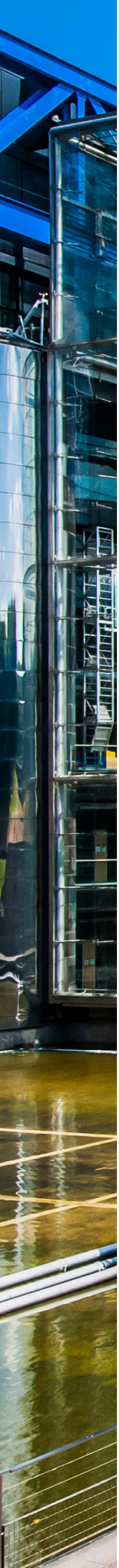
11th EPIZONE Annual Meeting

Welcome in Paris

by ANSES



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THANK YOU

We are very grateful to the sponsor of the 11th EPIZONE Annual Meeting
- ANSES

Also a big thank you to everybody else involved in the 11th EPIZONE Annual Meeting

- Administration Bureau EPIZONE at Wageningen University
- All members of the committees
- Keynote speakers
- Chair persons
- Oral presenters
- Poster presenters
- Participants

WELCOME

11th EPIZONE Annual Meeting: **Crossing barriers**

The French agency for food, environmental and occupational health & safety (ANSES) warmly welcomes all the participants of the 11th EPIZONE meeting here in Paris.

The theme of this year's event is "**Crossing barriers**", not only with regard to species barriers or to the barriers between different scientific disciplines, but also the crossing of geographical barriers. ANSES is committed to crossing barriers – those between public and private stakeholders, those which may exist between various scientific disciplines, and the barriers created by borders, since ANSES has numerous collaborations with institutes around the world.

ANSES undertakes monitoring, expert assessment, research and reference activities in a broad range of topics that encompass human health, animal health and well-being, and plant health. "Investigate, evaluate and protect" - this summarises the missions of ANSES, based on collaborative and impartial expert assessment of the health risks and benefits of chemical, biological and physical agents, often through the prism of the human and social sciences. ANSES works with approximately 800 external experts selected primarily based on their scientific expertise and on a review of potential conflicts of interest. The Agency also relies on a network of 11 reference and research laboratories located throughout France that are recognised in Europe and internationally a wide range of fields and disciplines and are located in geographical proximity to their respective sectors. It also assesses the effectiveness and risks of veterinary medicinal products, plant protection products, fertilisers, growing media and their adjuvants, as well as biocides, with a view to delivering marketing authorisations. ANSES also provides assessments of chemicals within the framework of the European REACH and CLP regulations.

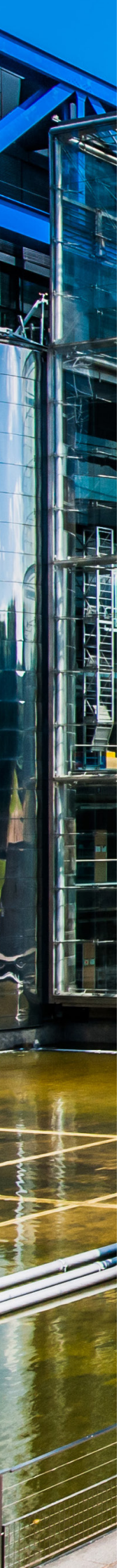
Like human health, animal health can be threatened by numerous pathogens (bacteria, viruses, parasites), some of which can also affect humans (zoonoses). ANSES contributes to the prevention and control of the major and emerging animal diseases affecting both farm animals and wildlife, and in doing so participates in the protection of public health. Via the French agency for veterinary medicinal products (ANMV), ANSES is also the competent French authority for the assessment and management of veterinary medicinal products.

Global climate change, as well as new forms of globalisation in trade, have led over the last few years to the emergence in Europe of exotic animal diseases with a high economic impact. The emergence of these infectious animal diseases remains a topical issue which has often mobilised the ANSES teams, for example : new variants of the bluetongue virus, tick-borne diseases, African swine fever, etc. However, other more established diseases also remain a concern for France and for Europe and warrant our sustained efforts.

Another challenge is to develop methods of screening, analytical diagnosis and control, in accordance with the European and international standards in force, while integrating new technologies for analysing genomes and proteins. The detailed analysis of host/pathogen interactions, as well as the development of new epidemiological methods, will enable ANSES to continue to combat emerging and re-emerging animal diseases, in line with the «One Health» concept promoted by the World Health Organisation.

I sincerely hope that this 11th annual meeting of EPIZONE will be an opportunity to cross barriers and to provide the "epizootic community" with effective ways of working together to plan new initiatives, to interact and to enjoy our time here together in Paris.

Dr Roger GENET



Committees

The 11th EPIZONE Annual Meeting is organised by ANSES,
French agency for food, environmental and occupational health & safety

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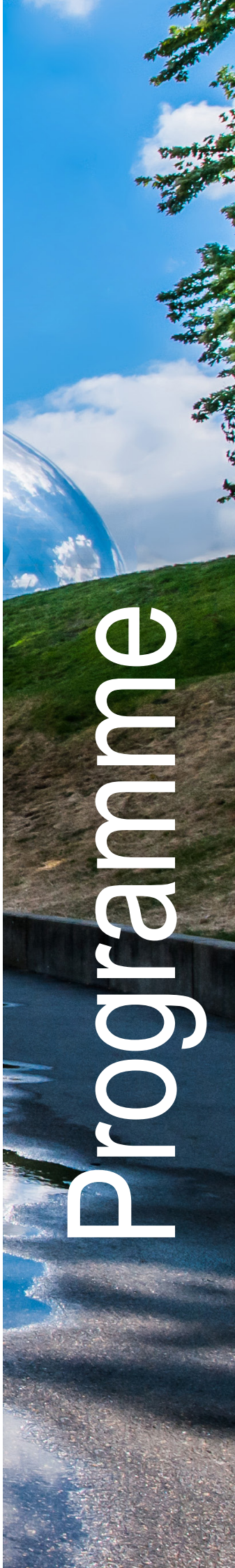
11th EPIZONE Annual Meeting
Crossing Barriers
Paris, France



Tuesday 19th September 2017

YOUNG EPIZONE MEETING

Room 1

08:00 ***Registration and welcome desks***

09:00 WELCOME ADRESSES

Wim van der POEL, Coordinator Epizone
Stéphan ZIENTARA & Marie-Frédérique LE POTIER, Scientific Committee, ANSES, France

10:00 ICE-BREAKING GAME

Laura ZANI, Friedrich-Loeffler-Institut, Germany

11:00 ***Coffee break***

11:30 WORKSHOP ON "COMMUNICATION SKILLS"

Baudouin JURDANT, Professor emeritus of science information and communication, Université Paris-Diderot, Lisbonne, Portugal

12:30 ***Lunch break at*** **The Loft,** ***into the*** **Cité des sciences**

INVITED SPEAKERS 'COMMUNICATION'

14:00 Clémentine SCHILTE, communication officer, center for translational science, Institut Pasteur, Paris

14:40 Brigitte RAULT, communication on animal experiments, office of animal experimentation, INSERM, Paris

15:20 Sophie FÉLIX, scientific journalism, communication and publishing, Université Paris-Saclay, Paris

16:00 Pauline MAISONNASSE, scientific popularization,IDMIT, Paris

11th EPIZONE ANNUAL MEETING

15:00 ***Registration and welcome desks***

19:00 ***Welcome cocktail***

Programme

Wednesday 20th September 2017

08:00 *Registration and welcome desks*

09:00 WELCOME ADRESSES - Amphitheatre

Dr Roger GENET, Director General, ANSES, France
Wim van der POEL, Coordinator Epizone
Stéphan ZIENTARA & Marie-Frédérique LE POTIER, Scientific Committee, ANSES, France

09:20 PLENARY SESSIONS - Amphitheatre

KEYNOTE: THE EMERGENCE, PERSISTENCE AND EVOLUTION OF BLUETONGUE VIRUSES IN EUROPE SINCE 1998 - Peter MERTENS

KEYNOTE: RIFT VALLEY FEVER: UNANSWERED QUESTIONS AND UNMET NEEDS - Jeroen KORTEKAAS

10:20 *Coffee break & poster session*

SESSION 1: ARTHROPOD-BORNE DISEASES (ARBOVIRUSES)

Chairs : Stéphan Zientara & Jose Manuel Viscainot - Amphitheatre

11:00 C1 - Natalia Golender - **Novel topotypes of bluetongue serotype 3 viruses in the Mediterranean Basin**

11:15 C2 - Gil Patricia - **First detection of a Mesonivirus in Culex pipens in five countries around the Mediterranean basin**

11:30 C3 - Mazigh Fares - **Pathological modelling of Tick-Borne Encephalitis Virus infection using primary human brain cells derived from fetal neural progenitors**

11:45 C4 - Grégory Caignard - **Interactomic high-throughput mapping for bluetongue virus in its natural hosts to identify new factors of pathogenicity and interspecies transmission**

12:00 C5 - Mathilde Gondard - **Tick-borne pathogens detection in Caribbean ticks using high-throughput microfluidic real-time PCR (DOMOTICK Project)**

12:15 C6 - Belen Borrego - **Propagation in the presence of favipiravir leads to selection of RVFV variants with attenuated phenotype in mice**

SESSION 2: CURRENT CHALLENGES INSIDE EUROPE

Chairs : Benoît Durand & Wim van der Poel - Room 1

C7 - Claude Saegerman - **Risk of introduction of Lumpy Skin Disease in France by the import of cattle**

C8 - Mieke Steensels - **Belgian introduction and re-emergence of HP H5N8 clade 2.3.4.4**

C9 - Marcel Hulst - **Identification of a Novel Reassortant of a Mammalian Orthoreovirus in Faeces of diarrheic pigs in the Netherlands**

C10 - Sylvie Lecollinet - **Case investigation of unexplained infectious meningoencephalitis in animals - a new rotavirus in horses?**

C11 - Llilianne Ganges - **Novel subunit vaccine based in the E2 glycoprotein fused to porcine CD154 prevents classical swine fever virus vertical transmission in pregnant sows**

C12 - Andy Haegeman - **Lumpy skin disease (LSD) vaccine evaluation using an optimized infection model**

12:30 *Lunch break & poster session*

13:45 PLENARY SESSIONS - Amphitheatre

KEYNOTE: LUMPY SKIN DISEASE EPIDEMICS IN EUROPE AND PREVENTIVE AND CONTROL MEASURES FOR AN EFFECTIVE CONTROL OF THE DISEASE - Tsviatko ALEXANDROV

KEYNOTE: PESTE DES PETITS RUMINANTS: STATE OF AFFAIRS AND FUTURE CHALLENGES - Arnaud BATAILLE

SESSION 3: ARTHROPOD-BORNE DISEASES (ARBOVIRUSES)

Chairs : Alejandro Brun & Martin Beer - Amphitheatre

14:45 C13 - Cindy Kundlacz - **Impact of Bluetongue virus on the MAPK/ERK pathway**

15:00 C14 - Oran Erster - **A wolf in lamb's clothing: Detection and isolation of a new infectious Bluetongue serotype from commercial vaccine**

15:15 C15 - Piet A. van Rijn - **Prospects of African horse sickness DISA vaccine platform based on deletion of 77 amino acids in NS3/NS3a protein**

15:30 C16 - Alessio Lorusso- **Antigenic mapping and interaction of recent Italian West Nile and Usutu viruses**

15:45 C17 - Francisco Llorente - **Characterization of serological cross-reactivity between mosquito-borne flaviviruses co-circulating in Europe**

16:00 C18 - Andrey Koltsov - **Development of the typing assays for several BTV serotypes in Russia**

SESSION 4: CURRENT CHALLENGES INSIDE EUROPE

Chairs : Christian Griot & Emmanuel Albina- Room 1

C19 - Kerstin Wernike - **Segment variation of Schmallenberg virus in malformed fetuses is the consequence of immune escape**

C20 - Thierry Morin - **Interest of phylogenetic data and specific diagnostic tools to support management and eradication of Viral Haemorrhagic Septicaemia and Infectious Hematopoietic Necrosis in France**

C21 - Najmul Haider - **Microclimatic temperatures of Danish cattle farms: a better understanding of the variation in transmission potential of Schmallenberg virus**

C22 - A. Alessandro Broglia - **Epidemiological analysis of the lumpy skin disease epidemics in Europe in 2015-2017**

C23 - Efrem Alessandro Foglia - **Prevalence and characterization of Encephalomyocarditis virus (EMCV) in Italy**
fever virus vertical transmission in pregnant sows

C24 - Cristina Jurado - **Risk factors for African swine fever persistence in Sardinia**

16:15 KEYNOTE: THE EUROPEAN UNION ANIMAL HEALTH POLICY, NOW AND IN THE FUTURE
Christianne BRUSCHKE - Amphitheatre

16:45 *Coffee break & poster session*

18:45 *Social Event*, Cité des sciences, Géode, Paris

Thursday 21th September 2017

09:00 PLENARY SESSIONS - Amphitheatre

KEYNOTE: FLOWPAD, A GENERIC MICROFLUIDICS PLATFORM FOR A WIDE RANGE OF APPLICATIONS
Remco den DULK

KEYNOTE: AVIAN INFLUENZA VIRUSES: FROM EPIDEMIOLOGY TO HOST JUMP
George GAO

10:15 KEYNOTE: SWARM INCURSION: A REVIEW ON THE 2016/7 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS EPIZOOTIC IN EUROPE
Timm HARDER - Amphitheatre

KEYNOTE: EXCHANGE OF PATHOGENS BETWEEN FARMED AND WILD FISH
Niels Jørgen OLESEN - Room 1

10:45 ***Coffee break & poster session***

SESSION 5: ANIMAL INFLUENZA VIRUSES

Chairs : Gaëlle Simon & Thierry van den Berg - Amphitheatre

11:15 C25 - François-Xavier Briand - **Genetic diversity of French highly pathogenic H5 viruses during 2015-2016**

11:30 C26 - Saskia A. Bergervoet - **Whole genome sequence analysis of potential between-farm transmitted low pathogenic avian influenza (LPAI) viruses**

11:45 C27 - Jean-Luc Guérin - **Detection of clade 2.3.4.4. H5N8 HPAI viruses in feather samples in ducks and geese: an assessment on field outbreaks**

12:00 C28 - Evelien Germeraad - **Development of a multiplex serological assay for Avian Influenza**

12:15 C29- Ana Maria Moreno - **Genetic diversity of influenza A virus in Italian pigs in the period 1998-2017**

12:30 C30 - Mariette F. Ducatez - **Influenza D virus circulation in Europe and Africa**

SESSION 6: AFRICAN SWINE FEVER

Chairs : Marie-Frédérique Le Potier & Dolores Gavier-Widen - Room 1

C31 - Grzegorz Woźniakowski - **Preparation of African swine fever isolates for CRISPR/Cas9 modification**

C32 - Laura Zani - **Deletion at the 5'-end of Estonian ASFV strains associated with an attenuated phenotype?**

C33 - Jan Hendrik Forth - **Detection of Asfarvirus-like sequences in Ornithodoros soft ticks**

C34- Veronica Delicado - **Spatio-temporal clusters of ASF with different stages of infection in Lithuania and Poland**

C35 - Jolene Carlson - **Soil as a vector for African swine fever virus in wild boar populations**

C36 - Dolores Gavier-Widen - **ASF-STOP: progress and updates from the first year of COST Action "Understanding and combating African Swine Fever in Europe**

12:45 ***Lunch break & poster session***

14:00 PLENARY SESSIONS - Amphitheatre

KEYNOTE: CROSSING BARRIERS : THE CASE OF RABBIT HAEMORRHAGIC DISEASE VIRUS 2
Aleksija NEIMANIS

KEYNOTE: CORONAVIRUSES, MASTERS IN JUMPING BETWEEN INTESTINAL, RESPIRATORY AND IMMUNOLOGICAL TARGETS - AN EVOLUTIONARY GAME
Hans NAUWYNCK

SESSION 7: CURRENT CHALLENGES INSIDE EUROPE

Chairs : Thierry Morin & Béatrice Grasland - Amphitheatre

15:00 C37 - Eugénie Bagdassarian - **Modulation of the host interferon response by the nonstructural polyprotein ORF1 of hepatitis E virus**

15:15 C38 - Justyna Miłek - **Gamma- and deltacoronaviruses in wild birds in Poland**

15:30 C39 - Thomas Brun Rasmussen - **Variations in the full-length genome sequences of porcine epidemic diarrhoea virus strain CV777 as determined by NGS**

15:45 C40 - Sarah Gallien- **Comparison of the horizontal transmission of a French InDel strain and a US non InDel strain of porcine epidemic diarrhea virus**

16:00 C41 - Sandra Barroso-Arévalo - **Immune system: an essential approach to the study of infectious diseases in honey bee colonies**

16:15 C42 - Geneviève De Keyser - **Detection algorithms in syndromic surveillance: a use case for animal rendering data**

SESSION 8: THREATS AT THE EUROPEAN BORDERS

Chairs : Labib Bakkali & Donald King - Room 1

C43 - Pascal Hudelet - **Can we eradicate FMD? An industry perspective**

C44 - Aurore Romey - **Use of lateral flow device for safe and cost-effective shipment of FMDV suspected samples**

C45 - Julia Henke - **Protection against transplacental transmission of Classical swine fever virus using live marker vaccine "CP7_E2alf**

C46 - Sandrine Blaise-Boisseau - **FMDV- Host interaction in a model of persistently infected bovine cells**

C47 - Wataru Yamazaki - **Highly sensitive detection of PEDV in environmental samples using a new virus concentration technique**

C48 - Céline Bahuon - **Separate determinants of West Nile virus virulence in mammals and birds**

16:30 CONCLUSIONS
Wim van der POEL, Coordinator Epizone
Stéphan ZIENTARA & Marie-Frédérique LE POTIER, Scientific Committee, ANSES, France

Keynote speakers

11th EPIZONE Annual Meeting
Crossing Barriers
Paris, France

Peter Mertens

Prof. Peter Mertens studied Virology at Warwick and Oxford Universities in the UK and as a Post-Doctoral Research Fellow at the University of Guelph in Canada in 1979. He returned to the UK in 1981, working at the UK's National Virology Centre, at the Pirbright Institute in Surrey untill 2016.
His group at Pirbright developed diagnostic assays and epidemiology studies that have been widely used to help identify and track the bluetongue outbreaks within Europe and elsewhere since 1998, contributing to the eradication of BTV-8 from the region in 2008-2009. His group also played a major role (with he Structural Biology Group in Oxford) in determination of the atomic structure of the bluetongue virus core-particle by X-ray crystallography, still one of the largest single molecular structure determined to date. In 2016 Prof Mertens became 'Chair of Virology' at the School of Veterinary Medicine and Science, University of Nottingham, establishing a new Orbivirus Research Group and coordinating a major new EU H20:20 grant 'PALE-Blu' concerning bluetongue virus, involving 19 partner institutions across Europe, the Middle East and Africa. Professor Mertens has written over 240 scientific papers on these viruses and has supervised over 30 Ph.D. students. He is a Visiting Professor in the Department of Veterinary Medicine at the University of Glasgow; the University of Minas Gerais in Belo Horizonte in Brazil; and at LUVAS Veterinary University in Hisar, India. Prof Mertens is an OIE Bluetongue expert and a member of the Reoviridae Study Group for ICTV

The emergence, persistence and evolution of bluetongue viruses in Europe since 1998*

Bluetongue virus (BTV) is the type species in the genus *Orbivirus* within the family *Reoviridae*. It is also an icosahedral virus, approximately 80 nm in diameter, with a three layerd protein capsid containing ten dsRNA genome-segments.
BTV is an arbovirus transmitted by biting midges (*Culicoides spp.*) causing 'bluetongue' (BT), an economically important disease of ruminants, that can be fatal, particularly in naïve sheep and some deer species (e.g. white tailed and brocket deer). At least 27 distinct serotypes of BTV have been identified to date, the identity of which is controlled by the specificity of interactions between major BTV-outer-capsid protein VP2 and the neutralising antibodies generated during infection of the vertebrate host. Consequently the BTV serotype is an important factor in vaccine strain selection and control strategies. BTV can be typed by serum neutralisation tests but these are slow (weeks). However, sequence analyses of genome segment 2 (coding for VP2) has shown variation that correlate with virus serotype. This has led to development of serotype specific qRT-PCR assays, for more rapid and reliable typing the virus, for surveillance and control programmes.
Both genome segment 2 and the rest of the BTV genome, also show sequence variations that reflect the geographic origin of the virus isolate (topotype). Consequently full genome sequencing of novel outbreak strains can be used to not only to type the virus but also to determine the origins of each genome segment and reveal reassortment events that have occurred during the evolution of each virus strain.
Using these techniques it is clear that there have been new incursions of BTV into Europe almost every year since 1998, involving multiple different strains and a total of 9 different serotypes (BTV-1, 2, 4, 6, 8, 9, 11, 14 and 16) arriving from either eastern, or western (African) origins, resulting in the deaths of many thousands of animals, trade restrictions and massive losses to the livestock industries. These novel typing methods have also helped to identify several additional 'novel' serotypes, including BTV-25 and 26 within Europe.
The first BT outbreak recorded in the naïve ruminant population of Northern Europe, was caused by a western strain of BTV-8 starting in the Netherlands in 2006. This was also the largest outbreak that occurred in the region, although it was effectively controlled by vaccination and the virus appeared to have been eradicated by 2010. However the same virus strain re-emerged in central France in 2015 and continues to circulate, although apparently with much reduced virulence.
Results concerning the emergence, persistence, and evolution of BTV in Europe will be presented and discussed. A new database providing information and sequence data concerning BTV is being constructed at: http://btv.glue.cvr.ac.uk

* Peter Mertens & Kyriaki Nomikou, The School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonnington UK.

Jeroen Kortekaas

Jeroen Kortekaas, PhD, is a senior scientist of Wageningen Bioveterinary Research (www.wur.nl/bioveterinary-research), located in Lelystad, the Netherlands. His laboratory studies exotic arboviruses in cell culture, laboratory animals, target animals and insects. His key expertise is vaccine development, with a primary focus on Rift Valley fever virus.

Rift Valley fever: Unanswered questions and unmet needs

Rift Valley fever virus (RVFV) is a mosquito-borne virus of the newly established order Bunyavirales (family Phenuiviridae, genus Phlebovirus) [1]. RVFV is highly pathogenic to domesticated ruminants, of which pregnant ewes and newborn lambs are the most susceptible. Humans can become infected via contact with tissues from infected animals or via mosquito bites. These infections generally result in acute, self-limiting febrile illness, whereas a small percentage of patients develop meningitis or hemorrhagic fever, of which the latter may have a fatal outcome [2]. RVFV was isolated from more than 20 mosquito species belonging to 10 different genera, of which specific species of the genera Aedes and Culex play a dominant role in transmission. Although the virus is currently confined to Africa and the Arabian Peninsula, susceptible livestock and mosquito species that are associated with transmission in endemic regions are globally widespread, explaining the fear for new outbreaks in previously unaffected areas.

Research on RVFV biology, epidemiology and pathogenesis has contributed significantly to our understanding of RVF epizootics and epidemics, but important knowledge gaps remain. Examples include the molecular basis of vector competence and the role of infected mosquito eggs and wildlife in the maintenance of the virus during interepidemic periods. Apart from filling knowledge gaps, there is an urgent need for more effective control strategies. Whereas vaccines are available in some African countries, these do not optimally combine efficacy with safety and logistical means to rapidly deploy these vaccines are lacking. As several highly promising next-generation vaccines are currently under development [3-6], the next task is to determine how these vaccines can be effectively applied in the field to prevent future epidemics.

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[6] H.M. Weingartl, C.K. Nfon, S. Zhang, P. Marszal, W.C. Wilson, J.C. Morrill, G.E. Bettinger, C.J. Peters. Vaccine, **32**:2345-2349 (2014).

Tsviatko Alexandrov

Dr Tsviatko Alexandrov is actually working as Head of the Animal Health Department of Bulgaria. Since 2005 he has been directly involved in all epidemics of infectious diseases which have occurred in Bulgaria including the LSD epidemics in 2016, the outbreaks of FMD in domestic livestock and wildlife in 2011, BT in 2014, CSF, AI, Newcastle disease and many others. He has passed through the pre-accession period of Bulgaria to the EU and was involved in the transposition of the EU legislation in the field of Animal Health. He is responsible for the animal health control and surveillance programmes and contingency plans of his country. In Sept 2011 – Mar 2012 he worked as a short term professional at the European Commission for the control of Foot-and-mouth disease (EuFMD) at FAO, later contracted for a numerous of projects and member of the EuFMD Special Committee for FMD Research & Programme Development. He was a contract manager for the Bulgarian food safety agency for the project ASFFORCE 311931 – 7th Framework Programme, Targeted research effort on African swine fever. Meanwhile he had been working with the EFSA Panel on Animal Health and Welfare on scientific opinions on FMD and SGP. In 2014 he defended a PhD on CSF and FMD epidemiology. Interested and experienced in wildlife health and management. He is a focal point for OIE wildlife and retired officer of the air forces of the Bulgarian Army.

Lumpy Skin Disease epidemics in Europe and preventive and control measures for an effective control of the disease*

Lumpy skin disease (LSD) is a high-impact pox disease of cattle caused by *Capripoxvirus* (the family *Poxviridae*). The disease is characterized by fever, skin nodules, lymphadenitis, lachrymation and nasal discharge and occasionally death. Substantial cattle production losses are typical of the disease and control and eradication measures are of substantial financial burden for cattle farmers, local veterinary services and governments.
Biting and blood-feeding vectors transmit the virus mechanically over short distances and cattle movements are usually associated with both short and long distance spread of the disease. Direct contact is not believed to be very efficient way to transmit the disease but may occur. Contact with contaminated material such as fresh skins and hides has been identified as a risk.
LSD is endemic in most African countries from where it has steadily spread northwards. In 2012-17 LSD has been reported from most Middle Eastern countries, Turkey, Greece and Bulgaria, the Balkans and the Northern Caucasus region.
On 13th April, 2016 Bulgaria reported the first ever outbreak of LSD in the country. Within three months, the disease spread throughout the country. The national strategy for limiting and controlling the spread of LSD included early detection, prompt implementation of total stamping out policy and vaccination of the whole cattle population in the country. In view of the several transmission pathways, strict controls of cattle movements, intensified clinical surveillance, vector control and desinsectation activities were also applied, supported by education and awareness campaigns. The last outbreak was reported on 1st August 2016. The combination of measures proved to be effective to limit the spread of the disease.

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Damyan Iliev, Petya Petkova, Georgi Chobanov, Anna Zdravkova and the whole BFSA team; European Commission, particularly Francisco REVIRIEGO, Dimitrios Dilaveris, Christophe Bertrand, Nicolas KRIEGER; The veterinary authorities of Greece, FYR of Macedonia, Serbia, Hungary; Nadav Galon, Kris De Clercq, the EuFMD team, Alessandro Broglia, Andriy Rozstalnyy and Daniel Beltran Alcrudo for all their hard work and constant support.

* Tsviatko Alexandrov[1], Eeva Tuppurainen[2] & Alexandra Miteva[1]
[1] Bulgarian Food Safety Agency
[2] Independent Veterinary Consultant for lumpy skin disease, sheeppox and goatpox

Arnaud Bataille

Arnaud Bataille is a researcher in virology at CIRAD, Montpellier, France, focusing principally on the Peste des petits ruminants virus *(PPRV). He investigates the transmission and evolutionary dynamics of PPRV with the aim of elaborating better control strategies. He contributes to the development of new diagnostic tools and of vaccines against PPR. He is deputy head of the OIE/FAO and EU reference laboratories for PPR at CIRAD.*

Peste des Petits Ruminants: state of affairs and the road towards eradication*

Peste des petits ruminants (PPR) is a highly contagious viral disease of small ruminants that causes mortality rates that may be as high as 90% in naïve populations. The disease is caused by *Peste des petits ruminants virus* (PPRV), which is an enveloped ribonucleic acid (RNA) virus with a monosegmented genome of negative sense, belonging to the genus Morbillivirus in the family *Paramyxoviridae*. This virus has only one seroptype but can be separated in four distinct phylogenetic lineages.

PPR is currently present in Africa, Middle East, and Asia. It represents a serious risk for the economy and food security in regions the disease is endemic. With multiple outbreak recorded in Turkey, Georgia and the Maghreb region, it is now at the door of Europe. In addition, a recent mass die-off of critically endangered Saiga antelopes in Mongolia has been associated with PPR infection, bringing a new light onto the impact of this pathogen on wildlife.

Inspired by the eradication of closely related Rinderpest virus, the World Organisation for Animal Health (OIE) and the Food and Agriculture Organisation (FAO) launched in 2015 a global campaign for the eradication of PPR. This effort will rely on massive vaccination campaigns based on widely-used, live attenuated vaccines. The campaign requires an important financial investment, but will clearly be highly economically and socially beneficial.

Here, we will review the knowledge of PPR epidemiology and the threat of this disease to Europe. We will discuss recent and on-going research of major importance for the success of the eradication campaign, notably on vaccine development, transboundary infection dynamics, and the role of wildlife in PPR transmission. Finally, we will examine the major gaps in our knowledge of this disease and how those could hinder the global eradication effort.

