cells is still unknown. Blood-feeding of *Culicoides* causes significant cellular influx and inflammation of the ruminant skin, hence saliva induced inflammatory responses and immune-modulatory effects may also indirectly impact on BTV infectivity.

Here we demonstrate for the first time that BTV infection of bovine peripheral blood mononuclear cells (PBMCs) is altered in the presence of salivary proteins collected from a BTV competent vector species; *Culicoides sonorensis*. *C. sonorensis* salivary proteins significantly enhance the release of progeny virus during BTV infection of PBMCs, compared to progeny virus produced by BTV-infected PBMCs in the absence of salivary proteins. Furthermore, flow cytometry analysis has highlighted that PBMC infection in the presence of *C. sonorensis* salivary proteins leads to a significantly higher proportion of monocytes expressing viral structural and non-structural proteins. This indicates that BTV replication is accelerated in monocytes, within PBMCs, in the presence of vector salivary proteins. It is unclear at present whether these changes in virus infectivity are due to a direct interaction between *C. sonorensis* salivary proteins and the BTV virion. Initial experiments have however indicated modifications in the cytokine milieu of PBMCs exposed to different infection and treatment conditions. BTV-infected PBMCs produced high amounts of interferon- whilst in comparison the presence of salivary proteins during BTV infection causes a marked decrease in the production of this cytokine. In contrast, the presence of *Culicoides* salivary proteins appeared to increase the production of interleukin-10 in both mock-infected and BTV-infected PBMC cultures.

These results indicate a novel role of *C. sonorensis* salivary proteins in altering BTV infection of bovine PBMCs, specifically monocytes, which may be attributable to modifications in cellular cytokine production. With BTV remaining an ever increasing threat to European and UK farming, the study of the interactions between *Culicoides* salivary proteins, BTV infectivity and the host immune response is vital to fully understand how BTV, and other *Culicoides*-borne viruses, cause disease with potential applications to novel methods of viral control.

## ORAL 49: Recall T cell responses to Bluetongue virus produce a narrowing of the T cell repertoire

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**Keywords: BTV, T cells, T cell epitopes, VP7**

In most viral infections the recall T cell responses elicited by prior virus infection is critical for immune protection. Not only the magnitude of this secondary response may affect protection but also the CD8 and CD4 epitope repertoires and the diversity of this repertoire. Bluetongue virus (BTV) infection of sheep elicits a T-cell response after acute infection that contributes to viremia control and could be relevant for cross-protection between BTV serotypes. However, memory development has not been studied and many cavities are present in this field. Here we have characterized the CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses during acute, primary memory and recall responses. During primary immune response, both CD4<sup>+</sup> and CD8<sup>+</sup> T cell populations expanded by 14 days post-infection. CD4<sup>+</sup> T cell populations showed a lower peak of expansion and prolonged contraction phase compared to CD8<sup>+</sup> T cell populations. Analyses of secondary response after BTV challenge showed BTV-specific expansion and activation of CD8<sup>+</sup> but not of CD4<sup>+</sup> T cells. The evolution of BTV-specific TCR repertoires (Rojas et al, 2011; *Vaccine* **29**:6848) was also characterized by IFN- $\gamma$  production in response to VP7-peptide stimulation. Striking differences in repertoire development were noted during time-course infection. During primary responses a broader repertoire was induced for MHC-I and MHC-II epitopes. However, during memory responses, a narrowed repertoire was activated toward a dominant motif in VP7 comprised between amino acids 175 to 291. These findings could have implications for vaccine design as the narrowing memory T cell repertoire induced after BTV re-infection could lead to the development of protective immunodominant TCR repertoires that differs between individual sheep.

### Reference

Rojas JM, Rodríguez-Calvo T, Peña-Rubio ML, Sevilla N. T cell responses to bluetongue virus are directed against multiple and identical CD4<sup>+</sup> and CD8<sup>+</sup> T cell epitopes from the VP7 core protein in mouse and sheep. *Vaccine* 2011; **29**:6848-57.

## ORAL 50: First identification and isolation of Camelpox virus in Israel

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**Keywords: Camelpox virus, Isolation, Real-time PCR, Molecular diagnostics**

Camelpox is an infectious disease specific to camels, which was diagnosed in Africa, Asia and the Middle East, in most countries where camels are bred. Disease outbreaks can have a major economic impact on camel husbandry professionals in affected regions. The disease is characterized by fever, enlarged lymph nodes and typical skin lesions, with severe cases diagnosed mostly among young animals and pregnant females. The etiological agent of the disease is the Camelpox virus (CMPV) of the genus *Orthopoxvirus*, family *Poxviridae*, which is closely related to Cowpox virus and Variola, the cause of smallpox.

Here we report on the first documented identification and isolation of Camelpox virus in Israel.

Field tissue and blood samples from animals showing clinical signs indicative of pox virus infection were examined using standard and quantitative PCR. Sample suspension was used to inoculate embryonated chicken eggs and was examined by electron microscopy. Both Real-time and standard PCR results were positive for Camelpox virus, and infected chicken eggs developed characteristic viral foci. Suspensions from the infected chicken eggs were used to further inoculate cultured Vero cells, and cytopathic effect was observed three days following inoculation. Both infected eggs and cell cultures tested positive for Camelpox by PCR. Electron microscopy examination confirmed the presence of Orthopox virus. Finally, sequencing of four different regions of the virus genome confirmed the virus identity. Following the first identification, samples from three different regions, in the Negev region (Israeli desert) and the Palestinian Authority, were tested and found positive for Camelpox.

Further study of the obtained isolates is currently underway.

## ORAL 51: DNA Vaccination Regimens Against Schmallenberg Virus Suggest Two Novel Targets For Immunization

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Schmallenberg virus (SBV) is a pathogen that has been shown to infect both wild and livestock species of ruminants. An arbovirus of the Orthobunyaviridae genus, SBV has been associated with disease symptoms ranging from fever to foetal malformation, and has been detected in European livestock for nearly 5 years. While inactivated and attenuated vaccine candidates have been shown to protect animals against SBV infection, little is known about the mode of immunity generated following vaccination; in particular, which components are responsible for conferring immunity against infection. Here, we attempt to address this question through the evaluation of multiple components of SBV through DNA vaccination.

As previous DNA vaccination experiments on bunyaviruses have shown that glycoprotein and nucleoprotein components can confer protection against viral challenge, the efficacy of various DNA vaccine constructs were evaluated for their ability to induce immune protection in IFNAR<sup>-/-</sup> mice. These constructs encoded for the soluble domains of the glycoproteins G<sub>N</sub> and G<sub>C</sub>, as well as the ubiquitinated and non-ubiquitinated forms of the nucleoprotein (NP). Following intramuscular vaccinations, the mice were challenged with a virulent strain of SBV, and clinical signs were monitored for two weeks. While both the ubiquitinated and non-ubiquitinated groups produced elevated levels of antibodies, only the non-ubiquitinated form generated protection from viral challenge. Furthermore, a construct encoding for the N-terminal ectodomain of glycoprotein G<sub>C</sub> also demonstrated significant protection from SV challenge. Viremia was also shown to be significantly decreased in both the NP and G<sub>C</sub>-ectodomain groups. These results suggest that SBV has at least two protective immunological targets paving the way to novel vaccination strategies. The extent of their immunological protection, as well as their hypothesized mechanisms of immunity will be discussed.

### Acknowledgments:

NGAA is supported by the BFU2015-64541-R (MINECO/FEDER), HB by MSCA H2020 no 660155.

## ORAL 52: Development of high resolution melt (HRM) analyses for DIVA and genotyping of Lumpy skin disease virus (LSDV) and Bovine ephemeral fever virus (BEFV)

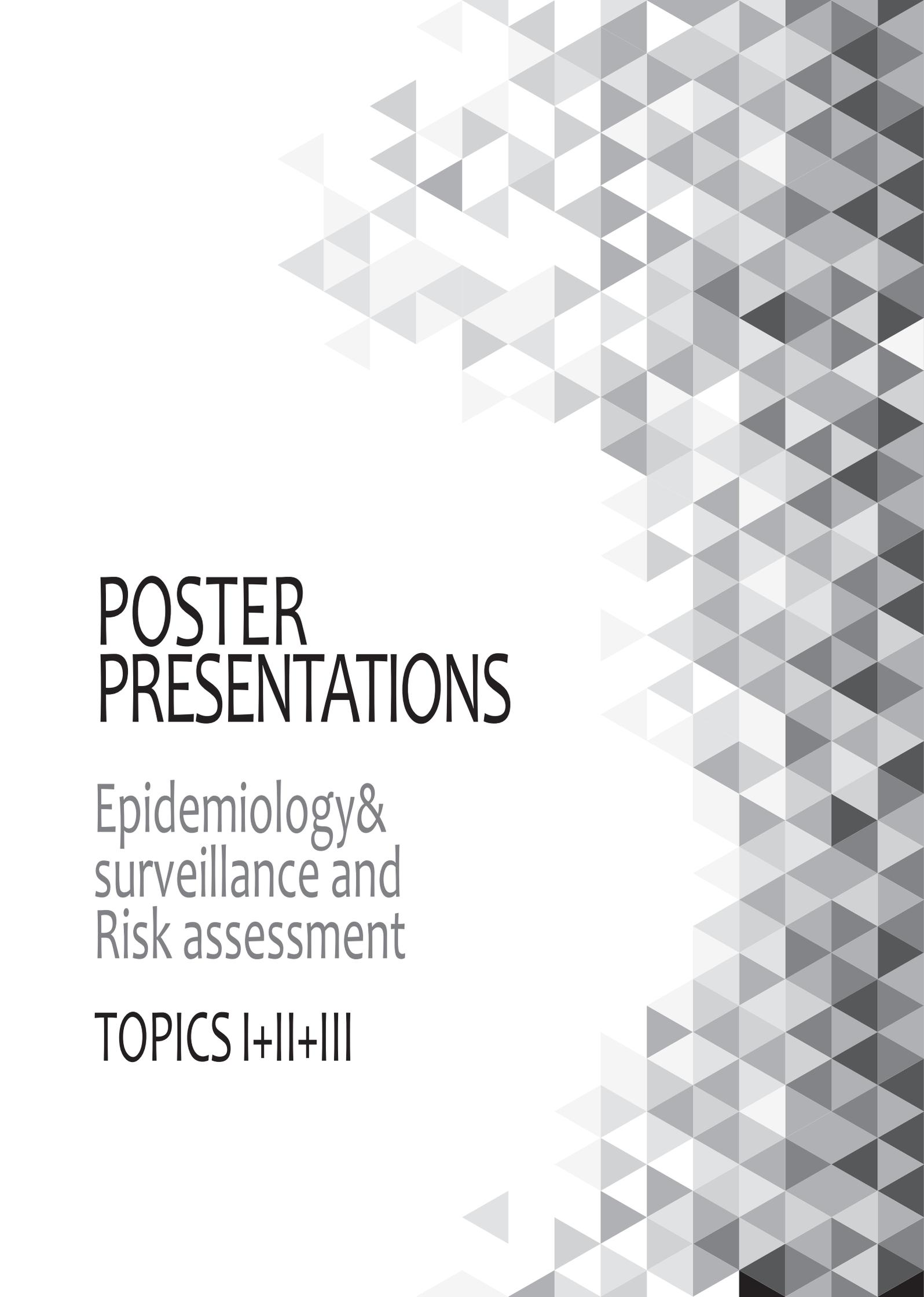
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**Keywords: DIVA, Lumpy skin disease, Bovine ephemeral fever, High resolution melt analysis**

Differentiation between infected and vaccinated animals (DIVA approach), and genotyping of related viral isolates, are fundamental requirements in order to conduct efficient disease control management, as well as to promote vaccine-related research and viral evolution studies. Here we describe the development of fast and accurate detection procedures for isolate identification of two important veterinary pathogens: lumpy skin disease virus (LSDV) and bovine ephemeral fever virus (BEFV). In the first study, differentiation between LSDV local virulent field strain and the LSDV attenuated Neethling vaccine was accomplished using qPCR followed by high resolution melt (HRM) analysis of a specific segment from the LSDV126 of the EEV gene family. This enabled rapid differentiation between vaccinated and infected animals without the need to sequence each isolate. This procedure also enables comparison of isolates from different geographical regions, thereby advancing our knowledge on the spreading of LSDV.

In the second study, a similar procedure was developed to distinguish between BEFV isolates from different origins, including the Australian vaccine strain. By using a unique algorithm developed specifically for this study, suitable regions within the BEFV glycoprotein G gene were identified for analysis. These regions share high similarity among isolates from the same region, and low similarity between isolates from different regions. HRM analysis based on these regions successfully differentiated between isolates from Israel, Turkey, South Africa, Japan and Australia, allowing rapid genotyping of field samples. This tool was then used to compare the geographic distances and HRM patterns of the different isolates. Correlation between geographic distance and melt point difference was not established, but the HRM pattern was consistent with sequence-based phylogenetic analysis in the examined gene regions. The application of this method in local BEFV diagnostics enables differentiation between vaccinated and naturally-infected animals, as well as tracing the origin of introduced strains that are new to this region. The tools developed in this work can be used to design differentiation procedures for various viral pathogens, both for diagnostic and research studies.



# POSTER PRESENTATIONS

Epidemiology &  
surveillance and  
Risk assessment

TOPICS I+II+III

## POSTER 1: Outbreaks and genetic characterization of porcine epidemic diarrhea (PED) virus in Korea

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**Keywords: PED, phylogenetic trees, mutation**

Porcine epidemic diarrhea (PED) has resulted in economic losses for swine farms in Korea since the reported outbreak in 1993. PED is notifiable disease in Korea. Infected farm is controlled by government and pigs in the infected farms are restricted the movement. Therefore, many farmers are inclined to dislike the exposure of diseases in their farms. Since January 2014, PED cases increased explosively in Korea. Although new vaccine was developed and commercialized in 2015, PED have continued to occur up to recently in Korea. PED has occurred in biased province according to official record. However, we realized that PED incidence was high and low in proportion to pig population of province and PED prevailed throughout the country since 2014 though the credible data of PED cases collected from 4 competent diagnostic laboratories.

Based on the phylogenetic trees of the full spike (S) protein sequences PEDV field strains and reference strains, including vaccine strains, the Korean strains could be divided into two groups (G1 and G2). G2 was further divided into G2-1 and G2-2. G1 consisted of vaccine strains, and European, Japanese, Korean and Chinese strains. G2-1 was grouped with only Korean field strains, with the exception of KH from Japan, and G2-2 consisted mostly of Chinese, several Korean, and US strains. Specially, most of G2-2 strains detected recently in Korea were highly similar to the US strains that spread in 2013. Therefore, we can assume that recent PEDV strains might have originated from the USA, and the possibility of importation of pig or livestock products and movement of contaminated people. The S genes used in this study were not homologous, with nucleotide (nt) and amino acid (aa) similarities of 89.7–99.8%. The ORF3 and envelope (E) protein sequences of Korean strains were divided into G1 and G2, and G2 was further divided into G2-1 and G2-2. Most Korean strains were grouped into G2-2 and showed 95.6–100% nt and 95.9–100% aa identity. These findings indicated that the prevalent PEDV strains in Korea are genetically diverse and can be divided into 3 groups according to their putative origin: Chinese-like (G2-2), Korean-like (G2-1), and vaccine-like (G1) viruses. We also found partial mutations in the COE region and SS6 neutralizing epitopes in spike protein.

Among positive selected mutations in S protein, 6 mutations were found in non-consecutive years during 2005, 2006, and 2008. Amino acid mutation at position 303 in similar period was unique and was not found in neighboring countries. We can assume that environmental change occurred during that period. The positive selection rate in the S protein and nucleoprotein was only 8.5%. The positive selected mutation was also even more conservative with few radical changes. This result suggests that PED viruses in Korean undergo a slow evolutionary process.

## POSTER 2: Analysing the role of virus populations in chronic infections with classical swine fever virus

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**Keywords: virus population, Next-generation sequencing, Classical swine fever virus**

Infections with classical swine fever virus (CSFV) which cause classical swine fever have a huge economic impact world-wide. Three different courses of the disease can be distinguished after infections with CSFV: the acute, the chronic and the prenatal form. Due to culling policies, the chronic form of infection has low impact in domestic pigs. However, it may play an important role in wild boar populations. Because of the rare occurrence, little is known about the impact of the virus and its interaction with the host in the development of chronic infections. To shed further light on chronic courses of CSFV infection, this study was designed to examine the virus population of chronically infected in comparison with acutely infected animals and the inoculum.

Deep sequencing using 454 or Illumina technologies was used to sequence samples of chronically and acutely infected animals as well as the inoculum. Consensus sequences were generated by *de novo* assembly and compared. For further insight, viral genome variants present in the samples were analyzed and compared. Furthermore, viral populations were analyzed by calculation of Manhattan distances taking all variants into account.

With a single exception, comparison of viral consensus sequences revealed no differences between viruses recovered from chronically infected or acutely infected animals and the respective inoculum. Only in the sample from an acute-lethally infected animal 4 substitutions were found. Additionally, 3 substitutions could be detected in a cell culture passage of one of the inocula which vanished in all animal samples. Population analyses revealed no differences in clusters of different forms of the disease except the acute-lethal form and the cell culture passage. Nevertheless, an increase in virus population diversity was observed over time within one animal which was expected due to high virus replication and a lack of proof-reading function. An analysis of mutational “hot spots” did not uncover any differences between different isolates, breedings, or outcome of the disease.

Based on the results it can be concluded that the virus population has no impact on the outcome of disease. Further work in chronically forms of classical swine fever should therefore focus on host factors. In addition, it could be demonstrated that the viral population remains constant after infection but becomes more diverse regarding low frequent variants. Furthermore, the analysis of an additional cell culture passage of the inoculum showed cell culture adaptations which are stable in animal infection.

## POSTER 3: Virome characterization of *Culex pipiens* in France through metagenomic analysis

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**Keywords: West Nile virus, *Culex pipiens*, virome, metagenomics**

*Culex pipiens* is a mosquito vector of West Nile virus (WNV), the causing agent of West Nile fever. Epizooties of WNV have taken place in France, mainly in French Camargue, and in some instances human cases have been diagnosed. However, beyond WNV, little is known on the viruses associated to *Culex pipiens*, above all on viruses non-pathogenic for humans or livestock. Such viruses could have a role in WNV epidemiology either limiting mosquito populations or modulating the chances of WNV infection of *Culex pipiens*. Since the human impact of an ecosystem usually has an influence on species diversity, we can imagine a negative impact of human activity on the diversity of viral communities. Here we describe the development of an effective methodology for exploring viral diversity using high-throughput sequencing. We present the first results of the virome of *Culex pipiens* from sites with two different level of human activity in Camargue, showing that our approach allows to identify new viruses and, in some cases, to obtain almost full-length viral genomes.

## POSTER 4: Evidence of asymptomatic West Nile virus infections in horses in Central Spain, 2011-2013

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**Keywords: West Nile virus, serosurvey, virus neutralization, Spain, horse**

West Nile fever (WNF) is a zoonotic viral disease which is emerging or re-emerging in different parts of the world. WNF affects horses, birds and humans, and the etiologic agent, West Nile virus (WNV) is an arthropod-borne virus (arbovirus) belonging to the genus *Flavivirus* (family *Flaviviridae*). WNV cycle involves different types of birds as natural reservoirs and a range of *Culex spp.* mosquitoes as transmission vectors. WNV has had great impact on public and animal health in recent decades, being a notifiable disease in humans and animals. In 2010 WNV foci appeared in horses in south Spain (Andalusia), where outbreaks occur every year since. More recently, in 2014, an outbreak in horses was declared in Central Spain, in the province of Ciudad Real, approximately 200 km away (north-east) from the closest foci in Andalusia. Later, in February 2015, the virus was detected in captive birds in the province of Avila, also in Central Spain. These two events represent the northernmost equine WNF foci and the northernmost virus detection in birds in Spain, respectively. In previous years, evidence of West Nile virus (WNV) circulation in central Spain had been obtained only from wildlife, but never in horses.

The purpose of this work was to carry out an epidemiological serosurvey on susceptible horse population in central Spain to retrospectively detect West Nile virus infections horses from 2011 to 2013, before the occurrence of the first outbreak in the area.

Serum samples from 369 Spanish Purebred horses aged between 1 and 24 years and non-vaccinated against WNV, collected between September 2011 and November 2013, were analysed by ELISA (competitive and IgM) and confirmed by virus neutralization. Specificity of neutralizing antibodies was established by parallel titration against another flavivirus of the same serocomplex (Usutu virus).

The analysis of the samples yielded 10 seropositive serum samples by competitive ELISA of 369 horses' sera analysed, 5 of which were confirmed as positive to WNV by virus neutralization (seropositivity rate: 1.35%). One of these WNV seropositive samples was IgM positive. Chronologically, the first positive samples confirmed by VNT, including the IgM-positive, corresponded to 3 sera collected in Madrid province in May 2012. Four additional sera collected in July, and 1 in December were also positive by the competitive ELISA in the same year and province (one of them confirmed by VNT). Later, in July and August 2013, 2 positive samples by competitive ELISA (one of them confirmed by VNT) were detected in Segovia province, respectively. From these results, we can conclude that WNV circulated in asymptomatic equine populations of central Spain before the first disease outbreak reported in this area, at least since 2012.

### Reference

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### Acknowledgements

This study was funded by INIA-MAGRAMA agreement CC08-020. The authors wish to thank the owners and the veterinary surgeons of the studs included in the study.

## POSTER 5: Has the evolving cattle transport network in Finland 2009-2015 changed the potential for disease spread?

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**Keywords: animal transports, connectivity, cattle, development in time**

The animal transport network is essential in determining the dynamics of contagious animal disease spread and the final size of an outbreak. We have earlier forecasted that by 2033 the animal transport network in Finland would be much more concentrated than in 2009, due a reduction in the number of farms and an increase in farm size. We forecast that the number transports in 2033 would be approximately half of those in 2009. In the current study, we compared this prediction with the animal transport data of 2015 and how the network related parameters, In-degree and Out-degree, have evolved from 2009.

In 2009 the animal transport registry contained 92233 records of cattle being transported between farms. We predicted that at least one end of most of these transports would still be operating in 2033. This would correspond a 12.6% (1.92% per year) reduction in animal transport registry entries between farms by the year 2015. Based on transport registry entries there was a 12.3% reduction in the transport volume of between 2009 and 2015, which corresponds well with our prediction. Simultaneously the average number of animals per transport between farms has increased by 20.4%. The number of yearly contacts per farm increased by 13.2% between 2009 and 2015. This was due to larger reduction in the number of farms (-22.5%) than in the number of contacts. Recurrence of contacts increased slightly (6.9%).

The In-degree and Out-degree distributions have not changed. Although the number of contacts per farm has increased, it has occurred partly between old contacts. The same number of new contacts has replaced the disappeared old contacts. In other words, the number of partners has remained the same. Our results suggest that there has not been a large change in the cattle production system between 2009 and 2015, if judged by the stability of In- and Out-degree level and variation. However, the increase in the contact rate per farm suggests that there is an increase in general spread potential between cattle farms in Finland. The increase in the number of contacts per farm is partly compensated by increased number of animals per transport. Otherwise the contacts between farms would have increased even more.

## POSTER 7: A cartographic tool for managing African Swine Fever in Eurasia: mapping wild boar distribution based on the quality of available habitat

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**Keywords: African Swine Fever, surveillance, wild boar distribution, land coverage, habitat**

African Swine Fever (ASF) is a highly contagious viral disease affecting domestic pigs (DP) that represents a threat to the swine industry with potentially devastating socio-economic and political consequences. Since the last entrance of ASF into continental Europe in 2007 via the Caucasus, the disease has spread up to 3,000km in just 10 years and affected 10 countries (Armenia, Azerbaijan, Georgia, Russian Federation, Belarus, Ukraine, Latvia, Lithuania, Estonia and Poland), in which the disease is still active. Wild boar (WB) appears to be a key factor in maintaining the disease in endemic areas and local spread across European Union (EU) borders. Therefore it is crucial to know their distribution to help predict and interpret the dynamics of ASF infection. We developed a standardized distribution map based on global land-cover vegetation (GlobCover) that quantifies the quality of available habitats (QAH) for WB across Eurasia. QAHs were estimated using a seven-level scale based on expert opinion and were found to correlate closely with georeferenced presence of WB (n= 22 362): the highest WB densities (74.47%) were found in areas with the two highest QAH, while the lowest densities (5.66%) were found in areas with the lowest QAH. A significant difference was obtained between the observed presence in each QAH and the expected presence based on surface area (multinomial adjustment 19,304.3 df6 p<0.001), thereby suggesting that the number of observations reflect habitat quality rather than surface size. Mapping notifications in 2007–2016 (WAHID) on the QAH map showed that in endemic areas, 60% of ASF notifications affected DP, mostly in agricultural landscapes, buffer monoculture areas and agroforestry areas (QAHs 1 and 1.75). In the EU, by contrast, 95% of ASF notifications occurred in WB within natural landscapes (QAH 2), above all in broadleaved deciduous forests and mixed broadleaved and needleleaved forests. The implications for managing ASF thus are different in endemic areas and EU in general, and a particular focus on QAH scenarios is needed to guarantee preventive intervention, early disease detection and rapid response.

QAH 2 should be considered as key zones where ASF could persist in WB populations, particularly in Baltic countries and Poland. The incidence and prevalence of infection should be monitored to assess the risk of endemicity or the appearance of animals that have survived ASF. Pig farmers should be warned about the risk of keeping pigs outdoors in these areas, since this practice may promote contact between WB-DP. Due to the potential spread of ASF into ASF-free countries, borders such as the EU-Ukraine border should be defined as zones of surveillance if QAH 2 vegetation corridors exist in areas where there are also high densities of DP on low-biosecurity farms.

QAH 1 and 1.75 should be considered as key interface areas where the potential for ASF transmission between DP-WB exists, above all in endemic areas where the density of backyard pig production is high. Here, priorities should include early diagnosis, continuous monitoring and the improvement of pig farm biosafety and hunting management. In QAH 1, a ban on the use of fresh grass or crops to feed DP is strongly recommended since these food sources may be contaminated by infected WB.

These results suggest that the QAH map is a useful epi-tool for defining risk scenarios and identifying potential travel corridors for ASF, as well as for improving the prevention, control and surveillance of ASF and other diseases that potentially affect swine and WB in Eurasia.

## POSTER 8: Evaluation of insect larvae as possible mechanical vectors for transmission of ASFV in wild boar populations

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**Key words: African swine fever virus, blow fly, maggots, mechanical vector**

African swine fever (ASF) is a contagious viral disease of domestic pig and European wild boar. In 2007, ASF virus (ASFV) was introduced into eastern Europe, reaching the EU in 2014 (Baltic States, Poland).

Considering the rapidly progressing course of disease with case fatality rates of up to 100 %, it is unclear why the virus keeps slowly spreading through the East European wild boar populations in the absence of the only known vector (*Ornithodoros* soft ticks) instead of quickly eradicating its natural hosts and, ultimately, itself. In addition to direct contact to infected animals with high virus load, pigs and wild boar can become infected by oral ingestion of ASFV-containing organic material in which the virus remains stable for weeks or even months. Thus, transmission might also be possible through carcasses and insect larvae developing on those, which are known to belong to the diet of wild boar.

To check the hypothesis of immature insect stages serving as mechanical vectors of ASFV, larvae of two commonly found necrophagous blow fly species, *Lucilia sericata* and *Calliphora vicina*, were bred in the laboratory on ASFV-infected wild boar tissue. After preset periods of development, maggots were removed and tested for replicating virus via titration on porcine macrophages and for viral DNA by qPCR, both in an unwashed status and following external cleaning.

First qPCR results show a very limited surface contamination of some maggots with ASFV-genome but no replicating virus could be detected in any single maggot or pool of maggots tested so far.

The study is meant to contribute to understanding the epidemiology of ASF in eastern Europe and to predicting the further spread of ASFV within the wild boar population.

## POSTER 9: MediLabSecure: Implementing a network of virology and entomology laboratories for a One Health approach of vector-borne and respiratory viruses in the Mediterranean and Black Sea regions

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**Keywords: Network, (re)emerging arboviruses, One Health, Entomology, Public Health**

As (re-)emerging viruses are threatening global health, the EU-funded MediLabSecure program aims at enhancing the preparedness to viral threats by establishing a One Health network of virology and entomology laboratories and Public Health institutions in 19 non-EU countries of the Mediterranean and Black Sea areas. The project aims to strengthen and enhance the integration of relevant emerging arboviral diseases surveillance between laboratories, including national diagnostic labs and universities, and central national surveillance systems. To achieve this, the MediLabSecure network comprises 55 laboratories addressing animal and human virology as well as medical entomology research issues (one laboratory per field of study and per country), as well as 19 public health institutions. The program is coordinated by 4 institutes based in 3 European countries.

Capacities and needs regarding diagnostic methods, biosafety and integration of laboratory and epidemiological surveillance for emerging vector-borne and respiratory viruses were assessed in each one of the network laboratories. Moreover, joint meetings allow the network partners and coordinating institutions to meet and exchange on the objectives and future steps of the project, their experiences, needs and expectations. Thanks to this, tailored training curricula were elaborated and the activities foreseen in the program were fine-tuned. Two types of actions have been implemented in the frame of the program: within- specialties training sessions and transversal workshops and meetings. These training activities enabled the laboratories and public health institutions to (1) implement harmonized and up-to-date diagnostic techniques for vector borne viral diseases such as West Nile, Dengue, Rift Valley fever, Crimean-Congo Hemorrhagic Fever, and Chikungunya (2) develop capacity building in medical entomology and vector surveillance and (3) foster interdisciplinary collaboration for surveillance integration in the framework of One Health.

By enhancing laboratories diagnostic capacities and regional inter-sectorial cooperation, the MediLabSecure network could represent the cornerstone of a corporate preparedness and response to vector-borne viral threats in the Mediterranean and Black Sea regions based on a One Health approach.

### Acknowledgements

We are very grateful to all the contact points of the four disciplines (animal virology, human virology, medical entomology and public health) in the 19 participating countries for their collaboration. The MediLabSecure Project is supported by the European Commission (DEVCO: IFS/21010/23/\_194).

## POSTER 10: Detection and full genome sequencing of Beta-CoV viruses related to Middle East Respiratory Syndrome from two bats in Italy

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**Keywords: MERS-like Beta-CoV viruses, full genome sequencing, bats, Italy**

Since the early 70's a variety of pathological conditions in domestic and wild animals has been attributed to Coronaviruses (CoVs) infections. Currently six different CoV strains are known to infect humans. Two of these belonged to Beta-CoV genus, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), and caused severe respiratory diseases with case fatality rates of 9% and 36% respectively. The evolutionary origin of SARS-CoV, which was firstly detected in 2002, involved bat hosts, possibly with civets as intermediate host and the source of human infection. The origin of the MERS-CoV is currently not well known although more recent studies point for camels as possible reservoirs or intermediate hosts. Bats have also been suspected as the source of MERS-CoV because of genetic likenesses between Beta-CoV found in bats and the MERS virus in humans. Recently, a CoV originated from an South African bat (SA bat) was identified as highly related to MERS-CoV, and a fragment of a CoV showing 100% of identity to MERS-CoV was found in a fecal sample from an Egyptian tomb bat (*Taphozous perforatus*). In this study, we described the detection and full genome sequencing of CoVs from two Italian bats (IT bats) of different species, *Pipistrellus kuhlii* and *Hypsugo savii*. Bat carcasses were obtained from the Modena Wildlife Recovery Center (North Italy). During necropsy, no macroscopic lesions suggesting infectious diseases but traumatic injuries were observed. Pools of viscera (lung, heart, spleen and liver) and intestine were tested separately using a pan-coronavirus one-step RT-PCR, which used neoCoV specific primers that amplified 180bp of the RdRp gene. For phylogenetic analysis, full genome sequencing was conducted directly on pan-coronavirus positive intestines using the Ion Torrent NGS technology. Complete genome sequences of MERS-CoV and Alfa- and Beta-CoVs from bats, human and camels (n. 131) were downloaded from ViPR system and a multiple sequence alignment was calculated using the MUSCLE algorithm. Maximum likelihood phylogenetic tree was performed using the ViPR implementation of the RAxML algorithm with the GTR model. Genetic relationships between Italian and SA bats and MERS-CoV were confirmed by comparison of the sequence distances of MERS-CoV and bat-BCoV 2c (SA bat and IT bats) using SSE v1.2. IT bats BCoVs showed 80% overall nucleotide identity across the whole genome to MERS-CoV. The nucleotide distances respect to MERS-CoV were higher in the genomic region encoding the S protein for all bat sequences including SA bat and in the ORF3 and ORF5 coding regions for the Italian sequences (fig.1A). Phylogenetic tree of the complete genome showed a monophyletic group formed by MERS sequences originated from humans and camels that are related to bat sequences. The most related sequences are those originated from SA, Italy and China (fig. 1B). Bats, with extensive geographical distribution and capability of flight have been documented as natural hosts of large number of diverse viruses such as lyssaviruses, paramyxoviruses and filoviruses. The genetic diversity of CoVs in bats exceeds that known for other hosts, which is compatible with bats being the major reservoir of mammalian CoVs. The emergence of MERS-CoV probably involved genetic exchanges between different viral ancestors that may have occurred either in bat ancestors or in camels acting as mixing vessels for viruses from different hosts.

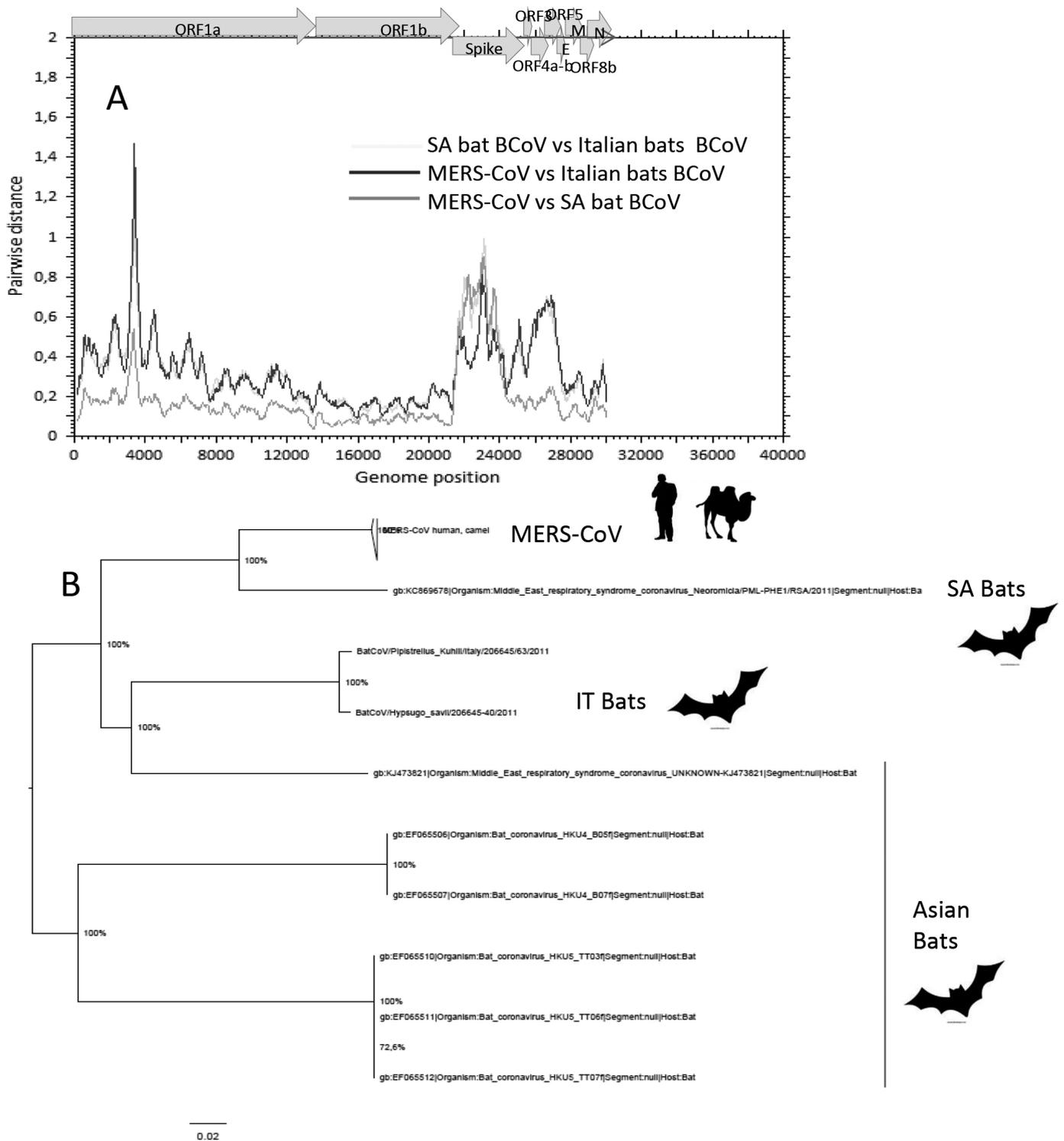


Figure 1 – Phylogenetic analysis of two Italian Bats BCoV strains. A) Comparison of the sequence distances of MERS-CoV and the bat strains highly related. B) Maximum likelihood tree based on the complete genome of field and reference sequences of the MERS-like clade.



# POSTER PRESENTATIONS

Diagnostics and  
Intervention strategies

TOPICS I+II+III

## POSTER 11: Generation of a recombinant structure of West Nile virus, in a baculovirus system, useful for diagnostic procedures

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**Keywords: WNV, VLP**

West Nile virus is a worldwide spread zoonotic arbovirus. The routine laboratory diagnosis of WNV infection is based on the identification of the virus by RT-PCR or by serological analysis of specific antibodies through VNT (virus neutralization test) as gold standard or IgM capture ELISA. Both techniques usually use a whole virus preparation as antigen. The growth and culture of WNV is only allowed in BSL-3 facilities, with all the drawbacks and efforts associated with that type of works.

Since WNV emerged in the New World (New York 1999), efforts of numerous scientific groups have focused on the development of recombinant structures that are safe and efficient for both vaccination and diagnostic purposes. Several groups used the envelope (E) protein or just its domain III (DIII), but the results obtained were not satisfactory. The objective of this work was to obtain a recombinant antigenic structure with better characteristics than E protein alone for achieving both goals in one solution: the safety and the accuracy in the diagnostic result.

Structurally, WNV virions are enveloped spherical particles containing 3 structural proteins (C, capsid; M, membrane and E, envelope) which form a icosahedral structure that encloses the viral RNA genome. The M protein (in its immature form, prM) and the E protein integrate the envelope of the virus enclosing the capsid, constituted by C protein only. The co-expression of the 3 structural proteins can yield noninfectious viruslike particles (VLPs). Several groups have successfully constructed VLPs for diagnosis or vaccination purposes with diverse viruses, some of which are flaviviruses as WNV however it is the first description of the use of baculovirus-derived VLP in WNV diagnosis.

cDNA sequences corresponding to the three structural proteins were amplified from WNV L1 NY'99 strain and cloned in the multiple expression baculovirus vector PBAC 4x-1. The expression of the different proteins was assessed by Western blot with polyclonal antibodies against M and C proteins and monoclonal antibodies against E protein. Expression of the proteins was carried out in sf900 insect cells that grow up in a serum-free medium. After the infection, the secreted structures were semipurified from the supernatants by ultracentrifugation in a sucrose cushion. Subsequently, they were loaded on the top of a sucrose layer gradient. VLP formation was confirmed by electron microscopy.

The ultimate goal of our work is the substitution of the virus in our diagnostic test by this recombinant structure. Preliminary results obtained from testing VLPs as antigen in ELISA were very promising, giving similar sensitivity and specificity indexes than if using the whole virus and higher than the E protein.

## POSTER 12: Infection Dynamics of Respiratory Viruses In Cattle Herds

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**Key words: Infection dynamic, Respiratory viruses, Ruminant**

The aim of this study is to recommend the optimum time for first vaccination of calves against respiratory disease complex by elicit the infection dynamics of bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (PI-3), bovine herpesvirus 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine adenovirus type 3 (BAV-3) and bovine coronavirus (BCoV) with serological examinations.

This study was carried out in a total of 10 dairy cattle farms which were grouped according to size of their animal population (large, medium, small). For serological studies, blood samples were collected from calves (n=94) born in a one month period from the studied farms. The animals were sampled during their 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 12th months of age. There was no vaccination applied in the herd against studied viruses. During first sampling, blood samples were also gathered from the dams to get information about their immunological status. Serum neutralization test (SN<sub>50</sub>) was used for following the changes in antibody titers.

A decline was shown in maternal antibody titers in the 2nd month for BRSV, BAV-3 and BHV-1 (97.8%, 91.4% and 25.5% respectively) and in the 3rd month for BCoV, PI-3 and BVDV (93.6%, 85.1% and 67% respectively). New infection with PI-3 and BVDV were detected at the first month of age and role of seronegative calves in circulation of infections was determined.

It was concluded that maternally derived antibodies started to decrease after the 1st month and the calves come across with the studied viruses for the first time after the 2nd month. On this basis, first vaccination can be applied between 2nd and 4th months of age and to enhance the effectiveness of the first dose of inactivated vaccine a booster is recommended. Moreover, it is suggested that after the booster dose a third vaccination at 6 months can be beneficial against the recirculation of respiratory viruses in the population.

## POSTER 13: Occurrence of Swine Enteric Coronavirus (SeCoV) infection during 2016 within Central Eastern Europe

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**Keywords: Porcine coronavirus; PEDV; TGEV, SeCoV**

An outbreak of disease, in a pig herd, characterized by diarrhea and vomiting occurred in Central Eastern Europe (CEE) in early 2016. A commercial RT-qPCR assay, testing for both porcine epidemic diarrhoea virus (PEDV) and transmissible gastroenteritis virus (TGEV) in fecal samples, gave a positive result. However, further analyses, by virus-specific RT-qPCR assays, provided results inconsistent with infection by either of these coronaviruses suggesting the presence of a distinct porcine coronavirus. Sequencing of amplicons, generated by RT-PCRs specific for the PEDV S gene, indicated a very close similarity to recently described chimeric viruses from Italy and Germany that have been termed swine enteric coronaviruses (SeCoV). The virus (with an RNA genome of ca. 28kb) was first identified in Italy in samples from 2009 but has not been detected since 2012 (Boniotti et al., 2016). Similarly, the virus from Germany, detected in archived samples from 2012, has not been detected subsequently (Akimkin et al., 2016). Further, overlapping, amplicons were generated by RT-PCR and in total some 9kb of the CEE SeCoV genome was sequenced, this corresponded to the 3'-portion of the viral genome encoding mainly the structural proteins. Comparison of the sequences showed that each of these three SeCoVs contain the S gene of PEDV within a backbone of TGEV but these three chimeric SeCoVs are clearly distinct from each other. It was demonstrated, for the first time, that pigs from within the SeCoV-infected herd in CEE seroconverted against PEDV but tested negative in a TGEV-specific ELISA that detects antibodies against the S protein. These results indicate that SeCoV is continuing to circulate in Europe and is present in animals displaying disease that is very similar to PED. Specific detection of the chimeric SeCoVs either requires development of a new diagnostic RT-qPCR assay or the combined use of assays targeting the PEDV S gene and another part of the TGEV genome.

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## POSTER 14: Induction of FMDV- specific T and B cell responses *in vivo* using calicivirus-based virus-like particles

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**Keywords: VLPs, vaccines, FMDV**

Foot-and-mouth disease virus (FMDV) is a highly infectious disease of cloven-hoofed animals and one of the most important livestock diseases in terms of economic impact. Inactivated FMDV is currently in use as a vaccine against the disease but safer and heterotypic alternatives are required. While the bases for protection involves neutralizing antibodies, a requirement for specific T-cell mediated cellular responses for efficient protection is increasingly being recognized.

Virus-like particles (VLPs) are good vaccine candidates because their inherent properties that mediate the induction of safe and efficient immune responses. In particular, VLPs derived from rabbit haemorrhagic disease virus (RHDV) have been shown to be good vaccine platforms. RHDV belongs to the family Caliciviridae which are nonenveloped, icosahedral viruses composed of 180 copies of a single capsid protein (VP60). Our research group has identified two independent locations within the VP60 protein that can accommodate foreign epitopes of up to 42 aminoacids in length, without affecting the ability of the resulting chimeric protein to self-assemble into VLPs.

In this study we have generated chimeric VLPs harbouring at an exposed insertion site a neutralizing B-cell epitope derived from FMDV type O (currently the most widespread serotype), located around positions 140 to 160 of capsid protein VP1 (loop G-H). Besides this, different potential T cell epitopes derived from the nonstructural 3A protein, which is highly conserved among different FMDV serotypes, were inserted into a second site internally located on the assembled VLP.

Using a murine model, we have compared the capacity of the different constructs to induce epitope-specific humoral and cellular immune responses. Thus, we found that modified VLPs can efficiently induce FMDV-specific neutralizing antibody responses as well as T-cell responses *in vivo* and suggest that these may be used as an alternative vaccination approach in swine.

## POSTER 15: Use of baculovirus pseudotyping to characterise the antigenic structure of AHSV-VP2

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**Keywords: AHSV, VP2, baculovirus pseudotyping, vaccine**

African horse sickness (AHS) is an infectious, non-contagious, insect vector-borne disease of equids with more than 90% mortality rates in infected horses. The disease is caused by African horse sickness virus (AHSV), which is mostly endemic in sub-Saharan Africa. However, occasional outbreaks have occurred outside Africa such as by serotype 4 (AHSV4) including Europe. The horse industry has a major role in the economy of mainland Europe and the United Kingdom. An AHSV outbreak in these non-endemic regions would have a considerable impact on sporting events, trade and animal health. The prevention of disease is mainly dependent on an efficient vaccination regime. However, the current live attenuated vaccines are not considered safe for use in non-endemic regions and various recombinant DNA vaccination platforms have been investigated to develop safer and more efficacious vaccines. Sub-unit protein vaccines expressed from recombinant baculoviruses are an option for generating safe vaccines due to the absence of pre-existing immunity, non-toxicity in mammalian systems and their inability to replicate in mammalian cells. However, baculoviruses can also be used to display foreign antigens in the surface of the baculovirion envelope (pseudotyping). The AHSV major outer capsid protein, VP2, has been shown to be the main-serotype specific antigen, carrying the virus neutralizing epitopes, hence VP2 is the optimum target for subunit vaccine studies. We have investigated the antigenic structure of AHSV4-VP2 further by expressing the full-length protein and eleven subdomains thereof using baculovirus expression and pseudotyping (surface display). Understanding the antigenic structure of the protein contributes to generating more efficient, safe vaccines. All eleven VP2 subdomains were displayed on baculovirus envelope surface, unlike the full-length VP2. BV surface display greatly improved the yield of these proteins when compared to their synthesis as individual proteins in insect cells. The antigenicity of these recombinant baculovirus constructs was investigated by several *in vitro* binding assays using AHSV4-VP2-specific virus neutralizing antibodies and vaccination studies in mice. In doing so, generation of a potential safe vaccine for AHSV was also explored.

## POSTER 16: Validation of a new cELISA & qPCR for the diagnosis of African swine fever

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African Swine Fever Virus (ASFV) is a highly virulent swine disease characterized by fever, hemorrhages and high mortality rates. ASF control and eradication programs require accurate and reliable diagnostic tests.

IDvet offers indirect ELISAs based on three recombinant ASFV antigens (P32, P62, and P72) for antibody detection in serum, blood filter paper and meat juice samples. This ELISA demonstrates excellent specificity, especially for wild boar samples.

IDvet has also developed two new tools, a competitive ELISA and a real-time qPCR, for ASF diagnosis.

**The ID Screen® African Swine Fever Competition ELISA** allows for the detection of anti-ASFV P32 antibodies. Specificity was evaluated through the analysis of 280 disease-free sera from domestic and Iberian pigs. Measured specificity was 100.0% (CI 95%: 98.7% - 100.0%). 8 positive reference sera from the ASF European Reference Laboratory (EURL-ASF, Madrid, Spain) were correctly identified as positive. Seroconversion was detected between 6 and 13 dpi. The test correctly identified sera from all genotypes tested, including genotype II.

The test was also evaluated by the EURL. Results indicate a diagnostic specificity of 99.4% (n=177) and a diagnostic sensitivity of 95.8% (n=213). Perfect agreement (k=0,95) with the IPT (Immunoperoxidase test) was obtained.

**The ID Gene™ ASF is a TaqMan ready-to-use real-time PCR assay** based on the simultaneous detection of ASFV and an endogenous internal positive control. It may be used for blood, serum, plasma, swabs and tissues samples. Results may be obtained in less than two hours (exaction in only 20 minutes, and amplification around 1hour).

DNA sample panel from the EURL was tested. The ID Gene™ ASF kit correctly identified all samples (14/14, including DNAs from genotypes I, II, V, VIII, IX, X) and did not show any cross-reactions with 31 other pathogens. The detection limit of the PCR was <10 copies, indicating high sensitivity.

The EURL-ASF reference panel consisting of 16 ASF lyophilised samples including experimental and clinical field samples, was also tested. DNA extraction was performed by magnetic beads (IDGene™ Mag Fast) as per manufacturer's instructions. All samples were correctly scored positive and negative.

To conclude, IDvet offers a full range of tools for the accurate and rapid diagnosis of African Swine Fever, either by serology or qPCR.