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Christianne Bruschke

Christianne Bruschke graduated in 1991 as veterinarian and started working in a mixed private practice in the north of the Netherlands. In 1993 she joined the Virology department of the Central Veterinary Institute in Lelystad where she did a PhD on pestivirology and was heading the laboratory for bovine virology from 1994 on. After the classical swine fever outbreak in the Netherlands she was seconded for a year to the Inspection Service for Livestock and Meat to support the updating of the Dutch contingency plans. A year later she returned to Lelystad as head of the unit Notifiable Diseases and 2 years later she became director of the Division of Infectious Diseases. In 2005 joined the Ministry of Agriculture and was seconded to the OIE where she led the global Avian Influenza Programme. In 2008 she returned to The Hague as deputy Chief Veterinary Officer and since 2010 she is the Chief Veterinary Officer of The Netherlands. She is the primary adviser to the Minister of Agriculture on all veterinary and veterinary public health issues.

The EU animal health policy, now and in the future*

The risk of introduction and spread of animal diseases has changed over the years due to globalisation, climate change and also due to changes in animal husbandry. Developments towards more intensive farming as well as a developments towards more extensive farming with a focus on animal welfare are found. The risk of introduction of animal diseases in the latter may be bigger however the consequences of introduction of a disease in the intensive husbandry may be bigger.
The current EU animal health legislation has been developed over many years and since the establishment of the common market, legislation has been extended with many new directives, regulations and decisions. The risks of outbreaks of animal diseases, zoonotic diseases, risks for human health coming from the agricultural sector and the one health concept receive increasing attention from professionals in the sectors as well as from society. The package of legislation that was developed over a long period was not appropriate anymore in the changing situation.

In 2007 the EU adopted a new modernised animal health strategy which was the basis for new animal health legislation in the EU which came into force in 2016 and will become applicable in 2021. The whole animal health legislation will be brought under one coherent framework together with legislation concerning trade, registrations, prevention etc.
Collaboration between EU member states is essential in animal health management. One of the basic principles of the new legislation is that prevention is better than cure whereas another principle is that risk-based policies will be implemented. In this risk based approach, assessments should be made to indicate whether animal diseases need to be regulated at EU level to be able to protect the Union against introduction of diseases or further spread within the EU. The new legislation will also lead to further implementation of the one health concept by focussing on collaboration between the different fields.
In the coming period, EU wide strategic discussions will take place at many different levels to further develop the secondary legislation (e.g. listing of animal diseases, specific disease eradication measures, trade issues etc.) where after the EU member states have 2 years to implement this in their national legislation.

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Remco den Dulk

Remco den Dulk is a system architect at CEA-Leti (Grenoble, France) specialized in lab-on-chip systems. He is leading a small team of specialists to integrate biological protocols of our clients and partners into an automated microfluidic format and is responsible for the development of technology building blocks and their integration into functional prototypes. He holds a MSc degree in physics from Delft University of Technology, a PhD degree from Eindhoven Technical University and has worked at various high-tech companies (Nanoledge, Philips Research, Vescent Photonics, Polymer vision, Effect Photonics) in The Netherlands, France and USA.

Flowpad, a generic microfluidics platform for a wide range of applications

The need for miniaturization and integration of laboratory procedures into fully automated lab-on-chip devices is ever-increasing. FlowPad is a generic microfluidics platform that responds to this need by providing a transportable development platform for automated sample-to-answer systems targeting a wide range of applications.

The Flowpad system consists of single-use microfluidic cartridges and an associated instrument. Pneumatically actuated valves and pumps are integrated in the polymer cartridges to direct fluids through channels and reaction chambers, which potentially contain embedded reagents. The instrument includes various hardware modules, such as pumping, heating and optical detection.

Within the wide range of applications that are addressed by our Flowpad development platform, pathogen detection takes an important place. In such applications, the sample preparation steps consist typically of concentration, purification and mechanical lysis of the microorganisms present in the sample. For the subsequent detection of the released bacterial DNA, various amplification techniques are used, such as qPCR, LAMP and RPA. Without intervention of the user, this system thus effectuates all steps from the raw sample to the answer.

George F. Gao

Gao is Director-General of Chinese Center for Disease Control and Prevention and professor of Institute of Microbiology, Chinese Academy of Sciences. His research interests include enveloped viruses and molecular immunology, mainly focusing on the enveloped virus entry and release, esp. interspecies transmission (host jump) influenza virus and coronaviruses. His research has recently expanded on public health policy and global health strategy. Gao has been elected as a member/ fellow of several academies, including Chinese Academy of Sciences and The Third World Academy of Sciences (TWAS). Gao is a recipient of several international and national awards, including TWAS Medical Prize (2012) and Nikkei Asian Prize (2014).

AVIAN INFLUENZA VIRUSES: FROM EPIDEMIOLOGY TO HOST JUMP

The emergence of avian influenza viruses has posed great public health concern worldwide. H5N1 subtype avian influenza virus (AIV) was first found in 1996 in China. Since then, human H5N1 infections have been continued reported and it has caused at least 859 human infections including 453 deaths. In 2013, a novel H7N9 AIV emerged, which has been regarded as the most dangerous AIV in the world, has caused at least 1557 human infections with 605 deaths until now. Of note, H7N9 has been evolved from low pathogenicity to high pathogenicity recently. In addition to H5N1 and H7N9, other AIVs such as H10N8 and H5N6, emerged and caused sporadic human infections. Here, we have performed the epidemiological surveillance of H5 and H7 subtype avian influenza viruses in China and showed the dynamics of virus genome evolution. The receptor binding property of virus is a major determinant for the host tropism, which enables interspecies transmission. The receptor binding specificity of influenza virus is determined by the viral hemagglutinin (HA) and we summarize recent crystallographic studies that provide molecular insights into HA-host receptor interactions that have enabled several influenza A virus subtypes to 'jump' from avian to human hosts.

Timm Harder

Timm Harder, Germany: «Highly pathogenic avian influenza"
Timm Harder is a veterinary virologist with a broad general interest in influenza viruses ranging from diagnostic improvements, molecular epidemiology, and pathogenicity, to applied preventive measures. He combines in-field and experimental approaches to study the evolution of and to deduct improved control measures against animal influenza viruses. He works at National Reference Laboratory for Avian Influenza, at Friedrich-Loeffler-Institut, in Germany.

Swarm incursion: A review on the 2016/7 highly pathogenic avian influenza virus epizootic in Europe

In autumn of 2016 an incursion of highly pathogenic avian influenza virus (HPAIV) of subtype H5N8 was first noted in a mute swan in Hungary. This event marked the start of an HPAIV epizootic among wild birds and poultry in most European countries that was unprecedented in its geographic extent (reaching, apart from Europe, also Western and Southern Africa) and in the number of wild bird species affected. Originally believed to be a homogenous virus population derived from Central Asian sources of HPAIV of the H5 goose/Guangdong (gs/GD) lineage, clade 2.3.4.4b, in-depth genetic studies revealed the presence of several different genotypes. At least five different reassortants were described so far of which two also include shifts in the neuraminidase subtype: N5 (various European countries) and N6 (Greece). Experimental infections in gallinaceous and anseriform poultry species confirmed a high virulence of these viruses also for Pekin ducks. However, no increased zoonotic potential was deduced from genetic analyses and ferret infection studies. A two peak course of the epizootic was evident in wild birds in Germany showing maxima in December 2016 and late February 2017. The first wave mainly affected diving duck species such as Tufted ducks and Common pochards. During the second wave Mute swans, various gull species and birds of prey dominated the species spectrum. The outbreaks receded during April but sporadic cases both in poultry and wild bird were still detected throughout June 2017. Poultry populations were severely affected in several countries including Hungary, Poland, Germany and France, amongst many others. Primary cases in poultry are believed to have been caused by direct or indirect contact with HPAIV derived from the infected wild bird population. However, secondary outbreaks in poultry resulting from farm-to-farm spread were reported as well.
The latest and largest outbreak of gs/GD-like viruses in Europe prompted a reconsideration of AI-preventive measures aiming in particular at limiting risks of incursion into poultry holdings. Effective biosecurity measures that maximize bio-exclusion and bio-containment are compromised by species-specific needs of commercial poultry housing and by an increasing market demand for poultry products derived from outdoor rearing systems. Further episodes of gs/GD-derived HPAI outbreaks must be expected to occur in Europe in the future, at least as long as new viruses of this lineage continue to evolve and spread within and from their epicenters in Southeast and Central Asia. Avoiding the establishment of an endemic status of these viruses in European poultry populations remains the ultimate goal of preventive and acute control measures.

Niels Jørgen Olesen

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Exchange of pathogens between farmed and wild fish*

Fish farming conditions usually imply very close contact and co-existence between farmed fish and a large number of wild fish species, sharing water and pathogens. Disease management in fish farming therefore is obliged to take environmental factors into consideration. But the extent and effect of disease interaction and pathogen exchange between wild and farmed fish populations is an area of research that is difficult to explore. By means of molecular epidemiology a number of studies on the transmission of the most important viral pathogens between farmed and wild fish populations have, however, been conducted.

Viral haemorrhagic septicaemia is a devastating notifiable viral disease in farmed rainbow trout in Continental Europe. In the last decades more than 100 wild fish species have been found to carry various genotypes of VHS virus and mutations of these viruses into high virulent isolates seem to be the origin of this disease, the disease appears to be introduced as few single events by feeding infected raw fish material. Also direct transfer of VHSV from wild fish to farmed turbot, Japanese flounder and cleaner fish as wrasse and lumpfish has been observed in Europe and East Asia. In the coastal waters of British Columbia, Canada, VHSV and infectious hematopoietic necrosis virus (IHNV) occur naturally in multiple species of wild fish. Upon introduction of Atlantic salmon, exotic to BC, into open net-pen marine aquaculture sites, these viruses have sporadically crossed species barrier causing disease and losses to the Atlantic salmon aquaculture industry.

Piscine reovirus (PRV) is widely distributed in both farmed and wild Atlantic salmon, and is believed to be ubiquity present in the Northern Atlantic. Under farm condition the virus can cause "heart and skeletal muscle inflammation (HSMI) a serious disease for the salmon industry. The role of farmed and wild fish in the transmission of the virus will be illustrated.

Infectious salmon anaemia (ISA) caused by an orthomyxovirus is also widely present in both wild and farmed Atlantic salmon. A low virulent wild type of the virus, ISAV HPR0, is present both in the wild and under farm condition, causing a sub-clinical transient gill infection. Under farming conditions the virus will from time to time evolve into a highly virulent HPRΔ form causing a systemic disease with high mortalities with very significant consequences for the industry.

Viral encephalopathy and retinopathy (VER) caused by a nodavirus infection appear to be a bottleneck for the sea bass production in the Mediterranean basin. The virus is present in both wild and farmed fish all over the area. Recently significant mortalities affecting wild fish species were reported in southern Italy and Tunisia. Phylogenic analysis revealed closed relationship of the isolated viruses with nodavirus from nearby sea bass farms, suggesting a persistent circulation of betanodaviruses and transmission between wild and farmed stocks.

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Aleksija Neimanis

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The case of Rabbit Haemorrhagic Disease Virus 2

Rabbit Haemorrhagic Disease (RHD) is a disease of wild and domestic European rabbits (*Oryctolagus cuniculus*) that typically manifests as acute, necrotizing hepatitis. RHD is caused by closely related, pathogenic lagoviruses of the Family *Caliciviridae*, namely Rabbit Haemorrhagic Disease Virus (RHDV) and the antigenic variant RHDVa, and the recently described RHDV2 or RHDVb. While RHDV2 has many similarities to RHDV/RHDVa, it has crossed several significant barriers, resulting in its rapid emergence in Europe and beyond.

RHDV2 has was first documented in France in 2010 and quickly spread to Italy and the Iberian peninsula, and then to northern Europe and more widely. Geographic barriers have posed little challenge, as RHDV2 has been detected on numerous islands including Great Britain, the Azores and the Canary Islands. In 2015, it jumped continents to Australia where it continues to spread. In 2016, it was also reported in a local outbreak in Canada. Rapid geographic spread is likely facilitated by the highly contagious nature of the virus, its persistence especially within organic material, and effective indirect spread via contaminated material carried by insects, other animals and humans.

RHDV2 was first detected in France after it caused RHD in domestic rabbits vaccinated against RHDV. Concurrent outbreaks were also seen in immunologically naïve wild rabbits. Because RHDV2 is antigenically distinct from RHDV, development of new vaccines specific for RHDV2 was necessary. While presumptive diagnosis of RHD can be made based on clinical signs and pathology, confirmation of RHDV2 requires analyses specific for RHDV2, as many diagnostic tests for RHD do not differentiate between viruses. RHDV2 is continuing to evolve and pathogenicity has increased. Continued monitoring for detection of new subtypes and regular evaluation of vaccines and diagnostic tools are therefore warranted.

Perhaps one of the most significant barriers that RHDV2 has crossed is that of host species. RHDV is believed to be species-specific, generally causing disease only in European rabbits while the related lagovirus European Brown Hare Syndrome Virus (EBHSV) causes similar disease in hares. RHDV2, however, has caused RHD-like disease in four different hare species to date. Cases range from sporadic, spillover events to more common infections depending on species involved and other epidemiologic factors. RHDV2 has also crossed an important age barrier. Unlike RHDV where susceptibility to disease begins from about 5 weeks of age, RHDV2 has the capacity to cause clinical disease and death in rabbits less than 5 weeks old.

Finally, there is a proposal by lagovirus researchers to break down another barrier- that of historical, but poorly adaptable, nomenclature. Given the continued characterization of new lagoviruses, the blurring of host species boundaries, and the increasing recognition of recombinant strains, a new, more robust lagovirus nomenclature has been proposed (Le Pendu et al, in press, Journal of General Virology). Within this proposal, all lagoviruses are grouped within a single species and RHD viruses fall within a single genogroup (GI). RHDV and RHDVa are grouped within the GI.1 genotype and RHDV2/b is classified within the GI.2 genotype.

Hans Nauwynck

Hans Nauwynck is Doctor in Veterinary Medicine who did his PhD and became nominated as professor at Ghent University in 1993. In 2004, he took the lead of the Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University and became Diplomate at the European College of Pig Health Management. At present, he lectures several courses on viral diseases in mammals, fish and shellfish. His research focuses on cellular and molecular pathogenesis of viral diseases in man and animals, with special emphasis on (i) the entry of the virus in its host cell, (ii) the invasion of the virus in its host through barriers, via leukocytes and along neurons and (iii) the escape of the virus from immunity. Better insights lead to research-driven development of new generation treatments and vaccines. He is (co-)author of 330 peer reviewed papers and owner of several patents. He is a regular invited speaker on international congresses.

Coronaviruses, masters in jumping between intestinal, respiratory and immunological targets - an evolutionary game

Coronaviruses belong to the Order of the Nidovirales. They have a primary tropism for intestinal and/or respiratory epithelial cells and as a result of their replication they may cause local damage, resulting in intestinal and respiratory problems. Besides, they may infect cells of the monocyte lineage. Normally, they do not replicate productively in these cells, but exceptionally they do and cause internal lesions.
The first barrier that they have to cross is the respiratory and intestinal mucus. Work in our laboratory demonstrated that enveloped viruses normally get stuck in the mucus by their charges and only when the bonds are enzymatically cleaved, they can spread further. Beautiful examples are neuraminidases and esterases in influenza- and coronaviruses. When viruses do not possess these enzymes, they need other tricks to reach and infect epithelial cells. Own work on feline enteric coronavirus resulted in a hypothesis that the sialic acid binding domain on the spike binds/detaches alternatively in a cis-trans manner with sialic acids that are present on the viral spike itself and sugars on top of the enterocytes. By doing so, the virus moves slowly into the firm layer of sugars, finding its way to the internalization receptor on the enterocytes. Although the mucins in the enteric tract and respiratory tract differ, the sugar trees that are on top of the protein core are similar. Therefore, we believe that the coronaviruses may penetrate both enteric and respiratory mucus layers in the same way. Therefore, mucus layers are most probably not responsible for the mucosa specificity of coronaviruses.
By changes in the spike, the virus may jump from enterocytes to the respiratory epithelial cells. A beautiful example is transmissible gastroenteritis virus (TGEV) that by a deletion in its spike switched its tropism from intestinal to respiratory tissues. The deleted virus was called porcine respiratory coronavirus (PRCV). The emergence of PRCV induced a population immunity that squeezed TGEV out of the pig population. Important in this context is the common mucosal immunity and the appearance of protective sIgA in the milk of the sow.
Several coronaviruses are able to target cells of the monocyte lineage and by doing so, they may eventually replicate in this type of cells and cause internal problems. A first group consists of viruses with originally an enteric tropism. Feline enteric coronavirus is a prototype. It replicates mainly in the intestinal tract and due to immune evasion, it persists for months-years. This allows the virus to accumulate mutations and to switch from an enterocyte tropism into a monocyte tropism. The consequences are disastrous. The virus causes pyogranulomas in serosae in which monocytes are the driving force behind the vicious circle of viral infections. Similar switches from local enteric tract to the entire body are more recently seen with coronaviruses in other carnivores, such as dogs, ferrets and minks. A second group of viruses consists of viruses with a respiratory tropism. Infectious bronchitis virus (IBV) is a prototype. Most IBV strains replicate in the respiratory tract. Monocytes may be infected but without progeny. However, certain strains succeed in replicating productively in these cells allowing the virus to spread and further replicate in internal organs such as kidneys leading to nephritis and high mortality. The human coronavirus MERSV is demonstrating a similar pathogenesis in certain human patients. Kidney involvement is causing a bad prognosis.
The abovementioned examples clearly demonstrate the very restless character of coronaviruses in their interaction with their hosts. Together with their species jumps and recombination games, coronaviruses can be considered as one of the most dangerous virus species in the world.

Oral presentations

11th EPIZONE Annual Meeting
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ORAL PRESENTATIONS
Session 1 - Arthropod-borne diseases (arboviruses)

C1: Novel topotypes of bluetongue serotype 3 viruses in the Mediterranean Basin

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Bluetongue viruses identified as belonging to serotype 3 (BTV-3) were isolated for the first time in Israel, from blood sample of diseased sheep in 2016. Initial identification was achieved by conventional pan-BTV-16 RT-PCR analysis, targeted to conserved regions of segment 2 (Seg-2). An additional BTV-3 was identified from archived virus isolate, which was isolated from blood sample of clinically ill sheep in 2013. Three BTV-3 isolates (one from 2013 and two from 2016) were sequenced and phylogenetically analyzed.

Analysis of Seg-2 sequences from different samples and their products (VP2 outer capsid protein 1) revealed that the identity between Israeli isolates was of 99.25-99.44% and 99.36-99.68% [nucleotide (nc) and amino acid (aa) sequences, respectively]. Israeli isolates were found to be closely related to the Cypriote BTV-3 isolate RSArrrr/03 (identity of 95.95-96.08% and 98.06-98.21% correspondingly). The Tunisian BTV-3 strain identified in 2016 was found to be closely related to BTV-3 from Zimbabwe (ZIM2002/01) with identity 98.4% and 99.18% correspondingly. Comparison of the same segment and its putative product (Seg-2 and VP2) from the Tunisian TUN2016 strain and Israeli isolates revealed identity of 93.93-94.1% and 97.2-97.27%.

Surprisingly, phylogenetic analysis of Seg-6 and its product (VP5 outer capsid protein 2) from the three Israeli BTV-3 isolates (nc and aa identity of 99.76-99.81% and 100%, respectively) and Tunisian TUN2016 BTV-3 strain (identity of 95.41-95.59% and 99.81% to Israeli isolates), were closely related to the Nigerian BTV-16 isolate (NIG1982/10) (identity of 96.52-97.00% and 99.81-100%). Of note, Seg-6 of above cited BTV-16 Nigerian strain is closely related to that of the ZIM2002/01 BTV-3 strain (98.23% and 100%), although their Seg-2 fragments are relatively distant (70.77% and 74.32%). Other Israeli and Tunisian BTV-3 genome segments showed wide nc and aa sequence variations.

The emergence of BTV-3 in Israel and in Tunisia shows the dynamic of spreading of BTV-3 into new regions. The high homology between BTV-3 Israeli/Tunisian viruses and Nigerian BTV-16 NIG1982/10 is a demonstration of the ongoing process of re-assortment, which, along with point mutations, contributes to the emergence of new BTV-3 topotypes.

C2: First detection of a *Mesonivirus* in *Culex pipens* in five countries around the Mediterranean basin

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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 Europe, mainly in France, Italy and Spain, 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. Like *Wolbachia* bacteria, such viruses could have a role in WNV epidemiology either limiting mosquito populations or modulating the chances of WNV infection of *C. pipiens.*
Through metagenomics approach, we characterized the virome of *C. pipiens* in five countries around the Mediterranean basin

Our results show for the first time conserved patterns in diversity and structure within the virome of *C. pipiens* with a large abundance of the *Mesoniviridae*, a relatively new family of mosquito-specific viruses in the *Nidovirales* order. To date, the few data available on *Mesoniviridae* show that they probably have a worldwide distribution and they can infect different species of mosquitoes. The sequences obtained allowed to assemble several full genomes of *Mesoniviridae.* Interestingly, phylogenetic analysis showed a highly similarity with strains of *Nam-Dinh* virus, a virus recently identified in Côte d'Ivoire (West Africa) and Vietnam but which had not yet been observed in Europe. In view of the importance of the Mesoniviridae dispersion throughout the world, we determined the relative abundance of this family in natural population of *Culex pipiens* in Camargue.

C3: Pathological modelling of Tick-Borne Encephalitis Virus infection using primary human brain cells derived from fetal neural progenitors.

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Tick-Borne Encephalitis Virus (TBEV), a member of the *Flaviviridae* family, is the most important arbovirus of health interest in Central-Northern Europe and North-Eastern Asia (1). It is responsible for febrile illness, and in a subset of cases, for neurological manifestation ranging from mild meningitis to severe encephalomyelitis (2). Although the primary route of infection is through the bite of an infected tick, alimentary infection through the consumption of TBEV-contaminated dairy products has also been documented (3). So far, TBEV-induced neuro-pathogenesis is poorly understood. To decipher the mechanisms by which the viruses damages the human brain, we used an *in vitro* model of primary neural cells differentiated from human fetal neural progenitors (hNPCs). Our results showed that neurons and glial cells are susceptible and permissive to TBEV infection. The virus replicates in a dose-dependent manner and impairs the two cellular types. Neurons first form clusters and gradually die. At 72 hours post-infection (*pi),* 25% of them were lost and, later in the course of infection, at 14 days *pi*, the loss had reached 70 percent. At that time, neurites had dramatically shrunk. Neuronal loss occurred, at least partly, through an apoptotic mechanism, as shown by cleaved caspase 3 and TUNEL-positive staining. On the contrary, astrocytes survive to the infection but a hypertrophy, suggestive of a reactive stage was observed. Analyzing the antiviral response, we further report an overexpression of viral sensors (RIG-I/TLRs,...), cytokines (CXCL10,11,...), and innate immune response factors (IFN-I/ISGs,...), indicating a strong activation of the antiviral response. Thus, our results show that the *in vitro* model we developed recapitulates the main events that occur in the human brain (4). We are currently using this model to go deeper in our understanding of TBEV-induced neuro-pathogenesis and to screen for antiviral drugs.

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C4: Interactomic high-throughput mapping for bluetongue virus in its natural hosts to identify new factors of pathogenicity and interspecies transmission

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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 belonging to the *Culicoides* genus. BTV induces a large panel of 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 studied extensively, we still have little understanding of the molecular determinants of BTV virulence.

We took advantage of functional proteomic approaches such as high-throughput yeast two-hybrid (Y2H) to map interactions between BTV and cellular proteins. As a starting point, this approach has been applied on two serotypes of BTV (BTV-8 and 27). These serotypes have been chosen for their recent (re)-emergence and differences in term of pathogenicity/interspecies transmission: only BTV-8 induces clinical signs in cattle whereas BTV-27 infects exclusively goats without causing any symptoms. All viral proteins encoded by BTV were used as baits to screen two cDNA libraries originating from cattle and *Culicoides*. Therefore, 43 Y2H screens were performed and 1,478 positive yeast colonies were analyzed, allowing us to identify a hundred of new virus-host interactions (Figure 1). A preliminary global analysis of these interactions has uncovered many signal transduction factors involved in the modulation of autophagy, apoptosis and the ubiquitin-proteasome system. Moreover, most of the BTV-host interactions identified by Y2H seem to be specific to either viral serotype and/or infected host. These cellular interactors are currently re-tested by several protein-protein interaction methods such as GPCA (Gaussia princeps protein complementation assay) and at functional level with the viral proteins encoded by BTV-8, BTV-27 and also other serotypes like BTV-1 and 4. Conserved protein interactions will be instrumental to design generic drugs against multiple serotypes. On the other hand, interactions that are highly specific of a particular virus would shed light on the molecular mechanisms responsible for its virulence/pathogenicity and transgression of cross-species barriers.

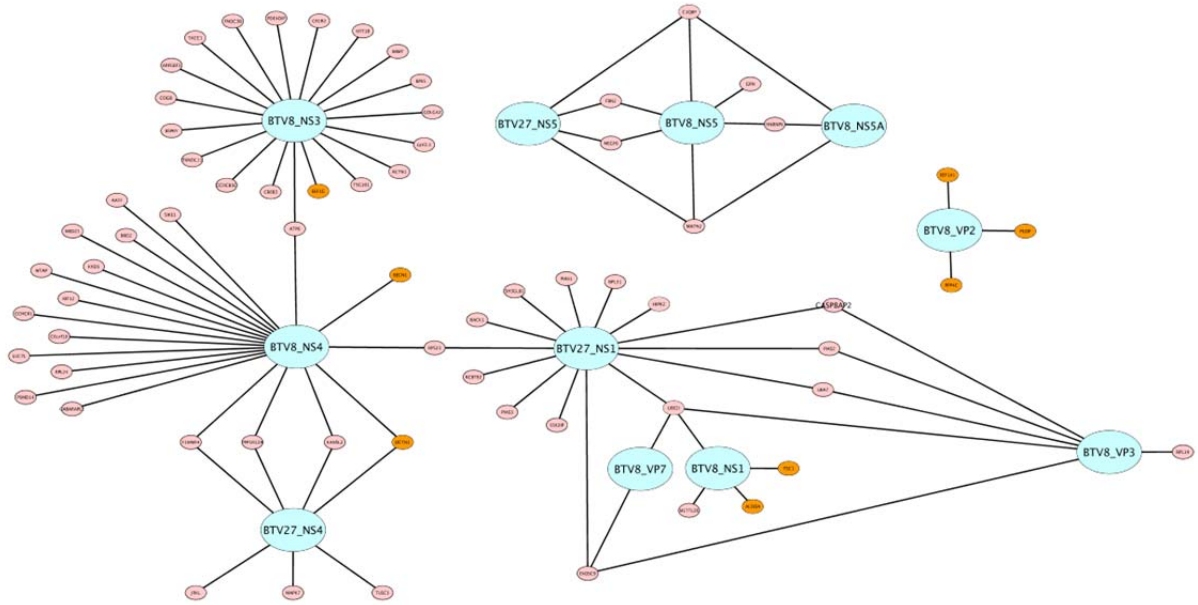

Figure 1: Mapping BTV-host interactions. Interactions were displayed using Cytoscape network analysis tool. Viral baits are shown in blue whereas bovine and *Culicoides* cellular preys are shown in pink and orange, respectively.

C5: Tick-borne pathogens detection in Caribbean ticks using high-throughput microfluidic real-time PCR (DOMOTICK Project)

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Among hematophagous arthropods, ticks transmit the greater variety of pathogens of public health and veterinary importance whose (re)-emergence is recognized worldwide. Whereas the main human and animal tick-borne pathogens are well characterised in the Northern hemisphere, very few is known concerning the diversity of tick species and tick-borne pathogens circulating within the Neotropical zone of the Americas, especially concerning the Caribbean area. Most of the epidemiological data on the topic are based on old records and focused on the main livestock pathogens such as Ehrlichia ruminantium, Babesia (bovis and bigemina) and Anaplasma marginale. These observations underline the need to develop high throughput diagnosis methods that will allow us to conduct large scale epidemiological surveys required to better anticipate the risk of (re)-emergence of tick-borne disease in such areas. In this context, the DOMOTICK project was designed to develop a new high-throughput real-time PCR method for a large scale screening of tick-borne pathogens potentially circulating in the Caribbean. This technology is based on high-throughput microfluidic real-time PCRs using Taqman probes (BioMarkTM dynamic arrays, Fluidigm Corporation), allowing the simultaneous detection of up to 95 pathogens across 95 samples of ticks (Michelet *et al*, 2014). The choice of pathogens included in this new high-throughput technology was based on a comprehensive analysis of the literature, and on a without a priori detection of new or unsuspected pathogens by RNA-sequencing on nucleic acids extracted from ticks collected in Guadeloupe and Martinique. NGS analysis suggests that these ticks may harbour more pathogenic microorganisms than the currently monitored 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 ; 14 parasites species, belonging to the genera Babesia, Theleria, Hepatozoon, Leishmania, Rangelia vitalii, Cytauxzoon felis ; and 32 arboviruses mainly belonging to viral genus of Orthobunyavirus, Phlebovirus, Nairovirus, Asfivivirus, Thogotovirus, Flavivirus, Coltivirus and Orbivirus. The high-throughput real-time PCR technology developed here have been validated on tick samples collected in Guadeloupe and Martinique. Finally, this new high throughput method will allow exploratory epidemiological studies on tick-borne pathogens circulating within Caribbean ticks collected on various vertebrate hosts through some Caribbean islands, such as Trinidad and Tobago, St Kitts, Barbados, St Lucia, and Cuba, thanks to the CaribVet network, and to local veterinarians.