## POSTER 17: Comparative evaluation of the performance of six ELISA tests for the detection of antibodies against African swine fever virus (ASFV)

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African swine fever (ASF) is a very complex disease for which no vaccine is available. Prevention and control of ASF is based on two main principles: early detection (based on epidemiological, clinical and laboratory findings) and strict sanitary measures. Therefore, the use of the most appropriate diagnostic tools updated to be applicable to all scenarios is critical for the implementation of effective control programs. Epidemiological serological data in the affected Eastern European countries have shown a significant increase in the incidence of seropositive animals, particularly evident into the wild boar population in some affected parts of the EU (European Union) affected countries, since the last period of 2015. These data suggest that some animals are surviving for over a month and may be able to recover from the infection and in certain cases even remaining sub-clinically infected as previously described in the Iberian Peninsula, Americas, and in Africa. Antibody detection techniques are, therefore, essential to get complete information that will assist control and eradication programs. For the detection of ASF antibodies, the current OIE-prescribed assays involve the use of an indirect ELISA test for screening followed by the confirmatory assays. However, comparative studies on samples collected from the EU affected countries have demonstrated the relatively lower sensitivity observed for the OIE-prescribed ELISA test compared to the confirmatory tests, such as the Indirect Immunoperoxidase test (IPT). Within this study the protocol of the indirect OIE-ELISA test has been optimized by adjusting incubation time, incubation temperatures, blocking buffers, concentrations of the antigen and the serum samples, as well as the type and concentration of the conjugate and substrate. Once established the optimized conditions, 110 experimental sera collected from infected domestic pigs representing a variety of clinical forms induced by ASFV isolates of different virulence and representatives of genotypes I, II and X, were analyzed. The experimental samples were tested in parallel using the OIE-modified indirect ELISA (OIE-ELISA v2) and five different ELISA tests for ASF antibody detection. The ELISA tests included in the comparative study were; i) the OIE indirect ELISA based on the ASFV semi-purified antigen as is described in the OIE manual (OIE-ELISA), ii) the blocking INGENASA-ELISA based on the use of Monoclonal antibody against the pP72 ASFV protein, and iii) three different "in house" recombinant indirect ELISAs for the detection of antibodies against the P30 (p30-ELISA), p54 (p54-ELISA) and p15-p30 (DUAL-ELISA) ASFV proteins. The results were compared with those obtained using the IPT as gold standard. By the IPT the number of positive sera was 99 (90%), whereas the number decreased to 75 (68%) with the DUAL-ELISA, 72 (65%) with the p54-ELISA, 69 (63%) with the OIE-ELISA v2, 68 (62%) with the p30-ELISA, 66 (60%) with the INGENASA-ELISA and 59 (54%) with the OIE-prescribed ELISA assay. Therefore, the sensitivity values ranged from 73% using the DUAL-ELISA to 60% using the OIE-ELISA. With the optimized OIE-ELISA v2 the sensitivity was 70%. To further investigate the reasons for this high number of false negative results, titers were determined by IPT in all positive serum samples and the results were compared with that obtained using the ELISA tests. For the 99 positive serum samples, there was 100% agreement between the IPT test and the six ELISAs when the sera had antibody titers higher than  $10^4$ . However, the results showed that the ELISA tests were clearly less sensitive ( $\leq 80\%$ ) to detect the infected pigs with antibody titres below  $10^5$  in the INGENASA and the OIE-ELISA, and below  $10^3$  in the case of the OIE-ELISA v2 and the recombinant assays. This was correlated with the ASF clinical infection. Despite the higher sensitivity showed by the DUAL and p54 ELISAs in the detection of earlier infections (acute forms of the disease), the INGENASA, p30 and the OIE-ELISA v2 provided higher sensitivity in detecting animals sub clinically infected. Although the sensitivity of the optimized OIE-ELISA v2 test was significantly higher than that obtained with the prescribed ELISA test included in the OIE manual, these results will require further confirmation using field samples (in progress). Antibody detection is essential for successful ASF surveillance and control activities. The optimized "in house" OIE-ELISA v2 is now an improved tool, very useful as less costly, for its use in countries with limited resources to better get the control of the disease.

*Funded by the EURL for ASF and the Spanish grant INIA AT2013-002 project.*

## POSTER 18: Generation of African swine fever virus recombinants lacking membrane proteins p22 or pE199L

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**Keywords: African swine fever virus, envelope proteins, targeted gene deletion, vaccine development**

African swine fever virus (ASFV), the hitherto sole member of the family *Asfarviridae*, causes a severe disease of domestic pigs and wild boar with mortalities reaching up to 100 %. It is endemic in major parts of Sub-Saharan Africa and in Sardinia. In 2007 a highly virulent strain was introduced into Transcaucasia, and further spread to Russia, Belarus, Ukraine, the Baltic countries and eastern Poland. ASFV is a complex virus with an approximately 180 kbp dsDNA genome containing around 150 open reading frames. The virus mainly replicates in the cytoplasm of porcine cells of the monocyte/macrophage lineage, and can be transmitted by soft ticks. Due to the lack of suitable reverse genetics systems, targeted mutagenesis of the ASFV genome is difficult, and up to now no safe and reliable live virus vaccines could be developed. Nevertheless, targeted deletion of virus genes which are relevant for virulence in swine remains one of the most promising strategies to develop efficacious vaccines against ASFV. If these genes are also required for efficient virus replication in cell culture, recombinant permissive cell lines providing the affected viral gene products in *trans* would enable or at least facilitate isolation and propagation of the desired virus mutants.

Therefore, we cloned the authentic or codon-optimized open reading frames of the outer envelope protein p22 (KP177R), and of the inner envelope protein pE199L of ASFV into plasmids permitting co-expression with neomycin phosphotransferase under control of the human cytomegalovirus immediate-early promoter/enhancer from bicistronic mRNAs containing an internal ribosomal entry site (IRES). These constructs were used to prepare constitutively p22- or pE199L-expressing derivatives of a thymidine kinase (TK) negative wild boar lung cell line (WSL-Bu) [G.M. Keil et al. 2014, Arch Virol 159:2421-2428]. To generate ASFV mutants, major parts of KP177R or E199L were replaced by combined expression cassettes for DsRed and vaccinia virus thymidine kinase flanked by LoxP sites in cloned genome fragments. After transfection with these plasmids *trans*-complementing WSL-Bu cell lines were infected with a TK-negative, GFP-expressing mutant of ASFV strain NHV [R. Portugal et al. 2012, J Virol Methods 183:86-89], and ectopic TK rescue mutants resulting from homologous recombination were selected under hypoxanthine-aminopterin-thymidine (HAT) medium, followed by plaque purification. The obtained KP177R-deleted mutant exhibited similar growth on complementing and non-complementing cells, indicating that p22 is dispensable for *in vitro* replication of ASFV. However, the available mutant still expresses the N-terminal 82 amino acids of the protein, and complete deletion is required for confirmation. In contrast, pE199L is obviously essential, since the corresponding ASFV deletion mutant replicated inefficiently even in cells expressing low amounts of the protein. Thus, improved cell lines have to be prepared to produce putative infectious single cycle (DISC) vaccines based on a deletion of E199L.

In ongoing experiments, we attempt to apply the described methods also to other ASFV genes (e.g. to CP204L encoding the secreted phosphoprotein p30), and to the highly virulent ASFV strain which currently circulates in Eastern Europe. After initial *in vitro* characterization, the foreign gene insertions will be removed from the generated ASFV mutants by Cre/LoxP recombination [C.C. Abrams & L.K. Dixon 2012, Virology 433:142-148], and attenuation as well as protective efficacy will be evaluated in swine.

## POSTER 19: Differential regulation of type I interferon and epidermal growth factor pathways by a Bluetongue virus virulence factor

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**Keywords: Bluetongue virus, virulence factor, MAPK/ERK pathway, viral immune evasion**

Bluetongue virus (BTV) is an arbovirus responsible for bluetongue (BT), a non-contagious disease that affects a wide range of wild and domestic ruminants. It is transmitted by blood-feeding midges of the genus *Culicoides*. BTV is remarkably variable in its host range and clinical manifestations ranging from asymptomatic infection to lethal hemorrhagic fever. This variability is due to several factors related both to the infected host and the viral serotypes and strains. Despite the fact that BTV has been extensively studied as a model to study the orbiviruses, we still have little understanding of the molecular determinants of BTV virulence. In our laboratory, we aim to investigate the role of the innate immune response as a BTV cross-species barrier as well as the specific molecular interactions involved in interspecies transmission and viral pathogenesis. We have developed functional proteomic approaches such as high-throughput yeast two-hybrid (Y2H) to map interactions between BTV and cellular proteins in a systematic way. In parallel, we have recently reported that BTV is able to escape the antiviral activity of type I interferons (IFN- $\alpha/\beta$ ) at multiple levels, from their synthesis to signal. The inhibition of IFN- $\alpha/\beta$  synthesis involves the nonstructural NS3 protein, however, none of the viral proteins encoded by BTV have been shown yet to interfere with the IFN- $\alpha/\beta$  signal.

Using luciferase reporter assays, we first showed that transient expression of BTV-NS3 is sufficient to abolish IFN- $\alpha/\beta$  signal, thus confirming the critical role of this protein in IFN- $\alpha/\beta$  signalling pathway. We also found that either BTV infection or transient expression of BTV-NS3 both increase cellular response to Epidermal Growth Factor (EGF), as assessed by Elk1 transactivation and phosphorylation levels of ERK1/2 and translation initiation factor 4E (eIF4E). Growth factor binding to their receptor activates a signalling cascade called MAPK/ERK that regulates multiplicity of cellular processes including proliferation, differentiation, survival and protein translation. Furthermore, inhibition of MAPK/ERK pathway with U0126 prevents viral protein expression in BTV-infected cells. This demonstrates that MAPK/ERK signalling is essential for the expression of BTV proteins and suggests that BTV manipulates this pathway to increase replication efficiency.

Altogether, our data provide molecular basis to explain the role of BTV-NS3 as a virulence factor and determinant of pathogenesis and demonstrate how BTV has evolved a single virulence factor to block type I interferon signalling and to activate simultaneous cellular response to growth factors.

## POSTER 20: Haematological parameters as a tool to evaluate efficacy of bluetongue virus vaccines in IFNAR(-/-) mice

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**Keywords: bluetongue virus infection, IFNAR(-/-) mice, vaccines, haematological analysis**

**Objective:** Bluetongue is an arthropod-borne viral infection of sheep, cattle and other ruminants transmitted by *culicoides* midges. The disease is caused by bluetongue virus (BTV), a member of the *Orbivirus* genus, which has been spreading in Europe and many other areas during the last years, being at least 27 serotypes identified. Vaccination results critical to avoid the viral expansion. Vaccination experiments in IFNAR(-/-) mouse model seem to be efficient, reproducible, cost-effective, prompt and comparable with those performed in natural host. In this work, we analysed blood parameters (cell count and percentages of lymphocytes, neutrophils, eosinophils, basophils, monocytes and platelets), clinical signs, viraemia and protection in vaccinated and non-vaccinated IFNAR(-/-) mice challenged with three different serotypes as BTV-1, BTV-4 and BTV-8. The main aim is to demonstrate the potential use of haematological parameters as a tool to evaluate efficacy of bluetongue virus vaccines in IFNAR(-/-) mice.

**Methods:** Groups of 5 IFNAR(-/-) mice were immunized by two consecutive injections of either BTV vaccines or phosphate-buffered saline (PBS) (non-vaccinated), administered 3 weeks apart. Mice were challenged with a lethal dose of BTV-1, BTV-4 or BTV-8. Mice were examined for clinical signs daily. Whole blood was collected in EDTA tubes from all animals at 3 and 5 d.p.i. Viraemia was analysed by plaque assay in Vero cells. Haematological parameters were analysed by using a BC-5300 Vet Auto Hematology Analyzer (Mindray, Shenzhen, China).

**Results:** All non-vaccinated animals showed viraemia and clinical signs during infection and died at different times post-infection according to the serotype used for the challenge. All vaccinated animals were protected and no clinical signs or viraemia were observed after infection. Non-vaccinated animals showed severe lymphocyte and monocyte depletion at 3 and 5 d.p.i., while those vaccinated maintained normal values. An increase in the neutrophil percentage and in their absolute count was also observed in non-vaccinated mice, more severe at 5 d.p.i. in the case of animals challenged with BTV-1 and 8, while blood from those vaccinated showed normal values. Last, a generalised decrease of platelets concentration was detected in non-vaccinated groups, as opposed to the levels found in vaccinated mice.

**Conclusion:** We observed that changes in blood parameters correlate with the presence of viraemia, and they are detected even before the onset of clinical signs. Infected mice showed lymphopenia, neutrophilia, and thrombocytopenia after infection with BTV, also identified in the natural hosts. In contrast, protected animals showed normal values. We conclude the study of haematological parameters is a good tool to test vaccine efficacy in the BTV infection IFNAR(-/-) mice model by means of a non-invasive method.

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# POSTERS

## TOPICI

Animal health in  
a changing world

Diagnostics and  
Intervention strategies

## POSTER 21: Viral shedders in a herd vaccinated against Bovine Viral Diarrhoea Virus without prior removal of persistently infected animals

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**Keywords:** Bovine viral diarrhoea virus, BVDV, cattle, persistent infection, vaccination

Bovine viral diarrhoea virus (BVDV) is one of the most important viral pathogens of cattle worldwide. It belongs to the genus *Pestivirus* within the *Flaviviridae* family. Infections with this virus in naive herds can result in severe disease and significant economic losses. To successfully control this infection at a herd level, cattle producers should implement key principles: stringent biosecurity, the identification and removal of persistently infected animals (PI), continuous monitoring and the possible implementation of prophylaxis (vaccination). However, incorrect use of vaccination, like incorrect dose or route, failure in booster vaccination, contaminated equipment, interaction with other vaccines etc., can lead to incomplete protection. The aim of the study was to evaluate the BVDV status of a herd vaccinated against BVDV with an inactivated vaccine without prior removal of PI animals.

A dairy herd of 2480 animals vaccinated against BVDV was thoroughly tested for BVDV infection. Before vaccination which started 6 years ago, there was no prior identification and the removal of the source of infection (PI animals). It was expected that a long term vaccination itself will enable the elimination of possible viral shedders. All animals were tested for the presence of BVDV genome and antigen by RT-PCR, real-time RT-PCR and antigen ELISA. Phylogenetic analysis was carried out from positive samples, vaccine strain and reference strains. The seroprevalence was evaluated using a commercial ELISA for the detection of antibodies against BVDV. Selected positive results were confirmed in the serum neutralization test.

Despite the lack of any clinical problems in animals of all production groups (calves, heifers, cows) indicating possible presence of BVDV infection, 19 individuals persistently infected with BVDV were identified in calves and heifers but not in adult cattle. All virus shedders were antibody negative and the genotype of isolated virus from all of them was BVDV-1b indicating a single source of infection. The vaccine strain used in the herd was of genotype BVDV-1a.

Despite a long lasting vaccination and high sequence homology of vaccinal and field strains of BVDV (82.7%), it was not possible to avoid the transplacental infections of foetuses and to stop the birth of persistently infected calves from vaccinated heifers although the protection from the clinical disease was accomplished.

## POSTER 22: Expression of recombinant Zaire Ebola virus proteins in Baculovirus system to develop new diagnostic tools

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**Keywords: Ebola virus, baculovirus, recombinant proteins, diagnostic tool**

### Objective:

Zaire Ebola virus (ZEBOV) is a filovirus species that cause the most severe disease with hemorrhagic fevers in humans and non-human primates. Ebola virus diseases have a case fatality rate up to 70%. The 2014 outbreak in African sub-continent caused epidemics with more than 11.000 deaths. Although fruit bats are considered the natural reservoir for EBOV in Africa, previous reports showed that ZEBOV can infect pigs. Therefore pigs could be a natural host participating in the transmission to other pigs and non-human primates by contact and/or aerosol. The absence of effective treatment and vaccination options enhances the need to develop novel multispecies antibody detection assays. Three out the seven viral proteins were selected to be expressed: the virus glycoprotein (GP), considered the most important protein involved in induction of neutralizing antibodies; the nucleoprotein (NP), the most abundantly expressed antigen during infection, and the highly immunogenic matrix protein VP40.

### Methods:

The gene segments encoding GP, NP and VP40 proteins of ZEBOV were generated synthetically and cloned into pUC57 plasmids (pUC57-GP, pUC57-NP and pUC57-VP40). These genes were amplified by PCR with specific primers and cloned into the transfer vector pFastBac-HTA. DH10BAC competent cells were transformed with the transfer vectors to obtain the recombinant baculovirus rBac-GP, rBac-NP and rBac-VP40, following the procedure described in Bac-to-Bac Baculovirus Expression System (Invitrogen). Recombinant BACs were used to produce GP, NP and VP40 recombinant proteins of ZEBOV in High five insect cells (H5) and they were purified by ProBond Purification System (Invitrogen). Additionally, the mucin-like domain (MLD) of GP (GP<sub>305-501</sub>) was also cloned and expressed under the conditions described above. Vaccinia virus recombinants expressing ZEBOV NP, VP40, and complete and secreted versions of GP were isolated by inserting the complete genes under the control of a synthetic early/late promoter. Those recombinants were used to immunize BALB/c mice.

### Results:

The recombinant MLD domain from GP, as well as NP and VP40 ZEBOV proteins were highly expressed in H5 cells and purified under native conditions while GP protein expression was detected but purification was not efficient. In all cases the highest expression was detected at 72 hours post infection. Proteins were recognized by Western Blot and Indirect Immunofluorescence assay employing a mAb specific of GP and commercial rabbit polyclonal Ab specific of GP, NP, and VP40. Serum samples from BALB/c mice immunized with recombinant vaccinia viruses expressing NP, or VP40 proteins also recognized these proteins.

### Conclusion:

Recombinant VP40, NP proteins and recombinant MLD domain of GP should provide a useful tool to detect specific serum antibodies by ELISA or immunoblot assays. Furthermore, these proteins will be used to develop a Luminex-based DIVA assay for serological detection of Ebola virus infection in multiple animal species.

## POSTER 23: Poultry vector vaccines: innovative serological assays for vaccination monitoring and DIVA testing

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**Key words: Vector vaccines, vaccination monitoring, Innovative ELISAs**

Vaccination is an essential tool for poultry disease control. For many years, vaccines have been either live attenuated or inactivated, with innovation coming from the use of multivalent vaccines.

Today, innovation in poultry vaccinology include immune-complex vaccines and vector vaccines. Vector vaccines are made from a vector microorganism of which the genome has been genetically modified to encode an immunogenic protein of the disease of interest. Vectors in poultry vaccines are commonly the Fowl Pox Virus (FPV) or the Herpes Virus of Turkey (HVT). One or more genes may be inserted to ensure stronger protection or to widen the spectrum of protection to more diseases. Benefits associated with this technology include bio-security, efficiency, ability to breakthrough passive immunity, and long-lasting immunity. In addition, vector vaccines may be used to as part of DIVA (Differentiation between Infected and Vaccinated Animals) strategies.

Conventional serological kits do not efficiently detect seroconversion to vector vaccines. As a result, IDvet has developed new tools to monitor vaccination with vector vaccines for Newcastle Disease (ND), Avian Influenza (AI) and Infectious Laryngotracheitis (ILT).

Live vaccines with different degrees of attenuation (Ulster, Hitchner, La Sota), as well as killed vaccines and vector vaccines are used to protect animals against ND. In recent years, the use of recombinant HVT-NDV vector vaccines, which induce antibodies against the F-protein, has become widespread. IDvet has developed two ELISA tests to monitor vaccination using these vector vaccines. The **ID Screen® Newcastle Disease Indirect** (NDVS) test offers very high sensitivity, low background and excellent agreement with HI. It may be used to efficiently monitor HVT-NDV-F vaccination, and detects antibody levels which are not detected by other commercial ELISAs. Given that vaccinated animals will only develop antibodies against the F protein, IDvet has developed a DIVA strategy in which vaccinated animals may be monitored using the **NDVS assay**, and naturally-infected animals may be detected using the **NDV-NP** (nucleoprotein) assay. (Only naturally-infected animals will develop antibodies against the ND nucleoprotein.)

Vector vaccines may also be used to immunize animals against highly pathogenic Avian Influenza H5N1. HVT-H5 vector vaccines induce immunity in day old birds, and their effectiveness is not affected by maternal antibodies. To monitor vaccination with this vaccine, IDvet offers the **FLUACH5 ELISA** (ID Screen® Influenza H5 Antibody competition). Given that vaccinated animals will only develop antibodies against the H5 protein, IDvet has developed a DIVA strategy in which vaccinated animals may be monitored using the **FLUACH5 ELISA**, and naturally-infected animals may be detected using the **ID Screen® Influenza A Nucleoprotein Indirect**. (Only naturally-infected animals will develop antibodies against the AI nucleoprotein.)

Preliminary results obtained with the DIVA ND and AI tests described above will be presented.

## POSTER 24: Comparison of two different Real Time PCRs for detecting Zika virus and results obtained on *Ae. albopictus* collected during Summer 2015 in Italy

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**Keywords: Zika virus, Real Time PCR, Aedes albopictus, Italy**

Zika virus (ZIKV) is an emerging mosquito-transmitted virus in the family Flaviviridae and genus Flavivirus. It was initially isolated in 1947 from blood of a febrile sentinel rhesus monkey during a yellow fever study in the Zika forest of Uganda and was subsequently isolated from a pool of *Aedes africanus* mosquitoes collected in 1948 from the same region. In the recent outbreaks that occurred in Latin America, *Aedes aegypti* is believed to be the main vector but other species of *Aedes* mosquitoes such as *Ae. albopictus* (tiger mosquitoes) are suspected to be a less efficient but competed vector of the virus.

Different Real time PCR protocols are available in literature. The most used protocols was originally proposed by Lanciotti et al. (Emerging Infectious Diseases, Vol. 14, No. 8, August 2008) applied on human specimens and recently defused thought molecule panel test for Zika virus organize by Centers for Disease Control and Prevention (CDC), Fort Collins, Colorado, in the aim of arbovirus testing proficiency program for 2016. Another, quite diffused in literature, protocol proposed by Faye et al. 2013 (Virol J. 2013 Oct 22;10:311. doi: 10.1186/1743-422X-10-311.) was describe to be developed and evaluated with field-caught mosquitoes in Africa.

The aims of this work were to compare the efficiency and sensitivity of these 2 Real Time PCR with reference samples and positive RNA kindly provided by CDC (Center for Disease Control and Prevention, Fort Collins, USA) and evaluate their performance on field-caught *Aedes albopictus* mosquitoes in Italy.

Briefly, samples were extracted with BioSprint 96 semi-automated workstation with One-For-All kit (Qiagen, Hilden, Germany) and cDNA synthesis was achieved using random hexamer (Roche Diagnostics, Mannheim, DE) and SuperScript® II Reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturers' instructions. Real Time PCRs in comparison were run on the same cDNAs in triplicate and C<sub>T</sub> results were confronted. To test relative efficiency (%) and sensitivity (LOD) of the 2 PCRs, a reference positive RNA were diluted up to 1:1M and PCR reactions were made in quadruplicate. Finally the two PCRs were applied on field captured mosquitoes in the 9 Provinces of Emilia Romagna region by modified CDC traps baited by CO<sub>2</sub> (CO<sub>2</sub> traps).

The three positive reference samples were correctly identified by the two protocols. In the efficiency (%) and sensitivity (LOD) evaluations both the methods detect in quadruplicate positive control diluted 1:100'000, both method shown a good linearity (R<sup>2</sup>=0.989 and R<sup>2</sup>=0.991) with efficiency of 103.1% and 118.3% for Lanciotti et al. and Faye et al., respectively. The mean Delta C<sub>T</sub> between the two methods was 2.2 cycles less for Faye et al. protocol.

Between June and October 2015, a total of 3312 *Aedes albopictus* mosquitoes were collected. Mosquitoes were grouped in 189 pools according to date, location and species, with a maximum of 150 individuals per pool. Both the Real Time PCRs in comparison were applied on tiger mosquitoes pools and no positive reaction were detected.

In conclusion both the methods tested show good sensitivity and get the 100% satisfactory results in molecular panel test. The method of Faye et al. seem to be slightly more sensitive but differences observed falls in the interval of 3 cycles where PCRs are usually considered equivalent.

The research of Zika virus in tiger mosquitoes collected during Summer 2015 confirm that Zika virus didn't circulate in our Region. Nevertheless, the very diffused *Ae. albopictus* is in Emilia Romagna Region where it caused an outbreak of Chikungunya virus (CHIKV) in 2007 was recently demonstrate (Di Luca et al. 2016 Eurosurveillance, Volume 21, Issue 18, 05 May 2016) to be susceptible to ZIKV infection. Our results unlighted the importance of keep monitoring arboviruses transmitted by Italian mosquitoes in entomological arbovirus surveillance plan.

Acknowledgements: The authors wish to thank Brandy J. Russell and Colleagues of U.S. Department of Health and Human Services, Center for Disease Control and Prevention (CDC), Fort Collins, USA for providing the material used in this study.

## POSTER 25: First occurrence of H9N2 avian influenza virus in poultry, Morocco 2016

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**Keywords: Avian Influenza virus, H9N2, molecular diagnostics, phylogenetic analysis**

Infections due to H9N2 avian influenza virus (AIV) are not classified as notifiable AI (NAI). However, this AIV subtype has been identified as responsible of morbidity and mortality cases in poultry in Asia and Middle East, where is present since the mid-1990s. Through molecular analysis, Eurasian H9N2 AIV isolates are classified in three main lineages, namely G1-like, Y280-like, and Y439-like. Moreover, H9N2 could infect sporadically mammals' species such as pigs and humans, representing a potential zoonosis threat. In this study, the first outbreak due to H9N2 AIV subtype in Moroccan poultry is described.

During surveillance activities of the poultry flock implemented by the Moroccan National Office of Food Safety (ONSSA, Morocco), cases of abnormal mortality in several broiler farms were reported by the end of January 2016. The outbreak took place in 61 flocks located in different regions of Morocco from January to end of March 2016. The mean daily mortality was 2.67% reaching in some cases 50%. Also, a drop of immunity and decreased in production performance in poultry (egg drop and loss weight) were observed.