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C6: Propagation in the presence of favipiravir leads to selection of RVFV variants with attenuated phenotype in mice

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Rift Valley fever virus (RVFV) is a mosquito borne bunyavirus causing an important disease in ruminants often transmitted to humans after epizootic outbreaks, thus being a very relevant pathogen both for animal and human health. Although the disease has been reported only in African countries with some incursions in the Middle East, there is some concern for its potential to spread to other geographical areas, including Europe. Currently there is no available treatment or licensed RVF vaccine in Europe, therefore the development of effective control strategies, both novel vaccines and new antivirals intended also for human use is an active field of research. Type-I Interferon-related treatments as well as experimental antivirals drugs have proven to be efficient in controlling RVFV infection both in vitro and in animal models. One of these drugs is favipiravir (T-705), already proved to display a potent antiviral activity against many RNA viruses. Because of its high therapeutic index and the few works reporting selection of resistant viruses, favipiravir appears as an advantageous drug for the treatment of several RNA virus infections.

In order to gain insight into the mechanism of action of favipiravir the virulent South African RVFV isolate 56/74 was subjected to serial passages in Vero cells in the absence or presence of different concentrations of favipiravir. At the highest doses tested, virus extinction occurred. Lower doses led also to apparent extinction, however viral infectivity could be recovered after further passaging. Virus isolated in these conditions showed a decreased sensitivity to favipiravir, different plaque size on mammal cells, impaired growth in C6/36 mosquito cells and attenuation in mice.

Our results suggest that a favipiravir-resistant RVFV variant showing a number of different features was selected. Further results on the characterization of this virus as well as its potential use for an efficient control of Rift Valley fever will be presented and discussed.

Acknowledgements
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Session 2 - Current challenges inside Europe

C7: Risk of introduction of Lumpy Skin Disease in France by the import of cattle

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Background: The lumpy skin disease (LSD) virus belongs to the genus *Capripoxvirus* and causes a disease in cattle with economic impacts. In November 2014, the disease was first reported in Europe (in Cyprus); it was then reported in Greece (in August 2015) and has spread through different Balkan countries in 2016. Although indirect vector transmission is predominant in at-risk areas, long-distance transmission usually occurs through movements of infected cattle.

Methods and principal findings: In order to estimate the threat for France, a quantitative import risk analysis (QIRA) model was developed according to international standard (OIE, 2004) and to assess the risk of LSD being introduced in France by imports of cattle. Based on available information and using a stochastic model, the probability of a first outbreak of LSD in France following the import of batches of infected live cattle for breeding was estimated to be between 4×10^{-5} and 3.3×10^{-3} (in 95% of cases).

Conclusion and significance: The development of a stochastic QIRA made it possible to quantify the risk of LSD being introduced in France through the import of live cattle. This tool is of prime importance because the LSD situation in the Balkans is continuously changing. Indeed, this model can be updated to process new information on the changing health situation in addition to new data from the TRAde Control and Expert System (TRACES, EU database). This model is easy to adapt to different countries and to other diseases.

Acknowledgements
This study was performed by an ad hoc working group of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). The authors thank Tuppurainen E, Maartens L, Antoniou SE, and Chobanov G for their critical advice provided during the risk assessment.

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C8: Belgian introduction and re-emergence of HP H5N8 clade 2.3.4.4.

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In Belgium, the first introduction of the HP clade 2.3.4.4. H5N8 virus occurred at the end of January with an outbreak in hobby poultry. This first wave of HP-H5N8 cases ended with a wild bird outbreak in a bird refuge center at the end of March. Compared to other European countries, the first introduction arrived tardily and comprised only a limited number of outbreaks saving the Belgian poultry sector.

After two months of silence, the virus re-appeared, again in hobby poultry, in the South of Belgium. Simultaneously, not far from the Belgian re-emergence site, the Great duchy of Luxembourg declared their first time introductions of the HP clade 2.3.4.4. virus into 3 small local hobby flocks small poultry flocks.

Serological and virological data gathered from surveillance of wild birds during and after the first outbreaks demonstrated the seroconversion and viral clearance by some wild bird species (*Anas platyrhynchos*) illustrating the risk of silent circulation inside the population, resulting in reappearance and possibly endemication of the disease, a worrying evolution for the poultry sector.

C9: Identification of a Novel Reassortant of a Mammalian Orthoreovirus in Faeces of diarrheic pigs in the Netherlands

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In the spring of 2015 pigs in a farm in the province of North Brabant in the Netherlands suffered from diarrhea. Inspection of diseased pigs by a veterinarian did not reveal overt clinical signs other than severe diarrhea. A pooled feral sample from the pig herd was diagnosed PCR-positive for porcine epidemic diarrhea virus (PEDV). A 0.2 µm filtered 10% w/v suspension of feces was used to inoculate Vero cells grown in medium containing trypsin to facilitate PEDV infection. In this Vero cell culture an unknown viral agent induced a cytopathogenic effect (CPE) that differed from the typical CPE induced by PEDV, i.e. large area syncytia's forming. The viral agent induced clumps of rounded cells that detached from the monolayer within 16h after inoculation. A similar CPE was observed when Vero cells were inoculated without trypsin. Next Generation Sequencing (NGS) was used to characterize this novel trypsin-resistant viral agent. Culture medium harvested from passage 2 of the Vero cell culture was concentrated and used for isolation of total RNA followed by preparation of a cDNA library and sequencing of this library in a single NGS (MiSeq) run. NGS reads were annotated by BLAST analysis querying all virus sequences in GenBank. BLAST analysis showed that RNA segments of the Mammalian Orthoreoviruses (MRV) were the dominant sequences in the medium from the Vero cell culture. For all 10 MRV segments a consensus sequence was assembled from NGS-reads and compared to existing MRV sequences posted in GenBank. Sequences of L1–L3, M1–M3, and S2-S4 segment of our novel MRV variant were highly homologous to sequences of serotype 1 and 3 MRV strains isolated from pigs. The S1 segment of our novel MRV showed a relatively low homology to S1 segments of these serotypes, including to the S1 segments of MRV3 strains recently isolated in the USA and Italy from pig feces scoring PCR-positive for PEDV. In fact, most homology was found to S1 segments of serotype 2 MRV strains isolated from humans and bats. The relevance, in relation to PEDV pathogenesis, of this novel MRV variant with a serotype 2 S1 segment is discussed.

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C10: Case investigation of unexplained infectious meningoencephalitis in animals - a new rotavirus in horses?

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Investigating infectious neurological disorders in animals and in humans is challenging; 15 to 20% of nervous diseases in horses are caused by bacteria, viruses or parasites, but in more than two thirds of investigated infectious neurological equine cases in France, no common pathogen (such as type 1 herpesvirus, West Nile virus, ...) can be detected. The RESPE, the French network for epidemiological surveillance of equine diseases, investigates between 49 and 85 neurological cases a year. With a high-throughput sequencing approach, we sought to characterize the pathogens present in the nervous tissue or the cerebro-spinal fluid in horses suffering from meningoencephalitis and reported to the RESPE. In July 2014, a 4-year-old trotter presented severe hyperthermia, jaundice and nervous disorders acutely evolving to death. Encephalitis was of viral etiology, as suggested by histology. RNAs present in the cerebrospinal fluid (CSL) and the cortex of this horse were sequenced by NGS and the complete genome of an animal rotavirus could be recovered. The identified rotavirus was genetically close to avian type A rotaviruses (94.2, 96.0 and 97.1% nucleotide homology with avian rotavirus A isolates for segments 2 (VP2), 8 (NsP2) and 11 (NsP5 and 6) respectively). The rotavirus was infectious and one isolate could be obtained after serial passages in MA104 cells. Moreover, immuno-histochemistry performed on fixed brain slides with anti-NsP3 antibodies undoubtedly confirmed rotavirus infection of inflammatory cells in the horse brain.

High-throughput sequencing identified an avian-like rotavirus capable of infecting horses with an unusual tropism for the central nervous system. Experimental infections will be performed to assess the neuroinvasive properties of the isolated rotavirus strains in mammals.

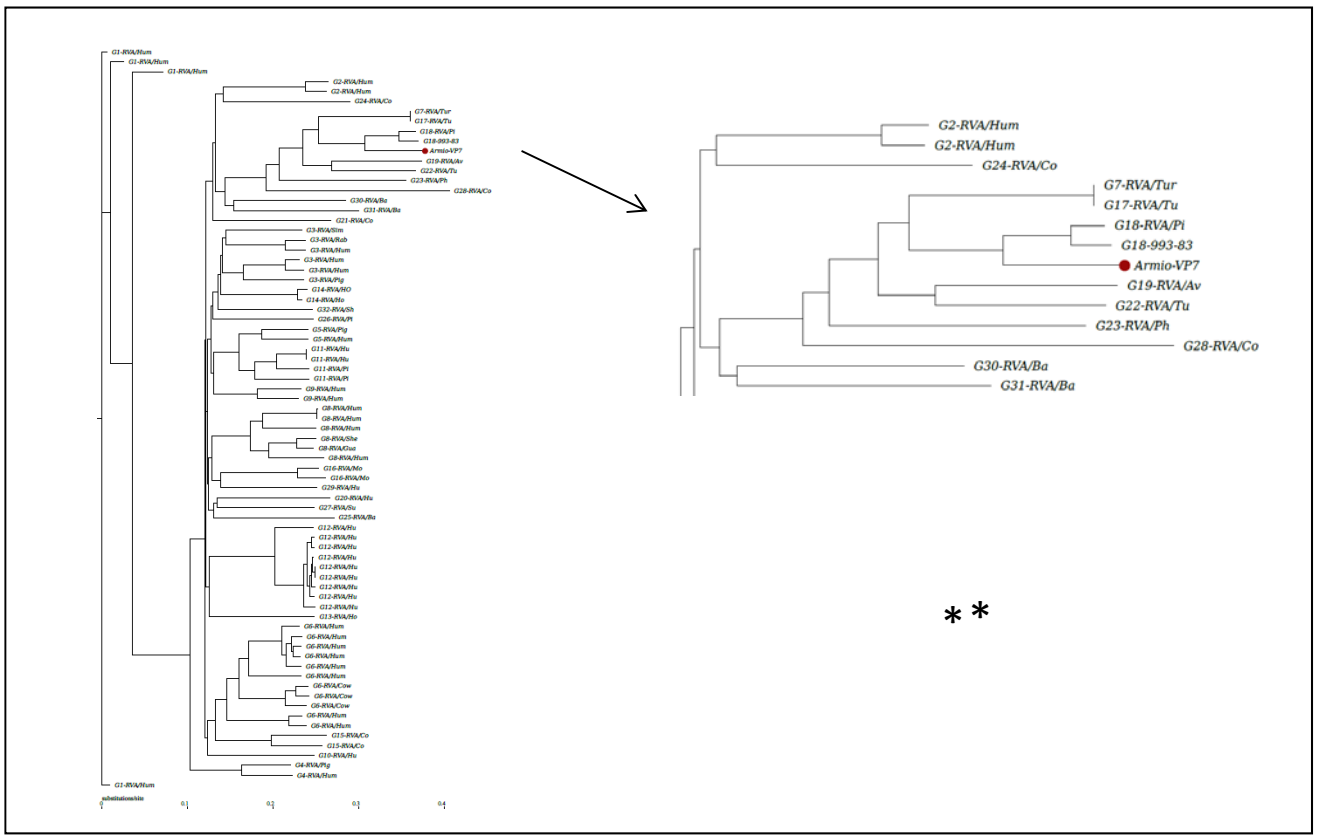

Figure 1: VP7 phylogeny of the avian-like rotavirus evidenced in the brain of a 4-year old trotter (Armio) in France, performed with RotaC$^{2.0}$automated genotyping tool for Group A rotaviruses (Maes et al, 2009) and immuno-labeling of rotavirus-NsP3 expressing cells (asterisks) in inflammatory lesions from Armio's brain

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C11: Novel subunit vaccine based in the E2 glycoprotein fused to porcine CD154 prevents classical swine fever virus vertical transmission in pregnant sows

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Classical swine fever (CSF) is one of the most important swine diseases, its etiological agent CSF virus (CSFV) belongs to the Pestivirus genus, Flaviviridae family. CSFV its remains endemic in South and Central America, the Caribbean region, Asia, and in some Eastern European countries. The routine vaccination for disease control mainly by using the live attenuated CSFV vaccines such as the lapinized C-strain is still carried out in these zones. Paradoxically, despite the intensive vaccination programs, CSF has not been controlled. This has been attributed to failures in the responses to vaccination, for example, quality and availability of the vaccine, and gaps in the cold chain, etc. Considering the complex epidemiology situation in endemic areas, the development of a safe vaccine candidate capable of inducing a strong viral protection, able to protect from the CSFV trans-placental transmission, is a priority for disease control. Thus, here we evaluated the effect of double vaccination in prevent CSFV vertical transmission in pregnant sows with a new subunit marker vaccine candidate based in the CSF viral envelope E2 glycoprotein fused to the porcine CD154.

A lentivirus-based gene delivery system was used to obtain a stable recombinant HEK 293 cell line for the expression of E2 fused to porcine CD154 molecule. Six pregnant sows at 64 days of gestation were distributed in two groups. Animals 1 to 4 (group 1) were vaccinated via intramuscular inoculation with 50 µg of E2-CD154 subunit vaccine. Group 2 (animals 5 and 6, control animals) were injected with PBS. Seventeen days later group 1 sows were boosted with the same vaccine dose. Twenty-seven days after first immunization, the sows were challenged with virulent CSFV (Margarita strain). Samples were collected to evaluate immune response and virological protection. Between 14-18 days after challenge, sows were euthanized and sera and tissues samples were obtained from foetuses.

Vaccinated animals remained clinically healthy until the end of the study; also, no adverse reaction was shown after vaccination. An effective boost effect in the neutralizing antibody response after boost-immunization and challenge was observed and protection against CSFV vertical transmission was found in the 100% of serums samples from foetus. Only two out of 208 samples (0.96%) were positive by qRT-PCR with Ct values around 36.

Besides, its DIVA potential and protection from vertical transmission, the novel CSFV E2 bound to CD154 subunit vaccine, is a promising alternative to the live-attenuated vaccine for developing countries.

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C12: Lumpy skin disease (LSD) vaccine evaluation using an optimized infection model.

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Lumpy skin disease is a pox disease of cattle (genus Capripox) characterized by fever and nodules on the skin. The disease can cause a reduction in milk production, sterility in bulls, abortion and damage to hides. This leads to significant loss of income but also to malnutrition. The disease, originally affecting cattle across Africa, has spread outside the continent with outbreaks in Israel, Egypt and Lebanon in 2012-13 and a current epizootic in the Middle-East, Turkey and has entered mainland Europe in 2015. Vaccination is the only effective way to control this vector-borne disease. However, disease-free countries hesitated to use live attenuated vaccines, currently the only types commercially available, on safety grounds and due to trade restrictions for live cattle and cattle products. Furthermore, data on efficacy and safety (side effects) are very scarce and only under field conditions which makes the choice of the most suited vaccine difficult. In order to address this problem a number of LSDV-based live attenuated vaccines were evaluated and compared under standardized conditions using an optimized challenge model. Clinical data and samples (Blood [EDTA, clotted, heparin], buccal swabs and biopsies, tissue and organ samples) were collected during the complete duration of the trial. The clinical data were analyzed and scored enabling the comparison of the animals on an individual and on a group level based upon a total clinical score. Different virological and immunological (humoral and cell mediated) parameters were followed using real-time PCR, sequencing, virus isolation, Immuno-peroxidase monolayer assay, virus neutralization, IFNg assay and LSDV ELISA. In general the LSDV-based live attenuated vaccines gave good clinical protection combined with a strong antibody response. Differences among the vaccines were seen for the seroconversion onset, the rate at challenge, and the IFNg reactiveness. For all vaccines tested a limited fever reaction was seen after vaccination around 7/8 dpv and the fever spike after challenge could not be completely blocked. A Neethling-like disease was observed after vaccination but before challenge in one analyzed vaccine. All the clinical and lab data were statistically analyzed in order to allow a final comparison of the vaccines regarding efficacy and safety.

Acknowledgements
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11th EPIZONE Annual Meeting
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Session 3 - Arthropod-borne diseases (arboviruses)

C13: Impact of Bluetongue virus on the MAPK/ERK pathway

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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. In the laboratory we study the effect of BTV on different cell signaling pathways. In addition to its antagonist role on type I interferons pathway, we have revealed a new function carried by the BTV-NS3 protein as a positive modulator of the MAPK/ERK pathway responds to growth factors such as EGF (Epidermal Growth Factor) and activates various phosphorylation events leading in particular to the activation of translation initiation factor 4E (eIF4E) and Elk-1 transcription factors involved in cellular survival, proliferation and differentiation (Figure 1A). Interestingly, due to its essential role in cell fate, the MAPK/ERK pathway is hijacked by many viruses. The use of the U0126 inhibitor known to block the phosphorylation of ERK proteins by MEK proteins, allowed us to refine the action level of the BTV-NS3 protein on this pathway, namely downstream of the EGF receptor and upstream or directly on the MEK1/2 proteins. We also measured NS3 expression in BTV-infected cells treated or not with U0126 and observed a strong reduction of NS3 detection in cells treated with the inhibitor suggesting that BTV replication is downregulated upon blocking MEK function (Figures 1B-1C). This demonstrates that MAPK/ERK signaling is essential for the expression of BTV proteins and suggests that BTV manipulates this pathway for its own replication. We could also show that the intrinsic ability of NS3 to activate the MAPK/ERK pathway is conserved throughout multiple BTV serotypes. In contrast, this activity is greatly reduced when using the NS3 protein from Epizootic Hemorrhagic Disease Virus (EHDV), another orbivirus. The MAPK/ERK pathway could be activated at several cellular compartments including the plasma membrane, Golgi apparatus and endosomes. A mutant form of BTV-NS3 protein ($NS3^{ALL131\text{-}133EEE}$), which lacks the viroporin activity and Golgi apparatus localization, is no more able to activate the MAPK/ERK signaling pathway. Whether the viroporin activity or Golgi targeting is important for MAPK/ERK activation is under investigation. Moreover, by using mutant forms of BTV-NS3 protein, we could not find any link between the activation of the MAPK/ERK pathway and the inhibition of the type I interferons pathway. The activation of the MAPK/ERK pathway by NS3 could be of benefit for BTV to ensure its own viral translation but could also explain the hyperinflammation observed in case of BTV infection. 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 to both increase MAPK/ERK signaling and inhibit type I interferons signaling.

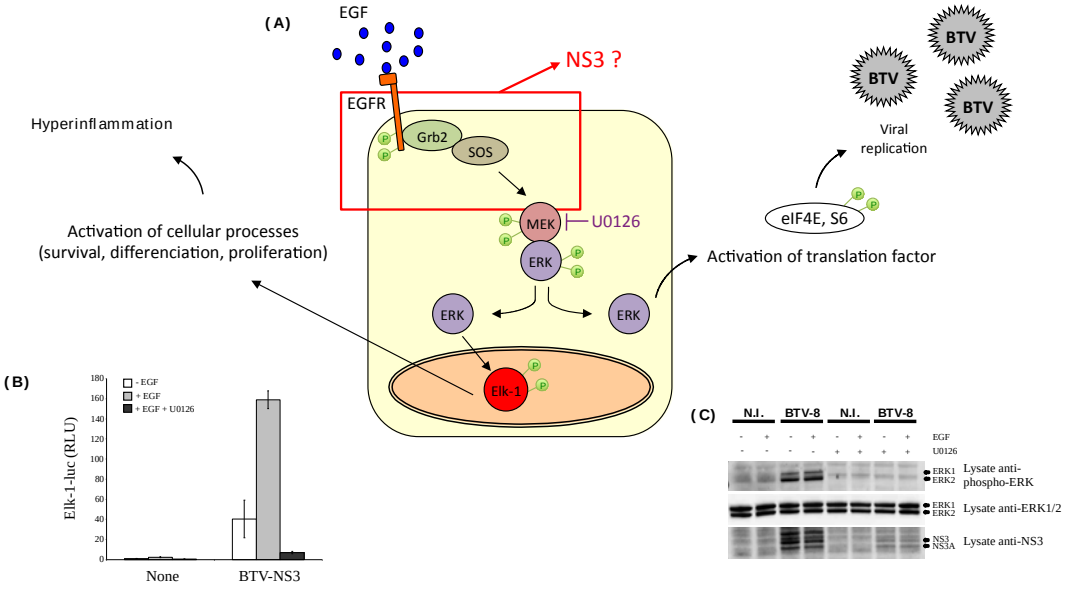

Figure 1 : **Potentiation of the MAPK/ ERK signaling pathway by the BTV-NS3 protein of serotype 8 (BTV-8).**
Schematic representation of MAPK/ERK signaling pathway **(A)**. After Epidermal Growth Factor (EGF) stimulation, MAPK/ERK signaling pathway is activated and regulates multiple cellular processes including proliferation, differentiation, survival and protein translation. Activation of the MAPK/ERK pathway was assessed upon EGF stimulation using a Elk-1 luciferase reporter assay **(B)** and/or anti-phospho-Erk Western blot **(C)** in cells overexpressing NS3 **(B)** or in BTV-infected cells **(C)** treated or not with the U0126 inhibitor.

C14: A wolf in lamb's clothing: Detection and isolation of a new infectious Bluetongue serotype from commercial vaccine

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Bluetongue virus is considered endemic in Israel since the 1940's and more than 10 different serotypes were identified from cattle and livestock (sheep and goats) during the last decade. In this report we describe the detection and isolation of a new Bluetongue (BT) virus serotype from batches of commercial vaccine. Routine vaccine inspection performed at the Kimron Veterinary Institute identified the presence of BT RNA in commercial batches of Sheeppox (SPV) and Lumpy skin disease (LSD) vaccines. Diagnostic PCR tests identified the presence of NS3 and VP1 genes. The sample obtained from the LSD vaccine was identified as BTV9, a serotype not yet identified in field samples from Israel. Despite repeated attempts, this sample could not be propagated in embryonated chicken eggs (ECE) or in cell culture, but a part of the BT9 VP2 gene was successfully sequenced. The sample obtained from the SPV vaccine was successfully propagated in cultured cells and in ECE. In order to identify the serotype of the isolated virus, the nearly-complete sequence of 6 segments (genes VP1, VP4, VP6, VP7, NS2 and NS3) was determined. The results indicated that the isolated BTV serotype was closely related, but different, from BT26, identified in Kuwait in 2011, suggesting that it is a new serotype, not yet described. Since its virulence and transmission properties are not known, controlled infection experiments were commenced. Preliminary results from the infection experiment show that the virus successfully propagates in healthy 8-months old sheep, with detectable viraemia 6-7 days post-infection. Further *in vivo* studies are currently underway. This study highlights the importance of rigorous vaccine inspection and provides genetic and clinical information on the newly-identified Bluetongue virus serotype.

C15: Prospects of African horse sickness DISA vaccine platform based on deletion of 77 amino acids in NS3/NS3a protein

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African Horse Sickness (AHS) is disease of equids with a mortality rate of up to 95% in naïve domestic horses, and is therefore on the list of notifiable diseases according to the World Organisation of Animal Health. The causative pathogen African horse sickness virus (AHSV) is a virus species in the genus *Orbivirus* of the family *Reoviridae*. Currently, nine AHSV serotypes have been defined showing limited cross neutralization. AHSV is transmitted by species of *Culicoides* biting midges and has become a serious threat for countries outside Africa, since *Culicoides* species endemic in a moderate climate are competent vectors for the closely related bluetongue virus. AHS outbreaks will result in large economic losses to the equestrian industry, and will have an enormous emotional impact on owners of pet horses in the developed world.

Conventionally live-attenuated AHS vaccines are commercially available, although these have several shortcomings. Therefore, research aiming improved AHS vaccines have been initiated, in particular with regard to safety and Differentiating Infected from VAccinated individuals (DIVA). Recently, reverse genetics for AHSV has been developed for liveattenuated AHSV. This research has also resulted in a new generation of AHS vaccine candidates, such as a platform for Disabled Infectious Single Animal (D ISA) vaccines.

Here, reverse genetics for virulent AHSV5 was developed as previously described for live-attenuated AHSV (van de Water et al., 2105). Virulence and non-virulence of these 'synthetically derived' AHSV strains was confirmed in horses. Further, prototypes of AHS DISA vaccine platforms were developed based on mutations in genome segment Seg-10 abolishing NS3/NS3a expression. Deletion of 77 amino acids codons in Seg-1 0 of fully virulent AHSV5 resulted in completely non-pathogenic, non- virulent AHSV in horses. AHS DISA vaccine candidates were further studied in horses for viremia, efficacy and Differentiating Infected from VAccinated individuals (DIVA). AHS DISA vaccine was not detected by panAHSV PCR tests adn cannot be detected by a newly developed Seg-1 0 panAHSV PCR test. AHS DISA vaccination will not raise antibodies targeting the immunogenic dominant region of NS3/NS3a protein (DIVA; Lavaida et al., 1995). Vaccination with AHS DISA vaccine candidates reduced mortality and viremia caused by lethal AHSV challenge, however, protection should be further improved. Since vaccine dose and virus backbone appeared important for seroconversion, there is ample room to increase the efficacy. Yet, the here presented DISA vaccine platform showed partial protection against lethal AHSV, whilst many important vaccine criteria are fulfilled, i.e. no adverse effects, no vaccine spread, applicability for all serotypes (van de Water et al., 2015), low production costs, DIVA, and safe use in cocktail vaccines making broad or prophylactic protection feasible. To fulfil remaining vaccine criteria, more expensive and ethically debatable horse trials are required. Ideally, one type of AHS vaccine should be developed which is acceptable and payable in different parts of the world and for different kind of horses.

C16: Antigenic mapping and interaction of recent Italian West Nile and Usutu viruses

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West Nile virus (WNV) and Usutu virus (USUV) are the most widespread mosquito-borne flaviviruses in Europe. Both viruses exist in multiple lineages (Lin) and the E protein mediates viral attachment as well as viral fusion to host cells; thus, most neutralizing antibodies (Ab) are generated against epitopes on this protein. Here, we investigated the interaction between WNV and USUV viruses at the antigenic level in vitro and in vivo. We also characterized the pathogenicity and infection dynamics of representative WNV and USUV isolates in mice.

Materials and Methods. A total number of 33 field strains isolated in Italy between 2008 and 2015, including seventeen WNV Lin1, eight WNV Lin2 and eight USUV strains, were sequenced and administered, after inactivation, to rabbits by an intramuscular route to obtain hyperimmune serum for each isolate. Homologous and heterologous serum-neutralization (SN) assays were performed. Quantitative analyses of the antigenic distances between strains were achieved using antigenic cartography. Low and high doses (HD/LD) of three Italian WNV isolates including one Lin1 isolate of 2008 (15803/2008, FJ483549), one Lin1 of 2012 (20652/2012, Livenza-like strain) and one Lin2 of 2012 (20168/2012) were then used in independent challenge experiments. Groups of 3-week-old (wo)-CD1 competent mice were first exposed to HD or LD of an USUV isolate (12543/2010, KX555624) followed by intraperitoneal (IP) inoculation with one of the WNV strains. Survival curves of 3wo-CD1 mice administered with the same WNV (LD) and USUV (HD and LD) isolates were also calculated. Furthermore, 3wo-CD1 mice were administered with a LD (WNV) or LD and HD (USUV) of the isolates and sacrificed, starting from 3-day-post infection (dpi), every two days to detect viral antigen and quantify viral RNA in internal tissues by immunohistochemistry (IHC) and real time RT-PCR (qPCR), respectively. Three-wo interferon α/β receptor knockout mice (IFNAR-/-) were also IP infected with a LD of the USUV isolate and sampled from 1 dpi.