Influenza A viral genome was initially detected in tracheal and oropharyngeal swabs collected from dead chickens by RRT-PCR for matrix gene (1) at the ONSSA's Regional Laboratory for Research and Analysis in Casablanca. However, the early subtyping to detect H5 and H7 hemagglutinin gene using EU recommended methods (1, 2) showed negative results. Due to the emergency situation and in the framework of scientific-technical assistance provided by the MediLabSecure network, positive RNA samples were delivered to INIA-CISA (Valdeolmos, Spain) where rapid molecular subtyping was carried out. Specific HA and NA subtypes RT-PCR tests (2-5) confirmed the viral infection by AIV H9N2 subtype. Results were immediately reported to the Moroccan veterinary authorities and specific vaccination was put in place to contain the virus spread. Full HA gene was sequenced from two samples, obtaining two identical sequences of 1691 nucleotides in length. Nucleotide sequence analysis revealed the highest similarity rate (96-97%) between the Moroccan H9N2 virus and Middle East H9N2 AIV isolates (Israel, Saudi Arabia, United Arab Emirates) reported between 2010-2014 in chicken and pheasant. Phylogenetic analysis, including over 200 H9N2 AIV sequences (Eurasian lineage) selected from *Influenza virus resource* (NCBI), clustered Moroccan virus within the G1 lineage and was most closely related to recent North African, Middle East and Pakistan H9N2 AIVs isolates.

In conclusion, the results of comprehensive molecular analysis confirmed H9N2 subtype as the causative pathogen of the AIV outbreak occurred in Morocco in January 2016. Nevertheless, despite its highest relatedness with Middle East and North African AIV strains, its definitive origin remains unknown. The lack of HA gene sequences of H9N2 viruses of the last two years makes this question certainly challenging. On the other hand, the mortality observed in Moroccan poultry by H9N2 infection could likely be associated with complications such as secondary bacterial and viral infections. For its control, strengthening sanitary barriers and preventive vaccination against the H9N2 virus were immediately implemented.

### Acknowledgements:

This study has been supported by MediLabSecure European project (IFS-2013-330961).

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## POSTER 26: Diagnosis of Classical Swine Fever: rapid, simple, innovative

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**Keywords: Classical Swine Fever Virus, double antigen ELISA, DIVA**

### Introduction

Classical swine fever (CSF) is a highly contagious disease affecting domestic swine and wild boar. Due to its highly variable and often unspecific clinical symptoms, extensive serological CSF surveillance programs are required. In the European Union, a strict 'stamping-out' strategy is applied in case of a CSF outbreak. Given ethical and socio-economic concerns, application of marker vaccines for emergency vaccination is a promising alternative in future outbreak scenarios. Successful marker vaccine use requires a reliable diagnostic test to differentiate infected from vaccinated animals (DIVA strategy). The most promising DIVA strategy, involving the recently approved Suvaxyn® CSF Marker, is based on the absence of a CSF Virus (CSFV) Erns-specific immune response. QIAGEN's new double-antigen ELISA pigtype® CSFV Erns Ab detects antibodies to the CSFV protein Erns and thus addresses an alternative target protein to conventional E2-specific antibody ELISAs. It also offers the possibility to be used as accompanying DIVA test for suitable marker vaccines. The aim of the present study was to validate the test for both applications.

### Materials and Methods

Sensitivity and specificity of pigtype CSFV Erns Ab were evaluated by testing a serum sample panel comprising CSFV antibody positive and negative sera in comparison to a commercially available E2-specific antibody ELISA. Furthermore, application as a discriminatory test was validated using sera taken from animals vaccinated with the marker vaccine Suvaxyn CSF Marker as well as sera from vaccinated and subsequently CSFV challenged pigs.

### Results

The new pigtype CSFV Erns Ab proved highly specific and sensitive in comprehensive validation with CSFV antibody positive and negative sera. Compared to a commercial E2-specific antibody ELISA, pigtype CSFV Erns Ab was more sensitive to sera obtained very soon after infection ( $\leq 21$  days). The novel test reliably detected Erns antibodies to a variety of isolates belonging to various CSFV genotypes. pigtype CSFV Erns Ab showed comparable specificity for sera obtained after vaccination with the marker vaccine when compared to the only other commercially available Erns-specific antibody ELISA, but demonstrated to be more sensitive for CSFV challenge sera obtained from marker-vaccinated and CSFV challenged pigs.

### Conclusion

The pigtype CSFV Erns Ab ELISA can be applied as antibody screening test in the context of CSF surveillance, but can also be employed as an accompanying differentiation test with Suvaxyn CSF Marker or another suitable marker vaccine.

## POSTER 27: How does pooling of serum or blood samples from young calves permanently infected with BDV-V affect the detectability of the real time PCR test? A field study in the context of the Belgian BVD eradication program

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**Keywords: BVD, PI, earnotch, pooling, PCR**

### Introduction:

Currently, antigen (Ag) ELISA on ear notch samples is the routine method to detect permanently infected (PI) newborn calves within the Belgian BVD eradication program. The purpose of this study was to evaluate if real time RT-PCR testing of pooled blood or serum samples obtained from young calves (< 40 days) could be used as an alternative method and to measure the risk of not detecting PI calves, due to the possible presence of maternal antibodies reducing the viral load in these samples. Therefore, we estimated the proportion of false negative results when testing pooled serum or blood samples by PCR, taking as reference the Ag ELISA performed on individual earnotch samples. We compared this proportion to the estimated percentage of undetected PI (~3%) reported by other authors (Fux, 2012; Oettl, 2010) when using Ag ELISA on earnotch samples.

### Materials and methods:

59 calves, previously detected as PI at birth in the routine BVD screening (August - December 2015), were monitored in this study. Each calf was sampled three times, to cover the period during which the detection of the virus is most critical: between day 10 and 15 after birth, around day 21 and around day 28. A fourth sample was taken at 45-60 days of age, to confirm the PI status. At each time point, full blood, serum and an ear notch sample were collected and tested with a commercial Ag ELISA. Three different PCR methods (one in house and two commercial kits) were performed on each blood and serum sample. Virus neutralization tests (VNT; BVDV-1 and BVDV-2) were performed on each serum. Afterwards, each blood and serum sample was diluted 1/20 (1/8) in blood or serum from a BVDV-free seropositive animal to mimic a pool size of 20 (8) samples and tested by PCR. The proportion (with 95% CI) of (false) negative PCR results obtained with pooled serum and pooled blood samples from the first three visits was estimated. We used a generalized estimating equation model (proc genmod, SAS 9.2.) to take into account the intra-class correlation of results obtained with successive samples from the same animals.

### Results:

All 59 PI animals were positive at each of the three visits when tested by Ag ELISA on individual ear notch. Three calves were negative by Ag ELISA for serum samples taken at the first visit and two of them were also negative for corresponding blood samples. PCR on individual blood samples were positive for all animals at all visits but one animal had a negative PCR result with the three PCR methods on serum samples from the first and/or second visit. Most calves were seronegative or had low VNT titers ( $\leq 1:60$ ) at all visits but a few animals had high VNT titer ( $\geq 1:240$ ), generally decreasing with time, probably linked to maternal antibodies. Some of the individual serum and blood samples which were positive but had high Ct values ( $>35$ ) and high VNT titers (1:120 – 1:640) could not be detected anymore when tested in pools and these pools results were considered as false negative. The proportion of false negative results with pooled samples, taking as reference the Ag ELISA method on individual earnotch was estimated to be between 1.13 and 2.26 %, depending on the sample matrix, the pool size, and the PCR method used.

### Conclusion:

A small percentage of PI calves, detected at birth by Ag ELISA on earnotch samples would not be detected when tested by PCR on pooled serum or blood samples taken during the first weeks after birth. However, it has been previously shown that a similar proportion of false negative results also occur with the Ag ELISA method on earnotch.

## POSTER 28: DetektiVir: Deployment of a generic diagnostic workflow for the de-novo detection of new viral pathogens

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**Keywords: pathogen detection, metagenomics, diagnostics**

Rapid and reliable detection and characterization is the basis for the control of viral infections. In recent years, numerous novel viral infectious diseases emerged that impair both human and animal health. Because no diagnostic methods are available for these new pathogens they often remain undetected and consequently, early restriction of potential outbreaks is not feasible. Here, we present a new workflow which includes the detection of novel pathogens and the subsequent development of molecular and serologic diagnostic methods.

One key approach for the detection of new emerging pathogens is the analysis by next-generation sequencing (NGS). These technologies allow unbiased sequencing of most nucleic acids in a sample. However, one major barrier for the use in day-to-day diagnostics is the lack of comprehensive and unified workflows for all steps from a sample to the final result. In particular, data analysis is often hampered by the lack of reliable and easy-to-use data analysis tools. The project DetektiVir is an interdisciplinary project that aims at closing this gap by deploying a new workflow which combines molecular nucleic acid-based virus detection by metagenomic sequencing with ad-hoc development of customized serological diagnostics and integration of dynamic database applications.

The centrepiece of the detection platform will be a data hub which allows the easy access of data and their analysis in a user-friendly environment. Sequences of potentially new pathogens will be used for a screening via qPCR and peptide arrays for the fast development of serological diagnostics. As a prerequisite for the deployment of the data hub we optimized the already established metagenomic workflow RIEMS (Reliable Information Extraction of Metagenomic Sequence datasets) (Scheuch, Höper et al. 2015) and developed an improved output with both a “computer-readable” and a more “human-readable” format.

The pipeline structure, the new output design and the first detection results of the optimized workflow will be presented and discussed.

### Acknowledgements

The project DetektiVir is funded by the German Federal Ministry of Education and Research within the framework “Civil Security - protection against biological threats and pandemics” ([www.sifo.de](http://www.sifo.de)).

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## POSTER 29: Immunomodulatory and antiviral effects of a functional diet for rainbow trout (*Oncorhynchus mykiss*)

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**Keywords:** fish, functional diet, viral hemorrhagic septicemia virus (VHSV), immune response.

The impact of viral infections constitutes one of the major problems for aquaculture, due to the lack of effective antiviral vaccines in the market. In this context, immunomodulation through the addition of functional ingredients in the diet may constitute a simple strategy to reduce the impact of viral infections in cultured fish. In the current study, we have studied the immunomodulatory and antiviral effects of a functional test diet in rainbow trout (*Oncorhynchus mykiss*). The levels of transcription of several immune genes were evaluated in skin, intestine and kidney of fish fed for 15 or 30 days with the functional diet, or fish that had been fed with a control diet. At this point, fish were challenged with virus hemorrhagic septicemia virus (VHSV), a common rainbow trout pathogen, and the viral load estimated through viral gene transcription. Our results show that the administration of the functional diet for either 15 or 30 days significantly modulated the levels of transcription of many immune genes in skin, intestine and kidney. Furthermore, fish fed with the functional diet had reduced viral loads demonstrating an antiviral potential of this diet.

## POSTER 30: Comparison of a commercial ELISA assay and hemagglutination inhibition test for swine influenza viruses antibodies detection

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**Keywords: Swine Influenza virus, serology, ELISA, hemagglutination inhibition test**

**Background:** Influenza A viruses (IAV) represent a major cause of acute respiratory disease in finishing pigs and is ubiquitous in swine populations all over the world. At present, four principal subtypes of IAVs are circulating among the Italian pig population: H1N1, H3N2, H1N2, and more recently, H1N1pdm 2009 (1). Serology is useful for the diagnosis of clinical disease, to assess the level of herd immunity and epidemiologic studies based on seroprevalence (2). According to OIE Manual 2015 (3) the gold standard test for detection of IAV antibodies is the subtype specific hemagglutination inhibition test (HI), able to detect both early and late antibody response to the virus. Moreover, additional serological tests have been described as the agar gel immunodiffusion test, indirect fluorescent antibody test, virus neutralisation, and ELISA. Since the antigenic diversity of IAV in swine is always increasing and requires the use of multiple H subtypes in the HI assay, there is a trend to use commercial ELISA kits for IAV antibodies detection, that mainly detect IgG antibody (3).

**Aim:** An explorative study to evaluate IAVs circulation in pig herds of a high-density area for swine (Cuneo province) is ongoing in Piedmont since 2014. Sera have been analysed with gold standard HI test and a competitive multi-species ELISA assay. Here we present the ELISA accuracy estimation in comparison with the HI.

**Methods:** A total of 1086 sera were collected from 43 herds and analysed with subtype specific HI assays (HI titer positivity cut-off  $\geq 20$ ) and with a competitive ELISA kit for the detection of anti-nucleoprotein antibodies of IAV subtypes (S/N% cut-off  $\leq 45$ ) against a highly conserved epitope of the Influenza A virus nucleoprotein. H1N1, H3N2, H1N2 and H1N1pdm strains were used as reference antigens in the HI assays, according to OIE procedures. In this study, ELISA performances were estimated by comparison with the HI test (positive for at least one subtype) by means of sensitivity, specificity, positive and negative predictive value (PPV e NPV) and the concordance between the two assays with Cohen K was assessed. Moreover the same comparisons were made between ELISA and each of the four subtype specific HI assays, separately.

**Results and discussion:** Out of 1086 sera tested, 864 were positive for at least one of the HI panel test and 222 negative. In the ELISA assay, 639 resulted positive, 54 inconclusive and 393 negative. In the statistical analysis, the inconclusive samples (about 5% of the total sera) were removed. ELISA performances vs HI test are expressed in table 1a and the concordance between the two tests was  $K=0.3961$  (95%CI: 0.3405-0.4516).

TAB.1	A) ELISA VS HI	B) ELISA VS H1N1PDM HI
SENSITIVITY	72.65% (95%CI: 69.46 - 75.68)	87.89% (95%CI: 83.25 - 91.62)
SPECIFICITY	79.34% (95%CI: 73.28 - 84.57)	46.65% (95%CI: 43.09 - 50.23)
PPV	93.11% (95%CI: 90.87 - 94.95)	35.21% (95%CI: 31.51 - 39.05)
NPV	43.00% (95%CI: 38.05 - 48.06)	92.11% (95%CI: 88.99 - 94.58)

Considering the four subtype specific assays separately, the best ELISA performances were reached in comparison with the H1N1pdm HI test (Tab.1b), while the best concordance was showed with the H3N2 HI assay ( $K=0.4601$ , 95%CI: 0.4059-0.5144).

Forty-four out of 213 sera seronegative in all the HI panel tests were positive in ELISA. One hypothesis for this result may be a seroconversion against an influenza virus not represented in the HI antigen panel used in this study, but also the different antibodies targeted by the two test might be taken into account. Moreover, 224 HI positive sera resulted negative when analysed in ELISA test and therefore were considered as false negative. The statistical analysis revealed that 50% of false-negative samples had a HI titer not greater than 20 and/or 40 in at least one of the subtype specific assays. The remaining false negative sera, had a HI titer distributed in a uniform manner among 80 and 5120 HI titers. These results may be indicative of an early immune response in some animals, not detectable by the commercial kit. It has to be taken into account the As assumed elsewhere, ELISA may not identify positive animals at the early stage of infection as effectively as the HI test, particularly when the virus is introduced to a naive swine population (4). The main merits of ELISA-based compared to HI serologic assay remain the ability to process a high number of samples at a time, a good turnaround time and less labor intensiveness.

In conclusion, the performances of the ELISA assay need to be improved, but the commercial kit can be used in serodiagnosis of SIV infection. However, caution may be used since the test could miss recently exposed animals.

We acknowledge the Italian Ministry of Health for supporting the research, under the Ricerca Corrente 2013 funding (grant code IZS PLV 07/13 RC) and Dr Foni E. of the OIE Reference Laboratory for Swine Influenza, IZSLER, Parma, Italy for the supply of reference antigens and antisera panel.

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## POSTER 31: Characterization of gut and gills CD8 $\alpha$ <sup>+</sup> MHC-II<sup>+</sup> dendritic cells in rainbow trout (*Oncorhynchus mykiss*).

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**Keyword: dendritic cells, CD8<sup>+</sup>, MHC-II,**

Although fish constitute the most ancient animal group in which an acquired immune system is present, the presence of dendritic cells (DCs) in teleost has only been briefly addressed and the identification of a specific DC subset in teleost remained elusive due to the lack of specific antibodies. In mice, CD8 $\alpha$ <sup>+</sup> DCs from lymphoid tissues have the capacity of cross-presenting extracellular antigens to T cells through MHC I, similarly to tissue derived CD103<sup>+</sup> DCs and the human CD141<sup>+</sup> DC population. Previous studies in our group, have identified a subpopulation of cells exhibiting functional characteristics of DCs and co-expressing MHC II, CD8 $\alpha$ , CD103 and CD141 in trout skin. These cells thus provide the first evidence of a specific DC-like subtype in teleost and support the hypothesis of a common origin for all mammalian antigen cross-presenting cells. In this work, I have identified this subpopulation of DCs in gills and gut. These cells also transcribe immune genes related with DC activities such as CD80/86, DC-SIGN, BAFF and CD11b and show a significant phagocytic capacity suggesting that this DC subset is also present in these tissues. Further studies will focus on determining whether there are phenotypic and functional differences among these cells in the different tissues.

## POSTER 32: Laboratory diagnosis of *Theileria equi* infection in equids imported into the Republic of Korea

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**Keywords:** Equine disease, *Theileria equi*, Korea, quarantine

*Theileria equi* (*T. equi*) is a tick-borne hemoprotozoan parasite that causes piroplasmosis in equids. The parasite replicates within erythrocytes and causes fever, anemia, icterus, hemoglobinuria, and, in some cases, death. Animals that recover from acute infection become persistently infected. These persistently infected animals pose a threat to naive populations, as they serve as reservoirs for iatrogenic or tick-borne transmission.

The growth of international equine sports and horse trading has increased the possibility of introduction of *T. equi*, which leads the necessity of stringent serological screening of horses prior to entry to non-endemic areas including the Republic of Korea. In this report we describe our laboratory diagnosis cases of *T. equi* infection in equids imported into the Republic of Korea.

Blood samples were collected from 2,863 imported equids undergoing post arrival quarantine during the period from 2013 to 2015. Serological tests against *T. equi* were screened first by Competitive Enzyme-Linked ImmunoSorbent Assay (cELISA) and then Indirect Fluorescent Assay (IFA) and Complement Fixation Test (CFT) were performed on the samples tested positive and doubtful by cELISA. Detection of the agent is assessed by microscopy of blood smears and the nested PCR (nPCR) assay for Equi Merozoite Antigen-1 (EMA-1) gene. The amplified products of the nPCRs were sequenced to establish genetic relationship of 18S rRNA and EMA-1 gene, and to perform phylogenetic analysis.

Among the 2,863 equid sera samples, 4 samples (equids originated in China) were positive and 3 samples (1 from the USA, 2 from China) were in borderline by cELISA. All of the samples tested positive and doubtful by cELISA were shown to be positive by IFA, while only 1 sample (horse originated in the USA) was positive reaction in CFT. Although all the blood samples that were positive by IFA were microscopically negative, nPCR assay detected expected amplicon with a size of 218 bp for *T. equi*. The phylogenetic analysis based on the 18S rRNA and EMA-1 gene showed that equid originated in the USA was closely related to the isolates from USA, Brazil and South Africa whereas equids originated in China were closely related to the isolates from Korea, China and Mongolia.

Therefore, it can be concluded that the infected equids were in the transitory phase, following acute infection by a *T. equi* isolate that was currently circulating in the exporting countries. The pre-export period under the quarantine control also assures this conclusion. The horses could have acted as an amplifier, and contributed to the spread of *T. equi* during quarantine period. Equine piroplasmosis is vector borne disease and constantly changing climates provides major challenges for controlling persistent infections. Due to the almost worldwide distribution of various vectors, it is very important to prevent the introduction of infected or carrier animals into non-endemic areas, which requires multiple diagnostic methods to obtain the most accurate information.

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# POSTERS

## TOPICI

Animal health in  
a changing world

Epidemiology & Surveillance  
and Risk assessment

## POSTER 33: Pestivirus infections in small ruminants flocks in Western Turkey

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**Keywords: Pestivirus, Small ruminants, ELISA, PCR, Seroprevalance**

Pestiviruses are economically important pathogens which causes diarrhea, poor performance, increase in the frequency of other infections and lethal conclusions at wild and domestic ruminants, pigs as well as camelids worldwide. Sheeps and goats play role for transmission of this infection among ruminants. In this study small ruminants in South Marmara region of Turkey are investigated against pestivirus infections. For that purpose 607 blood samples were collected from randomly selected 22 unvaccinated herds in the area, which is lack of sufficient information on the infection. During this term, 3 aborted animals in 2 different herds were encountered. Blood samples collected from 221 goats, 386 sheep as well as 3 tissue samples were tested against pestivirus antigens with enzyme-linked immunosorbent assay (ELISA). Pestivirus antibodies were investigated with neutralization assay after inactivation of serum samples from animals. Reverse transcriptase-polymerase chain reaction (rt-PCR) was applied to eighty (n: 80) blood samples and to 3 tissue samples. All of the blood samples were detected negative for pestivirus antigen while all of the tissue samples were positive by ELISA. Surprisingly ELISA positive 2 tissue samples were detected negative with panpestivirus-primers 324-326. All the tested blood samples (n: 80) were detected to be negative by rt-PCR. The geometric mean of antibody titers detected as 3.5 and the antibody titers ranged between 8 and 1024 ( $\log_2$ ). There were no antibodies detected at 6 of 22 herds. In virus neutralization test 199 (32,8%) samples produced positive result. Among these antibody positive samples (n: 199) 72.3% detected in herds in which abortion cases have been recorded in the past and remaining 27.6% were detected in herds without abortion history. It is concluded that small ruminants in the region are open to pestivirus infections. Molecular investigation of samples is currently underway. Because of some incompatibilities between PCR and ELISA results detected up to now, it is concluded that molecular identification of pestiviruses needs to be improved.

**Funding:** This study was supported by the Uludag University Research Fund (project no: OUAP(V) – 2014/19).

## POSTER 34: Use of a metagenomic approach to characterize influenza D viruses detected in cattle in north eastern Italy

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**Keywords: Influenza D, bovine, metagenomics**

Recent studies carried out in the United States of America, Europe and China have identified a new Genus within the *Orthomyxoviridae* family, tentatively named Influenza D virus (1), which was first identified in swine showing influenza-like symptoms. This pathogen was subsequently detected in cattle and also proved to replicate and transmit efficiently in ferrets (2). Retrospective studies identified Influenza D virus in samples collected between 2014 and 2015 from cattle and swine in two regions in northern Italy, namely Emilia Romagna and Lombardia; however, its spread throughout the country has not been assessed yet.

Here we report for the first time the detection of influenza D virus in cattle in the Veneto region and its characterization by metagenomic approach.

In north eastern Italy, in particular in the province of Venice and Treviso, two herds of beef cattle of about 2000 and 350 Charolaise cows respectively, experienced a respiratory syndrome characterized by nasal discharge, reduced food intake, conjunctivitis and lethargy.

The calves were introduced at the age of 12-13 months from France, 60 (Venice) and 15 (Treviso) days before the onset of the clinical signs. Field veterinarians suspected a viral infection and submitted nasal swabs and serum samples for differential diagnosis of main respiratory bovine pathogens. One out of three pools of nasal swabs collected among 9 subjects in the herd in Treviso tested positive for bovine respiratory coronavirus (BCoV). High microbial contamination was identified in two samples collected from the herd in Venice and *Mannheimia haemolytica* was detected in one of them.

Furthermore, the samples were tested for influenza D virus by using real-time (rt) RT-PCR as previously described (2). Six nasal swabs (3 from Venice and 3 from Treviso) were positive for Flu D. Representative Flu D positive swabs were sequenced on an Illumina Miseq platform using a metagenomic approach. Raw data were processed to remove adaptors and low-quality sequences. High quality reads were de novo assembled using SOAPdenovo (3) to produce a set of scaffolded contigs, refined with GapCloser (3). We then compared assembled sequences to a known influenza D virus reference (D/bovine/Italy/1/2014) to assess the quality of our assembly and its completeness.

Phylogenetic analyses were performed for each genome segment using PhyML 3.0 incorporating a GTR model of nucleotide substitution with a gamma distribution of among-site variation (with four rate categories,  $\Gamma_4$ ) and a SPR branch-swapping search procedure. A non-parametric bootstrap analysis was applied using 100 replicates. Haemagglutination inhibition test was carried out using D/bovine/Nebraska/9-5/2012 as antigen on serum samples collected from animals after more than two weeks of onset of clinical signs.

Serological tests revealed high antibody titres against influenza D virus for both cases.

The analyses of the seven gene segments demonstrated that the virus identified in the Veneto region clearly clustered with the Italian and North American influenza D viruses from swine and cattle, suggesting a common origin for these viruses. Interestingly, the influenza D viruses identified in Italy presented a low similarity with the French influenza D virus (4), which is the only other European country where this pathogen has been detected so far.

Bovine respiratory disease has a major economic impact on the cattle industry and the role of influenza D should be further investigated through surveillance studies.

### Acknowledgments

This project was partially funded by the Italian Ministry of Health (RC 05/14).