Results. A 3D antigenic cartography map (**Fig1**) has been successfully created and compared with phylogeny of the same isolates. Antigenic clustering was evidenced according to viral species regardless of Lin or year of incursion. In-vivo studies showed that starting from 6 dpi, LD WNV-infected CD1 mice succumbed within 11 dpi regardless the WNV isolate. All LD and HD USUV-infected CD1 mice survived the infection with no clinical signs and USUV RNA was never detected during the experiment. Previous infection with HD USUV resulted in 86% survival following HD challenge (group HD/HD) with WNV Lin1 15803, and 73% survival following HD challenge with WNV Lin1 20652 and WNV Lin2 20168; LD-WNV challenges (group HD/LD) resulted instead in total protection. Previous LD exposure to USUV resulted in 100% protection if followed by a HD challenge (group LD/HD) with WNV Lin2 20168 and 94% protection if followed by HD challenges with WNV Lin1 15803 or WNV Lin1 20652. Interestingly, 90% of succumbed mice in group HD/HD died within 2 dpi. A statistically significant difference between group HD/HD and LD/HD and between HD/HD and HD/LD has been, indeed, observed. Differences in the infection dynamics in competent mice have not been detected among the different WNV strains. The highest WNV RNA copy numbers were evident in the brain and in the spinal cord (up to 10^7 RNA copies Log10) starting from 5 dpi. LD infected-IFNAR -/- mice succumbed or were euthanized within 5 dpi. High RNA copy numbers were detected in mice sampled at 3 dpi (up to 10^6 RNA copies Log10 in the spinal cord), before the appearance of signs. **Discussion.** In this study, we showed that **i)** robust measurement of antigenic variation among selected flaviviruses shows close correlation between genetic and antigenic variation; **ii)** although distant in the antigenic map, previous exposure to LD of USUV confers almost full protection from lethal challenges with different WNVs; **iii)** further analysis is currently ongoing to unravel if preexisting USUV immunity exacerbated WNV infection in some mice; **iiii)** the pathogenicity and dynamic of infection was similar within the isolates of the two viruses regardless Lin or year of isolation.

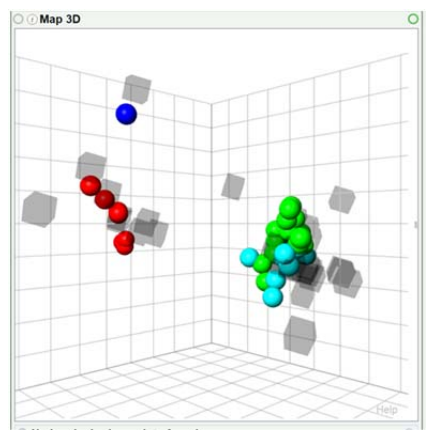

Figure 1: Red, USUV; Blue, JEV; Green, WNV Lin1; Light Blue, WNV Lin2.

Acknowledgements

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C17: Characterization of serological cross-reactivity between mosquito-borne flaviviruses co-circulating in Europe.

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In the last years, different mosquito-borne flaviviruses are co-circulating in Europe (1) and differential diagnosis of these viruses is crucial for surveillance and control. Serological diagnosis is difficult due to antigenic cross-reactivity among members of the flavivirus genus. Therefore, serological assays must be interpreted with care and confirmed by comparative virus-neutralization using the viruses known to circulate in the study area. However, this confirmation method is not completely specific, since some degree of cross-neutralization still exists between different flaviviruses even at this level.

To determine the level of serological cross-reactivity (including cross-neutralization) between West Nile (WNV), Usutu (USUV) and Bagaza (BAGV) viruses, which are known to co-circulate in game birds in Southern Spain (2), we analysed sera obtained from red-legged partridges experimentally infected with these three flaviviruses.

Samples were analysed by ELISA and virus-neutralization test. Two epitope-blocking commercial ELISA kits were compared for WNV detection (INGEZIM WNV COMPAC, from INGENASA, Madrid and ID Screen West Nile Competition Multi-species from ID-Vet, Montpellier) and virus-neutralization test (VNT) was performed in parallel with the three viruses by micro-VNT in 96-well plates. Cross-neutralizing antibodies detection was considered specific for a given virus when VNT titre was $\geq$4-fold higher than titres obtained for the other viruses.

The specificity for the detection of antibodies against WNV in the samples used in this work was higher for the INGEZIM WNV COMPAC kit than for the ID Screen kit. WNV diagnosis by VNT (considering in-parallel test with other flaviviruses) was more specific than the ELISA tests. Nevertheless cross-neutralization was observed not only between flaviviruses from the same serocomplex (WNV and USUV), but also between flaviviruses from different serocomplexes (WNV and BAGV; USUV and BAGV). Interestingly, comparatively VNT is highly sensitive and specific for the detection of WNV antibodies, but shows a very low sensitivity in the specific detection of USUV antibodies (with a high cross-neutralization with WNV).

These observations can imply important consequences in flavivirus surveillance because of the lack of specificity and cross-neutralization can limit differential diagnosis between flaviviruses. Furthermore, an underestimation of USUV prevalence can occur due to low sensitivity in the detection of specific antibodies, and consequently a high proportion of inconclusive results after VNT could be related to USUV infections.

Acknowledgements

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C18: Development of the typing assays for several BTV serotypes in Russia

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Among animal viruses, arboviruses are unique in that they are transmitted by blood-sucking arthropods (primarily mosquitoes and ticks) to vertebrates. The importance of arboviral infections has been illustrated by the dramatically increasing frequency of old and newly emerging arboviral disease problems. Bluetongue (BT) is vector-borne disease of severe economic consequence.

Twenty seven Bluetongue virus (BTV) serotypes have been identified in many tropical and temperate areas of the world, including some Europe countries, the United States and Russia. Identification of BTV serotype is important for vaccination programmes and for BTV epidemiology studies.

The outbreak of the BTV 16 serotype in the Republic of Buryatia in 1996 and outbreaks of the BTV 14 serotype in the Smolensk and Kaluga regions (2011-2012) have been identified in the territory of the Russian Federation. And in addition repeatedly seropositive animals were detected among imported livestock in 2008-2009.

The development of modern assays of identification and typing of the BTV serotypes which the most important in Russia was the main purpose of our work.

In the course of the work, we suggested a new scheme of laboratory diagnostic to identify the BTV serotype using molecular genetic methods. The nucleotype-specific RT-PCR assays were used for primary identification type of virus and to narrow the range of potential BTV serotypes. The second stage involves the accurately identification of the virus serotype using the serotype-specific oligonucleotide primers and DNA probes by Real-Time RT-PCR.

The analysis of the situation with BTV in the Russia Federation and neighboring countries, as well as countries, which export cattle to Russia, was allowed to determine the most epidemiologically significant virus serotypes for our country.

The quantitative RT-PCR test for identification of 27 serotypes, three PCR assays for identification nucleotypes A (4, 10, 11, 17, 20 and 24 serotypes), B (3, 13, 16 serotypes) and C (6, 14 and 21 serotypes) and the kits for identification of 1, 2, 4, 6, 8, 9, 11, 14 and 16 BTV serotypes by Real-Time RT-PCR were developed in VNIIVViM. Sensitive and specific nucleic-acid based typing assays have clear advantages in terms of speed, sensitivity and specificity over established serological tests.

Thus, the RT–PCR assays developed in this study provide a rapid, sensitive and reliable method for the identification and differentiation of the epidemiologically significant serotypes of the BTV in Russia, and will be updated periodically to maintain their relevance to current BTV distribution and epidemiology in the Russian Federation.

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C19: M-segment variation of Schmallenberg virus in malformed fetuses is the consequence of immune escape

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The insect-transmitted Schmallenberg virus (SBV), which emerged in 2011 in Central Europe, causes in adult ruminants no or only mild non-specific clinical signs for a few days but can induce premature birth, stillbirth, or severe malformations in the offspring when immunologically naïve animals are infected during a vulnerable period of pregnancy. SBV is the first European member of the Simbu serogroup of orthobunyaviruses and like in typical members of the genus *Orthobunyavirus*, Schmallenberg virions contain three segments of negative-stranded RNA genome. Within the M-segment of SBV, which encodes the viral glycoproteins Gn and Gc and the non-structural protein NSm, a region of high sequence variability is present in viruses detected in malformed fetuses. This region co-localizes with a previously identified key immunogenic domain which raises the question whether the high mutation rate is related to immune evasion mechanisms.

Here, the M-segment of selected SBV variants with point mutations only or with insertions of 2 amino acids or with in-frame deletions of 12, 138 and 204 amino acids, respectively, was analyzed. Mammalian cells were transfected with the different cloned M-segment cDNA plasmids of these SBV strains displaying nucleotide mutations, insertions or deletions, and the reactivity with a set of neutralizing and non-neutralizing SBV-specific antibodies was assessed. The first SBV isolate, which was initially isolated from a blood sample of a viremic cow in 2011, was used as reference strain.

The original SBV isolate reacted with each of the tested SBV-specific neutralizing antibodies while the virus strains displaying only a few amino acid substitutions in the M-segment could be stained by five out of six antibodies. Each strain with amino acid insertions or deletions within the hot spot of mutations did not react with any of the tested neutralizing anti-SBV antibodies clearly indicating that the observed sequence mutations within the N-terminal main immunogenic domain of glycoprotein Gc are related to targeted immune evasion.

In malformed fetuses exceptional high viral loads together with high antibody levels can be found suggesting a massive virus replication under the pressure of antibodies potentially leading to sequence adaptions within the relevant antigenetic SBV domains. However, these escape variants with a high mutation rate in the Gc-coding region are only observed in viruses present in fetuses whose mothers were infected during the vulnerable period of pregnancy. The viral genome detected in the blood of acutely infected, viremic animals is much more stable. As insect-transmitted viruses such as SBV have to adapt to two different hosts, namely the arthropod vector and the mammalian host, and undergo replication cycles in both hosts this high sequence stability of viruses detectable in the blood of acutely infected animals might be necessary for the transmission to the vector which is not the case for viruses present in malformed fetuses.

C20: Interest of phylogenetic data and specific diagnostic tools to support management and eradication of *Viral Haemorrhagic Septicaemia* and *Infectious Hematopoietic Necrosis* in France

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Oncorhynchus 1 and 2 novirhabdovirus are the causative agents of infectious hematopoietic necrosis (IHN) and viral hemorrhagic septicaemia (VHS) respectively. These severe aquatic diseases, which affect several fish species from fresh and sea water, are regulated in European Union in order to reduce the risks of dissemination by trade. In France, a monitoring and eradication plan (PNES) is currently being set up with the objective to obtain a free status for all the territory in the next 6 years. After a brief overview of the sanitary situation, this presentation aims to describe various actions carried out these past years but also currently in progress to improve the control of these diseases.

To better understand viral evolution and circulation, a Bayesian inference approach was applied to partial glycoprotein gene sequences of 88 Oncorhynchus 1 novirhabdovirus representative strains isolated in France over the period 1987-2015. The genetic diversity of these sequences showed mean nucleotide and amino-acid identities of 97.1 and 97.8% respectively and a viral population clustered into three groups with a clear spatial differentiation suggesting a predominant role of local reservoirs in contamination. Atypical "signatures" of some isolates underlined the usefulness of molecular phylogeny for tracking the spread of this virus. Similar approach was conducted with isolates of Oncorhynchus 2 novirhabdovirus.

To improve diagnostics, a “one-step” real time RT-PCR method specific to Oncorhynchus 1 novirhabdovirus adapted from Purcell et al. (2013) was validated. It includes an innovative strategy based on the use of a RNA bacteriophage as universal exogenous external control (EEC) for extraction and amplification steps. This method showed a high level of robustness with absence of cross reactions and will be transferred to the surveillance laboratories. More discriminative molecular tests are also under investigation with the objective to characterize the virulence associated to a novirhabdovirus strain to adapt sanitary actions. A potential target is the single amino acid change R116S in the NV non-structural protein of Oncorhynchus 2 novirhabdovirus, which we have recently demonstrated the key role in virulence for rainbow trout.

All these actions which sustain and consolidate the PNES will contribute to the improvement of the monitoring and control of fish novirhabdovirus on our territory.

C21: Microclimatic temperatures of Danish cattle farms: a better understanding of the variation in transmission potential of Schmallenberg virus

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Background: Insects inhabiting the surroundings of a cattle farm are exposed to microclimatic temperatures of the habitats surrounding the farm. Microclimatic temperatures are key drivers of the extrinsic incubation period (EIP), the speed by which an infected insect becomes infectious. The objective of this study was to quantify the variation of EIP of Schmallenberg virus among Danish cattle farms and identify possible spatial patterns of the EIPs.

Methods: We quantified 21 different land cover classes within a 500 meter radius of all cattle farms in Denmark (N=22092) using CORINE land cover and regrouped them into four major land cover types: dry meadow, wet meadow, hedges, and forest. We then obtained the meteorological temperatures and other parameters (solar radiation, wind speed, humidity) near the farm from the Danish Meteorological Institute (DMI) for the period of 2000-2016. Using recently developed microclimatic temperature prediction models for those four major land cover types, we calculated the hourly microclimatic temperatures of each farm based on their surrounding habitat types and meteorological parameters. We then modelled the daily EIP of Schmallenberg virus for each farm for each year of the period of 2000-2016 using both hourly DMI and hourly microclimatic temperatures and calculated mean EIP of 17 years for each farm. Finally, we plotted the average spatial pattern of farm level EIP for spring (May-June), summer (July-August) and autumn (September-October) in Denmark for the 17 years.

Results: Of the 22092 cattle farms, we were able to predict the hourly microclimatic temperatures of 22006 farms (99.6%) - the rest of the farms had habitats either not suitable for insects resting or the microclimatic model was not able to calculate the temperature of the surrounding land covers. We found a surprisingly large between-farm variation in EIP between farms on a specific day. For example, in the year 2016, the EIP of all farms varied (5^{th} and 95^{th} percentiles) from 9-19 days on May 1^{st}, 12-23 days on July 1^{st} and 11-21 days on September 1^{st} . The mean EIP of Schmallenberg virus [inter quantile range (IQR)] of all the cattle farms during spring, summer, and autumn for 17 years period were 16 [13-17], 15 [13-16] and 40 [38-42] days respectively, when using microclimatic temperatures. These estimated EIP values were much shorter compared to EIP estimated using DMI temperatures for the same periods of spring (29 [27-30]), summer (21 [19-24]), and autumn (56 [55-58]) days respectively. For the summer period, we observed a large area where farms with shorter EIP for Schmallenberg virus were grouped together, comprising southern Funen and associated islands, Lolland, Falster, and southern Zealand.

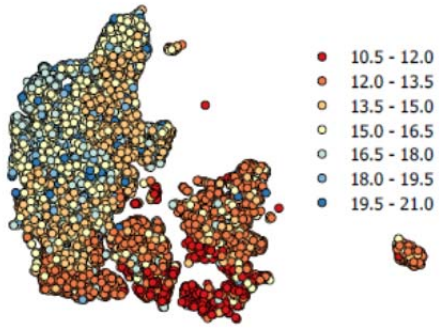

Fig: Map of Danish cattle farms showing the 17 years average EIP of Schmallenberg virus in the summer season. EIPs are generated from our virus development model using estimated microclimatic temperatures. Cattle farms with shorter EIPs are grouped together in the south-eastern part of the country.

Conclusion: Microclimatic temperature is highly important for understanding and predicting insect-borne virus transmission on Danish cattle farms. We were able to predict the daily farm level EIP of Schmallenberg virus for 17 years. We found large variation in EIP between farms and also a spatial pattern with a strong geographical trend suggesting that disease transmission may vary substantially between regions even in a small country like Denmark – and this could be useful for designing risk based surveillance for emerging and reemerging vector-borne diseases.

C22: Epidemiological analysis of the lumpy skin disease epidemics in Europe in 2015-2017

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Prior to the introduction of lumpy skin disease (LSD) to Europe, the European Food Safety Authority (EFSA) has performed a series of risk assessments, including assessment of the risk of introduction, spread, impact and effectiveness of different control measures such as different stamping out policies and vaccination strategies.

At the request of the European Commission and in collaboration with European countries affected by or at risk of LSD EFSA has performed an epidemiological analysis of the data collected so far.

An analysis of the temporal and spatial patterns of LSD epidemics and an analysis of the risk factors involved in the occurrence, spread and persistence of the LSD virus among the cattle population is presented. Vaccine effectiveness in Albania was estimated to be 70% at the farm level and 77% at the animal level (Figure 1). The analysis of possible adverse effects of vaccination in an unaffected country (Croatia) and opportunity maps for LSD vector survival are also presented and discussed.

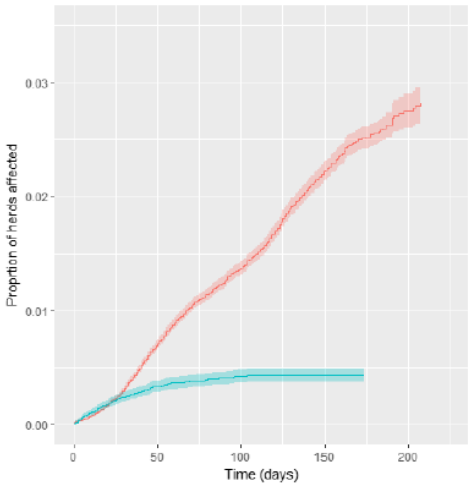

Figure 1: Cumulative proportion of vaccinated (blue) and unvaccinated (red) affected herds in Albania, according to follow up time starting with occurrence of the index case in each district.

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C23: Prevalence and characterization of Encephalomyocarditis virus (EMCV) in Italy

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Encephalomyocarditis (EMC) is a viral disease caused by a virus belonging to *Picornaviridae* family, which may induce myocarditis, encephalitis, and reproductive disorders, depending on host species and host aging. It has been demonstrated to be able to affect various mammals, despite swine is the most receptive between farm species. In Italy, the virus was first described in 1986 and, after sporadic detection during the following 10 years, since 1997 the disease occurred endemically in pig farms in northern Italy, causing fatal myocarditis. EMC outbreaks seem to be related to the local rodent population, which works as virus reservoir, causing a typical seasonal trend of the infections in farms and zoos during autumn/winter season.

This work aims to give an update on molecular/antigenic evolution of EMCV Italian strains and on prevalence of infection in pig farms.

The antigenic profile of 83 EMCV strains isolated between 2013 and 2016 was characterized by an in-house ELISA using a panel of 40 monoclonal antibodies (MAb) identifying seven different antigenic sites, some of them composed by multiple epitopes [1,2]. Only sporadic mutations occurred in two of the three sites involved in neutralization, except for the mutation in the epitope target of one neutralizing MAb, which exhibits low or null reactivity with most samples (77/83), as already observed in isolates collected previously. Occasional mutations were also identified in non-neutralizing sites, but none resulted permanent, substantiating the antigenic stability of the virus.

The phylogenetic analysis based on the VP1 coding gene classified all the isolates within a unique lineage (lineage B), comprising also isolates from Belgium and Cyprus [3,4]. The 76 pig strains isolated in Italian farms are organized in few distinguishable clades, with a nucleotide identity ranging from 88.9% to 100%. The analysis included three recent pig isolates from Spain and four strains isolated from primates in an Italian natural park, which resulted to cluster in two further specific clades.

To investigate on the diffusion of EMCV infection, a serosurvey was carried out on approximately 20,000 swine sera sampled during 2016 in about 700 different farms, located in Northern Italy. Sera were analysed by an in-house MAb-based competitive ELISA and seroprevalence levels were compared to those recorded in 2010 in a similar sample. The proportion of positive farms increased from 54% in 2010 to 76% in 2016, with a parallel increase of within-herd seroprevalence. Considering the 528 positive farms, the seroprevalence resulted higher than 50% in the 13% of them, between 50% and 20% in another 33%, between 20% and 5% in the 43% of considered farms and lower than 5% in the remaining 11%. Similar levels of seropositivity were detected in farms with and without clinical evidence of encefalomyocarditis, substantiating the subclinical circulation of EMCV.

Acknowledgments
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C24: Risk factors for African swine fever persistence in Sardinia

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African swine fever (ASF) is an infectious disease of swine, present in Sardinia since 1978. Since the early introduction of the disease, several control and eradication programs were established with limited success. Some researchers attributed the persistence of the disease in Northern and Eastern areas to certain socio-economic factors, the existence of some local and traditional farming practices (i.e. unregistered free-ranging pigs also named brado animals) and the high density of wild boar in the region [2], [3].

The scarcity of information on pig data has traditionally complicated the evaluation and study of ASF in Sardinia [4], [5]. However, as a result of the last eradication programs, more complete, accurate and reliable information on pig farms is available nowadays. In this work, we identify the main risk factors that have caused ASF persistence in Sardinia by performing a statistical modelling analysis based on available data from 2010 to July-2015 and the distribution of the Sardinian susceptible populations (domestic pig and wild boar).

Our results identified the main risk factors (9 risk factors) that are favouring ASF persistence on the island. Some of them have been pointed out as risk factors for the first time in this work. The most significant factors were the number of medium farms, the estimated wild boar density at high altitudes and the presence of brado animals. The obtained results also allowed the delimitation of the regions most at risk which were mainly located around east and central Sardinia. In addition, we proposed specific control measures focused on identified risks to mitigate them and finally achieve the eradication of ASF from the island.

Acknowledgements
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C25: Genetic diversity of French highly pathogenic H5 viruses during 2015-2016

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From November 2015 to August 2016, 81 outbreaks of highly pathogenic (HP) H5 avian influenza virus (AIV) were detected in poultry farms from South-Western France. These viruses were mainly detected in duck and geese farms with silent infection, but also in chickens or guinea fowl flocks. Preliminary analysis of the hemagglutinin sequences of the viruses identified in these outbreaks showed that they were all directly related with low pathogenic H5 virus had previously circulated in Eurasia, but not related to the highly pathogenic H5 lineage Gs/Gd/1/96 which has a zoonotic potential. The highly pathogenic feature of these H5 viruses was demonstrated experimentally by the in vivo intravenous pathogenicity index of 2.9 for the H5N1 HP isolate. Among the 81 documented cases, the HP H5 genes were associated with three different neuraminidases (N1, N2 and N9) [1]. Full genome sequencing of 18 viruses were obtained by Next generation sequencing (NGS) using Ion Proton system from direct field samples or after virus isolation on SPF embryonic eggs. Phylogenetic analyses of the eight viral segments confirmed that they were all related to avian Eurasian lineage. In addition, through analysis of "Time of the Most Recent common ancestor" (tMRCA), it was also estimated that the common ancestor of the French highly pathogenic H5 sequences detected in South-Western France could have emerged in early 2014 (± 1 year). This date is earlier than the first detection of H5 HP in poultry farms, It is not clear whether this common ancestor was already introduced in farms but kept undetected since this time, which could have been possible due to these H5HP viruses causing mostly silent infection, or whether this ancestor evolved in another epidemiologic compartment, then spread widely in duck farms in late 2015. In addition, the phylogenetic study of the different segments showed that several phylogenetic groups could be established. So, these results support the idea that at least 11 reassortment events did occur indicating that a large number of co-infections with highly pathogenic H5 and other influenza viruses did take place since the first highly pathogenic H5 virus emerged.

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C26: Whole genome sequence analysis of potential between-farm transmitted low pathogenic avian influenza (LPAI) viruses

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The recurrence of avian influenza (AI) outbreaks highlights the importance of global surveillance efforts to detect AI virus infections early on. In the Netherlands, intensive monitoring has provided extensive information on the spatiotemporal distribution of AI virus infections in free-living and domestic birds. Wild birds are the natural reservoir of AI viruses and the primary source of infection for poultry. Surveillance focuses on the early detection of AI viruses of subtypes H5 and H7, which have the potential to mutate from a low pathogenic AI (LPAI) variant to a highly pathogenic AI (HPAI) variant. However, introductions of LPAI viruses of other subtypes are also mon itored. Over the past decade, more than 200 introductions of LPAI virus infections have been detected on Dutch commercial poultry farms. In multiple cases, LPAI viruses of the same subtype have been isolated from different poultry farms within a short time frame. These outbreaks were either caused by separate introductions from the same reservoir source or by between-farm transmission. To determine the route of virus introduction to poultry, we compared whole genome sequences of viruses isolated from LPAI outbreaks in poultry. In addition, AI viruses from poultry and wild birds were analyzed using phylogenetic approaches to identify potential wild bird precursor viruses. Genetic data were combined with data from epidemiological studies to reconstruct the most probable route of introduction to poultry farms. Expanded knowledge on AI virus transmission likely reveals important risk factors for virus introduction to poultry, which can be used to improve global disease prevention and control strategies.

C27: Detection of clade 2.3.4.4. H5N8 HPAI viruses in feather samples in ducks and geese: an assessment on field outbreaks

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The pulp at the basis of immature feathers is highly vascularized. Highly Pathogenicity Avian Influenza viruses (HPAIVs) display a tissular pantropism, which implies that they could spread in feather pulp. The detection of HPAIVs from feather samples has been positively evaluated for HP Asian H5N1 viruses (1) or HPAI H7 viruses (2). These studies (very few however) suggest that viral loads detected in the pulp of immature feathers are equivalent or often higher than those detected on either tracheal or cloacal swabs. These results are also confirmed on carcasses of dead birds, suggesting a better preservation of virus in the feather calamus than in viscera (2). Almost all of these studies were carried out in the context of experimental infections.
In this study, we investigated field cases of H5N8 HPAI the suitability of feather pulp samples to detect clade 2.3.4.4. H5N8 HPAIV in ducks or geese. Seven flocks were included from January to March, 2017: 5 mule ducks flocks (5 to 13 weeks of age), 1 Pekin duck flock (8 weeks of age) and 1 geese flock (8 weeks of age). On each flock confirmed H5N8 positive, at least 10 non clinically affected birds were sampled: tracheal (TS) and cloacal swabs (CS) were taken, as well as immature wing feathers. On-farm investigations and processing of samples were performed in strict compliance with regulation and biosecurity procedures.
Samples were routinely processed: RNA was extracted from swabs or feather pulp and real-time RT-PCR was performed for M and H5 genes. An absolute quantification of M or H5 RNA copies was performed for each sample and the distribution of viral RNA loads was statistically analyzed.

In all flocks included in the study, loads of viral RNA detected in feather pulp were at least equivalent and in most cases up to 10^3 higher than those detected in either TS or CS. At the flock level, detection performances of feather pulp samples were significantly much better than TS or CS. IHC assays performed on feather follicles confirmed an intense viral staining.
These data, based on a selection of spontaneous H5N8 outbreaks, suggest that feather pulp samples should be considered as suitable samples for detection of clade 2.3.4.4 HPAI viruses. Further investigations are needed in experimental settings and on a wider range of viruses to clarify the relevance of their insertion in the diagnostic protocols of HPAI surveillance.

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C28: Development of a multiplex serological assay for Avian Influenza

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Avian Influenza (AI), also called bird flu, is a highly contagious viral disease that affects both domestic and wild birds and can be zoonotic. Avian Influenza viruses (AIV) are classified into subtypes based on two different surface proteins: haemagglutinin (HA) and neuraminidase (NA). In birds, 16 hemagglutinins (1-16) and 9 neuraminidases (1-9) subtypes have been found in numerous combinations [1].

Various serological methods are available to detect antibodies of AI; Enzyme-linked immunoassay (ELISA), Agar gel immunodiffusion (AGID) and Haemagglutination inhibition test (HI-test) [1]. All these tests require a large volume of serum and are time-consuming, which makes them costly. In this study, we investigated the potential of a multiplex serological assay that is based on Luminex technology. The intended assay enables detection of antibodies against all HA and NA subtypes simultaneously in one single assay and thereby serotyping the virus. This test will be more time- and cost-efficient and using small volume of serum than the already existing tests to detect antibodies against AI.

Using recombinant techniques multiple variants of the different HA and NA proteins were produced. The HA and NA proteins were covalently bound to spectrally different fluorescent beads resulting in 54 different beads. When serum and beads are mixed, the antibodies will bind to the coupled HA and NA proteins. Next, the beads are incubated with phycoerythrin labelled anti-chicken IgY and analyzed using the Magpix (Luminex). Using CCD imaging technology, the beads are identified and analyzed for the presence of specific antibodies.

The Luminex assay was validated using 87 individual chicken field samples. Confirmation of the HA results was performed using the HI-test. Results for NA were confirmed using an in-house ELISA that detects all NA-serotypes. In 92% of the samples, the results of Luminex were similar with the results of the HI-test and ELISA.

To conclude, the prototype assay based on Luminex technology looks very promising and is more time- and cost-effective than other serological tests for the detection of AI antibodies. Further optimization and validation is ongoing.

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C29: Genetic diversity of influenza A virus in Italian pigs in the period 1998-2017

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Swine influenza virus (swIAV) is one of the most important respiratory pathogens of swine and a significant zoonotic pathogen with public health relevance. In European pigs the co-circulation of three distinct subtypes is evidenced: Eurasian avian-like H1avN1, A/sw/Gent/1/84-like H3N2 (Gent/84) and A/sw/Scotland/410440/94-like H1huN2 (scot/94) (3). Since 2009 the novel pandemic virus A(H1N1)pdm09 was also detected. The simultaneous circulation of influenza A viruses (IAV) in different hosts and the risk of reassortment events pose great concern for its implications in human health and highlight the need of a deeper surveillance worldwide. In this study, we investigated the complete genomic characterization of 350 SwIAV strains isolated in Italy with particular attention to the detection of reassortant strains and genotype diversity.

Influenza surveillance between 1998 and 2017 was based on investigation of pigs showing respiratory disease and included genome detection, virus isolation, sequencing and genomic characterisation. Whole genome sequencing of 350 Italian swIAVs collected in this period was performed using Illumina MiSeq and Ion Torrent NGS platforms (1). Phylogenetic analysis was performed using MEGA6 including swine, avian and human IAVs retrieved from public databases.
Phylogenetic analysis showed high genome diversity and several different reassortment events that involved swine and human IAVs but no avian IAVs. A total of 18 different genotypes were dentified but with differences between subtypes (Figure 1). The major part of the isolates showed the characteristics of the contemporary circulating European genotypes for the H1N1 and H3N2 swIAVs. Contrasting epidemiological situation was observed for the H1N2 genotype with only 7% of strains showing the characteristics of scot/94 viruses and high percentage of multiple different lineages. Among these, the most prevalent lineage appeared to be the A/Sw/Italy/4675/2003-like H1huN2 (49.6%), first detected in 2003 and originated from reassortment between scot/94 and human seasonal H3N2 of 1997-98 (2). The introduction of A(H1N1)pdm09 in Italian pigs in 2009 determined the presence of different patterns of reassortment between this genotype and H1N2 and/or H1N1 Sw IAVs.

Reassortment events involving swine and human influenza viruses were frequent in Italian pigs but with different patterns. Some of these patterns were highly frequent, suggesting the presence of new gene constellations and well balanced HA-NA combinations, able to contribute to efficient replication and successful transmission among pigs. The frequent reassortment events between human and swine IAVs in pigs provide the conditions to investigate adaptive evolution of IAVs in different hosts and to acquire more information on their capability to cross host barriers and become a pandemic strain.

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Figure 1 – Gene constellations detected in Italy in the period 1998-2017. The lineage origin for each segment are reported in different colours

	tot	PB2	PB1	PA	N	M	NS	HA	NA	
H1N1	83									EU Avian- like H1av N1
	46									A(H1N1)pdm2009
	5									EU Avian- like H1av N1 Rpdm2009
	3									EU Avian- like RH1hu N1
	1									EU Avian- like RH1pdm2009 N1
	1									A(H1N1)pdm2009-EU avian like internal genes (IG)
H1N2	9									EU A/Sw/Scotland/410440/94-like H1hu N2
	4									EU A/Sw/Scotland/410440/94-like RH1av N2
	62									EU A/Sw/Italy/4675/2003-like H1hu RN2hu
	21									EU Avian- like H1av RN2sw
	9									EU Avian- like H1av RN2hu
	9									EU A/Sw/Italy/4675/2003-like H1huRN2 Rpdm2009IG
	6									A(H1N1)pdm2009 RN2sw
	1									EU Avian- like H1av RN2sw Rpdm2009
	2									EU A/Sw/Italy/4675/2003-like H1hu RN2sw
	2									EU H1av RN2sw pdm2009 IG
H3N2	85									EU A/Sw/Gent/1/1984 -like H3N2
	1									EU A/Sw/Gent/1/1984 -like H3N2 Rpdm2009

Avian- like H1avN1
A/Sw/Gent/1/1984 -like H3N2
A/Sw/Scotland/410440/94-like H1huN2
human seasonal H3N2 (1997-1998)
A(H1N1)pdm2009
A/Sw/Italy/4675/2003-like H1hu

C30: Influenza D virus circulation in Europe and Africa

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Influenza D virus (IDV) was recently discovered in the USA in a pig with influenza like symptoms (1). Like human influenza C virus (ICV), IDV harbored 7 genomic segments, whereas influenza A and B viruses have 8. So far, IDV or anti-IDV antibodies were detected in the USA, Canada, Mexico, France, Italy, Ireland, Benin, Togo, Morocco, Kenya, China and Japan, in either healthy or sick ruminants and pigs having respiratory signs (Figure 1, modified from (2)). The pathogenesis and transmission of this virus are not fully understood but recent experimental infections of calves showed that IDV can cause moderate respiratory disease (3 and Salem et al, Epizone's abstract) and that IDV is related to the bovine respiratory disease complex (4).

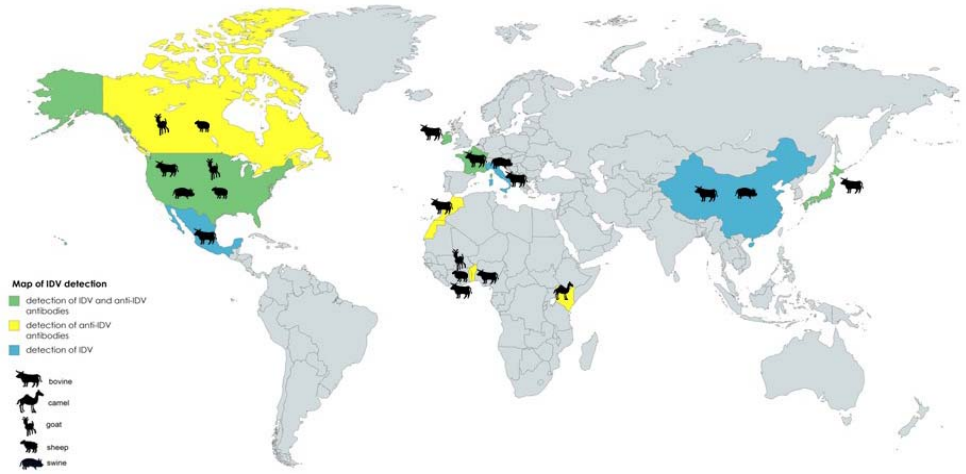

Figure 1: Map of countries where IDV or anti-IDV antibodies were detected. Countries where IDV, anti-IDV antibodies, or both have been detected are in blue, yellow, and green, respectively.

We carried out IDV surveillance in Europe and Africa. The seroprevalence of IDV in cattle in France and Ireland was very high (>70%) and sequenced viruses clustered either in the first lineage identified in the USA (D/swine/Oklahoma/2011-like viruses) or in a new genetic cluster (5). IDV also circulated in African ruminants: antibodies were detected in West and North African ruminants (seroprevalences ranging from 1 to 35%). The IDV seroprevalence increased over time, suggesting a recent emergence of the pathogen (2).

Further studies are warranted to fully assess IDV host tropism, IDV circulation in the world, and estimate the virus genetic diversity and date of emergence.

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C31: Preparation of African swine fever isolates for CRISPR/Cas9 modification.

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African swine fever (ASF) presents a serious threat in world-wide production of pigs. The aim of presented study is the construction and investigation of biological properties of recombinant African swine fever virus (ASFV) strain lacking A238L, EP402R and 9GL genes related to evasion or modulation of host immune response. The recombinant virus will be obtained using CRIPSR/Cas9 (Clustered ***R****egularly* ***I****nterspaced* ***S****hort* ***P****alindromic* ***R****epeats*) mutagenesis system. The first step of this study was conducted using previously collected infectious material originating from 264 cases of ASF in wild boars and 23 outbreaks in domestic pigs to select 12 most representative ASFV isolates. The collected isolates were propagated using pig alveolar macrophages (PAMs) and continuous 3D4/21 ATCC cells. All selected 12 isolates were tested using hemadsorption assay (HAD) and their titer reached from $10^{4.8}$ to $10^{6.0}$ hemadsorpitron units (HAU)/ml. Next the DNA of ASFV field isolates was extracted using High Pure Template Kit (Roche). The resulted DNAs were sequenced using next generation sequencing technology (NGS) on My Seq (Ilumina) machine. The obtained NGS sequences of ASFV field strains were assembled, and analyzed using Geneious R9 software (Biomatters, Auckland, New Zealand). The particular sequences were analyzed with application of appropriate databases including: Uniprot, Gatu (Genome Annotation Transfer Utility) and Viral Bioinformatics in Canada. For the further CRISPR/Cas9 modification the most representative ASFV strain from Poland territory has been selected. This world's first application of CRISPR/Cas9 system for edition of ASFV genome opens new horizons to explore the features of particular ASFV genomic regions as well as preparation of potential candidate for future vaccine against ASF.

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C32: Deletion at the 5'-end of Estonian ASFV strains associated with an attenuated phenotype?

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African swine fever (ASF) is a severe, multi-systemic disease of pigs. The disease was re-introduced into the European Union in 2014, affecting domestic pigs and wild boar in the Baltic States and Poland (Sánchez-Vizcaíno et. al. 2013). Every week, new cases are reported especially from wild boar. However, the geographic outbreak regions remain rather stable. Based on the experimental findings that the virus strains involved showed exceptionally high virulence, this epidemiological behavior was rather unexpected. 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 situation are far from being understood.
Among the different explanations is also virus attenuation. In fact, wild boar hunted in Northern Estonia were tested positive for ASFV-antibodies without showing obvious clinical or pathological signs of the disease. Last year, we reported about the biological testing of a re-isolated Estonian virus in different animal experiments. In a first trial, a non-cultivable field isolate from the above mentioned area in Estonia was used to inoculate three wild boar. All three animals got infected and were euthanized to gain infectious material which was the basis for the biological characterization in ten wild boar (Nurmoja et. al. 2017) and the following animal experiments (Fig.1).

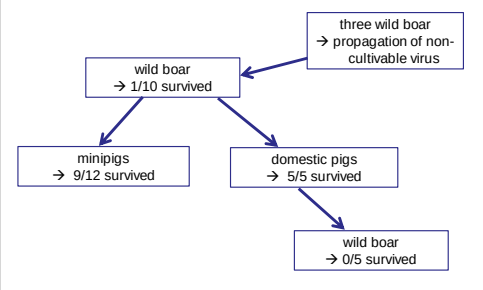

Figure 1: overview on the biological characterization

Since the survival rates and clinical courses were rather variable in the different trials, representative samples from each trial were full-genome sequenced on a MiSeq instrument (Illumina) to see if there is a genetic base for this variance. So far, full ASFV genome sequences could be assembled from all but the minipig trial. Compared to ASFV Georgia 2007/1 (FR682468.1), the alignments indicate a large 14 kb deletion at the 5'-end, and a genome re-organisation by duplication of 7 kb in all sequenced samples. The deletion and re-organisation site could be confirmed by both PCR and Sanger sequencing. In order to verify if the deletion can also be found in field samples, specimens of different Estonian locations were screened by PCR. Preliminary results show that the mutation is detectable in field samples but further investigations are required to examine the temporal and geographic distribution. Moreover, a correlation with antibody positive samples is conceivable.

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C33: Detection of Asfarvirus-like sequences in *Ornithodoros* soft ticks

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African swine fever virus (ASFV) is a large double-stranded DNA virus and the only known DNA arbovirus. In sub-Saharan Africa, the virus is transmitted in an ancient sylvatic cycle between soft ticks of the genus *Ornithodoros* and suids such as bush pigs (*Potamochoerus larvatus*) and African warthogs (*Phacochoerus africanus*) (Anderson et al., 1998). Infected ticks can carry the virus for many years and transmit it horizontally, vertically, and to susceptible suids during feeding (Kleinboeker et al., 1998).

The virus can be differentiated into 23 distinct genotypes according to the DNA sequence of its major capsid protein P72 (Gallindo et al., 2017). ASFV belongs to the order Megavirales and is the only recognised member of its family (*Asfarviridae*) and its genus (*Asfivirus)* (Takamasu et al., 2011). Recently, amoebae were found to harbour partially-related viruses, which were also assigned to the Megavirales (Reteno et al., 2015), but no closely-related virus is known.

After detection of uncharacterised viral structures in cell lines from the natural ASFV vector *Ornithodoros moubata* by electron microscopy (Bell-Sakyi et al., 2009), six cell lines were analysed at the RNA and DNA levels for viral sequences related to ASFV using next-generation sequencing. In total, about 26 kb of sequence information was detected with up to 85 % nucleotide identity to ASFV. These sequences are spread over the entire ASFV core genome and include mainly conserved genes with metabolic functions such as polymerase, topoisomerase and ribonucleotide reductase. Amino acid sequence analysis revealed significant homologies to known ASFV open reading frames.

Ornithodoros ticks from different geographical origins are currently being screened by qPCR for ASFV-like sequences, and further studies are being conducted on the nature of these sequences. Both the existence of a novel virus related to ASFV and the integration of Asfarvirus-like endogenous viral elements into the tick genome are considered.

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C34: Spatio-temporal clusters of ASF with different stages of infection in Lithuania and Poland

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After three years (2014-2016) of African swine fever (ASF) circulation in areas of Estonia, Latvia, Lithuania and Poland, we analyze the evolution of ASF spread patterns within the wild boar population with spatial statistics. While ASF can affect both domestic and wild pigs[1], 95% of notifications have occurred in wild boar in the mentioned countries, suggesting a self-sustained epidemic within the wild boar population[2,3].
The aim of this work is to identify areas with wild boar at different stages of infection: recent infection (<1.5-2 months since infection) and potential survivors that could lead to recirculation of virus (>1.5-2 months since infection). For this purpose, we first analysed the presence of spatio-temporal (ST) clusters, with SatScan and ArcGIS software, using the notifications of ASF cases in wild boar to the OIE from Lithuania and Poland, that includes information from 2014 to 2016. We used the estimated date of start of each notification, a spatial window of 41km based on a spatial point pattern analysis using the K-Ripley function, a temporal window of 6 months and an aggregation time of 7 days. Next, we classified the stage of infection in each cluster based on the ASF EU Reference Laboratory (RL) test results to quantify viral DNA (RT-PCR) and antibody titres (IPT). The data from the ASF EU RL represented almost 80% of the cases in Lithuania and Poland notified to the OIE.
There are a total of 606 point locations and 623 notifications with case information data from 2014 to 2016, in Poland and Lithuania. We identified 13 significant ST clusters ($p<0,05$), 8 of which were in Lithuania (clusters 1, 4, 6, 7, 9, 10, 11 and 12) and 5 in Poland (clusters 2, 3, 5, 8 and 13). The average radius/cluster was of 19 km (2-38) and the average number of cases/cluster of 17 (6-50). 363 animals with serological results were included within the clusters, with an average of 28 animals with serology/cluster (6-89). Of the 13 clusters identified, 6 presented animals with different stages of infection. The 3.6% of animals present serologic results that indicate an infection >1.5 months, all of which were wild boars hunted at the end of 2015 (a few) and in 2016 (>80%). Clusters 1 and 7 presented 69.2% of animals with an infection >1.5 months. There is a potential population of wild boars that could be persistently infected and be a potential source of infection for susceptible animals. With this study we show that when the serological information is present, it can be combined with spatial statistics to offer a more accurate picture of the evolution of ASF spread that could explain some of the transmission patterns in wild boar in the field that remain unclarified at present.

Acknowledgements
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C35: Soil as a vector for African swine fever virus in wild boar populations

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African swine fever (ASF) is a socioeconomically important, lethal hemorrhagic disease of suids that is notifiable to the OIE. There are no vaccines and the only methods of control are surveillance, biosecurity, and slaughter of infected animals. ASF virus (ASFV) is transmitted directly between infected swine and wild boar by the oro-nasal route, indirectly by ingestion of contaminated meat or through infected ticks. ASFV is a DNA virus that is extremely stable in meat products, excretions of infected swine, and carcasses, possibly contaminating the soil around them. ASFV was introduced to the Republic of Georgia in 2007 and has spread from the Caucasus to Eastern Europe, affecting both domestic swine and wild boar. It can become endemic in wild boar populations, complicating disease control and causing a constant threat of introduction to swine production. ASFV may remain infectious in chilled blood for over one year, in boned meat for several months, and in frozen carcasses for several years [1,2,3]. The spread of ASFV through carcasses is considered to be more significant than direct contact with mobile infectious animals[3,4].

We propose to characterize and investigate the role of contaminated soil as a vector in ASFV transmission. Wild boar and swine demonstrate rooting and digging behaviors[3]. It is important to understand if the ground under a carcass contains infectious ASFV. If so, how long is it infectious? What measures can remedy ASFV contamination, thereby reducing ASFV spread among wild boar?

The Estonian University of Life Sciences collected soil underneath virus-positive wild boar carcasses, and found ASFV DNA with Cq values of 29.8 to 38.5. This piqued our interest to look for infectious ASFV in such soil.

We have developed a protocol to detect ASFV in soil by qPCR and virus isolation. Preliminary results show virus titers in soil spiked with infectious blood from wild boar and stored at 4°C or 25°C generally decreased within the first 24 hours. Titers in the infectious blood itself were relatively constant under the same conditions. Despite decreasing titers, we found that ASFV remains viable in soil for up to 7 days. Soil with Cq values of 32 and lower contained infectious virus, which was subsequently cultivated on swine macrophages and titrated.

In conclusion, our results provide a first indication that ASFV could be transmitted to wild boar by contact with contaminated soil. We plan to evaluate the influence of longer incubation periods as well as different soil compositions and parameters such as pH and water content.

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C36: ASF-STOP: progress and updates from the first year of COST Action "Understanding and combating African Swine Fever in Europe"

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Combating and understanding African Swine Fever in Europe (ASF-STOP) is an interdisciplinary research network financed by the COST EU programme, which has as its main aim to stop African swine fever (ASF) from spreading further in Europe and protecting the European pig industry by combating ASF through a comprehensive, multi- and interdisciplinary approach. ASF-STOP started on 1st May 2016 and will end on 30th April 2020.

ASF-STOP enables researchers to interact and collaborate on the multiple aspects related to ASF. The decision-taking body is the Management Committee (MC), formed by 1 or 2 representatives from each of the 29 participating COST countries. Five working groups (WG1: ASF virus, WG2: ASF in wild boar, WG3: ASF in domestic pigs-pig industry, WG4: ASF infection dynamics and control, WG5: integration of knowledge and communication of results), work towards the achievement of the scientific objectives.

During its first year, ASF-STOP held:

i. Website launch for the dissemination of Action activities (https://www.asf-stop.com).
ii. International Launch conference, with eight scientific Sessions and including four invited speakers from USA, Canada, Uganda and Russia, in Pulawy, Poland.
iii. Workshop "Advances in Vaccinology", Pulawy, Poland.
iv. Workshop 'Researchers link European industrial partners', Pulawy, Poland.
v. Working group meetings in: Pulawy, Poland (all WGs), Pirbright (England; WG1), Brescia (Italy; WG3), and Lisbon (Portugal; WG2-4).

Five Short Term Scientific Missions (STSM) were conducted by young investigators and were hosted by expert centres/laboratories in various European countries. These were: "The role of wild boars in the dynamic of ASF spread among domestic swine in Sweden" (host: SVA, Sweden), "Study of Extracellular vesicles in the ASFV vector-host interface" (host: The Pirbright Institute, UK), "Further validation of alternative sampling methods for the passive swine fever surveillance in wild boar under field conditions" (host: Univ. of Life Science, Tartu, Estonia), "Application of fast molecular techniques for ASF virus detection" (host: Nat. Vet. Research Inst. Pulawy, Poland) and "Short training program on tools to census and monitor wild boar population status" (host: Spanish Wildlife Research Institute IREC, Spain).

Dissemination activities included participation with a ASF-STOP Session at the Global ASF Research Alliance (GARA) Scientific Workshop in Ploufragan (France) in September 2016 and dissemination at the 12th European Wildlife Disease Association (EWDA) conference in Berlin (Germany) in August 2016. More information is presented in the Action's website.

During its first year, ASF-STOP fulfilled the COST Policy aims, with the following representation of participating countries: Inclusiveness Target Countries (ITCs): 46%, Early Career Investigators (ECIs): 19%, and Gender balance: 31% females in MC, 57% females in leadership roles.

The presentation will summarise the main achievements towards the scientific, training and strategic objectives obtained during the first year of ASF-STOP.

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Session 7 - Current challenges inside Europe

C37: Modulation of the host interferon response by the non-structural polyprotein ORF1 of hepatitis E virus

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Zoonotic hepatitis E virus (HEV) is an emerging pathogen in Europe. The virus causes acute hepatitis in humans that are self-limited but can lead, in some patients, to complications, extra-hepatic manifestations and chronic hepatitis E. Zoonotic HEV can infect several animal species including pigs, wild boar, deer and rabbits and domestic pigs represent the main reservoir [1]. Direct contact with infected animals and the consumption of infected meat are risk factors for HEV exposure [2]. As HEV is largely present in pig farms in Europe, it represents an important concern for public health and food safety. However, little is known about the biology and pathogenesis of the virus and particularly about its interactions with the host immune response. The interferon (IFN) system is a key component of the host innate response against viral infections, and many viruses have developed different mechanisms to overcome its antiviral effects. Previous studies have shown that the IFN system is activated in response to HEV infection. However, the effect of HEV on IFN signa ling remains poorly understood. The first open reading frame of HEV (ORF1) encodes a polyprotein with non-structural functions that contains several putative domains such as a papain-like cysteine protease (PCP), a macro domain, a methyltransferase (Met) and a Y domain. These domains are homologous to other domains found in the "alpha-like" supergroup of viruses [3]. Interestingly, several reports have suggested that the PCP and macro domains of different viruses can modulate the host innate antiviral response. This study aims to determine whether the different domains of ORF1 are able to modulate the IFN system. To investigate this possible effect, expression vectors encoding different ORF1 domains were constructed. Reporter assays were then performed to evaluate the effect of the different domains on IFN signaling pathways. The results obtained have shown that the HEV PCP domain expressed with the Met and the Y domains (Met-PCP domain) is able to inhibit the activation of the ISRE promoter. Immunoblotting and immunofluorescence analysis have provided additional evidence that the Met-PCP domain interferes with the JAK-STAT pathway and is able to inhibit the phosphorylation of STAT1 but not STAT2. This study provides a better understanding of the strategies evolved by HEV to counteract the host antiviral responses and will help in the future to design new prophylactic agents to control the virus.

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C38: Gamma- and deltacoronaviruses in wild birds in Poland

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Introduction

Among coronaviruses (CoVs), the genera gamma- and deltacoronavirus were detected in avian species of different orders and occasionally also in mammals. Infectious bronchitis virus (IBV), turkey coronavirus (TCoV) or guinea fowl coronavirus (GfCoV), are the best known representatives of the gammaCoV genus and are responsible for economically significant losses for the poultry industry. However, an ever increasing diversity of gamma- and deltaCoVs is detected in wild birds. Wild birds could constitute the genetic reservoir of CoVs for poultry, potentially being the donors of new spike genes for generating new IBV, TCoV or GfCoV variants and additionally play a role in virus spreading.
The purpose of this work was to investigate the presence *of gamma– and deltaCoVs in a variety of wild bird species in the territory of Poland and to perform preliminary virus characterization.*

Material and Methods

A total of 479 pooled cloacal swabs (up to 5 from the same bird species and the same location) collected from *live wild birds between 2009 and 2016 in the framework of active* avian influenza surveillance were used in this study. Samples *were screened by a modified nested RT-PCR assay targeting the RNA-dependent RNA polymerase (RdRp) gene of all CoVs. In case of positivity the PCR products were directly sequenced with a c*apillary sequencer *and the results were analysed using BioEdit 7.2.5. Phylogenetic analyses were performed using MEGA v7.*

Results and conclusions

CoVs were detected in 10.2% of the 479 examined pools. Phylogenetic analysis revealed the presence of both gamma- and deltaCoVs in a variety of wild birds in Poland, as well as a great CoV intra-genus diversity. Of 49 positive CoV samples, 44 strains belonged to the gammaCoVs genus. They were detected predominantly in *Anseriformes* which included 27 pools of mallards, 6 of mute swans and 3 of common teals. In *Charadriiformes,* gammaCoVs were identified in 5 pools of black-headed gulls, 1 of herring gull and 1 of common gull. Additionally, gammaCoV was detected in one *Galliformes* species (pheasant) and this strain grouped together with IBV and TCoV. Five CoVs belonged to the deltaCoVs genus; four of them were found in birds from the *Charadriiformes* order (3 pools of black-headed gulls and 1 of common tern) and one in *Suliformes* (cormorant). The highest rate of positives was identified in 2009 and 2014 (14.6%) and the lowest in 2010 (4.2%). Sampling was conducted in areas of high wild bird concentration in Poland, especially in Pomorskie and Warminsko-Mazurskie provinces where the highest numbers of positives were found.
Our findings support the recent view that coronaviruses infect many different bird species and are more widespread than previously thought. CoV surveillance in wild birds of different orders provides knowledge about prevalence in these birds, which in our study still needs to be determined by testing individual samples of positive pools, and risk of transmission to poultry. Moreover, more genetic data are needed to understand CoV biology and evolution including the generation of new variants. Our future efforts will therefore focus on obtaining the complete genome of selected CoVs.

C39: Variations in the full-length genome sequences of porcine epidemic diarrhoea virus strain CV777 as determined by NGS

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The prototypic porcine epidemic diarrhoea virus (PEDV), strain CV777, was initially characterized in 1978 after the disease was first identified in the UK in 1971. This coronavirus has subsequently been widely distributed among different laboratories and has been passaged both within pigs and in cell culture. To determine the variability between different stocks of the PEDV strain CV777 (and its close relatives), sequencing of the full-length genome (ca. 28kb) has been performed in 6 different European laboratories, using different NGS protocols. Not surprisingly, each of the different full genome sequences were distinct from each other and from the published reference sequence (Accession number AF353511) but they are >99% identical. The unique and shared differences between the individual sequences have been identified. The region of the genome encoding the surface exposed spike (S) protein showed the highest proportion of variability including both point mutations and small deletions. The predicted expression of the ORF3 gene product was more dramatically affected in three different variants of this virus strain through either the loss of the initiation codon or the gain of a premature termination codon. The genome of one virus isolate had a substantially rearranged 5´-terminal sequence. This rearrangement was validated through the analysis of sub-genomic mRNAs from infected cells. This work has implications for understanding the basis of differences in virulence of this porcine coronavirus.

C40: Comparison of the horizontal transmission of a French InDel strain and a US non InDel strain of porcine epidemic diarrhea virus

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The porcine epidemic diarrhea virus (PEDV) emerged in Europe during the 1970s and further spread throughout the world. The pigs infected by porcine epidemic diarrhea (PED) are affected by important liquid diarrheas, vomiting and dehydration. Since the 1990s, PEDV prevalence declined significantly in Europe until 2014. In 2013, a severe PED epidemic struck the United States of America causing a loss of 7 million of piglets within one year. The disease spread throughout the country as well as in Canada and South America. This disease is still present endemically and its rapid spread motivates a closer surveillance of regarding PED viruses in Europe. Two types of virus strains were isolated from US cases, namely the InDel and the non InDel strains which are differentiated by insertion/deletion event occuring in the S1 nucleotide sequence of the S gene. In 2014, PED outbreak occurred in a pig farm in France, from which an InDel strain, named PEDV/FR/001/2014 (GB N°: KR011756), was isolated. This study aimed at comparing, in experimental conditions, the pathogenicity and transmission between this InDel strain and a non InDel strain isolated from a PED-affected piglet in 2014 in the USA.

The study has been carried out in Anses animal facilities according to the regulations on animal experimentation. Four rooms were used with 10 weaned pigs per room separated in two groups of five. In room 1 and 2, one pig of each room was inoculated orally with 5 ml of an inoculum of FR/001/2014 titrating 108 copies of viral genome/ml. Similarly, one pig in room 3 and in room 4 was inoculated orally with 5ml of an inoculum of a non InDel strain, US/2014, titrating 108 copies of viral genome/ml. Each inoculated pig was in direct contact with 4 pigs and in indirect contact with the 5 other pigs housed in a neighbor pen (40 cm apart, solid partition between the pens) to assess the indirect transmission of the virus. The experimental trials lasted 49 and 72 days after inoculation (dpi) for the InDel and non InDel strains respectively. The virus genome load was quantified by RT-qPCR in blood, feces and air samples and seroconversion assessed by ELISA.

All the pigs showed clinical signs with the non InDel strain while only the direct contact pigs and the inoculated pigs showed clinical signs in the InDel strain group. For the two strains, the inoculated and the direct contact pigs shed virus in the feces: from 24 hours after inoculation to 53 dpi for the non InDel strain and from 48 hours after inoculation to 49 dpi for the InDel strain. The indirect contact pigs shed virus in feces only in the non InDel strain group. These pigs began to shed virus in feces at 24 hours after inoculation until 51 dpi. With both strains, the fecal excretion was intermittent for some pigs. Indeed, some pigs showed a second shedding periods lasting from 4 to 11 days for non InDel strain (pigs in direct and indirect contacts) and from 2 to 11 days for Indel strain (pigs in direct contacts). All pigs which had showed a PEDV infection seroconverted except one direct contact non InDel pig. The viral genome was detected in air samples from 1 to 71 dpi with the non InDel strain and from 2 to 35 dpi with the InDel strain.

The results of this study evidenced direct contacts as the main transmission route for PEDV InDel strain. Although viral genome was detected in the air samples, airborne transmission of the virus to indirect contact pigs was not effective in our experimental settings with the InDel strain. In contrast, the airborne transmission was evidenced for the non InDel strain. The quantification of the transmission highlights a propagation rate significantly higher with the non InDel strain than with the InDel strain. The estimation of transmission parameters is used for modelling the PEDV InDel strain and non InDel transmission in a larger population.

C41: Immune system: an essential approach to the study of infectious diseases in honey bee colonies

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The decline in Honey bee population is a threat to both to the beekeeping sector and to agriculture and global biodiversity. There is therefore a need for greater knowledge of factors involved in these losses and how honey bees deal with them [1]. Among others, pathogens are capable of inducing colony losses relatively easily, such as in the case of the joint action of Deformed Wing Virus (DWV) and *Varroa destructor* [2, 3]. Due to their immunosuppressive effect which aids in triggering colony disorders, the study of honey bee´s immune system may be an effective tool to control diseases in apiaries [4].