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## POSTER 35: Diversity and evolution of Avian Influenza (AI) viruses in poultry and wild birds

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Over the past decade, surveillance programmes for Avian Influenza (AI) virus infections in the Netherlands have provided extensive information on the spatiotemporal distribution of AI virus infections in poultry and wild aquatic birds. Wild birds are the natural reservoir of AI viruses and suspected to be the source of AI virus infections in poultry. Surveillance focuses mainly on the early detection of AI virus infections of subtypes H5 and H7, which have the potential to mutate from a low pathogenic AI (LPAI) variant into a highly pathogenic AI (HPAI) variant. However, the introduction of AI viruses of other subtypes is also monitored. Here, we provide an overview of all AI virus subtypes that have been detected by virus specific antibody detection or virus isolation in poultry and wild birds in the Netherlands from 2006 to 2015. Results show that poultry as well as wild birds are frequently infected with LPAI viruses. However, the subtype distribution differs between the two groups, indicating that LPAI virus transmission from wild birds to poultry is not random and likely depends on viral factors that determine host range restriction. In multiple cases, LPAI virus infections of the same subtype have been detected on several poultry farms at approximately the same time, suggesting that these viruses have acquired the capacity to be efficiently transmitted from wild birds into poultry and/or between farms. The whole genome sequences of more than 300 AI viruses isolated from poultry and wild birds have been determined to perform phylogenetic analysis. In this study, we aim to identify specific mutations in the AI virus genome that correlate with an increased chance of LPAI virus introduction in poultry, within-farm spread of LPAI viruses, and transmission of LPAI viruses to other poultry farms. Increased knowledge of LPAI virus transmission is important to control virus spread and reduce the probability of mutation of LPAI viruses into HPAI viruses.

## POSTER 36: Seroprevalence and Genetic Characterization of Hepatitis E virus (HEV) in Korean wild boars (*Sus scrofa*)

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**Keywords: hepatitis E virus, Korean wild boar, seroprevalence**

The hepatitis E virus (HEV) is an emerging zoonotic pathogen with worldwide distribution. In Korea, several outbreaks of human HEV infection due to the consumption of infected wild animals have been reported and swine and wild boars (*Sus scrofa*) are known as reservoirs of HEV infection. The aim of this study was to investigate the nationwide seroprevalence of hepatitis E virus (HEV) infection and the genetic characterization of HEV among Korean wild boars (*Sus scrofa*) from 2011 to 2015.

A total of 1,053 wild boar hunted in nine provinces were examined for antibodies against HEV by using commercial ELISA kit. Overall, 33.9% of serum samples from individual boar were seropositive for HEV.

Phylogenetic analysis of the ORF2 region revealed that the strains clustered within genotype 4, subgenotype 4a with 98.6-99.6% nucleotide sequence identity and were closely related to HEV strains previously detected in Korean wild boars. These results suggest that HEV genotype 4a is circulating in wild boars in Korea and have potential risk factor for HEV transmission to human.

This is the first report on the seroprevalence of HEV infection in wild boars in Korea. The consumption of undercooked wild boar meat may expose humans to a high risk of HEV.

## POSTER 37: Creating an acceptable and effective BVD eradication program for the Netherlands

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**Keywords: BVD, Eradication, control measures, surveillance**

Bovine Viral Diarrhoea (BVD) is an infectious disease generally most severe in young stock. Infections in bovines can be divided in two types: Transient Infection (TI) and Persistent Infection (PI). A TI normally results in relatively mild symptoms and recovery after around 10 days. In contrary, PI animals remain extremely infectious during their full life span. These animals have a very high mortality during the early days of their lives. Up to 70% of PI animals do not reach the production age (2 years). The surviving PI animals will, if they give birth, create new PI calves. BVD infection in susceptible pregnant dams gives a high probability of abortion (up to 80%) and birth of PI calves, depending on the state of gestation at infection. Consequently, the presence of a PI-animal results in substantial production losses for individual farmers (estimated at €30 to €60 per cow).

BVD is endemic in the Netherlands and one of its most significant reproductive disorders in cattle. Approximately 1-2% of cattle are PIs. Since many European countries are in the process of BVD-eradication, the export potential of the Netherlands is decreasing. In combination with the direct economic impact, eradication of BVD has become an important for the Dutch cattle industry.

In the absence of a European eradication strategy, we look for a cost-effective strategy, with as few imposed measures as possible. To investigate the most effective control strategy, we developed a stochastic model for BVD transmission and eradication. In the model, the animals are classified as Maternal Immune (M), Susceptible (S), TI, PI and Recovered (R). Within herd transmission is performed by the Gillespie algorithm. We included all Dutch farms distributed over the following herd types: dairy, young stock raising, veal calves, bulls, other beef, suckling cows, cattle traders, cattle collection centres and small cattle holdings. We categorised age structure that covers production status for each herd type individually. Transmission between farms can occur through live animal movements. Moreover, the model includes the current certification program for BVD, which is based on ear biopsies, serology and/or bulk milk (BM) screening.

In the model, we determine the key elements influencing the efficacy of a control strategy, based on specific risk factors (such as veal calve import), restriction of live animal movements and the impact of non-dairy farm types on national prevalence. Furthermore, we estimate the time until the disease free status is achieved and economical costs of eradication.

## POSTER 38: Molecular epidemiology of bovine viral diarrhoea virus (BVDV) in Spain between 2007 and 2015, spatial and temporal distribution of BVDV-1 subtypes

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**Keywords:** bovine viral diarrhoea virus, cattle, genotyping, spatial and temporal clustering, molecular epidemiology

### Introduction

Bovine viral diarrhoea virus (BVDV) causes major economic losses in domestic ruminants worldwide. Two distinct genotypes of BVDV, BVDV-1 and BVDV-2 have been described. In Europe, BVDV-1 is by far the most frequent, with different subtype's predominance by countries. In Spain, previous studies showed BVDV-1b as the most prevalent subtype with no evidence of BVDV-2<sup>1,2</sup>, but recently BVDV-2 has been reported for the first time<sup>3</sup>. Studies investigating the molecular epidemiology of BVDV will provide valuable information about the diversity of the viral strains present in the Spanish cattle population. Main objectives are to: 1) identify the genotypes of BVDV circulating in Spain from 2007 to 2015; 2) study the spatial and temporal clustering for each genotype, and 3) investigate the trends of diversity of the genotypes along the time.

### Materials and methods

Cattle sera from 164 viremic animals, previously diagnosed by antigen ELISA, were randomly selected from a sera bank collected from 2007 to 2015. For genotyping, a one-step RT-PCR which amplifies a 288 bp of the 5' untranslated region (5'-UTR) previously described<sup>4</sup> was carried out. Products of RT-PCR were directly sequenced.

The sequences obtained were aligned, and those 100% identical were grouped as nucleotide sequence types (ntST). In order to classify the sequences in the study into different genotypes and subtypes, a maximum likelihood tree with a bootstrapping of 1000 replicates was made with ntST and the reference sequences. In addition, to investigate the spatial and temporal clustering of the sequences, two neighbour-joining networks were constructed, one representing the regions and the other one the years of sampling. For the network analysis all sequences previously described in the Genbank from Spain were also included, all of them being collected from 1999 to 2002.

### Results

Main results revealed a high genetic diversity, identifying 88 ntST out of 164 total sequences obtained. All of them belonged to genotype 1. Among subtypes, 1b was the most frequent, representing the 79.9% of total sequences obtained (131/164) and the 77.3% of all ntST (68/88). Other subtypes found were 1f (7.9% total sequences and 11.4% ntST), 1e (6.1% total sequences and 2.3% ntST), 1d (4.3% total sequences and 6.8% ntST), and 1h (1.8% total sequences and 2.3% ntST).

No regional or temporal clustering was observed for each subtype, with the exception of subtype 1h that was only found in one region (Castilla y León) and in one year (2014). In fact, some frequent ntSTs were detected in up to six different regions out of 10 included in the study. Regarding the evolution of the diversity of subtypes, 1b and 1e were found from 1999 to 2015 and from 1999 to 2013, respectively. Eight ntST were present since 1999-2002 to 2007-2015, revealing a certain stability of predominant sequences. Subtypes 1d, 1f and 1h were identified for the first time in 2007, 2008 and 2000, respectively.

### Conclusions

The results obtained in the network demonstrate the predominance of subtype 1b in Spain since 1999, which seems to be enzootic. The recent introduction of subtypes 1d and 1f highlights the importance of periodic genotyping of BVD in order to obtain relevant epidemiological information about the evolution of the different subtypes along the time.

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## POSTER 39: Influenza A epizootiology at the wild bird/swine interface: surveillance in wild boars and free-range Iberian pigs in Spain

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**Keywords: Influenza A, Wild boar, Iberian pig**

Spain is recognized by the OIE as a priority region for Influenza swine surveillance because meets the main risk factors for spread and emergence of Influenza A virus (IV) in swine: high populations of swine, poultry and waterfowl, high prevalence of IV infection in industrial pigs, potential for reassortment between virus circulating in pigs and birds due to husbandry methods and high rate of swine movements and exports. In addition, up to 10% of Spanish pig population belongs to Iberian breed which are mostly bred in a free-range system. The wild-boar is other member of *Sus scrofa* species which is susceptible to swine influenza viruses. Habitats where wild boars and free-ranging Iberian pigs coexist closely with migratory wild birds constitute an excellent natural model for studying the interface avian/mammal of Influenza epidemiology in their natural environment and the ecology of Influenza viruses in their natural reservoirs.

The aims of this study are to assess the incidence of Influenza infection and to study the nature of influenza viruses in two populations de free-range *Sus scrofa* animals in locations where wild migratory birds are abundant. Of especial interest is the potential role of Eurasian Cranes (*Grus grus*) as High Pathogenic Avian Influenza virus (HPAIV) transmitter. In fact, a number of cranes were found infected with HPAIV H3N8, responsible of several outbreaks in wild birds and poultry in Western Europe in 2014-15. Eurasian cranes are particularly abundant in Extremadura region and compete actively for food with wild birds and Iberian pigs during wintering season.

The sampling was conducted from September 2015 to April 2016 in south west Spain, mainly in the provinces of Badajoz, Cáceres, Córdoba and Huelva, areas that account for most of the Spanish Iberian pig and wild boar populations. Intensive and extensive (montanera) farms of Iberian pigs were included in the study. A total of 1018 serum samples of Iberian pigs from 63 farms of different locations were tested. Proximity to wetlands or resting areas of migrant or resident wild birds was taken into account. By ELISA, 55.5% of farms were Influenza A positive. By HI tests, 54% of sera were specific to pdmH1N1, 83% to swH1N1, 8% to swH3N2 and 12% to swH1N2. Interestingly, a significant number of serum samples did not react with any of the reference virus strains used in the HI and NI tests. Up to eight samples (lung tissues and oral samples) were RT-PCR+ to IAV. With respect to wild-boars, 10% (35/362) of serum samples were positive to pdmH1N1 or swH1N2 and 1.4% (4/279) of tissue samples were RT-PCR+ to IAV. Significant differences regarding IAV subtype distribution between Iberian pigs and wild-boars as well as between Iberian pigs and industrial white pigs have been found. Up to now, no evidence of avian influenza virus transmission from wild birds to wild boars or Iberian pigs has been found.

### Aknowledgements:

This study was funded by the Center for Research in Influenza Pathogenesis (CRIP) as part of the National Institute of Allergy and Infectious Diseases (NIAID)-Centers of Excellence for Influenza Research and Surveillance (CEIRS, HHSN272201400008C). We thank J.L. Cortés DMV, INGULADOS SL and Fundación Alfonso X el Sabio for support and for providing us with samples from animals.

## POSTER 40: 2016 H7N7 Highly Pathogenic Avian Influenza outbreaks in Italy

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**Keywords: Avian Influenza, HPAI, outbreaks, wild birds, poultry**

Following the 1999–2000 H7N1 Highly Pathogenic (HP) Avian Influenza (AI) epidemic, involving over 16 million birds, no HP viruses were detected in domestic poultry in Italy until 2013. In August 2013 a H7N7 HPAI virus was identified in a free-range layer farm belonging to a large production company with numerous farms in several Italian regions. The affected farm was located in close proximity to important resting sites for migratory wild birds. The prompt adoption of strict control measures limited the spread of the disease, preventing the virus from spreading to neighbouring Densely Populated Poultry Areas (DPPAs). Only 6 farms were infected and the epidemic was contained in less than a month. After an interval of over a year, a H5N8 HPAI virus was isolated in a single fattening turkey farm in December 2014, in proximity to the same wetland areas as the 2013 outbreaks. The virus was closely related to the H5N8 strain that circulated in wild birds in Europe in 2014-2015, with repeated incursions into domestic poultry.

On 30 April 2016, a H7N7 HPAI virus was confirmed in an organic free-range layer farm located in the same area as the 2013 outbreak. Control measures provided in Directive 94/2005/CE were promptly applied, and enhanced surveillance activities were implemented in the DPPAs. Epidemiological data and phylogenetic analyses indicated that the virus likely originated from wild birds as a Low Pathogenicity (LP) AI strain, introduced into the farm through direct contacts. Phylogenetic analyses indicated the H7N7 HPAI virus regrouped with viruses belonging to different subtypes identified in wild and domestic birds in Europe, Africa and Asia between 2010 and 2015. The haemagglutinin (HA) gene had low genetic similarity with other HPAI viruses previously identified in Italy and Europe, and the H7N7 LPAI virus detected on 15 April 2016 in an ornamental birds farm located in the same province as the outbreak (sequence similarity < 93%). Further analyses of HA sequences showed the co-circulation of at least two distinct highly pathogenic viruses with a different insertion at the HA cleavage site. Moreover, the HA cleavage site motif typical of an LPAI virus was identified in swabs collected from live birds.

A HPAI H7N7 virus was also identified on 16 May 2016 in a fattening turkey farm located within the Protection Zone of the previous outbreak. The epidemiological investigation did not allow identification of the possible source of the virus, and contact tracing led to the preventive culling of a fattening turkey farm belonging to the same production company and located within the Protection Zone. Control measures included enhanced surveillance in turkey farms belonging to the same company. In the phylogenies of the eight gene segments, the virus resulted closely related to the H7N7 HPAI virus identified during the previous outbreak.

The limited number of HPAI outbreaks recorded in Italy after the large epidemic of 1999-2000 points to an improved coordination of efforts to contain the spread of AI in the poultry sector, possibly in addition to a shift in the ecology and dynamics of the disease. However, the occurrence of repeated introductions of both HPAI and LPAI strains from wild birds, along with the potential for successive mutation within domestic poultry, highlights the need of a targeted improvement of biosecurity, and the importance of developing a surveillance framework to enable prompt detection of asymptomatic AI infections in wild birds before spillover can threaten domestic farms.

## POSTER 41: Identification and isolation of ORF virus from sick sheep in the Republic of Tuva in 2015

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**Key words: sheep, ORF, virus, PCR, cell culture**

In August 2015 in the Republic of Tuva disease of sheep, characterized by development of erythema, vesicles, pustules and scabs on the skin of ears and legs has been registered (Figure 1).

The scabs from these animals were collected for isolation and identification of causative agent. For this aim three lambs were experimentally infected with the suspension of scabs. The mucocutaneous borders of the lamb's lips along the labial commissures were scarified and inoculum was loaded by cotton swabs.

The animals were subjected to daily clinical examination, every two weeks the skin smears from its lips were collected. At 4-5 days after infection on the lamb's lips vesicles appeared that have moved to the stage of pustules across one day. The pustules dried up, forming grayish-brown scabs. After 2 weeks after infection the healing of lesions observed. The suspension of scabs from the lips of the infected animals was used for inoculation of sheep kidney cell culture. After 7 days post inoculation the cell monolayer was harvested and second passage was performed. In the cell monolayer of second passage specific cytopathic effect was not observed. The skin smears from three infected lambs were examined by Real-Time PCR in accordance with the protocol developed by G. Venkatesan et al., 2014 [1]. DNA of ORF virus was detected in the skin smears collected from the lambs at 7 – 28 days post infection. Also DNA of ORF virus was detected in the scabs collected from infected animals and in the lysate of the cell monolayer harvested after second passage.

Thus, the pathogen, caused in 2015 disease of sheep in The Republic of Tuva, was isolated on lambs and sheep kidney cell culture and was identified as Orf virus.

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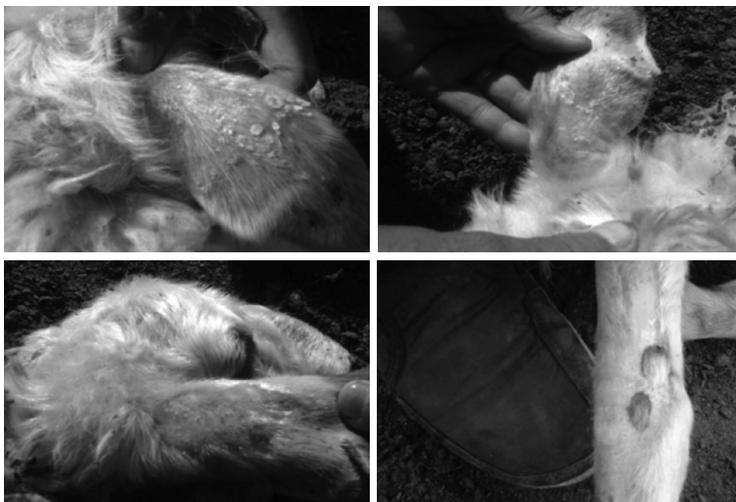


Figure 1. Clinical signs of ORF in sheep from the Republic of Tuva. A, B – scabs on the skin of sheep's ears; C – pustules on the skin of sheep's ears; D – pustules on the skin of sheep's leg.



# POSTERS

## TOPIC II

Threats at the  
European borders

Diagnostics and  
Intervention strategies

## POSTER 42: Blocking of Type I and Type II IFN Pathway by Peste des Petits Ruminants Virus (PPRV)

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Peste des petits ruminants virus (PPRV) is the causative agent of an economically significant and highly contagious disease of small ruminants, peste des petites ruminants (PPR). PPRV belongs to the family *Paramyxoviridae*, genus *Morbillivirus*, which includes important pathogens as Measles virus (MeV) in humans or Rinderpest virus (RPV) in animals. There is only a single serotype of PPRV but it is genetically grouped into four distinct lineages (1, 2, 3 and 4) on the basis of partial sequence analysis of fusion protein (F) gene. The P gene of *Morbillivirus* encodes for the P protein, and also for three non-structural proteins: the V and W proteins, that are produced by co-transcriptional insertion of additional G residues into a fraction of the mRNAs transcribed from the P gen, and the C protein, that results from translation of an alternative open reading frame. Previous studies with MeV and RPV have shown that these non-structural proteins play a role in blocking IFN pathway signaling. In the case of PPRV only V protein seems to interfere with both type-I and type-II IFN signaling pathways. However, there is no evidence of the inhibitory activity of C, P and W proteins.

To improve our understanding of the mechanisms involved in the ability of PPRV proteins to block IFN action, we have studied the inhibition of the activation of IFN stimulated response elements (ISRE) and the inhibition of the activation of gamma activated sequence (GAS), using luciferase reporter assays, by V, C, P and W proteins. First, we have shown that ICV'89 and India/94, two virulent PPRV strains, and the vaccine strain Nigeria/75, are highly effective in blocking the action of type I IFN by significantly reducing the activation through the ISRE promoter. In the same way the three strains of PPRV can inhibit IFN II-triggered activation of GAS promoter. Thereafter, we have cloned and sequence for the first time the PPRV W protein, needed for subsequent assays. *In vitro* experiments demonstrated that V appears to be the dominant inhibitor of IFN signaling. The W effect was weaker than the inhibition observed with the V protein but still significant. Finally, P protein seems to weakly block IFN activity and C shows no blocking effect on the stimulation through the ISRE promoter. Furthermore, overexpression of PPRV P, V and W proteins also inhibits IFN- $\gamma$ -induced activation of the GAS promoter. The effect of each protein in STAT1/2 phosphorylation and/or nuclear translocation is currently under study. In summary, this study highlights the ability of PPRV proteins to block the IFN response as a mean to control the host immune response.

## POSTER 43: Expression and characterization of bunyavirus glycoprotein ectodomains as tools for diagnostic development

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**Keywords: RVFV virus, recombinant expression, glycoprotein ectodomains, diagnostic tool**

Bunyavirus comprises a large family of viruses including important zoonotic pathogens for which novel control and diagnostic tools are needed. Most of them are also arthropod-borne virus with capacity to infect both arthropod and mammalian host cells. Bunyavirus share similar genomic organization into three ssRNA(-) segments (Large, Medium and Small). The L-segment encodes the viral RNA-dependent RNA polymerase (RdRp) while the S-segment encodes the nucleoprotein and host-virulence factors. The M-segment encodes the viral envelope glycoproteins Gn and Gc, responsible for virion architecture and cell tropism, and are the main target for neutralizing antibodies upon infection. These features make glycoprotein antigens suitable to develop both diagnostic and control tools. In this work we used different expression systems to produce soluble glycoprotein ectodomains. As a model system the predicted sequences of Rift Valley fever virus Gn and Gc glycoprotein ectodomains were amplified from cDNA and cloned into plasmids allowing for bacterial, insect and mammalian cell expression. Expression of recombinant ectodomains was detected by Western Blot and Indirect Immunofluorescence assay employing specific mAbs. The antigenicity of the recombinant proteins expressed in the different formats was compared using panels of anti-RVFV sera from experimental infections.

## POSTER 44: Validation of a new competitive ELISA for the detection of EHDV antibodies

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**Keywords : EHDV, diagnosis, serology, competitive ELISA**

### Introduction

Epizootic hemorrhagic disease (EHD) of deer is an arthropod-borne Orbivirus that causes infection in wild and domestic ruminants. In domestic ruminants, EHDV may induce clinical and pathologic signs similar to those of bluetongue (BT), but can also be asymptomatic. Laboratory diagnosis is most often based on the detection of anti-EHDV antibodies in serum. This study summarizes validation data obtained for the **ID Screen® EHDV Competition ELISA**.

### Methods, results and discussion

The **ID Screen® EHDV Competition** allows for the detection of anti-VP7 antibodies in serum or plasma. It is a competitive ELISA based on the use of a VP7 recombinant protein as coated antigen and an anti-VP7 monoclonal antibody, HRP labelled, as conjugate. Results are obtained in about 1h30.

### Specificity

Serum samples from cattle (n=383), domestic deer (n=172), sheep (186) and goats (124), were tested. These samples originated from France and Europe and were collected before 2004. As a result, they are considered free of antibodies to Orbiviruses. Samples had also tested negative for BT using the IDScreen® Blue Tongue Competition ELISA kit. All samples were found negative with the ID Screen® EHDV ELISA, giving a measured specificity of 100.0% (CI<sub>95%</sub>: bovine [99,0 – 100%]; domestic deer: [97.8 – 100%]; sheep [98.0 – 100%]; goat [97.0 ; 100]).

### Sensitivity

42 samples from deer, experimentally-infected with EHDV US serotypes 1 or 2, were tested. All samples were found positive with the **ID Screen® EHDV cELISA**, including samples classified as weak positive by the Agar Gel ImmunoDiffusion technique.

Samples from a reference panel (The Pirbright Institute, UK) comprised of 5 samples from serotypes 1, 2, 7, 8 (EHDV1 RR91, EHDV1 PJ70, EHDV 7, EHDV 8, EHDV 318 (2)) were tested, as were samples from serotype 6 (ANSES, Maisons-Alfort, France). All samples were found positive.

Different seroconversion kinetics from experimental infections were tested ; seroconversion was detected between 4 and 15 dpi.

### Exclusivity

20 samples from a reference panel (The Pirbright Institute, UK) raised against different BTV serotypes (3, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 16 strain UN95 and 2 OBP) were tested. All samples were positive for BTV antibodies. 19 samples were found negative by the EHDV ELISA, and only one sample was found doubtful.

Bovine (n=153) and ovine (n=67) field samples containing anti-BTV antibodies from natural infection and/or vaccination (origin : France and Europe) were tested. These samples had been found positive with the ID Screen® Blue Tongue Competition ELISA. 217 out of 220 samples were found negative, giving a specificity of 98.6% (CI<sub>95%</sub>: [96,1 – 99,5%]). These results demonstrate the excellent specificity of the ELISA, even in the presence of high levels of anti-BTV antibodies. However cross-reactivity can be observed, especially with samples with high VP7 BTV titers.

### Conclusion

The **ID Screen® EHDV Competition ELISA** is a robust, easy-to-use, highly sensitive and specific ELISA for the detection of EHDV antibodies, even in the context of high BTV seroprevalence.

## POSTER 45: Assessment of different “Peste de Petits Ruminants” challenge models in Goats

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**Keywords: Peste des Petits Ruminants, PPR, Challenge, Viraemia, Clinical signs**

### Introduction

Peste des petits ruminants (PPR), is an OIE Listed, acute contagious disease caused by a *Morbillivirus* in the family *Paramyxoviridae*. It affects mainly sheep and goats and occasionally wild small ruminants.

PPR was first identified in West Africa, in the 1940's and has progressively spread to East Africa, most of the Middle East, and parts of Asia including much of the Indian subcontinent.

The clinical disease resembles rinderpest in cattle. It is usually acute and characterised by pyrexia, serous ocular and nasal discharges, erosive lesions on different mucous membranes particularly in the mouth, diarrhoea and pneumonia.

While the disease is frequently dramatic in endemic countries, experimental inoculation in controlled conditions does not always allow reproduction of the disease.