This work describes the study of honey bee immune system, conducted in an experimental apiary over a 1-year period. The expression levels of four related immune-genes were measured (defensin, domeless, dorsal and relish) and their relationship with the load of two viruses (Deformed Wing Virus and Black Queen Cell Virus (BQCV)) and one parasite (*Varroa destructor*). Temporal trends of these variables were also analyzed, taking into account seasonal variations.

Negative correlation between dorsal expression and viral load has been evidenced, as well as in the case of defensin expression and DWV-varroa complex. A positive correlation between relish expression and DWV load was also detected. Some seasonal trends were detected in defensin, domeless and relish expression. Our findings show the importance of evaluating immune gene expression and shed light on the role of immune system during infectious diseases. It may, therefore, be a useful tool for controlling colony losses associated with viruses and varroa.

Acknowledgements

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C42: Detection algorithms in syndromic surveillance: a use case for animal rendering data

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Investigating mortality data for their usefulness in syndromic surveillance (SyS), we took a journey from testing several algorithms in a theoretical manner, on simulated outbreaks and past calculating sensitivity, specificity and precocity as indispensable markers. Leaving trends and seasonality behind, short-term periods were compared resulting in a multivariate monitoring method. And finally, data were aggregated in time and in space on a local level, rendering maps showing where mortality was increased, booting inquiries of what caused this change.

The mortality data for the period 2011-2016 are delivered by the Federal Agency for the Safety of the Food Chain (FASFC) and originate in rendering company Rendac. Rendac collects cadavers at homes, farms or companies. For our investigation the focus lies on cattle for their economic value, and for the personal drama if large amounts should be rendered for disease ratification. Next to classic surveillance conducted by the farmer himself, the veterinarian, or based on regular data of monitoring programs, SyS can add an additional layer of vigilance towards exceptional health events [1]. Therefore it was important to find a way to implement this type of surveillance. As the density chart of data aggregated by date, animal type (cattle weight classes) and location gives away a very high frequency of less than five rendered animals around the same time in one location, it is possible to determine a cut-off which combines detection of really important events with a reasonable cost of a low false alarm rate.

In particular, we investigated time series of numbers of rendered animals obtained by aggregating over certain types and locations, into weekly or daily counts. Several univariate and multivariate detection algorithms were tested on these count series in order to assess their usefulness for the detection of anomalies in the form of outbreaks, i.e. certain contiguous periods with abnormally large counts, possibly the consequence of an ongoing disease. Seasonality and correlation are accounted for by pre-processing the series using a Poisson regression adjustment and differencing. The univariate algorithms include Shewhart, EWMA, Holt-Winters and CUSUM methods known from statistical process control and propounded by the VETSYN R library [2]. However, we found that the way VETSYN cleans the counts in periods with an alarm is inadequate and propose our own modifications. Multivariate algorithms, based on the Hotelling T^2 statistic and dimensionality reduction using Principal Components, allow to scan for outbreaks with a particular signature. For example, if a disease is known to affect the mortality of younger animals more than mature animals, this knowledge can be used to improve the performance of the detection algorithm. The performance of both uni- and multivariate algorithms were studied in two ways. As the observed 2011-2016 Rendac data was assumed to be outbreak-free, applying the algorithms directly yields estimates for their specificity for various algorithm parameters, in particular the chosen detection limit. However, to estimate their sensitivity and precocity (average time to detection of an ongoing outbreak), we first introduce artificial outbreaks of various durations, shapes and magnitudes to the count series [3]. A large array of such situations were tested.

In conclusion, our investigation did not yield a clear-cut answer as to which algorithm is best under all conditions, but confirms that detection methods should be shaped for the data, and a thorough a priori investigation of the data is a necessity.

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Session 8 - Threats at the European borders

C43: Can we eradicate FMD? An industry perspective

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Foot and mouth disease (FMD) is a highly contagious disease that affects all cloven-hooved animals. It remains endemic in many countries in most parts of Asia, Africa and the Middle East.

We know from the successful precedents of smallpox and rinderpest, diseases can be eradicated. However, there are certain criteria for a disease to be considered eradicable, and FMD does not fit all of them. For this reason, international organizations and governments have set objectives of FMD control rather than eradication. Guidelines to achieve FMD control are laid out in the Progressive Control Pathway for FMD (PCP-FMD), which is a stepwise methodology for a risk management and cost effective approach to FMD control.

Obstacles in the way of FMD control are numerous. Developing countries often lack resources and infrastructure to implement efficient control plans. Because of the complexity of FMD vaccine production and the lack of independent control, a lot of insufficient quality vaccines are available on the market, leading to failure to achieve the needed level of immunity in a majority of susceptible animals.

At a country or regional level, FMD can be eliminated, as exemplified in Europe, the Philippines, or South America. The foundations that need to be established to achieve this goal rely on strong veterinary services and infrastructures, implementing national programs, with regional coordination approaches, public /private partnerships, proper funding and resources. Authorities have to put in place a strong set of standards for their vaccination programs. Also, FMD control cannot be achieved without livestock movement control, biosecurity measures and massive use of high quality vaccines -potent, purified, stable, with well-suited and, when possible broad-spectrum strains.

C44: Use of lateral flow device for safe and cost-effective shipment of FMDV suspected samples

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Foot-and-Mouth Disease (FMD) is one of the most economically devastating diseases affecting cloven-hoofed animals. This disease induces high morbidity and is characterized by vesicles, with subsequent erosions in the mouth, on the muzzle, feet, or teats. Its causative agent is Foot-and–Mouth Disease Virus (FMDV) belonging to the *Aphtovirus* genus in *Picornaviridae* family. It consists of a non-enveloped particle containing a positive-sense single-stranded RNA genome. Seven immunologically distinct serotypes (O, A, C, Asia1, SAT 1, 2 and 3) and several subtypes are described worldwide. Identification of circulating strains is an essential step towards the global eradication of FMD. However, the cost of sending FMD samples due to shipping conditions is a major obstacle to submission of samples to reference laboratories. In this study, we developed a low cost and safe method for shipment of samples from FMD suspected cases, based on the use of FMDV lateral flow device (LFD, penside test routinely used in the field for rapid immunodetection of FMDV).

Seven FMDV strains (representative of the seven serotypes) were deposited onto LFDs (FMDV-Ag Svanodip®). After 30 min, LFDs were soaked in 0.2% citric acid bath for 15 minutes. Strips were then completely disassembled and grounded. Sensitive cells were incubated with the grinding suspension. Appearance of CPE (cytopathic effect) was monitored during 48 hours. In parallel, viral RNA was extracted from the grinding suspension. rtRT-PCRs targeting FMDV genome (IRES and 3D coding region) were performed. VP1 coding region was amplified by conventional RT-PCR and the resulting amplicons were sequenced. Viral RNA extracted were then chemically transfected into cells for live virus rescue. Cells were monitored for appearance of CPE and the rescue virus characterized by antigen capture ELISA. This protocol was evaluated on three positive field samples available in the laboratory.

After treatment of positive LFDs in a 0.2% citric acid bath, FMDV was efficiently inactivated. Viral RNA was however detected by 3D and IRES rtRT-PCR. VP1 coding region was sequenced, showing 100% identity with the homologous virus strain used in each experiment. Live virus was rescued after transfection of RNA extracted from LFD. The serotype involved was confirmed by ELISA. After applying this protocol to positive field samples, viral genome was also detected, serotype was characterized and VP1 was sequenced showing 100% of homology with the original sample. However, FMD live virus was rescued only from two out of three RNA extracted from field samples.

After live FMDV collection onto LFD strip and citric acid treatment, the virus is hence totally inactivated. Viral RNA is however still detectable by rtRT-PCR and the virus strain can be characterized by sequencing of the VP1 coding region. In addition, live virus can be rescued by transfecting RNA extracted from treated LFD into cells. Evaluation and validation of this process on field samples will be continued, particularly by improving RNA transfection method. This protocol should help promoting submission of FMD suspected samples to reference laboratories by reducing the cost of sample shipment and thus characterization of FMDV strains circulating in endemic regions.

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C45: Protection against transplacental transmission of Classical swine fever virus using live marker vaccine “CP7_E2alf”

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Classical swine fever (CSF) is one of the most important diseases in swine with a large impact on the pig industries worldwide. Outbreaks of CSF are notifiable to the OIE, and prophylactic vaccination against CSF virus (CSFV) is prohibited in the European Union. Recently, the vaccine Suvaxyn® CSF Marker (Zoetis) was licensed as a first live attenuated marker vaccine by the European Medicines Agency (EMA) and is available for emergency vaccination in case of outbreaks. The vaccine virus “CP7_E2alf” is based on the cytopathogenic Bovine viral diarrhoea virus (BVDV) strain “CP7” expressing the E2 glycoprotein of Classical swine fever virus (CSFV) strain “Alfort/187”.
One question which remained to be answered was whether the vaccine is able to provide sufficient protection against vertical transmission with a relevant challenge strain. We therefore sought to confirm the protective effect against the moderately virulent strain “Rösrath” (Germany 2009).
Eight pregnant, pestivirus negative sows were randomly allocated either to the control group (two sows) or the vaccinated group (six sows) to demonstrate protection against transplacental infection according to OIE guidelines.
On day 44 of gestation, the sows were intramuscularly vaccinated with a single dose of “CP7_E2alf” (Suvaxyn® CSF Marker, Zoetis, Batch: T24070) while the control group remained unvaccinated. Twenty-one days after vaccination, both groups were challenged with CSFV “Rösrath”. Sows were sampled at 7 and 9 days post challenge to detect viremia and seroconversion.
According to the OIE guidelines, the sows were euthanized humanely approximately 1 week prior to farrowing. All sows and their fetuses were examined grossly at necropsy. An initial screening for antigen and anti-E2 antibodies by ELISA demonstrated the sera of fetuses from vaccinated sows were free of CSFV. Preliminary results show all naïve controls were negative for anti-E2 antibodies, but positive for CSFV antigen by serum ELISA, confirming the validation of the study. Blood samples and organs (tonsil, lung, lymph nodes, spleen and kidney) were collected. Serum samples will be further tested with commercial ELISA kits for antibodies against CSFV Erns and neutralization assays with Alfort/187, while pooled organ samples will be tested with PCR and virus isolation for CSFV.
These first results comply with the OIE manual’s validation tests. The decision to vaccinate within the EU depends upon the risk assessment of marker vaccines in domestic swine, and so far these studies demonstrate the safety and efficacy of Suvaxyn® CSF Marker (Zoetis) in pregnant sows and their offspring when using a recent, moderately virulent CSFV strain.

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The research project leading to these results has received funding through the vaccine manufacturer, Zoetis. The authors would like to thank the animal caretakers involved in the study. Our special thanks go to Theresa Schwaiger, Ulrike Wilhelm, Ulrike Kleinert, Laura Zani, Simone Leidenberger, Charlotte Schröder, Christian Loth, Ralf Redmer and Kore Schlottau for helping us with sampling of piglets and necropsy.

C46: FMDV- Host interaction in a model of persistently infected bovine cells

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Foot-and-Mouth Disease (FMD) is one of the most economically devastating diseases affecting cloven-hoofed livestock. This disease is characterized by vesicles, with subsequent erosions in the mouth, on the muzzle, feet, or teats. Its causative agent is Foot-and–Mouth Disease Virus (FMDV) belonging to the *Aphtovirus* genus in *Picornaviridae* family. The virus consists of a non-enveloped particle containing a positive-sense single-stranded RNA genome. Seven immunologically distinct serotypes (O, A, C, Asia1, SAT 1, 2 and 3) and several subtypes within the same serotype are described worldwide. Following acute infection of ruminants, 15-50% of animals become persistently infected regardless of their immune (vaccinated) status. The underlying mechanisms of FMDV persistence remain however almost unknown but several studies have shown evidence for host/virus co-adaptation during persistence as well as modulation of innate immune response against FMDV.

In the framework of the "Transcriptovac" ANIHWA project, we aim to study host-FMDV interactions and their modulation in the context of persistent infection by using bovine epithelial cell models to identify virus and cell gene signatures associated with persistence. For this purpose we have established FMDV type O persistent infection in epithelial bovine cells MDBK (Kopliku et al, 2015). Persistent viruses (FMDVOp) have been collected for both phenotypic and genotypic characterization. In parallel, FMDV-host protein interaction map has been realized using the yeast two-hybrid (Y2H) system to screen a cDNA prey library derived from MDBK cells with 13 FMDV "bait" proteins. This Y2H screening identified 313 interactions corresponding to 18 candidate interacting bovine proteins. These candidate interactions are currently confirmed. Furthermore, since persistence takes place *in vivo* namely in epithelium of dorsal soft palate (DSP), similar studies have been carried out, using primary epithelial bovine cells derived from dorsal soft palate cells (DSP) in comparison with alveolar pneumocytes (AP), derived from lung (site of FMDV replication, no persistence described in this tissue in vivo).

Two models of persistent infection in the bovine host (MDBK or DSP) have been thus developed so far. Mutations affecting viral proteins known to modulate the antiviral response during acute infection and appearing during persistence are under study (luciferase reporter test). The candidate interactions are also undergoing biochemical validation. The impact of the mutations identified in the FMDVOp on these interactions is also investigated. Transcriptomic analyzes are also planned in order to identify differential cellular genetic expressions, potential "signatures" of persistence. The identification of modulated cellular signaling pathways during infection (acute or persistent) may indeed contribute to the development of better control strategies for foot-and-mouth disease.

Acknowledgements
This work is performed in the framework of the "Transcriptovac" ANIHWA project.

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C47: Highly sensitive detection of PEDV in environmental samples using a new virus concentration technique

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Background:
Porcine Epidemic Diarrhoea (PED) is a highly infectious viral diseases of pigs, causing vast economic losses throughout the world. In Japan, the last epidemic of PED had occurred on 2013-2016, with 25 million Euros damages. Movements of infected animals and their products, contaminated persons, objects and contaminated feed have been suspected as the causes of both domestic spread and invasion from foreign nations. Despite of the importance of identifying sources/transmission route of the PEDV spread, it is often confounded due to incomplete epidemiological evidence. Currently, several researchers have been developed molecular based assays such as PCR and LAMP (loop-mediated isothermal amplification) for rapid and sensitive detection of PEDV. Isolation/detection of the PEDV from environmental samples is however, rarely successful due to very low contamination level in the samples.

It is necessary to develop a simple and sensitive virus concentration technique from the environmental samples to enhance the epidemiological investigation and early containment of the PEDV spread. A "pansorbin-trap" method has been recently developed in combination with antisera and *Staphylococcus aureus* producing protein A on its surface to effectively concentrate a small number of norovirus (NV) in food samples. In Japan, the technique has been widely used to identify the causative food in the NV food poisoning outbreaks. In this study, we have applied for the Pansorbin-trap method to concentrate the PEDV in the environmental samples to enable identifying source/transmission route of PEDV spread.

Materials and methods:
One PEDV vaccine strain (P5V) and two clinical isolates were artificially spiked in 50mL of PBS (pH 8.2) containing 0.1% of PEDV negative pig fecal samples (assumptive swab samples in a pig farm/slaughterhouse). A pansorbin-trap method was carried out for the PEDV concentration as follows. The supernatant was collected from a fecal mixture by centrifugation at 3,670*g* for 30min. Next, 25µL of PEDV-negative pooled sera with 18.4-35.2 mean titers of PEDV-neutralizing antibody against the three strains was mixed well, and then, 300µL of inactivated *S. aureus* cell suspension was added in the supernatant. After incubation at 37 degree for 30min, the pellet consisting of the complex of PEDV particle-antibody and bacterial cells was collected by centrifugation at 3,670*g* for 20min. The pellet was dissolved in 200µL of PBS. Finally, PEDV RNA was extracted with Trizol, and eluted with commercial RNA extraction kit in 50µL of DW. The extracted RNAs with/without the pansorbin-trap procedure were quantitatively compared by real-time RT-PCR assay.

Results and Discussion:
The pansorbin-trap method successfully detected PEDV at least 250 times in concentration rate, and constantly retrieved PEDV above 25% in recovery rate in all three PEDV strains. The pansorbin-trap method required for less than 2 h from the beginning of initial centrifugation to the final RNA elution. Further application of this technique is under planning for the highly sensitive detection of other viruses. The pansorbin-trap method appears to be valuable in providing useful information to control programs for various animal viruses, as well as identifying the source/route of the spread.

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C48: Separate determinants of West Nile virus virulence in mammals and birds

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Introduction: West Nile virus (WNV) is a neurotropic flavivirus mainly transmitted through mosquito bites and whose reservoir hosts are wild birds. Equids and humans are incidental dead end hosts and can develop severe neurological symptoms in 1-10% cases. Up to now much remains to be explored about the virulence level and determinants of WNV strains circulating in Europe. In 1998 a highly pathogenic strain, the IS-98-ST1 strain, had been isolated from a white stork in Israel (Lucas *et al*., 2004). In 2008, a low pathogenic strain, responsible of 11 human cases and 78 equine cases, the Italy 2008 (It08) strain, was isolated (Sotelo *et al.*, 2011; Barzon *et al.*, 2014).

Objectives: We aimed at deciphering the determinants of WNV strains circulating in Europe by evaluating the impact of the introduction It 08 genomic fragments in an Israel 1998 infectious clone backbone, in WNV virulence for birds and mammals.

Materials and methods: An infectious clone, based on the WNV lineage 1 IS-98-ST1 strain, was previously constructed (Bahuon *et al*., 2012). Chimeric constructions were generated by replacing the 6551-8025 region (82 last nucleotides of NS4A, NS4B, and 344 first nucleotides of NS5) in chimera 3, or the 8025-10825 region (2372 last nucleotides of NS5 and 428 first nucleotides of the 3'UTR) in chimera 4, of Is-98-ST1 by the homologous regions of It08 by enzymatic digestion and ligation. The properties of recombinant viral particles were assessed *in vitro* and *in vivo*, and in animal models (mammal and bird (Dridi *et al*, 2013)).

Results: *In vitro* infection assays in Vero cells showed that chimera 4 displayed much smaller lysis plaques than Is98, It08 and chimera 3 at day 3 post infection (p.i) but infections kinetics in Vero cells did not evidence chimera 4 replicative defects.

Survival curves in mice were similar for all viruses tested. However RT-qPCR analysis showed that viral loads in the blood at 3 days *p.i* and in the brains upon animal death were significantly lower for chimera 4, suggesting that chimera 4 lead to a delayed viremia and to a hampered crossing of the blood-brain barrier in this model mammal.

Infection of 1-day old chicks via the intra cranial or the subcutaneous routes resulted in survival curves and viral loads in the blood, feather follicles and brains of infected chickens that were similar for It08 and chimera 3 (lower loads and higher survival rates), and for Is98 and chimera 4 (higher loads and lower survival rates).

Conclusions: Our study suggests that the impact of WNV genomic changes differ according to the studied host (*e.g* dead-end mammals or model bird). These results confirm that WNV virulence is driven by the genetic determinants of the WNV strain but also by the host. The molecular determinants studied here seem to modulate the replicative capacities of the virus or its capacity to escape the host immune system.

Acknowledgements
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POSTERS

Topic 1 - Arthropod-borne diseases (arboviruses)

P1 1: Use of feathers for detection of West Nile Virus and Bagaza Virus in surveillance programs

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Flaviviruses form a group of arthropod-borne viruses that include important pathogens such as Yellow Fever Virus (YFV), Usutu Virus (USUV) or West Nile Virus (WNV). Their incidence and geographical distribution have increased in the last decades. WNV and Bagaza Virus (BAGV) are good examples of flaviviruses that have recently emerged in Europe. Surveillance programs are essential to track their distribution and to assess disease risk. Previous studies have demonstrated the usefulness of feathers for the detection of a variety of viruses[1,2]. Blood samples are of limited utility to monitor flavivirus infections in their vertebrate hosts due to the short viremias that they produce. The current work focuses on the potential use of feathers for WNV and BAGV detection.

In this study, viral RNA load in blood and feathers were compared in experimentally infected birds. Samples were collected at different days post-inoculation (dpi) from WNV-infected house sparrows, common coots and red-legged partridges, and from BAGV-infected red-legged partridges, grey partridges and common pheasants. Real-time RT-PCR methods were used to detect viral RNA in blood and feathers.

All inoculated animals developed viral load in blood during a short period (1-6dpi). In feathers, however, differences among species were observed: while in house sparrows and common coots WNV RNA detection was inconsistent, with a low proportion of animals yielding positive results in wing feathers (sparrows) or mature rump feathers (coots), red-legged partridges showed a long-lasting viral RNA load in immature rump feathers. On the other hand, BAGV-infected red-legged partridges, common pheasants and grey partridges showed a high and long-lasting viral load in immature feathers. The low proportion of WNV positive feathers in house sparrows and coots when compared to phasianid birds could be explained either by lower overall virus replication or by the type of collected feathers. In fact, comparing viral loads in different types of feathers in BAGV-infected grey partridges, we observed that while the viral load in flight and mature rump feathers was low or absent, it was consistently high in immature feathers.

This study confirms that pulp from growing feathers offers a long-lasting window of viral RNA load detection for BAGV and WNV in the phasianid birds analyzed. Considering that the collection of feathers from live birds is easy, fast and noninvasive, causing minimal discomfort, it potentially constitutes a useful strategy in surveillance programs for early detection of viral infections. Even more, this approach could allow virus detection in symptomatic birds, where usually only antibodies can be identified. Whether this is true for other susceptible bird hosts awaits further investigations.

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P1 2: Bulk-tank milk sampling to assess the circulation of Bluetongue in a free area: the experience of northeast Italy

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Following the epidemic of BTV serotype 8 (BTV-8) occurred in France in 2015, and the large number of beef cattle imported from that Country, Veneto region (north-eastern Italy) implemented an extraordinary surveillance programme. Bulk-tank milk (BTM) was used to assess whether BTV might have been introduced through animal movements. At the end of August 2016, BTV serotype 4 (BTV-4) was detected in Veneto region. A new surveillance plan was therefore implemented and BTM testing was used to assess whether BTV was spreading within a densely populated territory. Hereby the results of BTV tests on BTM are reported to indicate the potential usefulness of this sample matrix in promptly identifying potential introduction of the disease in unaffected areas, and its further circulation.
In 2015, BTM samplings were performed between 1 October and 15 December in dairy farms located in an area with high density of beef cattle imported from France. In 2016, BTM was sampled in areas considered at higher risk of BTV spreading, between 6 September and 30 November. In both periods, 40 ml of BTM were sampled on a bi-weekly basis in each farm, with the addition of antimicrobial preservative. BTM samples were tested by means of a non-competitive ELISA kit (ID Screen® Bluetongue Milk Indirect, ID.Vet310, Grabels, FRANCE), specific for the detection of BTV antibodies in milk. Further ELISA and PCR analyses were performed on individual blood samples taken from farm with BTV-positive BTM samples.
In 2015, 4128 BTM samples collected from 1264 farms were tested. In 107 farms, tests did not result negative. Further investigations revealed that animals from 97 of those farms had been vaccinated against BT, and therefore the tests were assumed being negative. The remaining 10 farms were re-tested. Blood samples were taken from 372 animals, and were tested through ELISA and PCR. All of the samples were negative. In 2016, 2475 farms were tested at least once. BTM samples collected from 750 farms tested positive; due to the evolution of the epidemiological situation, single blood samples were taken only from 205 farms. BTV-4 was detected in 126 of those farms, located in 84 municipalities of six provinces, 65 of which (77.38%) were newly affected. Of the 126 BTV cases detected through BTM, 42 were farms with 15-50 animals, 35 with 51-100 animals, 28 with 101-200 animals, 16 with more than 200 animals, and 5 with less than 5 animals.
Analyses performed in 2015 did not detect any BTV circulating in the area. In 2016, the epidemiological situation was different as BTV-4 was already circulating in the region. BTM samplings proved to be economically viable, as the procedure required single blood testing of only 0.8% and 8.3% of farms tested with BTM, in 2015 and 2016 respectively. Furthermore, due to short operative time required for sampling, BTM testing allows to rapidly define the affected area. Although vaccination could negatively affect BTM results because of the persistence of antibodies against BTV in milk, the inclusion of BTM in routine surveillance could represent a valuable resource to investigate BTV circulation in unaffected populations.

P1 3: Vaccination of cattle with BTVPUR® containing BTV-4 provides full protection for one year

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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.
BTVPUR is a range of inactivated vaccines against BTV serotypes, registered in the EU under a "multi-strain" dossier, allowing formulation of monovalent vaccines or bivalent combinations among the different registered serotypes BTV-1, 4 and 8. In sheep and cattle, efficacy of BTVPUR vaccines is substantiated through an official claim with 1 year duration of immunity. Hereafter we present the experimental data supporting the BTV-4 duration of immunity in cattle.

Material & Methods

Fourteen BTV naïve calves were randomly allocated to 2 groups. One group was vaccinated twice (vaccine at low antigen content), 3 weeks apart, while the other group served as control. Twelve months after completion of the vaccination, all animals were challenged with a virulent BTV-4 isolate. Animals were monitored for serology (VNT), rectal temperature, clinical signs and viraemia (real-time RT-PCR) up to 28 days after challenge.
Further, in a parallel experiment, 7 other vaccinated animals (not challenged) were boosted with a single injection, 12 months after their initial vaccination, to confirm the immunogenicity of single annual revaccinations with BTVPUR.

Results

After challenge, in the control group, an increase of rectal temperature was observed in most animals, and all were found viraemic at high titre.
Conversely rectal temperatures in the vaccinates remained significantly lower and none of them was ever found viraemic (significant prevention of viraemia).
Further, BTV-4 neutralizing antibody titres 3 weeks after the 12 months revaccination were significantly higher than those observed after the initial vaccination, demonstrating the immunogenicity of single annual revaccinations.

Conclusions

One year duration of immunity of BTVPUR against BTV-4 in cattle was demonstrated. These results, together with previous ones show that the BTVPUR vaccine range is an effective and flexible tool to clinically and epidemiologically control BTV outbreaks in EU both in sheep and cattle.

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P1 4: Serological status for BTV-8 in French cattle prior to the 2015 re-emergence

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Despite being undetected in Europe since 2010, Bluetongue virus serotype 8 (BTV-8) re-emerged in August 2015 in Central France (1). To gain insight into the re-emergence on the French territory, we analyzed seroprevalence in cattle, before the detection of BTV-8 in 2015, in several areas affected more or less early by the current outbreak. A serosurvey was thus conducted in the winter preceding the re-emergence (2014/15) in seven French departments including the one where the virus was first detected. 10,066 animals were sampled in 444 different herds. Between-herd seroprevalence revealed the presence of seropositive animals in almost all herds sampled (97.4%). The animal-level seroprevalence averaged at 44%, with a strong age-pattern reflecting the cumulative exposure to both natural infection and to vaccination. A multivariate analysis allowed separating the respective effects on the seropositivity risk of the exposure to vaccination and to natural infection. A higher proportion of seropositivity risk was attributed to vaccination (67.4%) than to exposure to natural infection (24.2%). The evolution of seroprevalence induced by the two main risk factors in 74 mainland departments was reconstructed between the vaccination ban (2013) and the re-emergence (2015). We showed a striking decrease in seroprevalence with time after the vaccination ban, due to population renewal, which could have facilitated viral transmission leading to the current outbreak situation.

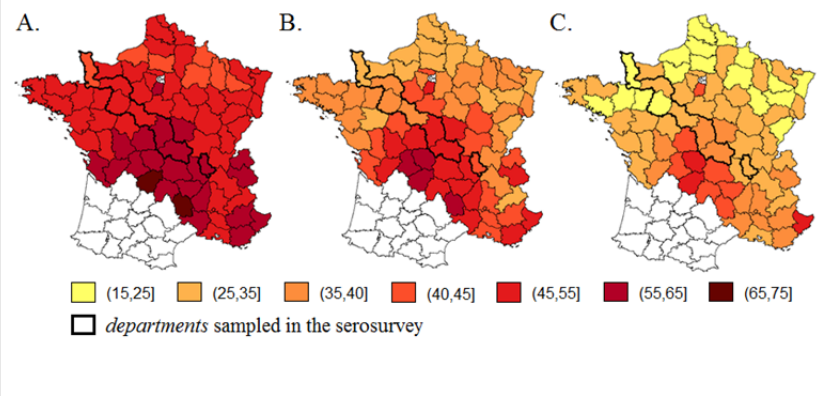

Figure 1: Seroprevalences (%) reconstructed between 2013 and 2015.
In winters: 2012/13 (A), 2013/14 (B), 2014/15 (C). (Scale: 1:20,000,000).

Acknowledgements
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P1 5: Rate of introduction of 36 vector-borne disease agents in the European Union

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The rate of introduction of vector-borne disease agents (VBD-agents) in the European Union (EU) through movement of livestock or pets was assessed to provide scientific advice to policy makers and risk managers. The selection of 36 VBD-agents to be assessed was based on the following criteria: the pathogens needed to be arthropod-borne; replicate in one of the following livestock or pets species: cattle, sheep, goats, swine, equines, dogs or cats; not reported yet or currently present only in 1 region in the EU (northern-, eastern-, southern- and western EU*); and not transmitted by tsetse flies, which do not occur in the EU.
A semi-quantitative Method to INTegrate all relevant RISK aspects (MINTRISK model), which was further modified to a European scale into the EFSA-VBD-RISK-model, was used for the risk assessment. First, the rates of entry, the level of transmission, and the probability of establishment were calculated separately; and then these three probabilities were combined into an overall rate of introduction. The probability of each step of the risk pathway was calculated choosing from a low, moderate or high uncertainty level. Data inputs from trade databases (e.g.: EUSTAT, TRACES), and disease notification systems (e.g.:OIE, WHO, TESSY) as well as transmission and disease detection, prevention and control parameters from systematic literature reviews and expert opinions were used to obtain the required data inputs to complete the model. Then the model sampled values from different triangular distributions according to the chosen uncertainty levels. Based on the reported parameters only eight of the 36 VBD-agents had an overall rate of introduction in the EU estimated to be above 0.001 introductions per year. These were Crimean-Congo hemorrhagic fever virus, bluetongue virus, West Nile virus, Schmallenberg virus, Hepatozoon canis, Leishmania infantum, Bunyamwera virus and Highlands J. virus. It should be noted that a minimum movement of 100 animals from infected areas into the EU was always included in the model, to accommodate for illegal movements or underreporting of disease occurrence. Due to the uncertainty related to some parameters used for the risk assessment or the instable or unpredictability disease situation in some of the source regions, it is recommended to update the assessment when new information becomes available. Since this risk assessment was carried out for large regions in the EU for many VBD-agents, it should be considered as a first screening. If a more detailed risk assessment for a specific VBD is wished for on a national or subnational level, the EFSA-VBD-RISK-model is freely available for this purpose.

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P1 6: Demonstration of full protection of cattle against BTV-2 challenge provided by a BTVPUR AlSap® vaccine

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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.

BTVPUR® is a range of inactivated vaccines for sheep and cattle against BTV serotypes, registered in the EU under a "multi-strain" procedure, allowing combinations among the different registered vaccines serotypes BTV-1, 4 & 8. Currently, the BTV-2 serotype is integrated in a BTVPUR AlSap® 2-4, which is registered in the EU only for protection of sheep against these two serotypes. Hereafter we present the experimental data supporting the BTV-2 efficacy in cattle for a BTV-2 possible future addition in the BTVPUR multistrain dossier.

Material & Methods

Twelve BTV naïve calves were randomly allocated to 2 groups, based on age. One group was vaccinated twice (vaccine at low antigen content), 3 weeks apart, while the other group served as control. Three weeks after the second vaccination, all calves were challenged with a virulent BTV-2 isolate. Animals were monitored for serology (VNT), rectal temperature, clinical signs and viraemia (real-time RT-PCR) up to 28 days after challenge.

Results

Very few clinical signs were observed following the challenge, suggesting that BTV-2 is lowly pathogenic for cattle. All controls were however found viraemic at high titers. Conversely none of the vaccinates was ever found viraemic.

Conclusions

Despite the apparent low pathogenicity of BTV-2 in cattle, the high vireamia titres observed in the controls demonstrate that cattle may play an important epidemiological role in the maintenance or spread of BTV-2.

In this context, vaccination of cattle using a vaccine allowing a complete prevention of viraemia may help to control BTV-2 outbreaks and potentially to reduce the impact of BTV-2 in sheep.

These results demonstrated the complete efficacy of the BTV-2 tested vaccine against viraemia in cattle. These data may in the future allow integration of BTV-2 in the BTVPUR multi-strain dossier.

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P1 7 :Monitoring of ZIKA virus in mosquitoes in Sardinia, Italy, 2016

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Zika virus (ZIKV) is a Flavivirus transmitted by infected mosquito bites and it causes an infection characterized by fever, skin rash, conjunctivitis, joint inflammation and pain. The main vector of ZIKV is *Aedes aegypti*, but also *Ae. albopictus* is considered a potential vector species. Considering the establishment of *Ae. albopictus* and more recently, the new introductions of exotic mosquitoes species as *Ae. koreicus* and *Ae. japonicus*, Italy has been classified as having a moderate likelihood of local transmission of ZIKV. During 2016 a total of 94 cases of imported infections were confirmed in Italy, included one case notified in July in Sardinia. Due to these reasons, during 2016 an entomological surveillance plan has been activated in Sardinia to evaluate the new introductions of invasive mosquitoes species, to define the abundance of *Ae. albopictus* and his seasonality, and to investigate about the presence of ZIKV in all mosquitoes species captured. For this aim, a BG Sentinel Mosquito Trap was positioned in urban areas of the major cities (12 sites) and in the most important border areas, ports and airports (17 sites). In details a total of 29 sites were included in this survey: 8 traps were positioned in Cagliari, 4 in Oristano, 2 in Tortolì, 2 in Nuoro, 6 in Olbia, 4 in Alghero, 1 in Sassari, 1 in Porto Torres and 1 in Santa Teresa di Gallura. A supplementary trap worked for 3 days, inside a pilothouse of a coal ship, moored in Porto Torres port and coming from Russia. Mosquitoes were collected fortnightly from April to December. All mosquitoes sampled were morphologically identified at the species level under a stereo microscope using taxonomic keys and then were assayed by Real Time RT PCR for detection of ZIKV RNA. A total of 3,089 mosquitoes bel onging to 10 species were collected and none invasive mosquitoes species was found. The most abundant species were *Cx pipiens s.l.* and *Ae. Albopictus*, both captured throughout the survey period. From supplementary trap none mosquitoes was captured. A total of 584 pool were analyzed and none evidence of ZIKV was reported. Although ZIKV presence was not detected during our study, because Sardi nia is located in the middle of Mediterranean basin with well established *Ae. albopictus* population and has high intensity of touristic and trade flows, these data could be provide epidemiological information to evaluate the risk of introduction of ZIKV and other arboviruses in Ital y.

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P1 8: Concurrent Infection of Bluetongue (BT) and Peste des Petits Ruminants (PPR) in Awassi Sheep in Jordan

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Bluetongue (BT) is a disease of ruminant livestock that is caused by the bluetongue virus (BTV). This virus is transmitted to livestock by selected species in the genus *Culicoides* (Diptera: Ceratopogonidae). BTV recently has been spread throughout large portion of Europe including northern areas of the continent (Saegerman C, 2008). Although, BTV and PPR have been reported in the Middle East, There have been no reports of concurrent infection in sheep and goats. In 2009, some sheep farmers in AL Mafraq area complained of mortalities in young lambs, in one farm reached (up to 25%) in lambs younger than three months and 5% in lambs about 4 months. Some adult sheep had swollen heads, and edema mainly in the lips, with few sheep with lameness and fever (41 C). The main gross pathological lesions were erosive and ulcerative stomatitis, seen mainly in the mucosa of lower lips, gingival, dental pads, tongue, hard and soft palates. Hyperemia was also seen in most of the oral cavity of the examined animals. The small intestines were congested and the adjacent mesenteric lymph nodes were enlarged, edematous, necrotic and hemorrhagic. The lungs were edematous, heavy and severely congested. The trachea and the major bronchi were hyperemic and full with froth. Petechial and ecchymotic hemorrhages were widely distributed in the epicardium, and in the endocardium with no obvious coagualtive necrosis in the heart. Three to four hemorrhagic spots were seen in the base of the pulmonary artery in three animals; two lambs and one adult sheep. In addition, diffuse extensive hemorrhages were seen in the aorta in some of the examined animals. Histopathological examination of the PA revealed sever hemorrhage covering two third of the its wall with some moderate to severe inflammation. There were also low to high numbers of inflammatory cells (mononuclear cells) invading some of the blood vessel walls, mainly the arterioles, beneath the epithelium of the tongue; Mild to severe perivascular infiltration of mononuclear cells was also seen. The endothelial cells were hypertrophic seen mainly in the small arterioles, taking large areas of their lumen. Severe congestion and mild to severe hemorrhage was seen in the tongue tissues. The small intestine showed massive coagulative necrosis affecting the epithelium, crypts and was infiltrated with mild numbers of lymphocytes. A very pronounced feature was extensive necrosis and depletion of the Peyer's patches in many of the sections examined. The mesenteric lymph nodes and spleen had severe hemorrhages and extensive widespread coagulative necrosis and depletion of the lymphatic nodules. In the lung tissues, diffuse and severe bronchopneumonia was seen with diffuse necrosis, hemorrhages, edema and congestion which were associated with mononuclear infiltrates and binucleated/trinucleated and syncytial giant cells in some sections. RT-PCR was conducted on 30 blood samples collected from suspected cases/with clinical signs comptable to BT, PPR and FMD (22 from sheep , 7 goat and 2 from cattle). Among tested samples, all sheep samples (Mafrag area) showed positive RT-PCR result for BTV serotype 4 (2150 bp), and PPR positive result (368 bp). None of these tested samples showed FMD positive band. Goat samples taken from another area (Jarash) showed positive RT-PCR results for BTV serotype 8 (562 bp); Two samples showed positive RT-PCR results for both BTV serotypes 4 and 8. Two cattle samples showed also BTV infection from the same area. Recent sequencing of PPR from infected tissues revealed that lineage IV is found in the examined tissues. Thus, we conclude that the presence of the two diseases BT and PPR together in the same animals.

P1 9: Mapping protein-protein interactions between tick-borne flaviviruses and their different mammalian hosts

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Tick-borne viruses represent a major threat to human and animal health, against which effective and sustainable strategies for prevention and control must be sought. Nonetheless, current understanding of the interactions between tick-borne viruses and their various host species is fragmentary, thus limiting conception of innovative approaches. Insight into virus-host relationships can be gained by large-scale mapping of virus-host protein interactions by high-throughput proteomics. As regards tick-borne viruses, such interactions are likely to be important for viral carriage in mammalian and arthropod hosts and interspecies transmission. In Europe, two tickborne flaviviruses, tick-borne encephalitis virus (TBEV) and Louping Ill virus (LIV), are responsible for neurological disease in humans and sheep, respectively. The aim of our study is to investigate interactions between TBEV and LIV, which are of concern to human and veterinary health, respectively, and their different mammalian host species, humans and ruminants. To this end, the network of protein-protein interactions established between viral proteins and proteins encoded by cDNA librairies of *Homo sapiens* and *Bos taurus* is being resolved using the yeast two-hybrid method. Once putative interactions between viral and host proteins have been identified, these will be validated at biochemical and functional levels *in vitro* in appropriate cell lines. Moreover, interactions between selected viral proteins and innate immune pathways are being explicitly addressed. Comparison of the interactomes in different vertebrate hosts is likely to illuminate the molecular bases of viral pathogenesis for these viruses.

P1 10: Isolation and Molecular Characterization of Bluetongue Virus in Sheep and Goats in the States of São Paulo and Rio De Janeiro – Brazil

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Bluetongue is an infectious, and may be contagious disease transmitted by *Culicoides sp*. The bluetongue virus (BTV) belongs the family *Reoviridae*, genus *Orbivirus.* It was recognize 27 serotypes by ICTV, however, described in the literature there are 29 BTV serotypes and they have been identified in tropical zone, subtropical zone and some temperate regions of the world. Sheep, cattle, goats and various species of wild ruminants are affected, but in Brazil, its clinical signs are usually observed in deer and some breeds of sheep and include a high rate of mortality, reproductive problems, weight loss and indirect losses as a result of export restrictions. The aim of this study was to verify the presence of BTV in sheep and goats of different breed, from 16 properties in southeast Brazil. A total of 646 animals, which age ranged from 3 to 36 months, were examined from 2007 to 2012. Blood, crusted lesions of animals with vesicle and postmortem tissue samples were analyzed by real-time reverse-transcription-polymerase chain reaction (RT-q PCR). The detection of antibodies in serum samples was performed by agar gel immunodiffusion assay (IDGA). Out of 646 samples, 15% (96) were positive by RT-q PCR and antibodies were detected in 23,4% (151/646). The positive samples in RT-q PCR were propagated in embryonated chicken eggs and also submitted to sequencing which revealed BTV- 4 in five properties in São Paulo State, corresponding to four sheep and one goat flocks and two sheep flocks in Rio de Janeiro State. Also BTV-12 and BTV-22 was detected in one sheep flock in São Paulo State. Molecular studies for typing of BTV isolates in Brazil are very important to evaluate of epidemiological situation thus contributing to sanitary measures of control; reducing the risk of introduction of new BTV strains to minimize the negative impact of the disease and to improve the exchange of products of animal origin.

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P1 11: Mosquito-borne pathogen surveillance in Germany: first results from the years 2015 and 2016

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Increasing international trade and travel and changing climate support the spread and development of invasive and native mosquito species. As some of these can serve as vectors of pathogens, the mosquito monitoring project "CuliMo" (Culicid Monitoring) was launched in 2015 in Germany, with the aim to update knowledge about the present mosquito fauna and circulating pathogens.
40.000 mosquitoes of 17 species collected within the project in 2015 and 2016 were screened for viruses and filarial worms. Mosquito identification was done mainly morphologically, but in case of species complexes or damaged individuals by molecular methods. Mosquitoes were pooled according to sampling date, site and species before screening for alpha-, flavi- and orthobunyaviruses as well as filarial worms using different PanPCR assays (Eshoo et al. 2007, Chao et al. 2007, Lambert et al. 2009, Kronefeld et al. 2014).
One mosquito pool, each, was tested positive for Sindbis virus and Usutu virus. Signals for filarial worms were detected in 34 samples.
The pool positive for Sindbis virus corresponded to a single *Culex torrentium* specimen, sampled 2015 in central Germany. Phylogenetic analyses showed the close relationship of this strain to the German prototype strain from southwestern Germany (Joest et al. 2010). The virus was isolated in cell culture.
The Usutu virus positive pool consisted of 50 mosquitoes of the *Culex pipiens* complex collected 2016 in northwestern Germany.
178 samples tested positive in the generic flavivirus RT-PCR. Subsequent sequencing and phylogenetic analysis demonstrated a high similarity with mosquito-only flaviviruses.
As for the filarial worms, numerous pools showed signals in the PanFila-PCR, but species could not yet be identified.

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P1 12: First serological evidence of BTV-1, BTV-2 and BTV-3 in south-eastern Brazil

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The *Bluetongue virus* (BTV), genus *Orbi virus*, family *Reoviridae*, presents icosahedral particles with 60 to 85 nm in diameter. Its genome consists of ten segments of dsRNA and presents high genetic variability, with 29 BTV serotypes already identified in several countries. The virus is transmitted by mosquitoes of the genus *Culicoides*, and induces the bluetongue disease, which can affect several ruminant species. Bluetongue is a disease of mandatory notification, and restricts movement and trade of animals in virus-free areas. In Brazil, infected cattle are usually asymptomatic with long periods (months) of viraemia. However, there is still a gap in the knowledge of the diversity of serotypes that occur in Brazil. In this study, 1,598 animals (all bovine females), one per farm, from different production systems were evaluated, representing the seven cattle production circuits of the State of Sao Paulo (the fourth largest beef producer in Brazil). The criterion for selection of the serum sample was the positive result in the competitive ELISA for the detection of anti-BTV antibodies. Reagent sera were tested by virus neutralization (VN) with serial dilution of 1:10 to 1:1,280. The prevalence of neutralizing antibodies (percentile, [95% confidence interval]) was obtained to BTV-1 (22.15%, [15.72-27.92]), BTV-2 (31.03%, [26.65-37.98]), and BTV-3 (18.96%, [12.42-24.90]). These results confirm that the Brazilian territory has favourable conditions for the occurrence of the bluetongue disease, since specific neutralizing antibodies for several BTV serotypes were detected in asymptomatic cattle. To date, this is the first report of the occurrence of the BTV-1 and BTV-3 in the State of Sao Paulo, and to our knowledge this is the first report of BTV-2 in Brazil.

P1 13: Estimation of spread of Bluetongue virus serotype 1 in sheep at the end of the 2014 epidemic in Umbria, central-Italy

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Introduction

Bluetongue (BT) is an infectious non contagious, vector borne viral disease of domestic and wild ruminants caused by Bluetongue virus (BTV) and transmitted by midges of the genus *Culicoides*. Clinical signs of BT are more severe in naïve animals, and are most commonly observed in sheep, although they are also described in cattle and other species. Starting from early summer 2014, BTV-1 massively circulated in central and southern Italy, causing 1531 confirmed outbreaks at the end of the 2014 epidemic. In Umbria, a region located in central Italy, the first BTV-1 outbreak was notified in August 2014 in a sheep flock; the virus then spread rapidly all over the region. During this epidemic, BT outbreaks were mostly notified based upon recognition of clinical signs in sheep and, therefore, subclinical forms of infection are likely to pass unnoticed. It is thus possible that the total number of BTVinfected animals within a single herd was underestimated. As part of a Regional Research Project, a study was carried out to evaluate the prevalence of the BTV-1 infection in a representative number of Umbrian sheep flocks after natural infection.

Materials and Methods

Between January 2015 and February 2015, 1.008 sheep originating from 13 flocks, 6 located in the province of Terni and 7 in the province of Perugia, were bled and serum samples were tested for the presence of BTV-1 antibodies by virus neutralization. Seroprevalence at regional and provincial level (BTV-1 positive animals/total animals tested) and 95% confidence intervals were determined and analyzed through Beta distribution. **Results** The overall regional sero-prevalence was 32.6%. In the province of Terni seroprevalence ranged from 15.6% to 82.3% whereas in the province of Perugia from 14.1% to 46.1% Mean sero-prevalence in the province of Terni (39.8%) was significantly higher than that of the province of Perugia (24.6%, $p<0.05$).

Conclusions

The estimation of BTV-1 virus spread after an epidemic event provides useful information on virus circulation in a given area.
This survey shows that BTV-1 did not spread uniformly in two provinces considered, probably due to different eco-biological factors.
However in both cases more than 60% of the sheep population is still naïve. This latter datum is critical as it underlines the importance of massive vaccination to protect sheep after BTV-1 natural infection.

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P1 14: Evaluation of the surveillance strategy used to detect WNF infection in sentinel equines during 2016 in Bulgaria

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West Nile fever is an arboviral infection characterized by two types of transmission cycles: rural (sylvatic) cycle maintained by wild birds (reservoir hosts) and ornithophilic mosquitoes and urban cycle in which domestic birds, bridge vectors and mammals are involved. Migratory birds are considered to play an important role for the introduction of the infection and the development of natural foci located in the wetlands.

Two of the biggest migratory routes in Europe (Via Pontica and Via Aristotelis) pass through the territory of Bulgaria. Thousands of hundred birds migrate along Via Pontica and Via Aristotelis leading to large concentration of migratory birds at the stop-overs. We identified the major stop-overs in the wetlands and big rivers and classified the populated areas in close proximity as risk areas for WNF annual introduction, taking into consideration the WNF epidemiological situation on the Balkan Peninsula.

In 2016, we implemented a sentinel based surveillance program in 11 risk areas within the country. Equines, backyard chickens and dogs permanently situated in the risk areas have been regularly tested for presence of NAbs during the active vector season. To assess the strategy used for the classification of the risk areas we compared the frequency of WNF infection between equines from the risk areas and equines located in non-risk areas at the end of the surveillance period. The aim of the study was to determine the presence or absence of a difference between the two groups. A total number of 500 equines have been included in the study: exposed group/equines from the risk areas (n=193) and unexposed group/equines from non-risk areas (n=307). Sample calculation was based on 90% power, 95% CI, ratio 1:2 (exposed:unexposed), 15% expected prevalence in the exposed group and 5% expected prevalence in the unexposed group. All positive and doubtful samples in ELISA (screening test) have been further examined by SNT. Serum samples with low titer (≤1:10) and negative samples on SNT have been tested for the closely related USUV by SNT.

The percentage of equines with WNF specific antibodies was notably higher in the exposed group (41.5%) compared to the unexposed (9.4%). The estimated odds ratio 6.3 (95% CI: 3.8-10.5, p-value<0.0001) confirmed our hypothesis for high probability (0.86) of being infected with WNF in the risk areas. The observed association between the geographical location and the frequency of the infection in equines suggests that future outbreaks are likely to occur in the risk areas and supports the strategy for their inclusion within the scope of the WNF surveillance program. Furthermore, the presented results could serve as an indirect proof for WNF infection among the resident human population and could be used to increase the sensitivity of the early-warning system for WNF human cases.

P1 15: Surveillance instruments in the Netherlands Do we detect BTV crossing our borders?

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Bluetongue (BT) is an OIE listed, *Culicoides*-transmitted viral disease of ruminants, and its introduction leads to implementation of control measures which impact the agro-economy due to trade limitations and costs for surveillance, prevention, and treatment. GD Animal Health is commissioned to execute yearly surveillance for the absence of BT in accordance with OIE and EU guidelines. To do so, the Netherlands is divided into twenty compartments in each of which at least 14 unvaccinated cows born in or after 2010 and that are at least eight months old are tested for the presence of antibodies against BT virus (BTV). Since 2009, seropositive unvaccinated cattle have not been detected, indicating that BTV did not circulate.

Since 2012, the Netherlands has been officially BTV free, however, in view of the current BT situation in Europe, incursion of BTV is considered a realistic scenario. Early detection of a BTV incursion is highly important to contain the outbreak and to prevent further spread. This study provides an overview of the different surveillance components for early detection of BT.

The Dutch Animal Health Surveillance System (AHSS) encompasses different surveillance instruments to detect incursions of diseases such as BT. One of the AHSS instruments is a telephone helpdesk for veterinarians and farmers, staffed with specialized GD veterinarians. In the period 2012-2016, on average 0.9% of disease related questions (of about 4000 phone calls per year) concerned BT. In case BT cannot be excluded, the Netherlands Food and Consumer Product Safety Authority (NVWA) is notified and a team of experts from both NVWA and GD visits the farm and initiates diagnostic investigations. A total of 35 cases of suspected BT were investigated by expert teams in the period 2012-2016.

Since 2015, syndromic surveillance on routinely collected bulk milk production data has been implemented. A drop in milk production provides a non-specific signal indicating a change in animal health which is followed up by GD epidemiologists and veterinarians. In July 2016, a regional drop in bulk milk production was detected but after follow up turned out to be related to periods of local heavy rainfall resulting in poor pasture conditions. Although this signal was not related to an infectious disease, it demonstrated the ability of the instrument to produce a signal which could indicate a disease outbreak.

Another active surveillance component to monitor the absence of BT is serological surveillance on export cattle. Although export testing is not designed as a surveillance instrument for early detection, the results provide additional evidence for the absence of BTV circulation.

Animal disease surveillance in the Netherlands uses various instruments, each with their own objectives (i.e. early detection or proving absence of disease) advantages and drawbacks. However, the combination of these instruments results in an adequate system for detection of BTV incursions.

P1 16: DISA vaccines for Bluetongue: An overview of a novel vaccine approach

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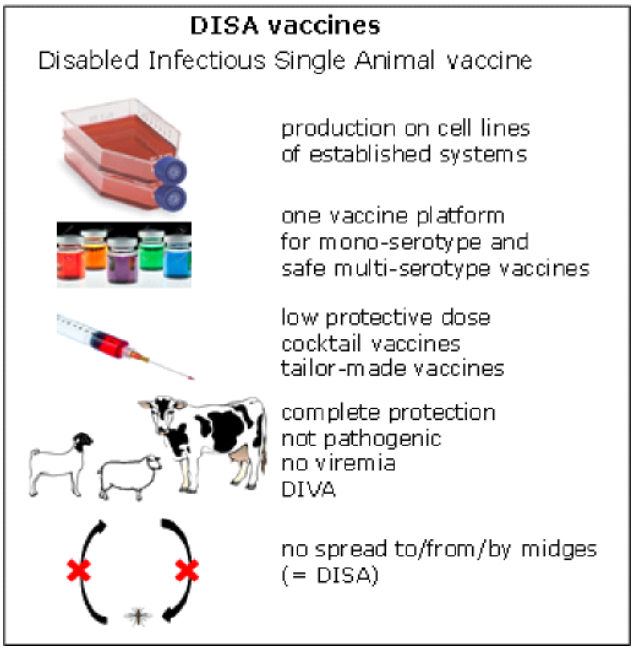
In general, conventionally live-attenuated virus is the most optimal vaccine because vaccination imitates natural infection without causing the disease resulting in maximal protection due to a complete antigen repertoire and full blown immune responses. Still, such replicating virus vaccines could have shortcomings regarding residual virulence, reversion to virulence and do not exhibit DIVA.

Bluetongue (BT) is a notifiable ruminant disease according to the World Organisation of Animal Health with various rates of morbidity and mortality depending on virulence and susceptibility of the ruminant species. The causative pathogen bluetongue virus (BTV) is a virus species in the genus *Orbivirus* of the family *Reoviridae*. Currently, >27 BTV serotypes have been defined showing limited cross neutralization. BTV is transmitted by species of *Culicoides* biting midges and has become a serious threat for countries in a moderate climate due to several factors, including global warming.



We have developed BTV harbouring a deletion in genome segment Seg-10 and is therefore deficient for functional NS3/NS3a protein (van Gennip et al., 2014). NS3/NS3a deletion BTV is **grown on standard cell lines**. Deletion BTV is **not virulent** (Feenstra et al., 2014a), and replicates in the target ruminant **without causing viremia** (no uptake by midges), resulting in **rapid** and **complete serotype specific protection** (Feenstra et al., 2014b, 2014c), and is named Disabled Infectious Single Animal (DISA) vaccine (**Patent WO2014185784**). Vaccine safety is further augmented, since propagation of BT DISA vaccine is abolished in midges (Feenstra et al., 2015a). The deletion in Seg-10 and thus the absence of NS3/NS3a protein enables differentiating infected from vaccinated animals (the **DIVA** principle) (van Rijn et al., 2012, 2013; Tacken et al., 2015). The **BT DISA vaccine platform** have been applied for multiple serotypes by exchange of Seg-2 encoding VP2 protein critical for serotype specific protection, such as the 'European' BTV serotypes 1, 2, 4, 6, 8, 9, or 16 (Feenstra et al., 2015b). The immune response following a single standard dose of BT DISA vaccine is **robust** as measured with 1/10 and 1/100 of the used protective dose, and i.m. and s.c. as suitable vaccination routes (van Rijn et al., 2017). BT DISA vaccines are the solution to combat Bluetongue disease in a cheap, safe, effective and controllable program for different field situations (Feenstra and van Rijn, 2017). BT DISA vaccines can be used as mono-serotype vaccine or can be **safely combined** to achieve broad protection or to combat specific multi-serotype field situations in a tailor-made fashion (in progress; EU-project PALE-Blu).

11th EPIZONE Annual Meeting
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POSTERS
Topic 2 - Threats at the European borders

P2 1: Molecular epidemiology of foot and mouth disease virus (FMDV) in Chad

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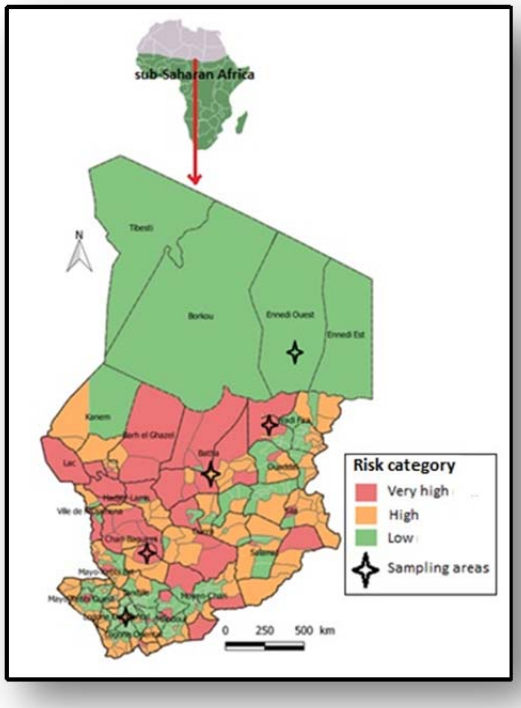

Figure 1: Risk of FMDV spread in Chad (based on data collected in 2016)

Foot and mouth disease (FMD) is a highly contagious viral disease affecting domestic and wild artiodactyl animals. Its causative agent is foot-and–mouth disease virus (FMDV: *Aphtovirus, Picornaviridae)*. Seven immunologically distinct serotypes (O, A, C, Asia1, SAT 1, SAT 2 and SAT 3) and many subtypes are described worldwide. FMD is one of the most economically devastating diseases of livestock. It is enzootic in many parts of the world including sub-Saharan Africa. Most studies on FMD are carried out in countries where control measures are implemented. On the other hand, in regions such as sub-Saharan Africa, where FMD is endemic and new strains are likely to spread due to animal movements, there are very few published studies on FMDV molecular epidemiology. In Chad particularly, no studies have been conducted to investigate circulating FMDV strains.

This work aims to understand the transmission process of FMDV in the pastoral area of Chad, based on a stratified sample of livestock herds (fig. 1). Susceptible animals (cattle, sheep, goats, and camels) were sampled according to the a priori risk of FMD spread in Chad, evaluated by a qualitative risk analysis combining the risks of its introduction and dissemination.

In total, 2,195 sera and eight epithelium samples were collected from October to December 2016 in six districts (Batha-Ouest, Batha-Est, Ennedi-Ouest, Wadi-fira, Chari and Lac Wey). Five out of the eight samples tested positive by real-time RT-PCR targeting the FMDV IRES region or the FMDV 3D polymerase coding region. Further analyses targeting specifically the VP1 coding region showed SAT2 type for four samples out of these five FMDV positive samples. Finally amplification and sequencing of the VP1 coding region of these four SAT2 positive samples was carried out to characterize more precisely the strains. Preliminary results were obtained for one sample, confirming the presence of a SAT2 virus, closely related to FMDV SAT2 viruses isolated in Egypt in 2012. Serological analyses are pending.