Hereafter we report the assessment of 4 different conditions of experimental PPR virus challenges in goats.

### Material and Methods

**Animals:** Sixteen, PPR naive, 10-months-old female goats (Saanen breed) were randomly allocated to 4 groups, balanced for bodyweights. Two groups (G1, G2) were housed separately in a high containment animal facility, while the 2 other groups (G3, G4) were housed similarly, in another high containment unit.

**Challenge:** 2 different isolates, one from Ivory Coast 89 (CI89) and the other from Morocco 2008 (Mo8) were multiplied in cell culture and freeze-dried. Each freeze-dried isolate was then resuspended in DMEM to a concentration of 5,000 TCID<sub>50</sub>/mL. Goats were then inoculated on Do as follows:

G1: CI89; 2 mL Intra Nasal (1 mL per nostril, with a spray nozzle)	G2: CI89; 2 mL Intra Venous
G3: Mo8; 2 mL Intra Nasal (1 mL per nostril, with a spray nozzle)	G4: Mo8; 2 mL Intra Venous

**Monitoring:** Daily, from Do to D14, all animals were monitored for clinical signs and rectal temperatures, and were sampled for assessment of viraemia (EDTA blood samples) and viral excretion (ocular swabs). Serum samples were taken at regular time points to assess seroconversion.

### Results

**CI89 IN challenge:** clinical signs were very mild and virus was rarely detected in blood samples or swabs.

**CI89 IV challenge:** clinical signs were mild and virus was rarely detected in blood samples while more frequently detected in ocular swabs.

**Mo8 IN challenge:** frank, moderate, clinical signs were observed, starting 7 days after challenge. A moderate weight loss (- 2 Kg) over 14 days was observed. From day 6, virus was detected at moderate titres in blood samples and high titres (low Ct values) ocular swabs.

**Mo8 IV challenge:** severe clinical signs were observed, starting 5 days after challenge. Signs were such intense that all goats of that group were euthanized on ethical ground on D11. Within 11 days after challenge, an average bodyweight loss of 5 Kg was observed in that group. From day 4, virus was detected at moderate titres in blood samples and high titres (low Ct values) in ocular swabs.

All groups were found seropositive from day 8.

### Conclusion

These results show that, in the condition of the study, the CI89 strain induces mild clinical signs. Conversely, Mo8 was able to induce moderate (IN route) to severe (IV route) clinical signs. The IV route of inoculation increased the level of both the clinical and virological parameters, whatever the challenge strain.

Overall the results showed that the Mo8 is more virulent than CI89, both clinically and virologically in European Saanen goats.

The results further suggest that an incursion in Europe of a PPR virus isolate, similar to the one isolated in Morocco in 2008, might have a devastating impact on the small ruminant population.

## POSTER 46: Rescue and characterization of attenuated Rift Valley fever virus (RVFV) expressing bluetongue virus (BTV) antigens

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**Keywords: Rift Valley fever virus, Bluetongue virus, reverse genetics system**

Rift Valley fever virus (RVFV) is an Arbovirus that belongs to the *Bunyaviridae* family. It causes a zoonotic illness affecting mainly ruminants. The disease is endemic in most of the sub-Saharan Africa, Egypt and Saudi Arabia. Nowadays, it is considered an emerging threat in non-endemic countries due to the potential spread of the virus and the presence of competent mosquito vectors in those areas. The possibility of manipulating the RVFV genome by means of reverse genetic systems paved the way to the generation of attenuated RVFV encoding and expressing heterologous genes. Using a reverse genetics system we obtained recombinant RVFV expressing the NS1 protein of bluetongue virus (BTV), a virus also affecting ruminants. In this work we show the generation of this recombinant virus as well as the characterization of its replicative phenotype and NS1 expression in cell cultures. The possibilities of using this viral vector as a bivalent vaccine against both RVF and BT disease are discussed.

## POSTER 47: Experimental Foot-and-Mouth Disease in sheep: validation of a virulent challenge model

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**Keywords: FMD, challenge, sheep, clinical signs, viraemia**

### Introduction

Foot-and-Mouth Disease (FMD) is a highly infectious disease of cloven-hoofed animals. Unlike FMD in pigs and cattle, which is clinically overt, FMD in sheep is frequently mild and often inapparent. A limited number of challenge experiments are described in the literature. They confirm the inconsistent clinical expression, with in general limited FMD lesions.

The purpose of this study was to assess, in sheep, the ability of 3 different challenge strains of FMD to induce clinical signs and viraemia, using the dose and route of inoculation considered suitable for cattle.

### Material and method

Six female sheep, of approximately 10 months of age, were randomly allocated to 3 groups of 2 sheep. Three virus suspensions, containing 10.000 cattle ID<sub>50</sub> per 0.2 ml of a cattle-passaged FMD virus stock of either A22 Iraq, O1 Manisa or SAT2 SAU serotype were prepared. In each group, both sheep were intradermally inoculated into the tongue (2\*0.1 ml) with one of these virus suspensions.

For all sheep, rectal temperature was recorded a day before infection and then daily for 8 days. The sheep were monitored daily from challenge to 8 days post-infection (dpi) for clinical signs of FMD. Blood samples were taken up to 7dpi for virus titration to detect viraemia. FMD specific lesions were scored 4dpi under anesthesia and 8dpi at necropsy.

### Results

Some transient and moderate hyperthermia (> 40°C) were observed in all sheep from 1 to 4 dpi. Partial anorexia was frequently observed regardless of the group. Other signs like lameness and polypnoea were occasionally observed.

All sheep infected with O1 Manisa and SAT2 SAU presented feet lesions 4dpi (2-4 feet affected). In sheep infected with A22 Iraq, 1 sheep presented similar feet lesions while the other sheep did not present any FMD lesions. At necropsy, all sheep showed tongue lesions (inoculation site). Moreover, all sheep, from all groups, presented lesions of at least 3 feet.

All sheep became viraemic shortly after challenge. Viraemia was detected for 2 to 3 days and only moderate levels of virus were detected in all animals.

### Conclusion

The study results demonstrated that all 3 challenge strains of FMD (A22 Iraq, O1 Manisa, SAT2 SAU) are able to consistently induce FMD clinical lesions (generalisation) and viraemia in sheep. This study further demonstrated that the intra-dermal tongue challenge route with 10.000 cattle ID<sub>50</sub> is suitable for sheep, at least for the 3 strains used in this study. Furthermore the induced infection resulted in more severe clinical lesions compared to field observations.



# POSTERS

## TOPIC II

Threats at the  
European borders

Epidemiology & Surveillance  
and Risk assessment

## POSTER 48: Risk assessment of seven vector-borne diseases for the Netherlands

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**Keywords: Risk assessment, Vector-borne diseases, Introduction, Impact**

### Introduction

Incursions of vector-borne animal diseases (VBADs) in recent years in North-Western Europe, such as bluetongue serotype 8 and Schmallenberg, have shown the need for preparedness for these diseases. Therefore, the risk of seven VBADs was assessed for the Netherlands with the aim to prioritize diseases for preparedness and to identify common parameters that contribute most to the risk.

### Material and methods

The VBADs selected for the risk assessment were tularaemia (*Francisella tularensis subsp. holarctica*), bovine babesiosis (*Babesia divergens*), epizootic haemorrhagic disease (EHD serotype 6), Crimean-Congo haemorrhagic fever (CCHF), Rift Valley fever (RVF), West Nile (WN), and African horse sickness (AHS). These include protozoan, bacterial and viral diseases that are either transmitted by ticks, biting midges or mosquitoes, five of which are zoonotic.

The MINTRISK calculation methodology presented at the Epizone Annual Meeting in 2015<sup>1</sup> was used for the risk assessment. This method provides a questionnaire in which semi-quantitative answers need to be given and the uncertainty in the answers is to be made explicit. Using Monte Carlo simulation a semi-quantitative estimate of the risk is given, along with the risk contribution of the different answers and uncertainty intervals. MINTRISK is subdivided into six sections (entry, transmission, establishment, spread, persistence, and impact) and results can also be obtained for each section separately. Results of the first three sections are combined to generate the overall probability of introduction, whereas the results of the other three sections contribute to the overall impact of disease taking into account economic, socio-ethical and environmental consequences.

The answers to the questionnaire were based on literature study, which were then discussed with disease experts, after which the risk assessment was finalized.

### Results

The risk of CCHF is negligible, because no competent vector is present in the Netherlands. The overall risk of all other VBADs was estimated to be moderate to high and no definite ranking could be given. However, when presenting the results of the risk assessment in risk profile diagrams indicating the overall probability of introduction and the overall impact of the VBADs, clear differences were observed. Tularaemia and bovine babesiosis have a high probability of being introduced, but impact is low, whereas the probability of introduction of RVF, EHD and AHS is low, but impact is high due to livestock trade restrictions (AHS and EHD) or zoonotic potential (RVF). WN has the highest overall risk with a high probability of introduction and moderate impact. Common parameters that contributed most to the risk include trade volumes, vectorial capacity and overwintering of the infection. Improved knowledge on these parameters will narrow the uncertainty intervals of the estimated risk.

### Conclusions

The overall risk of most VBADs that we evaluated was the same despite clear differences in their probability of introduction and impact. Hence, the risk profile diagrams provided more useful information for risk management than the overall risk scores. Of the seven VBADs evaluated, WN got the highest overall risk score for the Netherlands with a high probability of introduction and moderate impact. The MINTRISK model provided insight into the main elements contributing to the risk of each VBAD, thus indicating which parameters can be targeted for risk management and which require further study.

<sup>1</sup> De Koeijer, A., Hennen, W., Dhollander, S., De Vos, C., 2015. MINTRISK, a Method for INTeGrated RISK assessment of vector-borne livestock infections. 9<sup>th</sup> Annual Meeting of EPIZONE, 2-3 September 2015, Montpellier, France.

## POSTER 49: Detection of deltacoronavirus with genetic relationship with other avian and mammalian counterparts in quails

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**Keywords: deltacoronavirus, quail, enteritis, RdRp, genetic characterisation**

Coronaviruses (CoV) are worldwide enveloped viruses with the largest RNA genome identified so far in viruses (27- 30 kb) that infect birds and mammals. Subfamily *Coronavirinae* is divided into four genera: alpha-, beta-, gamma- and deltacoronavirus. Alpha- and betacoronaviruses have been isolated from mammals; the most representative member of gammacoronaviruses is infectious bronchitis virus (IBV), etiological factor economically devastating disease of chickens but they have also been detected in beluga whale, Asian leopard cat and in several species of wild birds. Viruses which belong to recently proposed genus *Deltacoronavirus* (2010) were found primarily in mammals but also in wild terrestrial and aquatic birds. Here we describe the detection of deltacoronavirus in the farm of quails in Poland.

In March 2015 organs samples from 7-day-old quails as well live and dead-in shell quail embryos were delivered to our laboratory for disease diagnosis. The flock consisted of around 2000 birds. Quails were hatched on the farm, however the eggs from an unknown source were bought. The first clinical signs in 2-5 day-old quails were observed. Affected birds showed symptoms of enteritis, uneven growth, depression and dull feather and a lots of them do not survived longer than 10-12 days. Increased embryo mortality was also noted. During the four weeks of observation the mortality reached about 30%. The most severe lesions were located in lungs, livers and intestines and they were used for virus identification. Nucleic acids (RNA and DNA) were extracted from delivered samples and different tests which enable detection of most popular poultry pathogens i.e. IBV, adenovirus, astrovirus, reovirus were applied but they gave negative results. The coronaviral RNA dependent RNA polymerase (RdRp) was detected in intestines and livers of affected birds. The RdRp gene fragment about 590 base pairs long were sequenced. The highest nucleotide identity of 90.5% between the Polish quail coronavirus PL/G032/15 strain and deltacoronavirus detected in *Amazona vinacea* in Brazil was found. The nucleotide homology to the RdRp of deltacoronavirus identified in north-American and Chinese pigs were 83.1-83.6%, Asian leopard cat DeltaCoV - 83.1% and Chinese ferret bagger DeltaCoV - 83.7%. Deltacoronaviruses detected in wild birds showed similarity in RdRp fragment in the range of 65.8 – 76.6%. Interestingly, in the analyzed region relatively high homology of 70.0% to the betacoronavirus of European hedgehogs identified recently in Germany was also found.

It was previously thought that only gammacoronavirus as IBV constitute a major health problem for poultry but the newest reports suggest that some birds including quails may share both gamma- as well as delatacoronaviruses. Our finding confirm that deltacoronaviruses could infect quails as we detected them in diseased birds in Poland. Phylogenetic analyses showed that the newly detected strain, quail DeltaCoV PL/G032/2015, was related to avian strain identified in parrot in Brazil but also to porcine strains from USA and China. However, whole-genome sequence is needed to determine the real relationships with other coronaviruses as well as the virus origin. Moreover, in spite the virus was detected in sick birds further investigation should be focused whether the sole infection with quail DeltaCoV PL/G032/2015 results in disease. Given the risk of inter-species host switching, and further adaptation to new hosts, detection of quails infected with CoVs related to other avian and mammalian CoVs should warn the potential for the emergence of new threatening viruses.

## POSTER 50: Illegal trade of avian species in Spain: health implications

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**Keywords: wildlife, control, risk assessment**

### Introduction and objectives

Wildlife trade is the biggest risk factor for the global spread of emerging infectious diseases and zoonosis. Spain is an entry point for species from Africa and America, and although there is a special body within the Police Force, the Environmental Protection Service (SEPRONA) effectively in charge of illegal wildlife seizures, the trade of illegal wildlife in Spain has an estimated turnover of around 450.000 euros, turning the risk of entry of illegal wildlife, and with it the risk of introducing diseases, non-negligible. We present the first part of a novel study to evaluate the risk of introduction of diseases through illegal wildlife trade, starting with the analysis of seizures of avian species and their products and their health status.

### Material and methods

The databases on wildlife seizures of SEPRONA and CITES (Convention on international trade in endangered species of wild fauna and flora) were analysed to obtain information on the commodities, their potential geographical origin and the destination at which they were seized. Species were grouped by taxonomical order. Both the number of seizures as well as the quantities were considered. The OIE WAHID database and the scientific literature were searched for the health status.

### Results

The data collected yields information on 5513 avian seizures in 1874 operations from 1992 up to 2014, the majority of which (5240 birds in 1763 operations) belong to live birds of 22 different orders. Other commodities include feathers, dead and naturalized birds. The orders of live birds most frequently seized were *Psittaciformes* (75% of live birds and 63% of total operations), *Accipitriformes* (6% of birds and 11% of total operations) and *Falconiformes* (6% of birds and 11% of total operations). The origin of seizures was derived from the CITES database only and includes 5 orders. Almost 50% of operations originate from Morocco, followed by Algeria (20%). However, one of the *Psittaciformes* species with a high number of seizures corresponds to *Psittacula krameri*, the origin of which was Senegal and Mauritania. Other origins include Cuba, Colombia or Brazil. The destination of seizures was derived from the SEPRONA database only. The main destinations were Cadiz, Madrid or the Canary Islands. Diseases that could be introduced through the traded birds include avian influenza, Newcastle disease and West Nile disease.

### Discussion and conclusions

Live wild birds are the largest commodity traded, which also poses the highest health risk. Compared to the information contained in the HealthMap Wildlife Trade website, our database contained information on many more orders. Even if Algeria or Morocco constitute important countries of origin for Spain, often the species seized originate from other regions like West Africa. Surveillance of wild bird diseases is generally very scarce, but some information can be found for those with a higher public health significance.

### References

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HealthMap Wildlife Trade website (last visited May 2016) <http://www.healthmap.org/wildlifetrade/>



# POSTERS

## TOPIC III

Current challenges  
inside Europe

Diagnostics and  
Intervention strategies

## POSTER 51: Isolation small ruminant lentivirus from gout in Russia

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**Key words: Small ruminant lentivirus (SRLV), isolation, ovine synovial membrane cell, polymerase chain reaction (PCR)**

### **Purpose**

The problem of spreading SRLV in Russia is being more actually. Isolation of SRLV is more difficult than isolation virus of acute disease. One of the problems is that SRLV isn't breed into the animals. They are only persisting in the sensitive cells as the cDNA [P.Agrimi].

The basic of phenomena of syncytium is fusion of plasmalemmas of infected SRLV neighboring cells. The cells (syncytium) consist of from 3-4 to tenths nucleus and they are variable size [Shulak, B., 2006]. The most effective method of isolation SRLV is the co-cultured cells infected animal with sensible cell culture [Sidelnikov G., 2009].

One of the most sensible cell cultures is the primary cell culture of ovine synovial membrane. It is universal cell system that using for isolation, accumulation and determination of titer of SRLV [Baryshnikova E., 2011].

In that article the results of isolation new isolate Kaluga SRLV using method of co-cultured ovine synovial membrane cell with leukocytes from a SRLV-seropositive gout.

### **Materials And Methods**

We used a method of growing tissue explants for obtain primary culture of the ovine synovial membrane cell [Baryshnikova E., 2011]. For that we used the 5-6-days lambs.

The virus was isolated from Nubian breed seropositive gout from Kaluga region of Russia. The clinic symptom was the an increase in the carpal joint. The leukocytes of peripheral blood were obtained using diacoll. After that the leukocytes co-cultured with the primary culture of the ovine synovial membrane cell. For detection cDNA of new isolation of SRLV we used PCR.

### **Results**

The primary culture of the ovine synovial membrane cell was obtained. The 90 % monolayer of cells was obtained during 7-8 days. The fibroblast cells were medium size with granular cytoplasm and clearly defined boundaries

After 7-9 days of co-cultivating ovine synovial membrane cell with leukocytes from a SRLV-seropositive gout we saw the syncytium. The nucleus of infected cells arranged in a circular-oval outlets or had irregular shape clusters. The infected cell culture we detected in PCR. The positive result of PCR confirmed the genome of new isolate SRLV .

### **Conclusion**

During this work we obtained new isolate SRL named Kaluga from SRLV-seropositive gout. The biological properties were characterized using the primary culture of the ovine synovial membrane cell. Using molecular- biological methods we confirmed the presence of the pathogen.

## POSTER 52: Preliminary genetic characterization of SRLV in Trentino Alto-Adige region, Italy

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**Keywords: lentivirus, CAEV, genotypes**

Caprine arthritis encephalitis virus (CAEV) and Maedi-Visna virus (MVV), now referred to as Small Ruminant Lentiviruses (SRLV), are retroviruses belonging to the Lentivirus genus. Five distinct genotypes (A to E) have been detected and characterized up to date. Genotype A is associated with the classic MV pathology in sheep and genotype B with the CAE complex in goats. Diagnosis of SRLV is based on clinical-epidemiological and serological data. In 2007, the Autonomous province of Bolzano, started a compulsory CAEV eradication program in goats and sheep based on annual serological surveillance using commercially available indirect ELISAs for detection of anti-CAEV antibodies, independently of the infecting genotype. A similar surveillance programme was implemented in Trentino Alto Adige region on a voluntary basis. The initial seroprevalence in Bolzano province for goats was 13.5% and decreased to 0.5% in 2014. In the same year, a pilot study was conducted to test the use of newly available ELISAs able to differentiate the antibody response to different genotypes. This generated discordant data probably caused by the genetic variability of the circulating SRLVs, which needs to be characterized by sequencing. To this purpose, 36 blood samples were collected from 29 goats and 7 sheep in 3 different farms. One farm located in Bolzano province was a mixed farm with goats and sheep, the other two farms reared only goats and were located in Trento province. All farms were seropositive using the IDVET screening ELISA test. Additionally, genotyping ELISA tests showed positive results for the A and B genotypes, or both. Peripheral blood mononuclear cells (PBMCs) were isolated by gradient density centrifugation from all sampled animals. DNA isolation was performed with the DNeasy Blood and Tissue Kit (Qiagen AG, Hombrechtikon, Switzerland). Extracted DNA was quantified using Nanodrop and PCR reactions performed with at least 400 ng of DNA using semi-nested sets of PCR primers located in the pol, env and gag genes. PCR positive samples were identified by a 1% Agarose gel. Bands of the expected size were obtained with different primers' combinations. Positive samples for one or more PCRs were selected for sequencing. PCR products were first separated on a 1% low melting gel, excised and cloned with the Topo TA Cloning kit (Life Technologies Europe B.V. (Invitrogen), Zug, Switzerland). After transformation into One Shot Top 10 chemically competent *E. coli* bacteria were grown on a LB-amp-X-Gal plate overnight at 37°C. Four to ten colonies were picked and grown overnight at 37°C in LB medium supplemented with 100 µg ampicillin/ml. Plasmid DNA purification was performed with the QuickLyse Miniprep Kit (Qiagen AG, Hombrechtikon, Switzerland). Sequencing was performed by Microsynth (Balgach, Switzerland).

Thirty samples were subjected to PCR. Nine out of 30 samples were positive using specific primers for the gag gene and 2 out of 30 were positive using primers for the env gene. No positive samples were detected for the SU5 gene region. One positive sample for both the gag and env genes was selected for sequencing.

The sequence analysis revealed that the virus belonged to the classical B1 genotype closely related to other Italian strains. Preliminary data presented herein show the detection of PCR positive goats indicating that primers and PCR protocols used are a valid tool for the target area.

We will continue this program with the final aim to fully genetic characterize the circulating strains. Such studies will permit us to develop new diagnostic tools specific for these strains, therefore improving the quality of the ongoing surveillance and eradication efforts.

### Acknowledgements

Italian Ministry of Health: RC 14/14

Epizone short term mission between IZSve and University of Bern in 2016.

## POSTER 53: Safety of a bivalent inactivated BTV-4/BTV-8 vaccine and immunogenicity of a monovalent inactivated BTV-8 vaccine in goats

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**Keywords: BTV, vaccine, goat, safety, immunogenicity**

### Introduction

Bluetongue virus (BTV) causes an infectious, non-contagious disease in wild and domestic ruminants. It is transmitted between ruminant hosts through the bites of certain species of *Culicoides* midges. BTV cycle can be interrupted by the systematic vaccination of susceptible species. While the vaccines commercially available in Europe are registered for use in sheep and/or cattle, none of them is registered for use in goat. The absence of claim for this minor species raises questions from the veterinarians and farmers regarding the safety and efficacy in case of “off-label” use of the vaccines in goats. As the competent authorities may encourage the inclusion of goats in the vaccination programs intended to deal with BTV outbreaks, it is important to provide safety and efficacy data of the vaccines when used in this species.

In this paper, we report the results of a safety and immunogenicity study conducted in young goats using respectively an over-formulated BTV-4/8 bivalent vaccine and a monovalent vaccine formulated at low BTV-8 antigen payload of the BTVPUR ALSAP range (Merial).

### Material and method

Twenty BTV sero-negative goats aged less than 5 months were randomly allocated to two groups of 10 animals each (G1 and G3), on the basis of their bodyweight. An additional group (G2) of 10 sero-negative goats aged less than 6 months was set-up.

Group G1 was vaccinated on D0, by the subcutaneous route, with 2 mL (“double dose”) of an over-formulated inactivated bivalent vaccine containing BTV-4 and BTV-8 components. This group was re-vaccinated on D14 and D28 (“repeated dose”) with 1 mL of the same vaccine.

Group G2 was vaccinated by the subcutaneous route with 1 mL of an inactivated monovalent vaccine containing low BTV-8 antigen payload on D0 and on D28.

Group G3 was injected with physiological saline on D0, D14 and D28, with the same volumes as group G1 and served as unvaccinated control.

The goats of groups G1 and G2 were monitored as follows:

- individual clinical examination and recording of rectal temperature prior to each injection, daily for 4 days following each injection, then on the 7<sup>th</sup> and 14<sup>th</sup> day after each injection,
- monitoring of local reactions until D49, and
- recording of bodyweight gain on D49.

Specific BTV-8 antibody titres were measured in the sera collected in goats of groups G2 and G3 on D-1, D14, D28 and D49.

### Results

The safety of the administration of an overdose (D0) and repeated administration of one dose (D14 and D28) of the bivalent BTV-4/BTV-8 over-formulated vaccine was demonstrated in goats aged less than 5 months with:

- very moderate and transient temperature increase following the administration of a repeated dose,
- occasional apathy and anorexia one day after vaccination,
- limited local reactions that almost disappeared 2 to 3 weeks after vaccination,
- no impact on bodyweight gain on D49.

The BTV-8 serological titres obtained in goats vaccinated with the vaccine containing a low BTV-8 antigen payload demonstrated that one injection was not sufficient to induce a serological conversion in goats. Conversely, two injections of this vaccine 4 weeks apart provided a clear serological conversion in all goats.

### Conclusion

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

Based on serological results obtained with a vaccine containing a low BTV-8 antigen payload, the vaccination program to be recommended in goats using BTVPUR ALSAP vaccines is two injections 4 weeks apart.

BTVPUR ALSAP is a registered trademark of Merial.

## POSTER 54: Comparative study of protection in pigs immunised by different routes and doses with the naturally attenuated African swine fever virus isolate OUR T88/3 and role of immunomodulatory cytokines

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**Keywords: ASFV, Vaccine, intramuscular, intranasal.**

African swine fever (ASF) is a devastating disease of domestic swine, with a mortality rate of up to 100% for highly pathogenic isolates. The apparently unstoppable spread of ASF throughout the trans-Caucasus region and Russia since its introduction in 2007, as well as the recent incursions into Eastern Europe has highlighted the need for an effective vaccine to help in the efforts for disease control.

Despite the safety concerns raised by the use of a live attenuated vaccine such as the possibility of reversion to a virulent form or their capability for causing subclinical or chronic forms of ASF, to date, these vaccines have been the only form capable of conferring a reliable and effective protection against experimental infections with homologous and occasionally heterologous virulent isolates of ASFV. The present study is an extension of previous works where protection induced by intramuscular administration of the attenuated OUR T88/3 genotype I isolate was demonstrated.

In the work reported here, comparative assessments were utilised in order to demonstrate differences among various combinations of doses ( $10^3$ ,  $10^4$  and  $10^5$  TCID<sub>50</sub>/ml) and routes of immunisation, including the commonly used intramuscular route along with intranasal route.

The results showed that intranasal immunisation of pigs with low and moderate doses ( $10^3$  and  $10^4$  TCID<sub>50</sub>/ml) provided complete protection (100%) against lethal challenge with a homologous high virulent ASFV isolate. Moreover protected pigs had either no detectable or minimal quantities of viral genome in blood at termination. In addition, only mild and transient adverse clinical reactions and mild lesions, consistent with secondary bacterial infections, were observed before and after challenge. However, in group of pigs immunised intranasally with  $10^5$  TCID<sub>50</sub>/ml and in all groups immunised intramuscularly ( $10^3$ ,  $10^4$  and  $10^5$  TCID<sub>50</sub>/ml); the rates of protection conferred were lower ranging from 33% to 66%.

Regarding immune protection mechanisms, along with the possible role of humoral immune response, our results suggested that survival of pigs after challenge was associated with a balance between pro- (TNF $\alpha$  and IL-1 $\beta$ ) and anti-inflammatory cytokines (IL-10), without participation of IFN $\gamma$ . In contrast, animals that died showing an acute form of ASF displayed an imbalance linked to an exacerbated increase of IL-10 along with an anomalous antibody response.

## POSTER 55: Adaptation of African swine fever virus field strains to productively replicate in WSL cells is not necessarily associated with larger deletions within the viral genomes

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### ASFV field isolates, adaptation to replicate in WSL cells, NGS sequencing

African swine fever (ASF), caused by African swine fever virus (ASFV), is an economically important disease which causes significant losses for small pig holders but also for the porcine meat industry in affected areas. The course of the infection in domestic pigs and wild boar depends on specifics of the ASFV strain. Highly virulent isolates cause a peracute or acute disease with 100% mortality within 4 to 7 days whereas low pathogenic or apathogenic isolates result in a mild disease or even inapparent infections. ASFV is a large and complex, enveloped virus with a double stranded DNA genome. The genome sizes vary, strain dependent, between 170 and 190 kbp.

Although significance and functions for many genes have been studied so far, much more still need elucidation. For doing that, cell culture adaptation appears to be a prerequisite since the natural host cells, monocytes and macrophages, are difficult to work with as primary cultures. In the past, this process was frequently associated with considerable genome modifications including large deletions.

We adapted field isolates (Armenia 2007, Benin 97, Kenya1033, and Sardinia 2015) to productively grow in WSL cells. Full protein coding genome sequences were so far determined for Benin 97, Kenya1033, and Sardinia by NGS. For the present, sequence comparisons revealed that the genomes of the respective WSL-adapted virus do not contain larger deletions by comparison to respective or related field strains or to other virus adaptations to Vero cells. Detailed analyses will be presented.

## POSTER 56: Development of quantitative RT-PCR for identification of 27 serotypes of bluetongue virus

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**Key words: Bluetongue virus, serotypes, RT-PCR**

Bluetongue virus (BTV), an arthropod-borne Orbivirus in the family Reoviridae, is a major pathogen of ruminants. Twenty seven BTV serotypes have been recognised worldwide. Isolation and amplification of the virus in embryonated chicken eggs, *Culicoides* cells, tissue culture or inoculations of susceptible ruminants are traditionally requires for virus identification. Traditional diagnostic methods are slow and can be inconclusive. Now RT-PCR is one of the methods that most commonly used for BTV diagnosis. Advantages of real-time PCR assays over traditional PCR methods are include rapidity of testing, quantitation of the virus loading and the reduced opportunity for contamination.

Bluetongue virus serotype 14 (BTV-14) was responsible for the outbreak in 2011 in Smolensk region of Russia. Moreover PCR kits presented in Russia are not capable for detecting some BTV serotypes. Thus, development of new real-time RT-PCR assay to identify all 27 serotypes of BTV was needed for fast, reliable, and sensitive detection of BTV RNA in animal samples in Russia.

Our work was aimed at selection of “oligonucleotide primers - DNA-probe” for the specific quantitative RT-PCR test to identify all serotypes of bluetongue virus. Primers and FAM-labeled TaqMan-probe specific for bluetongue virus were selected from the consensus sequence of the genome RNA 10-segment of reference strains of BTV-1 to 27. For test validation the reference strains of the 25 BTV serotypes (except serotype 25 and 27), the field isolates (serotype 14, 8, 16) and heterologous viruses (AHSV, EHDV) were used. The qRT-PCR assay presented here reliably detected BTV with high sensitivity and specificity. Our qRT-PCR test was strictly BTV-specific and no amplification was observed in all heterologous samples tested. The qRT-PCR assay proved to have a sensitivity of approximately 0,75 lg TCD 50/cm<sub>3</sub>.

Thus, the primer-probe combination selected in this study is provide a rapid, sensitive and reliable method for detection of all 27 known BTV serotypes.

## POSTER 57: Evaluation of insecticide treatments against *Culicoides* biting midges populations in sheep farms in northern Sardinia

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**Keywords: Culicoides, Insecticide treatments, Vectors, Bluetongue**

Nowadays 5 species of *Culicoides* are implicated in Bluetongue virus (BTV) transmission in Italy. Several incursions of BTV occurred in Sardinia island since 2000, with heavy outbreaks. However, few field studies have been conducted to evaluate the impact of chemical control on vector populations.

The aim of this study was to evaluate the efficacy of insecticides treatments to contain the vector populations.

The study was conducted during 2014, in farms located in Tula municipality, in Sassari province (northern Sardinia, Italy). Seven farms were randomly selected among all farms affected by BTV during 2013. In 3 of these farms, insecticide treatments (using larvicide and adulticide products) were carried out, whereas the remaining 4 farms were maintained unsprayed as untreated control. Against *Culicoides* adults a product of low mammalian toxicity based on deltamethrin (2.5% of active ingredient) was used. The insecticide treatments were carried out in and around animal housing. The products used for the larval control were 2 commercial formulations, one based on diflubenzuron (2% of active ingredient) and one on *Bacillus thuringiensis* var. *israelensis* (H-14) (containing 3,400 ITU/mg). All treatments were made with doses of insecticides recommended by the manufacturers. The insecticide treatments were performed 4 times, on 17 July, 4 September, 6 and 29 October. *Culicoides* collections were made between the 24 May and the 4 November of 2014. In each farm an Onderstepoort blacklight suction trap was positioned close to the night sheep shelter, provided by wide opened windows. Adult *Culicoides* were collected twice a week, from the dusk until early morning. All *Culicoides* were identified at species level. Before treatments the numbers of *Culicoides* collected were compared between farms. Successively, the total *Culicoides* captured from the first treatment to the end of trial was compared between treated and untreated farms. To analyze the differences among treated and untreated farms, data of different *Culicoides* species were compared using a repeated measures analysis of variance (ANOVA), performed with the Generalized Linear Mixed Model (GLMM) procedure. The Tukey's test was used to separate significantly different means.

Before starting treatment no statistical differences were observed between treated and control farms, regarding the mean of the population densities of total *Culicoides*, *C. imicola*, *C. newsteadi*, *C. punctatus*, *C. pulicaris* and *Obsoletus* Complex. However at the end of the study the mean number of total *Culicoides* was significantly different counting 32,462 specimens in control farms and 2,382 in treated farms. Treatments have decreased the number of *C. imicola* with 17,613 in control farms and 1,055 in treated farms. Specimens of *C. newsteadi* were 9,377 and 685 in control and treated farms, respectively. The total of *C. punctatus* was reduced from 286 to 25, whereas no differences were observed for *C. pulicaris* with 49 adults in untreated farms and 17 in treated farms. In addition *Obsoletus* Complex resulted 1,644 in control farms respect to the 159 observed in treated farms.

Our preliminary results suggest the possibility to control the *Culicoides* populations by using adult and larval insecticides. Certainly these data need to further evaluations, expanding the experiments in more farms and in areas with different bio-climatic characteristics. The decreasing of the number of BTV vectors could limit the probability of the transmission of BTV, and reduce the economical impact of BT in livestock sector in Sardinia.

This study was financially supported by Provincia di Sassari, Settore Ambiente - Agricoltura, Servizio Igiene e Profilassi (Project: Febbre catarrale ovina – Monitoraggio e lotta a *Culicoides* spp. sul territorio della provincia di Sassari).

## POSTER 58: Pioneering solutions for BTV control: Multi-strain vaccines and Antigen bank

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**Keywords: BTV, vaccine, multi-strain, antigen bank, serotype**

### BTV Epidemiology

Worldwide 27 bluetongue virus (BTV) serotypes have been identified. Since 1998, regular outbreaks occurred in Europe, involving mainly serotypes 1, 2, 4, 8, 9 and 16.

BTV causes severe economic losses due to mortality & morbidity but also due to trade restriction in affected countries.

Vaccination has proven its efficacy in the control of BTV. However, the control of the disease is facing 3 challenges: no cross protection between serotypes, simultaneous contamination of countries with multiple serotypes and unpredictable epidemiology. Therefore vaccine composition needs to be flexible. In case of outbreak, vaccine availability needs to be secured by high reactivity and by fit for purpose quantities.

### Merial has developed two innovative approaches to meet these field needs

**BTV multi-strain:** Merial BTVPUR (serotypes 1, 4, 8), the first BTV multi-strain vaccine, has been approved in EU in April 2016.

The multi-strain approach is defined in the European regulations (Directive 2009/9/EC) for certain immunological veterinary medicinal products containing antigenically variable viruses (foot-and-mouth disease, avian influenza and bluetongue), and allows the authorization of different options of strains/ combinations of strains through a single scientific dossier – provided the relevant data are presented.

Merial has built on the 4 full EU registrations of BTVPUR AISap (BTVPUR AISap 1, 8, 1-8 and 2-4), in order to create a single multi-strain dossier in compliance with EU regulation. This multi-strain dossier contains the strains most relevant today in EU (BTV 1, 4 & 8) intended for sheep & cattle, which can be formulated as mono-strain vaccine or as bivalent combination marketed under the trade name BTVPUR. This multi-strain dossier can be enriched later with other serotypes in order to enlarge flexibility to the end user regarding vaccine composition.

**BTV antigen bank:** Merial BTV1 & BTV4 antigen bank, the first in the world, has been created in France in 2015.

Bank of antigen is a provision to the governments of significant quantity of long term stable BTV antigens, produced, stored by Merial and ready for formulation. In case of outbreak, the bank can be triggered at any time by the government requesting the immediate formulation by Merial of a vaccine ready to use in concerned areas. This approach is the guarantee for a BTV free country to quickly control an outbreak and/or a quick action to continue exportation of live animals.

### Conclusion

The BTV vaccine offer must be adapted to the complex, unpredictable and fast evolving epidemiology. The multi-strain vaccine offers a flexible composition, adjustable to the epidemiological situation in the targeted region. The antigen bank system offers a high reactivity to deliver the expected vaccine in case of urgent need in the field. The combinations of both approaches will improve flexibility and reactivity, by provision of BTV antigen bank based on the serotypes of BTVPUR multi-strain vaccine, offering monovalent or bivalent combinations of several serotypes, available in a short time in case of outbreak.

## POSTER 59: Zulvac® SBV, an inactivated vaccine for sheep and cattle against Schmallenberg virus disease Change of the vaccination schedule for sheep from 2-shots to 1-shot

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**Keywords: Schmallenberg, inactivated, vaccine, sheep, 1-shot**

In November 2011, a new virus, the Schmallenberg virus (SBV) belonging to the genus Orthobunyavirus was identified in Germany. SBV is transmitted mainly by biting midges from the *Culicoides* genus and can infect cattle, sheep and goats. SBV rapidly spread throughout the whole Europe causing mild transient disease in adults (i.e. drop in milk production, fever, diarrhea). Main losses are associated with SBV infection during pregnancy that can lead to congenital infection, causing stillbirth, abortion and fetal abnormalities due to SBV ability to cross placental barrier.

In February 2015, the European Commission granted a centralized marketing authorization for the Zoetis vaccine Zulvac® SBV. This veterinary medicine approval is valid throughout the European Union (EU).

The vaccine Zulvac® SBV is a suspension for injection intended for active immunisation of cattle and sheep against SBV. The active substance is the inactivated SBV strain BH80/11-4 adjuvanted with aluminium hydroxide and saponin as adjuvants. The proposed route of administration is subcutaneous in sheep and intramuscular in cattle. The recommended dose is 1 ml in sheep and 2 ml in cattle.

The initial vaccination schedule for use in sheep was 2 doses given 3 weeks apart. Further studies were conducted to change the vaccination schedule from 2 doses to 1 dose. The proposal to change the vaccination schedule to one-shot was based on the demand in the field of a vaccination schedule that can offer a more rapid protection of the vaccinated animal and reduce handling of the animals.

In order to support the efficacy of such revised vaccination schedule in sheep, Zoetis conducted three laboratory efficacy studies:

1. Onset of immunity in 3 month-old lambs (below the minimum age)
  2. Duration of immunity in 3 month-old lambs (below the minimum age)
  3. Efficacy in pregnant ewes vaccinated prior to pregnancy
1. The administration of Zulvac SBV (at minimum potency) according to the proposed vaccination schedule (1-shot) to lambs of the minimum recommended age was able to prevent viraemia when the vaccinated animals were exposed to virulent SBV at 21 days after vaccination. An onset of immunity of 21 days is therefore demonstrated.
  2. The administration of Zulvac SBV (at minimum potency) according to the proposed vaccination schedule (1-shot) to lambs of the minimum recommended age was able to reduce viraemia when lambs were challenged 6 months after vaccination. A duration of immunity of 6 months with an efficacy claim of reduction of viraemia caused by SBV infection is demonstrated by the results of this study.
  3. The administration of Zulvac SBV (at minimum potency) according to the proposed vaccination schedule (1-shot) to breeding sheep before pregnancy resulted in reduction of viraemia and transplacental infection associated with infection by Schmallenberg virus during the first trimester of pregnancy.

The main benefit of the change of the vaccination schedule in sheep is that, in practice, only one shot is required to achieve protection in comparison to the initial two-shot schedule. This is of particular relevance for a vaccine like Zulvac SBV that is likely to be used under emergency/outbreak situations.

The proposed change in the vaccination schedule in sheep has the benefit of reducing the number of vaccinations required to achieve the protection claimed in the SPC which positively impacts on animal welfare as it reduces animal handling thus reducing animal stress as well as other associated risks (e.g. user risk, risk of adverse reactions). Also, the economic costs of vaccination are reduced (e.g. less handling, fewer vaccine doses). This is of particular interest in the context of the potential field use of Zulvac SBV (e.g. vaccination campaigns).

**In September 2015, the Committee for Medicinal Products for Veterinary Use (CVMP) adopted by consensus positive opinions for the change in the vaccination schedule in sheep as it was considered that the overall benefit-risk balance of the product remained positive.**

## POSTER 60: Pathological evaluation of pigs immunised with the low virulent African swine fever virus OURT88/3 using different routes and doses

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**Keywords: ASFV, vaccine, clinicopathological evaluation**

The aim of this study was to carry out a pathological evaluation in the framework of experimental vaccinations of pigs with low virulent African swine fever virus (ASFV) genotype I isolate OUR T88/3. The present study extends previous studies on protection induced by intramuscular administration of OUR T88/3. Here, different combinations of doses ( $10^3$ ,  $10^4$  and  $10^5$  TCID<sub>50</sub>) and routes of immunisation of OURT88/3, including the intranasal route which had not been previously tested, were evaluated. Three weeks after immunisation, pigs were challenged intramuscularly with a virulent ASFV isolate from the same genotype I (OUR T88/1). All protected pigs were euthanized 19 days after challenge.

The different doses administered intranasally gave rise to two clinical groups: protected pigs that developed transient clinical reactions (immunised with  $10^3$  and  $10^4$  TCID<sub>50</sub>, 100% of protection) and pigs which developed chronic forms of ASFV (immunised  $10^5$  TCID<sub>50</sub>, 66% of protection). Most of the protected pigs had recurrent viraemia from 14 days post-immunisation (dpi), but just in some pigs included in the groups where 100% of protection was achieved ( $10^3$  and  $10^4$  TCID<sub>50</sub>), viraemia persisted until the end of the study at 19 days post-challenge (dpc). Transient and intermittent mild to moderate joint swellings along with mild pulmonary lesions were the only remarkable clinical sign and lesions observed in some protected pigs immunised intranasally with  $10^3$  and  $10^4$  TCID<sub>50</sub>. However, protected pigs immunised with  $10^5$  TCID<sub>50</sub> displayed the most intense clinical courses (joint swelling, laboured breathing, conjunctivitis) along with the most severe macroscopic lesions mainly located in joints (serofibrinous/purulent peri-arthritis), skin (erosions/ulcers in nose, flanks and limbs) and cardiorespiratory system consistent with the presence of secondary bacterial infections (fibrinous pleuritis, fibrinonecrotic pleuropneumonia, fibrinous pericarditis).

In contrast, and although percentages of protection were lower (50-66%) in pigs immunised intramuscularly with  $10^3$  and  $10^4$  TCID<sub>50</sub>, this route of immunisation did not lead to protected pigs suffering chronic ASF. Viraemia was not detected after 7 dpc in most of the pigs, and any viraemia before then was less than  $10^3$  copies/ml or not detected at all. Transient mild to moderate joint swelling was the only clinical sign observed in most of the protected pigs together with occasional mild pulmonary lesions.

Independently of dose and route of inoculation, all tissue samples from protected pigs analysed were negative to ASFV by qPCR. Histopathologic evaluation confirmed cardiorespiratory macroscopic lesions in protected pigs immunised intranasally as well as a generalized activation of germinal centres in lymphoid organs of protected pigs immunised intramuscularly. Two pigs, both immunised with the highest dose ( $10^5$  TCID<sub>50</sub>) by different routes, were euthanized at 5 dpc after developing poor health conditions. High viral loads were detected by qPCR in samples taken from skin lesions in nose and around joints. Viral infection on such samples was corroborated by immunohistochemistry. Macrophage-like cells appeared as the main virus target cells. However, viral loads were very low or not detected in blood and tissue samples from internal organs. These results suggest that low virulent isolates involved in chronic forms of ASFV may display tissue tropism different to most of the virulent isolates involved in fatal forms of ASF where lymphoid organs, liver, lungs and kidney have been described as the main targets for virus replication.

In conclusion, intranasal immunization is presented as a feasible alternative for the protection of pigs against ASFV. However, although pigs immunised with low and moderate doses by intramuscular route displayed lower protection, the low or non-existent viral loads together with the asymptomatic clinical courses observed in protected pigs, suggested this route as the most feasible and safe for immunisations against ASFV.

## POSTER 61: Detection of Immunoglobulins of isotype G and M for African swine fever serodiagnosis

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**Keywords: African swine fever virus, antibodies, ELISA**

African swine fever (ASF) is an infectious disease that causes high mortality rates in domestic pigs and in consequence important economic losses. At present, there is no vaccine available against ASF virus (ASFV) and control and eradication programs are based mainly in sanitary measures and early diagnosis of the infection. Serological methods have been shown to be very useful and convenient for screening large populations of swine. Since there is not vaccine against the virus, the detection of ASF-specific antibodies is a direct indicator of an infection. The VP72 capsid protein is known to be an abundant and immunogenic viral antigen. Therefore, in this study we develop an enzyme-linked immunosorbent assay (ELISA) based on the semi-purified viral VP72 protein, for detection of ASF-specific immunoglobulins of isotype M (IgM), as an indicator of a recent infection. The assay was based on a capture format, using an anti-swine-IgM antibody to coat the plates and a monoclonal antibody against the VP72 protein as the detector reagent. Serum samples from experimentally infected pigs at PIR were analyzed. In parallel, the same sera were analyzed for detection of IgG antibodies specific for VP72 and results of both assays were compared with those obtained using a commercial ELISA test (INgezim PPA Compac, Ingenasa).

A total of 338 serum samples from pigs used in vaccination/challenge experiments at BSL3 facilities at PIR, were analyzed in this study. The animals (n=29) were immunized with an attenuated Benin strain and serum samples were collected at days 0, 2, 4, 7, 10, 15, 21, 28, 38, 43, 47 and 59 post infection (pi). They were boosted 21 days later with the same virus. On day 40, they were challenged with virulent Benin 97/1. The results showed that IgM seroconversion against VP72 occurred between day 7-10 pi and remained in the serum for about a week. After this, the antibodies dropped until undetectable levels. On the other hand, the IgG antibodies appeared about a week later and remained in serum for months. When the results of this two ELISAs were compared to those of the commercial ELISA, a competition ELISA that detects both IgG and IgM, the concordance was very good, indicating that at early days, only the IgM antibodies are detected. Although the commercial ELISA is highly sensitive, the detection of IgM and IgG separately, could be very useful in epidemiological studies. Finally, the clinical signs observed in the animals showed that there was a high correlation between the IgM seroconversion and protection, with those pigs with an increase in the IgM response survived the challenge with ASFV.

In conclusion, these preliminary results indicate that the new developed ELISA is able to detect the presence of IgM in pig serum as early as day 7 pi. This is a good indicator of a recent infection, especially useful in screening experiments during epidemiological studies.

### Acknowledgements

The research was funded by the EU, Seventh Research Framework Program FP7-KBBE-2207-2013 under grant number n° 311931 (ASFORCE).

## POSTER 62: Searching for the reservoir(s) of the novel zoonotic variegated squirrel bornavirus 1

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**Keywords: VSBV-1, squirrel, zoonosis**

A recently discovered novel zoonotic bornavirus, designated variegated squirrel bornavirus 1 (VSBV-1), is responsible for fatal encephalitis in three squirrel breeders and a zookeeper. Viral RNA was detected in brain samples from the patients as well as in different organ samples of a variegated squirrel (*Sciurus variegatoides*) kept by one of the deceased patients. This entire coding sequence of the novel virus forms a distinct phylogenetic cluster, which is related to the species *Mammalian 1 Bornavirus*.

The aim of this study was to search for the reservoir(s) of VSBV-1 in squirrels of different species from additional breedings, zoological gardens and wildlife and to characterize the viruses genetically. For this purpose, a non-invasive, in-vivo sampling method based on oral swab collection was established.

A total of more than 500 oral swab samples were collected from squirrels of different species originating mostly from Germany, but also the Netherlands and screened by VSBV-1 specific RT-qPCR. In addition, blood samples were tested for the presence of anti-VSBV-1 antibodies by indirect immunofluorescence assay using VSBV-1 infected cell culture. RT-qPCR positive animals were euthanized and during necropsy the gross pathology was evaluated. In addition, brain samples were investigated by RT-qPCR to prove the diagnostic sensitivity of the oral swab screening procedure. Furthermore, positive animals were analysed by histopathology, immunohistochemistry and whole-genome sequencing, with subsequent phylogenetic analysis.

In total, 24 VSBV-1 positive squirrels out of seven different holdings within Germany and the Netherlands were identified by RT-qPCR, including variegated squirrels (*S. variegatoides*) as well as squirrels of the subfamily Callosciurinae originating from different holdings. None of the animals showed clinical signs. In histopathology, intranuclear inclusion-bodies were found in several neurons and in some animals mild signs of a non-suppurative meningitis and/or encephalitis were detected. In all positive animals viral RNA was similarly found in both the swab and brain samples, but the highest viral loads were detected in brain samples analogous to viral antigen detection in the entire brain. Therefore, the non-invasive sampling method seems to be suitable for rapid screening of live animals. All sera from PCR-positive squirrels contained also VSBV-1 specific antibodies. Phylogenetic analysis of the entire coding regions of the new VSBV-1 strains showed clusters, which could be connected to the corresponding holdings.

In conclusion, this study shows the presence of VSBV-1 specific RNA and antibodies in multiple squirrels of different species. Further studies will be necessary to characterize this novel zoonotic bornavirus and to evaluate its host origin and specificity.

## POSTER 63: The enemy at the gates: efficacy of vaccination for Bluetongue virus serotype 8 performed shortly before challenge

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**Keywords: Bluetongue virus serotype 8, vaccine, sheep, animal trade, emergency vaccination**

Vaccination of susceptible animals is an efficient strategy for controlling Bluetongue virus (BTV) spread and economic consequences thereof. In case of BTV, vaccines are asked to protect animals from clinical disease and prevent viraemia, thus limiting secondary cases.

In this study we evaluated the efficacy of a commercially available inactivated BTV-8 vaccine (BTVPUR AISap 8, Merial) administered in sheep shortly before challenge with wild type virus (low passaged north-European BTV-8).

Twenty-four sheep were divided into four groups of six sheep each and vaccinated two weeks before challenge (Group A), one week before challenge (Group B) and concurrently with challenge (Group C). The remaining group consisted of six non-vaccinated-infected animals (Group NVIA). Vaccinated animals received a booster injection 28 days later with respect to the first vaccine administration.

Clinical signs were recorded. Serological and virological tests were performed before and after challenge (up to 42 and 77 days after challenge, respectively). The results of the study show that one single administration of vaccine performed 2 weeks before challenge (Group A) is able to completely prevent viraemia and RNAemia in challenged animals. Conversely, Group B and C showed viraemia and RNAemia after challenge. Overall, our findings may have direct consequences for the management of an unexpected BTV-8 outbreak in sheep and for the legislation on sheep trade from BTV restricted areas. Indeed we proposed that, at least for sheep, the period from vaccination to animal movement could be shortened up to 14 days after the single first injection of inactivated vaccine with potential saving of important economic resources.



# POSTERS

## TOPIC III

Current challenges  
inside Europe

Epidemiology & Surveillance  
and Risk assessment

## POSTER 64: Complete genome analysis of Schmallenberg virus isolated in Poland in 2012

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**Keywords: Schmallenberg virus, genome characterization, NGS, Poland**

Schmallenberg virus (SBV) is a novel insect-transmitted *Orthobunyavirus* that infects ruminants. Following its first identification in Germany in 2011, SBV has rapidly spread to many other European countries. SBV was identified in Poland from blood of animals which developed viraemia, stillborn calves, calf and lamb brains, and insect vector *Culicoides* spp. The SBV genome comprises three RNA segments of negative polarity, referred to as small (S), medium (M), and large (L). Despite the fact that a few years have passed from the first identification of SBV, there is not much work done on molecular characteristics of its genome. The aim of this study was to characterize the whole genome sequence of two Polish SBV field isolates 130/6 and 1018.

Analysed strain 130/6 SBV was isolated from brain tissue of stillbirth lamb from Podkarpackie province. Virus was propagated in BHK-21 cell culture. The viral RNA was extracted after third passage, while the RNA of a second strain 1018 was isolated from the serum of infected cow from Śląskie province. Libraries for the next generation sequencing were prepared using NEBNext Ultra RNA Library Prep Kit for Illumina. All libraries were checked on a BioAnalyzer 2100 using Agilent High Sensitivity DNA Kit. Illumina sequencing was performed with MiSeq Reagent Kit v2 mode PE250. Bioinformatic analysis was carried out using Trimmomatic (filtering reads, trimming adapters) and CLC GenomicWorkbench (mapping reads to reference sequences and De novo assembly of NGS data). Alignments of the nucleotide consensus sequences and deduced amino acid (aa) sequences and comparative sequence analysis were carried out with MEGA v. 5.03 and BioEdit v. 7.0.9.0 software.

Comparative sequence analysis of the Polish strains and the first virus isolate BH80/11-4 (GenBank accession numbers: HE649912 – HE649914) isolated in Schmallenberg, Germany revealed a high stability for the viral S segment with a length of 815 bp with only 2 nucleotide differences (99.7% sequence identity - ID) for 130/6 and none substitution for 1018 (100% ID). One of the changes, resulted in the amino acid change. L segment (6841 bp long) of the virus genome was also characterized by a high stability. 14 nucleotide substitutions were found in field isolate 130/6 (99.7% ID), but only 2 of them resulted in nonsynonymous mutations (amino acid changing). For 1018 strain 9 substitution were identified (99.8% ID), and only one of them caused the change in aa sequence. The most variable SBV segment was the segment M (4251 bp), with 34 nucleotide substitutions (99.2% ID) in 130/6, resulting in the changes of 23 amino acids. In 1018 strain there were 35 changes (99.1% ID) which resulted in the change of 6 amino acids. Amino acid changes in the deduced sequences of Polish strains are presented in the Table.

The results of this study confirmed that the most variable region of the virus genome is M segment, which is also responsible for the pathogenicity of the SBV. High sequence identity, of three segments of the Polish field isolates 130/6 and 1018 with nucleotide sequence of German reference strain from 2011, indicates a small rate of evolution, which may simplify the diagnostic testing and the implementation of any vaccination scheme.

BH80/11-4	S segment	M segment														L segment		
position	111	431	476	514	535	567	573	575	584	600	608	629	690	714	737	121	1159	1934
aa	Ser	His	Ser	Glu	Val	Gln	Arg	Phe	Pro	Ser	Glu	Asn	Glu	Pro	Thr	Asp	Glu	Glu
130/6 1018	Asn	Arg	Pro	Lys	Phe	Lys	Gln	Ser	Ser	Leu	Lys	Asp	Lys	Leu	Ala	Asn	Gln	Asp
		M segment cont																
position		738	746	822	844	1017	1084	1254	1270	1340	1352	1402	1403					
aa		Pro	Lys	His	Lys	Gly	Val	Gly	Ala	Lys	Gly	Phe	Lys					
130/6 1018		Thr	Glu	Arg	Glu	Asp	Ile			Glu	Ser	Leu						
			Glu					Arg	Thr	Glu		Glu	Asn					

Table Amino acid changes in deduced sequences of whole genome Polish SBV isolates compared to the BH80/11-4 GenBank reference sequences. AA positions refer to BH80/11-4 sequence.

The study was financed by the research project from The National Centre for Research and Development (No. PBS2/A8/24/2013).

## POSTER 65: Current Schmallenberg virus epidemiological situation in Poland - serological and entomological surveillance

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**Keywords: Schmallenberg virus, serology, *Culicoides***

Schmallenberg virus (SBV) emerged in Poland in the summer of 2012, affecting up to 3% farm and free-living ruminants. Congenital abnormalities were also reported in cattle and sheep. To monitor epidemiological situation in livestock since 2013, a broad scale serological surveillance was introduced. Midge vector of SBV were collected across the country, and entomological then virological studies were performed to assess vector competence.

Livestock serum sampling was conducted during national bluetongue virus (BTV) monitoring program. A total of 15,778 samples from cattle sheep and goats were collected between 2013 and 2015 and the numbers of the samples were calculated using country population statistics estimating 20% seropositivity with 95% confidence level. Serum samples originated from 15 out of 16 Polish provinces and were tested using multi-species ID Screen Schmallenberg Virus Competition Test (IDvet). *Culicoides* vector abundance was investigated by examination of midges trapped in 24 evenly distributed in the country Onderstepoort UV light traps. Over 4 thousand pools (97399 individuals) *Culicoides* females from the most abundant species were divided according to species and four parity status. In-house optimized real-time RT-PCR was performed to detect SBV-S segment and a fragment of 18S midge gene as internal control. RNeasy Mini Kit (Qiagen) in the automatic station Qiacube (Qiagen), AgPath-ID One-Step RT-PCR Reagents (Ambion, Applied Biosystem) kit in Step One Real-Time PCR system (Life Technologies) were used for virus RNA detection. Positive samples were retested by rt-RT-PCR, and if negative, the analysis was repeated from new extraction.

The overall seroprevalences were 35.4%, 58.7% and 29.6% in 2013, 2014 and 2015, respectively. In all years, the percentage of seropositive animals was higher among cattle (50.6%) than in sheep (24.0%) and goats (26.3%). Seroprevalences increased on average by 15.3% at the province level between 2013 and 2014. Between 2014 and 2015, the seroprevalence decline by 21.1% at the province level was observed. The estimated risk factors for SBV seropositivity included age, gender, species, year of sampling, ruminant densities and province.

*Culicoides obsoletus/scoticus* and *C. punctatus* were predominant vectors of SBV in Poland. As presented in the table approx. 1% of positive pools was detected. In the previous study in 2012, 10% of tested pools was positive for SBV RNA. The mean Ct values were also higher than in 2012 (Ct29.8). The significant decrease of the proportion of SBV positive midges and of the mean load of SBV RNA suggest clearance of the virus from the vector. However, SBV was identified in nulliparous culicoides (virgin females which have not taken any blood meal yet) in all years, what may indicate independent from ruminant virus circulation in the midge population. Possible “overwintering” shows detection of SBV positive pools at the same locations in the consecutive years.

Year	Number of tested pools	Positive pools in at least one analysis	Positive pools confirmed in repeated analysis	Mean ct
2013	1404	13 (0.9%)	2 (0.1%)	39.4
2014	1569	18 (1.1%)	13 (0.8%)	34.7
2015	1384	14 (1.0%)	10 (0.7%)	37.1

SBV after transmission in 2012 continued to spread reaching the peak seroprevalence in 2014. Observed decline in 2015 may be a result of antibodies decay in adult animals after first epidemic wave, while young animals had limited risk of infection due to low SBV infection rate in the vector.

The study was financed by the research project from The National Centre for Research and Development (No. PBS2/A8/24/2013).

## POSTER 66: Schmallenberg Virus in Sardinia 2013-15: results of entomological monitoring

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**Keywords: Schmallenberg virus, Culicoides, Surveillance.**

Schmallenberg virus (SBV) is a virus belonging to genus Orthobunyavirus of Bunyaviridae family. SBV infects ruminants and can lead to congenital malformations in foetus or stillborn. Species of *Culicoides* are considered vectors of SBV. In this regard RNA of SBV was detected in *C. scoticus*, *C. obsoletus* and *C. chiopterus* in North Europe and in other countries of Northern Italy. In December 2012, SBV was detected for the first time in Sardinia in numerous ovine flocks.

The aim of this study was to monitor *Culicoides* populations in flocks affected by SBV during the epidemic of 2012 in Sardinia. To do this we selected 4 sheep flocks located in 4 different municipalities of the island (Dorgali, Girasole, Mores and Sassari), in which the presence of SBV antibodies was confirmed.

In each site, near to animals night shelter, a light trap to catch *Culicoides* was positioned. The captures have been effectuated at 20 days intervals during the period included between May and November from 2013 to 2015. Light traps were activated before sunset and off the next morning. Successively *Culicoides* adults have been morphologically identified to the species level. Samples of parous females (females that have been completed at least a gonotrophic cycle) of more abundance species, were submitted for detection of SBV by Real Time RT- qPCR. Pools of maximum 25 parous females according to species, location and date were created.

In addition, to evaluate if SBV re-circulated throughout the period 2013-2015 in Sardinia, a serological investigation on other 94 flocks was carried out together with a passive surveillance plan by which the cases of malformed lambs, suspected to be SBV infected, were recorded on the total Sardinian sheep population.

During the entomological monitoring 85 catches were effectuated and 124,940 *Culicoides* were sampled. The most abundance species was *C. imicola* (76,550 adults) resulting in 61% of the total of *Culicoides* identified. Furthermore *C. newsteadi* (32,186) and *Obsoletus* complex (3,008) were present with the 26% and 2% respectively.

Successively 2,058 parous female of *C. imicola* (107 pool), 1,277 of *C. newsteadi* (72 pool), 92 of *Obsoletus* complex (21 pool), 60 of *C. punctatus* (15 pool), 29 of *C. pulicaris* (16 pool), 37 of *C. circumscriptus* (12 pool) and 5 of *C. paolae* (5 pool) were submitted to bimolecular analyses to SBV detection. All sampled analyzed resulted negative by PCR.

Serological survey on the flocks found that a low rate of sheep born after 2012 were SBV positive in the period from 2013 to 2015. By passive surveillance, sporadic cases of malformed stillborn lambs were found after 2012, however virological investigations did not confirm the presence of SBV.

Results obtained by entomological monitoring during the period 2013-15 confirmed how observed in the serological survey and passive surveillance. In fact also the sporadic cases of clinical disease of SBV in Sardinia after the epidemic of 2012 have never been confirmed by PCR.

Moreover our results confirmed even the difficulty to find SBV in the competent vectors. These difficulties were observed in a retrospective study effectuated about *Culicoides* sampled in Sardinia during summer of 2012, in which SBV was detected just in 2 out of 727 pool of *C. imicola* analyzed.

## POSTER 68: Seroprevalence of small ruminant lentiviruses in Belgian sheep and goats

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**Keywords: Seroprevalence, Visna/Maedi virus, Caprine arthritis encephalitis virus, Small ruminant lentivirus, AGID, Belgium**

Visna/Maedi virus (VMV) and caprine arthritis encephalitis virus (CAEV) are members of the group of small ruminant lentiviruses (SRLVs). Both result in progressive and persistent infections in sheep and goats. These closely related retroviruses are responsible for neurological disorders and chronic lesions in lungs, joints and mammary glands. Aside from their impact on the animal welfare and their fatal outcome, SRLVs cause economic losses due to reduced lamb weight, reduced milk production, early culling and restrictions to animal trade. The most important route of transmission is the ingestion of infected colostrum and milk by lambs but the virus can also spread via respiratory secretions when animals are housed intensively for prolonged periods.

Since no therapies are available against SRLVs, the control of the disease relates mostly on the early detection of infected animals with diagnostic tools such as agar gel immunodiffusion (AGID) and ELISA. As for several other European countries, the seroprevalence in Belgium for these diseases is unknown. Therefore, we performed a nationwide SRLV survey in the Belgian sheep and goat population.

We aimed to collect samples from a total of 100 sheep and 100 goat farms all over Belgium. Only farms that did not participate in the voluntary MVV/CAEV control program and had at least 3 goats or 5 sheep were eligible to participate. A stratified sampling approach proportional to the number of goat and sheep holders per province was applied and maximally 7 animals per farm were sampled. All randomly selected sheep and goat owners were contacted by phone and asked to participate to the study. A short questionnaire was submitted to the volunteers to collect information about flock size, animal health condition and age, potential contact with goat or sheep and the housing facilities.

Although 100 sheep and goat farmers confirmed their participation, we received samples from only 86 sheep farms and from 74 goat farms. Samples were received between November 2015 and May 2016. A total of 546 sheep and 393 goat sera were tested using two commercial immunodiffusion kits, the AGID-CAEV p28 kit (Pourquier, France) and the Maeditect kit (Apha Scientific, UK). For sheep, a between herd seroprevalence of 17.4% was found. The mean within herd seroprevalence at those farms that had at least one positive animal was 51.4%, but varied between 14.3% and 100%. An overall seroprevalence in sheep of 9.9% was observed. In the goat study, 14.9% of the farms had at least one seropositive animal and the mean within herd seroprevalence was 40.9% with a similar variation as found for sheep. Only 6.4% of all goat samples were found seropositive for SRLVs. Currently, we are performing a risk factors analysis to see if associations with seroprevalence can be found.

This study is the first to investigate the SRLV seroprevalence in the sheep and goat population of Belgium. Despite the fact that there is a voluntary control program for SRLVs in Belgium, we observed that SRLV are present on 15% to 20% of non-certified farms.

## POSTER 69: Molecular Characterization of African swine fever virus (ASFV) isolates circulating in the Eastern European Union countries 2014-2016

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African swine fever (ASF) is endemic in sub-Saharan Africa and in Sardinia (Italy), while multiple countries of Eastern Europe and the Caucasus have been affected since its introduction from East Africa in 2007. Within the European Union (EU), after Lithuania made in January 2014 the first notification of ASF cases in wild boar, and Poland followed in February, the disease has spread in Estonia, Latvia, Lithuania and Poland, mainly in the wild boar population. According to the Animal Disease Notification System (ADNS) a total of 82 outbreaks in domestic pigs and 2,575 cases in wild boar have been notified in the affected countries in the period from January 2014 to 1<sup>st</sup> April 2016. Within the duties of the European Union Reference laboratory for ASF (EURL) (council directive 2002/60/EC) it is included the characterization of the ASFV isolates circulating within the EU by the most up-to-date methods available to allow greater understanding of the epizootiology of ASF in the affected areas. To this end, it has been carried out the genetic characterization of 463 ASF viruses from samples received at the EURL selected from different hosts, diverse geographical locations and from different cases or outbreaks occurred in the affected EU countries since 2014. In detail, this included 387 samples collected from cases occurred in wild boar and 76 obtained from domestic pig outbreaks. The molecular analysis included the sequencing of the C-terminal end of the p72 coding gene, the central variable region (CVR) within the B602L gene, and the intergenic region (IGR) located between the I73R and I329L genes characterized by the presence of tandem repeat sequences (TRS). All ASF viruses characterized from Estonia, Latvia, Lithuania, and Poland clustered, within the p72 genotype II and showed a 100% of nucleotide homology with all compared ASFV isolates from Eastern Europe across the 478-bp C-terminal end of the p72 coding gene. 100% nucleotide homology among all sequenced viruses was identified by the analysis of the IGR, characterized by a TRS insertion identical to that present in ASFV isolates from Belarus 2013 and Ukraine 2012. It deserves to comment at this point that IGR sequencing of Russian ASF viruses (collected from 2009 to 2015) performed by Goller et al., (2015) revealed the co-circulation of these two variants since 2012, with and without the TRS insertion across the country, showing that TRS insertion (TRS+) prevails among current disease outbreaks. Finally, the sequencing of the CVR of all ASF viruses analyzed until July 2015 revealed the presence of the pattern of 10 copies of amino acid tetramer repeats characteristic to that of the ASFV circulating in the Caucasus regions since 2007 and in the EU territory since 2014. However, a new CVR variant (GII-CVR-2) was identified on July 2015 in Tartu region, Estonia, within the wild boar population. This was characterized by the deletion of three amino acid tetramer repeats. This variant has been detected only in Estonia in samples obtained from seven wild boar in Tartu region, where both genetic variants (GII-CVR-1, GII-CVR-2) are co-circulating over the time. It would be interesting to follow the geographical evolution of the two genetic variants to know if one of them finally overcomes the other one or, in contrary, both are able to keep circulating randomly and reach new territories.

# LAST MINUTE POSTERS



## POSTER 71: Molecular reconstruction of Newcastle disease history in Nigeria

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Since its first records in the 1950s in West Africa, Newcastle disease virus (NDV) has been submitted to several surveillance campaigns that have provided large datasets of samples.

In this study, we analysed 137 complete fusion gene sequences of NDV obtained from poultry sampled between 2006 and 2011 from nine West African countries: Nigeria ( $n=94$ ), Cameroon ( $n=6$ ), Ivory Coast ( $n=7$ ), Mali ( $n=7$ ), Burkina Faso ( $n=2$ ), Mauritania ( $n=1$ ), Niger ( $n=6$ ), Benin ( $n=2$ ), and Togo ( $n=2$ ).

In order to understand the transmission dynamics of NDV in Nigeria and the neighbouring countries, we conducted phylogenetic analyses by considering the temporal and the spatial evolution of the virus: (i) phylodynamic analyses at a regional scale to highlight the evolution of virus mutations over time that may reflect viral adaptations; and (ii) phylogeographic methods to infer the geographical history of NDV genotypes as well as to estimate the rate of virus transmission and spatial spread in the region.

By combining spatial and genetic analyses and using Bayesian stochastic search variable selection (BSSVS) procedure, we identified frequently invoked routes to explain the diffusion process. We revealed the pathways of introduction and transmission of NDV from neighboring countries to Nigeria. We also analysed the Nigerian diffusion pathways in relation to national commercial poultry networks.

By implementing molecular clocking, we estimated the date of virus diffusion and showed longstanding presence of NDV in West Africa, revealing how virus diffusion may enable endemic maintenance.



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Peter	Mertens	IAH	UK
Rodolphe	Michiels	CODA-CERVA	Belgium
Olympia	Mino	QEFII HELLAS	Greece
Miroslav	Mojžiš	State Veterinary and Food Institute / Veterinary Institute Zvolen	Slovakia
Kristian	Møller	DTU National Veterinary Institute	Denmark
Maria	Montoya	The Pirbright Institute	UK
Ana Maria	Moreno	IZSLER, Brescia	Italy
Sandra	Moreno	INIA-CISA	Spain
Inés	Moreno	MAGRAMA	Spain
Alexander	Morrow	DEFRA	UK
Mercedes	Mouriño	Zoetis Spain S.L	Spain
Laure	Mouton	MERIAL S.A.S.	France
Maria Jesús	Navas Sánchez	IRTA-CReSA	Spain
Raquel	Nieto Martínez	INIA-CISA	Spain
Ana Isabel	Núñez	IRTA-CReSA	Spain
Ann Sofie	Olesen	DTU National Veterinary Institute	Denmark
Javier	Ortego	INIA-CISA	Spain
Aykut	Ozkul	Veterinary Faculty, Ankara University	Turkey
Virginia	Pelayo	INIA-CISA	Spain
Daniel	Perez Núñez	CBMSO-CSIC-UAM	Spain
Elisa	Pérez Ramírez	INIA-CISA	Spain
Tamas	Petrovic	“Scientific Veterinary Institute” “Novi Sad”	Serbia
Giulia	Pezzoni	IZSLER, Brescia	Italy
Philippe	Pourquier	IdVet	France
Giselle	Rangel	INIA-CISA	Spain
Ana	Ranz	INGENASA S.A	Spain
Thomas Bruun	Rasmussen	DTU National Veterinary Institute	Denmark
Chelliah	Ravi Rajan	DEFRA	UK
Belén	Rebollo	INGENASA S.A	Spain

First Name	Surname	Institute	Country
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Ulla	Rikula	Finnish Food Safety Authority Evira	Finland
Fernando	Rodríguez	IRTA-CReSA	Spain
M <sup>a</sup> Elena	Rodríguez Valin	INIA-SGPCP	Spain
José Manuel	Rojas	INIA-CISA	Spain
Sandro	Rolesu	OEV-IZSSA, Sardegna	Italy
Luis José	Romero	Magrama	Spain
Paloma	Rueda	INGENASA S.A	Spain
Pedro	Sánchez-Cordón	The Pirbright Institute	UK
José Manuel	Sánchez-Vizcaíno	Veterinary Faculty, Universidad Complutense de Madrid (UCM)	Spain
Luis	Santos	MERIAL S.A.S	Spain
Antonio	Sanz	INGENASA S.A	Spain
Patricia	Sastre	INGENASA S.A	Spain
Kore	Schlottau	FLI	Germany
Carsten	Schroeder	QIAGEN Leipzig GmbH	Germany
Claudia	Schulz	FLI	Germany
Noemi	Sevilla	INIA-CISA	Spain
Liisa	Siivhonen	Finnish Food Safety Authority Evira	Finland
Alejandro de Jesús	Soler	INIA-CISA	Spain
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Elena	Sotelo	CZ Veterinaria S.A.	Spain
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Thierry	van der Berg	CODA-CERVA	Belgium
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Angel	Venteo	INGENASA S.A	Spain
Júlia	Vergara Alert	IRTA-CReSA	Spain
João Tiago	Vieira	IZSVE, Venezia	Italy
Rubén	Villalba	Laboratorio Central de Veterinaria (LCV)	Spain
Damien	Vitour	ANSES, Maisons-Alfort	France
Darima	Yanzhieva	NRIVaMR	Russia
Alexey	Zaberezhnyy	Y.R.Kovalenko All-Russian Research Institute of Experimental Veterinary Medicine	Russia
Laura	Zani	FLI	Germany
Stéphan	Zientara	ANSES, Maisons-Alfort	France



# LAST MINUTE MODIFICATIONS

## CANCELLATIONS

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### KEYNOTE SPEAKER

Mr. Jordi Figuerola has been substituted by Keynote speaker Mr Josue Martínez de la Puente.

### ORAL

**O17.** Concurrent Infection of Bluetongue (BT) and Peste des Petits Ruminants (PPR) in Awassi Sheep in Jordan (N.Hailat, Jordan) 28th September, 17:00h, EPIZONE 1 ROOM.

The programme will remain as follows:

- 17:00-17:15h**     **O18.** Improvement of the protective immune response in swine by co-inoculation of synthetic RNA and an FMDV vaccine. (B.Borrego, Spain)
- 17:15-17:30h**     **O19.** Efficacy and immune responses of DNA and MVA vaccines against Rift Valley fever in sheep (G.Lorenzo, Spain)
- 17:30-17:45h**     **O20.** Replication and shedding of Middle East respiratory syndrome coronavirus (MERS-CoV) in experimentally inoculated pigs and llamas (J.Vergara-Alert, Spain)
- 17:45-18:00h**     **O21.** Structural protein VP2 of African horse sickness virus is not essential for virus replication *in vitro*. (P.van Rijn, Netherlands)
- 18:00-18:15h**     **O22.** The presence of pre-formed antigen in the inoculum of experimental MVA-VP2 vaccines for African horse sickness virus plays an important role in the vaccine immunogenicity. (J.Castillo-Olivares, UK)

### POSTER PRESENTATION

**P6.** The potential role of wild pigs in the spread and maintenance of African swine fever at the wildlife-livestock interface in Uganda (K.Stahl, Sweden) 28TH September, 12:30h EPIZONE 2B ROOM

### POSTER

- P67** Infection of *Ornithodoros erraticus* by African swine fever virus: impact on the tick bacteriome • MICHAUD, Vincent
- P70** The effect of external temperature on the multiplication of the African swine fever virus Georgia2007/1 in the tick *Ornithodoros erraticus* – VIAL, Laurence

## ADDITIONS

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### POSTER

P 71 Molecular reconstruction of Newcastle disease history in Nigeria. BATAILLE, Arnaud.



# EPIZONE



EPIZONE European Research Group (ERG) is the international network of veterinary research institutes working on epizootic animal diseases including those which may have zoonotic potential. It plays a key role in research on prevention, detection and control of animal diseases and zoonoses in order to reduce the risks and harm to animal health and the risks to public health in the EU and beyond.

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