Filling the gap of knowledge concerning the FMDV strains circulating in Chad could both contribute to a better selection of vaccine strains but also to an update of the available molecular epidemiology data of FMD virus in sub-Saharan Africa in general.

P2 2: Two-Plasmid System to Increase the Rescue Efficiency of Paramyxoviruses by Reverse Genetics: the example of rescuing Newcastle Disease Virus

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Within paramyxoviruses, conventional reverse genetics require the transfection of a minimum of four plasmids: three to reconstruct the viral polymerase complex that replicates and expresses the virus genome delivered by a fourth plasmid. The successful transfection of four or more plasmids of different sizes into one cell and the subsequent generation of at least one viable and replicable viral particle is a rare event, which explains the low rescue efficiency, especially of low virulent viruses with reduced replication efficiency in cell lines. In this study, we report on an improved reverse genetics system developed for an avian paramyxovirus, Newcastle Disease Virus (NDV), in which the number of plasmids was reduced from four to two. Compared to the conventional method, the 2-plasmid system enables earlier and increased production of rescued viruses and, in addition, makes it possible to rescue viruses that it was not possible to rescue using the 4-plasmid system.

P2 3: BVDV Transmission Between Countries By Imported Animals

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Introduction

Pestivirus genus includes Bovine viral diarrhea virus (BVDV)- 1, BVDV-2, Border disease virus (BDV), Classical swine fever virus and atypical pestiviruses. BVDV species are economically important pathogens and include various subtypes. There are twenty-one subgenotypes (1a to 1u) identified for BVDV-1 and four subgenotypes (2a to 2d) for BVDV-2. These subtype variety can have a negative influence on diagnostics and efforts for prevention. BVDV can be transmitted either horizontally or vertically both by transiently and persistently infected animals. At this point virus-infected semen, contaminated fetal calf serum and live animal marketing are the case conditions, which come into question and discussed for transmission of the virus across national boundaries.

Aim

Purpose of this study was testing samples from imported animals and their calves to determine the risk of animal movement on virus transmission across international borders and their role on viral heterogeneity.

Materials & Method

Imported animals included in two different BVDV free herd at different provinces and their calves are sampled for BVDV detection. Blood samples of heifers and 744 calves were tested against pestivirus antigens with a commercial enzyme-linked immunosorbent assay (ELISA), which is specific to E^{rns} glicoproteins. From the animals detected positive by antigen ELISA, second blood samples collected and re-tested to identify the persistent infection status. For virus isolation, all the ELISA positive samples were inoculated onto Madin Darby Bovine Kidney (MDBK) cell line and after 3 blind passages indirect immunoperoxidase monolayer assay (IIPMA) was applied. Reverse transcriptase-polymerase chain reaction (rt-PCR) was applied both to blood samples and cell culture supernatants. After 288bp DNA product was amplified by panpestivirus-primers 324 and 326, differentiation of BVDV-1, BVDV-2 and BDV was performed by rt-PCR and confirmed by sequence analysis.

Results

All the heifers were negative but 9 calves born from imported heifers in BVDV free herd and 5 imported calves were found to be positive using the BVDV antigen ELISA. Only 7 of these ELISA (+) calves could be re-sampled and 3 of them detected as persistently infected. From samples subjected to virus isolation by three blind passages, non of them displayed cytopathogenic effects. Samples were further tested by IIPMA and virus propogation was demonstrated. From all tested 744 blood samples, 8 non-cytopathogenic (ncp) virus isolated. Although direct analyses from blood samples gave negative results, rt-PCR analyses from cell culture supernatants of 8 ncp virus isolate detected as BVDV-1.

Conclusion

- Despite pre-import screening, transmission of BVDV between countries with non-PI heifers pregnant to PI foetuses is possible.
- This situation creates additional risks for introducing the infection into non-infected areas.
- New strategies are needed for control of BVD virus at the market of importations, especialy for countries having eradication programes.

Funding: This study was supported by the Uludag University Research Fund (project no: HDP(V) – 2016/26).

P2 4: A simple method to evaluate the number of doses to include in a bank of vaccines. The case of Lumpy Skin in France

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The preparedness for a good and rapid response against some exotic diseases includes the availability of vaccines. The objective of this paper is to describe a simple method to estimate the size that a vaccine bank should have to manage an emergency vaccination campaign against Lumpy Skin Disease (LSD) in France.

Methods: To determine the size of the vaccine bank, the following factors were taken into account: 1) The average speed of the LSD through the affected area, estimated by Mercier et al. (2017) in 7.3 km / week. 2) The time required to obtain a full vaccination coverage, and 3) The cattle density in the area. It was assumed that all cattle present in the area will be vaccinated using a single dose per animal.
An Excel file with a macro was used to simulate ten thousands scenarios of LSD spread in France. To simplify the calculations, the French departments were considered as squares, and the index case was located randomly in the department. If the radius around the index case exceeded the department, it extended through the other departments in the region. Results were validated using a more complex model developed in R (R Development Core Team., 2013), which reproduced the real geography of France.

Results: The median of the vaccine doses need by both methods are significantly different (between 19% and 23% more vaccines are needed according the simple method). But both methods fit quite good when determining the number of doses that would be necessary in 90% of the cases. For a 7 weeks period (radius of 51.1 km), 750 000 doses of vaccine would be enough for 90% of the simulations, while for the more refined method the number of doses was 680 000 (difference of 10%).

Discussion: The method is simple and easy to use, and can be adapted to different conditions and diseases. It fits reasonable good with a more sophisticated method that takes into account de farms actually present in the zone. No jumps of the disease to long distances have been considered. At the department level, the results present more important differences (in some cases the number of vaccine needed are two folds higher using one method than using the other one).

Acknowledgements
This study was performed by an ad hoc working group of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES).

Reference:
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P2 5: Validation of a new ELISA for the detection of LSDV antibodies

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Introduction

Lumpy skin disease virus (LSDV) is the etiologic agent of LSD. Initially located in northern and central Africa, LSDV merged into Europe recently. Severe and permanent damage to hides results from the skin lesions. Lesions in the mouth, pharynx and respiratory tract commonly occur, resulting in a rapid deterioration in condition and sometimes severe emaciation, which can persist for months. Serious economic losses can follow outbreaks that have a high morbidity. As there is no specific antiviral treatment available for LSD-infected cattle, vaccination is the most effective option for controlling the spread of lumpy skin disease. Diagnostic tools are essential for the detection and the monitoring of LSD, and can play an important role to prevent the spread of the disease. IDvet developed an ELISA for LSDV antibody detection (from vaccination or infection).

Material and Methods

The ID Screen® CPV Double Antigen ELISA allows for the detection of anti-CPV antibodies. It is based on the use of purified CPV proteins for both coating and HRP conjugate. It can be used with serum or plasma of domestic and wild ruminants and any other susceptible species. Results are obtained within 2h30. The immunoperoxidase monolayer assay (IPMA) and viral neutralization (VNT [1]) were performed at CODA-CERVA (Belgium), the Indirect ImmunoFluorescence Antibody Test (IFAT) was performed at FLI (Riems).

Results and Discussion

Specificity was evaluated through the analysis of 1050 disease-free sera from cattle sampled in non-endemic and non-vaccinated areas (France, Germany, Belgium). Measured specificity was 99.7% (CI95%: 99.2% - 99.9%).

- 11 field samples from cattle vaccinated with a commercial live attenuated Neethling strain vaccine against LSD, and sampled 5 months post-vaccination were tested with ELISA, IPMA and VNT: 8 samples were found positive with the ID Screen® ELISA, 2 samples positive by VNT and all were negative by IPMA.
- 75 field samples from cattle vaccinated with a LSD commercial live attenuated Neethling strain vaccine (OBP), sampled about five months post-vaccination were tested: the ELISA picks up 44 samples, whereas the IPMA picks up 40 samples 71% of samples give similar result, 17% positive only in ELISA and 12% positive only in IPMA .
- 48 field samples from cattle vaccinated with a LSD commercial live attenuated Neethling strain vaccine (Lumpyvax®, Intervet/MSD), sampled two months post-vaccination were tested: the ELISA picks up 36 samples and the IPMA picks up 12 samples.
- 5 animals showing clinical signs of Parapox virus infection in the mouth region, and positive by IFAT, were tested ; no cross-reactivity could be ascertained with the ELISA.

Conclusions

The ID Screen® Capripox indirect ELISA specificity is excellent. The kit successfully detects antibodies against LSDV either in vaccinated or infected animals (data not shown), and seems to have at least equivalent sensitivity compared to IPMA and better compared to VNT. The kit is easy to handle, with ready-to-use reagents, and allows for high throughput screening without requiring high level containment facilities.

Acknowledgments

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P2 6: Abundance and seasonality of biting midges at a continental scale in Europe

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Background:
Culicoides biting midges are hematophagous insects of veterinary importance able to transmit viruses such as bluetongue virus and Schmallenberg virus to livestock. The aim of this work was to describe the average temporal abundance of biting midges at a continental scale, by comparing the seasonal variation at six different latitude ranges from southern Spain to northern Sweden.

Materials and methods:
We gathered *Culicoides obsoletus* group trap data collected by national surveillance programs in nine European countries (Spain, France Germany, Switzerland, Austria, Denmark, Sweden, Norway, Poland) from 2007-2013. In total, 904 farms were sampled for *Culicoides* midges using UV light traps. We divided Europe into 6 latitudinal bands of 5° width and calculated the average weekly midge abundance as well as the average annual cumulative sum of biting midges for each of these bands. We plotted the results to visualize the spatial and temporal patterns at a continental scale.

Results:
The midge season began earlier in southern Europe (week 10) compared to northern latitudes (week 19). The season also lasted longer at southern latitudes and became progressively shorter towards the northern latitudes. Abundance peaks occurred during spring and summer at all six latitudinal ranges. Despite the steadily shorter vector season in the north of Europe, the annual cumulative sum of biting midges increased towards the north. Norway and Sweden with the shortest vector periods, had the highest number of the *C. obsoletus* group, reaching a cumulative sum of 455.000 midges on average annually, with some traps collecting more than 110.000 in one night.

Discussion:
Temperature drives the start and length of the midge season at different latitudes, but it does not seem to drive the increasing abundance towards the north. A possible explanation could be that midge abundance may be more related to breeding site availability and soil moisture than temperature.

Conclusion:
This is the first time quantitative seasonal abundances for biting midges have been aggregated and jointly analyzed for the whole of Western Europe. By ignoring the fine scale variation in abundance between farms and regions, we have isolated and quantified an overall south-north trend in the temporal abundance of biting midges. These results may facilitate decision making by e.g. the EU member states when objectives and joint decisions are needed for prevention and control of midge-borne infections on a continental scale.

P2 7: Simple, quick and cost-efficient: a universal RT-PCR and sequencing strategy for genomic characterisation of foot-and-mouth disease viruses

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Foot-and-mouth disease (FMD) is a major contributor to poverty and food insecurity in Africa and Asia, and it is one of the biggest threats to agriculture in highly developed countries. As FMD is extremely contagious, strategies for its prevention, early detection, and the immediate characterization of outbreak strains are of great importance. The generation of whole-genome sequences enables phylogenetic characterization, the epidemiological tracing of virus transmission pathways and is supportive in disease control strategies. This study describes the development and validation of a rapid, universal and cost-efficient RT-PCR system to generate genome sequences of FMDV, reaching from the IRES to the end of the open reading frame. The method was evaluated using twelve different virus strains covering all seven serotypes of FMDV. Additionally, samples from experimentally infected animals were tested to mimic diagnostic field samples. All primer pairs showed a robust amplification with a high sensitivity for all serotypes. In summary, the described assay is suitable for the generation of FMDV sequences from all serotypes to allow immediate phylogenetic analysis, detailed genotyping and molecular epidemiology.

P2 8: Detection of *Chlamydia trachomatis* in samples from sheep with an enzootic abortion.

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Chlamydia trachomatis (CTR) and *Chlamydia psittaci* (CPS) are widespread well-known bacterial agents of infection in humans and animals. Chlamydia livestock can be invoke along with the generally recognized pathogens as bacteria and *C. trachomatis*, which makes it a real threat to the health of people in contact with infected livestock. Previously described, that Chlamydia can infect livestock in model experiments and in farms and cause abortions. In our study blood, scrape from the mucous membranes of the vagina from 55 aborted sheep tested for *C. trachomatis* and *C. psittaci* antigens by using both Chlamydia monospecific antibodies Ig-CTR and Ig-CPS respectively.
DOT-ELISA with Ig-CPS showed 30 (54,5 %) positive samples, with Ig-CTR we determined all this positive and 18 (32,7%) additional positive samples, where we can suppose presence *C. trachomatis* antigens only.
To confirm the presence of *C. trachomatis* DNA in clinical samples, containing chlamydia antigens, in PCR we thoroughly studied the material from two sheep (healthy and aborted) with the commercial kits (AmpliSens *Chlamydia trachomatis*-Eph, Russia; «RealBest DNA *Chlamydia trachomatis*» VectorBest, Russia). In the samples of aborted sheep compared with the samples of healthy sheep, *C. trachomatis* specific DNA fragments was clearly revealed.
The results of antibody-based assays strongly correlated with those obtained PCR.
On the next step we determine the *C. trachomatis* genovars of *C. trachomatis* in clinical samples of aborted sheep by the method based on the determination of the nucleotide sequence of the VD2 variable region of the *omp1* gene encoding the major outer membrane protein (MOMP), using primers momp-fw1-4 and Momp-rv1-4 (Quint et al., 2007). After sequencing of the short amplification fragments and comparing with the data of NCBI (https://www.ncbi.nlm.nih.gov/), we established that study samples of *C. trachomatis* belong to the *C. trachomatis* E genovar.
In the clinical material from the aborted sheep, a bacterium *C. trachomatis* genovar E, previously considered pathogenic only for humans, was found. Our study will be continue to establish the fact of overcoming the interspecies barrier and the high adaptive abilities of *C. trachomatis*.

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P2 9: Genealogy of bovine coronavirus in Brazilian herd of catlle

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The bovine coronavirus (BCoV) is one of the main etiological agents of gastroenteritis in calves, causing significant economic losses to cattle. Considering that for the design of preventive measures it is necessary to understand the pattern of circulation and evolution of this virus, the present study aimed to characterize genealogically BCoV based on the N gene. A total of 111 fecal samples of dairy and beef calves, with or without clinical signs of diarrhea from five Brazilian states during the period from 2008 to 2015. Of the 111 samples analyzed, 15.31% (17/111) were positive. BCoV was detected in 21.95% (9/41) of the animals with diarrhea and in 10% (7/70) of the asymptomatic animals. BCoV was identified in calves from rural farms located in all sampled regions. Genealogy analysis demonstrated that the Brazilian BCoV sequences for the gene encoding the N protein was segregated into 2 distinct clusters, and the samples from this study were closely related to Asian strains. These results contribute to the molecular characterization of BCoVs and demonstrate that the virus is widespread in Brazil.

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P2 10: Vaccination of goats with PPR-VAC confers a full protection against a PPR virulent challenge

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Introduction: Peste des petits ruminants (PPR) is a OIE-listed disease of sheep and goats, caused by PPRV, a Morbillivirus, from the *Paramyxoviridae* family. The disease, initially reported only in West Africa has now spread and is currently endemic in most of Africa (including the Maghreb), the Middle East, South Asia and China. Because of its high economic impact, PPR is considered one of the major livestock diseases. PPRV is mainly excreted through oculo-nasal discharge and spreads by contact between animals. Typical signs are fever, oculo-nasal discharge, diarrhea and dyspnea, associated with necrosis of the digestive and respiratory tracts. Morbidity and mortality rates reach up to 100% and 90% respectively. Goats are usually more susceptible than sheep.

Material and Methods: Twenty PPRV naïve goats were randomly allocated to 2 groups, balanced on weights. One group served as controls whereas the other vaccinated with a single dose of PPR-VAC (BVI, Gaborone, Botswana*). Twenty one days later, the 20 goats were infected intranasally with a virulent PPRV strain (Morocco 2008). Monitoring of clinical signs, weight, viral excretion (real-time RT-PCR [1]) and serological response (competitive ELISA) were performed throughout the 14 days post challenge (dpc).

Results: Control goats presented typical and severe clinical signs of PPR: fever, anorexia, nasal and ocular discharge, nasal mucosa lesions, diarrhea and weight loss. The intensity of clinical signs was maximal at 11 dpc. At that time, 4 control goats were euthanized on ethical ground. All control goats excreted high titres of PPRV genome after challenge.
Conversely, very few and mild clinical signs were observed in the vaccinated goats and they did not lose weight. Moreover, the vaccine completely prevented viral excretion in all vaccinates.

Conclusions: These results confirm the suitability of our challenge model for the clinical and virological assessment of PPRV infection.
Further, these results demonstrate that vaccination with PPR-VAC® fully protects against clinical signs and weight loss (clinical protection), and completely prevents viral excretion (epidemiological control).

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*: PPR-VAC is manufactured by the Botswana Vaccine Institute in cooperation with Merial.Merial is now a part of Boehringer Ingelheim.

P2 11: Validation of commercial real-time RT-PCR kits for rapid and specific diagnosis of classical swine fever virus, following the guidelines of the French AFNOR standards NFU47-600

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The AFNOR standards NF U47-600-1 and 600-2 define the general requirements and recommendations for the development and validation of PCR methods for detection of animal pathogens. The AFNOR standard NF U47-311 describes the dossier to be provided by the PCR manufacturer and the controls carried out by the national reference laboratory (NRL). The NRL defines the specifications that the method has to comply with in regards to a specific animal disease. For classical swine fever (CSF), the NRL has established the minimal criteria that the method has to reach for specificity and sensitivity. Samples corresponding to the minimum level of detection have been prepared and are available for PCR manufacturers, as a panel of samples to assess specificity (inclusivity/exclusivity) and sensitivity.

Classical swine fever is a notifiable disease. As the European Union has become free of CSF in domestic and wild pigs populations, it is of high importance to dispose of very sensitive and reliable diagnostic methods for rapid detection in case of new introduction. The etiological agent of Classical Swine Fever (CSF) is a small, enveloped single stranded RNA virus, belonging to the *Pestivirus* genus of the *Flaviviridae* family. The CSF virus (CSFV) is closely related to others pestivirus like Bovine Viral Diarrhea Virus (BVDV) or Border Disease Virus (BDV) that can also infect apparently healthy pigs.

Two real-time RT-PCR kits, developed respectively by ADIAGENE (Adiavet CSF Real Time) and BioSellal (Bio-T kit® CSF) were evaluated in comparison to the published method by Haines et al (2013).

The producers were asked to introduce an internal control to check the RNA extraction efficacy. The different criteria assessed were:

1. Analytical specificity (inclusivity / exclusivity) using a set of pestivirus strains and of other virus infecting pigs,
2. Analytical sensitivity using a RNA transcript to determine limit of detection (LOD) of the RT-PCR and CSFV spiked in negative blood/organs to determine the LOD of the full method (RNA extraction + RT-PCR),
3. Diagnostic sensitivity and specificity using samples from naturally or experimentally infected pigs/wild boars as well as samples from the CSF LRUE ILPT.

The reproducibility of the assays was confirmed by the analysis of a batch-to-batch panel control.
When the kit fulfils all the general and specific requirements, the CSF NRL delivers an initial certificate of compliance. For final authorisation of marketing, each new batch has also to be approved by the NRL as described in the AFNOR U47-311.

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P2 12: Lumpy skin disease detection and control in serbia in 2016

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Lumpy skin disease (LSD) is a viral disease of cattle caused by capripoxvirus. LSD is endemic in Africa and Middle East, but it is exotic in Europe. The disease mainly spread to infection-free areas by transport of infected animals and by vectors. In just one year period, starting from August 2015, the disease was spread from Turkey to the territory of 6 Balkan countries (Greece, Bulgaria, FYR Macedonia, Serbia including Kosovo, Montenegro and Albania). After the first clinical suspicion on 4th of June in settlement Ljiljance, municipality Bujanovac, the first LSD case in Serbia was laboratory confirmed on June 7th 2016.

At the beginning of the outbreak, only the early detection and stamping out method was used for control of the disease. Due to the long incubation period of the infection and transmission of the virus by vectors, even the fast laboratory diagnostic and stamping out procedure for the whole herds with detected infected animal, in a time frame of only 48 to 72 hours, was on place during that period, the restrictive measures for animal movement were not enough to stop the virus transmission. After the first 7 -10 days of LSD outbreak, when new cases were rapidly arisen all over the infected district, and due to the experiences of LSD control in Greece and Bulgaria, the vaccination strategy was decided to be included to control LSD, and OBP LSD vaccine was introduced in the country. The whole country were divided in three parts: part A (two first infected districts were vaccinated with 50,000 doses received from EU for emergent vaccination), part B (endangered districts near Bulgarian border and around Kosovo, and up to approximately 100 – 200 km from infected districts, as well as biggest cattle farms all over the country - vaccinated with 400,000 vaccine doses purchased by Veterinary Directorate in July 2016), and part C (regions around Belgrade, parts of central and northern part of Serbia - vaccinated with additional 600,000 vaccine doses purchased by Veterinary Directorate, during July/August 2016).

The measures for control of disease included: stamping out procedure of all cattle in herd with confirmed case of LSD, regardless of category, age and health status of animals; destruction of animals products; safe disposal of carcasses, by-products and waste; disinfection and disinfestation; control of vectors; animal movement restriction and intensive active surveillance in infected and protection zone. Since the decision was made for vaccination of cattle in the entire country with vaccine based on Neethling strain, measures to combat the disease were redefined. After conducting vaccination, in case if the clinical manifestation and laboratory confirmation of the disease arose in the previously vaccinated herd, and if more than 28 days passed after the vaccination, only the clinically sick animals were euthanized and safely disposed (modified stamping out).

The laboratory diagnosis of LSD cases was based on virus detection in biopsy samples of skin nodules and EDTA blood samples. For laboratory detection of LSDV, the qPCR methodology (Bowden et al., 2008) and conventional PCR methodology (Ireland end Binepal, 1998), were introduced in the labs shortly before the first onset of disease. Both of these methods are capripox virus specific, but since Serbia is free of sheep and goat pox, there was no need to include capripox virus differential methods. Mostly, the mentioned qPCR methodology, as highly sensitive, fast and robust method was used for detection of all LSD cases. Due to the possibility of mild or systemic post-vaccination reactions in vaccinated animals, after the implementation of the vaccination of cattle against LSD, application of new laboratory diagnostic procedures was necessary for differentiation of field from vaccine virus strain. For that purpose, the nested PCR

protocol with RFLP analysis (Menasherow et al., 2014) was introduced in labs. Since this protocol is time consuming and prone to cross contamination, the qPCR DIVA protocol (Vidanovic et al, 2016) to distinguish field from vaccine LSDV strain is developed and introduced in the labs.

In total, LSD outbreak in Serbia in 2016 resulted with 225 outbreaks/cases, 709 euthanized and safely disposed animals, and 875,380 vaccinated animals starting from first occurrence of disease on 4th of June until last LSD case on 1st of October 2016 (118 days). During LSD outbreak, the whole veterinary service showed that is highly competent and capable to deal with animal disease emergent situations and to successfully control the disease.

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P2 13: Preliminary serology and virology data after vaccination of animals with Neethling strain of LSDV

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Lumpy skin disease (LSD) is a viral disease of cattle caused by capripoxvirus, transmitted mainly by mechanical vectors, and characterised by severe losses, especially in naive animals. LSD is endemic in many African and Asian countries, and Middle East, but it is exotic in Europe. For the efficient control of LSD outbreak in Serbia in 2016, the vaccination of all cattle in the country with Neethling vaccine strain of LSD virus was a necessary measure. At the time of vaccination, the used LSD vaccine was not registered in EU and very limited data existed on efficacy and safety of this vaccine for cattle of the breeds that are usually raised in Europe. To evaluate the immune response and persistence / shedding of vaccine virus in vaccinated animals, a small experiment has been conducted.

In total 20 animals of Holstein-Friesian breed from 2.5 to 9 years old, vaccinated with a single dose of OBP (Onderstepoort Biological Products, South Africa) LSD vaccine with Neethling strain, were tested for the presence of virus neutralization (VN) antibodies in blood sera and vaccine (Neethling) virus presence in blood, nasal and oral swabs. The presence of vaccine virus was tested by real-time PCR (Bowden et al., 2008) in EDTA blood samples, nasal and oral swabs of all 20 animals sampled every day from the day of vaccination (0 days post vaccination – dpv) until 14 dpv. For serology testing, blood sera were sampled from all 20 animals every day from 0 dpv until 14 dpv, and then on days 21, 28, 42, 56, 70, 90, 120 and 180 dpv. The VNT was done with LSD virus isolated in Serbia during 2016 outbreak (SERBIA/Bujanovac/2016 – full genome sequence KY702007) on MDBK cell line.

The VN antibodies were detected in 11 out of 20 vaccinated animals. The VN antibodies become detectable from 11 dpv (in 1/20 animal) until the end of experiment - 180 dpv (in 6/20 animals). The VN antibodies were detected in 7/20 animals on 21 dpv, and on 28 dpv VN antibodies were detected in 11/20 animals. On 90 dpv VN antibodies were detected in 10/30, on 120 dpv in 9/20 and on 180 dpv in 6/20 animals. Six months after the vaccination, out of 11 animals that seroconverted, 6 (55%) animals still had detectable VN antibodies. All of this 6 animals show first detectable VN antibodies on 12 dpv. The titer of VN antibodies varied from 2 (1 $\log_2$) to 32 (5 $\log_2$).

The Neethling vaccine virus strain were detected in EDTA blood of 5/20 (25%) vaccinated animals. The first detection of vaccine virus in EDTA blood was on 6 dpv in one animal. The vaccine virus was detected in 3 additional animals on 7 dpv, and on 11 dpv it was detected in the blood of fifth detected viremic animal. In one animal the vaccine virus was detected constantly (every day) in EDTA blood from 6 dpv until 14 dpv (last day of testing). In 2 animals the vaccine virus was detected mostly constantly (almost every day) in EDTA blood from 7 dpv until 14 dpv, and in additional 2 animals it was detected just on 7 dvp and 8 dpv, or from 11 dpv until 14 dpv. In two animals vaccine virus was detected in nasal swabs from two consecutive days of sampling: on 11 dpv and 12 dpv for the first and on 12 and 13 dpv for the second animal. The only one vaccine virus positive oral swab was detected from that second animal on 13 dpv.

The preliminary date obtained in this experiment show that viremia of vaccine virus could be detected in some (25%) of vaccinated animals. The vaccine virus was also detected during short time (2 days) in nasal and oral swabs of 2/20 and 1/20 vaccinated animals indicating possible sheading of vaccine virus. Further study is needed to check the infectivity of the vaccine virus in nasal/oral swabs. Virus neutralizing antibodies could be detected as early as from 11 or 12 dpv, but not in all vaccinated animals. In 6 (55%) out of 11 animals that show detectable VN antibodies, i.e. in 6 (30%) out of 20 vaccinated animals, VN antibodies could be detected at least up to 6 months after vaccination. Further study is needed to obtain the knowledge on the duration of VN antibodies presence, and on the immunity of vaccinated animals.

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P2 14: Animal Health crisis in the Indian Ocean: Foot and mouth disease virus in Mauritius and Rodrigues in 2016

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Foot-and-mouth disease (FMD) is a contagious viral animal disease affecting domestic and wild artiodactyls (cattle, pigs, goats, pigs, African buffaloes ...). This disease is widespread throughout the world and is endemic in parts of Asia, Africa, the Middle East and South America. It is more rarely found in islands. The causative agent is a virus of the genus *Aphtovirus* within the *Picornaviridae* family and termed FMDV (Foot-and-Mouth Disease Virus). This virus has significant genetic and antigenic variability with seven immunologically distinct serotypes (O, A, C, Asia 1, SAT 1 to 3) each subdivided into several subtypes. In July-August 2016, an outbreak of FMD was reported in southeastern Africa on the Mauritius and Rodrigues Islands. Upon request, the National Reference Laboratory (NRL) in Maisons-Alfort implemented an emergency diagnosis aiming at detecting and characterizing the virus involved in this epizootic without delay. Epithelium, blood and serum samples collected from 10 bovines at Valley des Pretres and Cité la Cure in Mauritius and 3 epitheliums collected in Rodrigues were sent to the NRL. The following analyzes were performed on epithelial samples: (i) viral genome detection by real-time RT-PCR targeting the FMDV IRES region or the FMDV 3D polymerase coding region , (ii) typing by conventional RT-PCR targeting the VP1 coding region for serotypes O, A, SAT1 and SAT2, (iii) viral isolation using two epithelial cell lines (IBRS-2 and ZZ-R 127). Antigen-capture ELISA was performed on isolated virus to confirm the serotype involved and amplification then sequencing of the coding region for capsid major protein VP1 was carried out to characterize more precisely the strain involved.

FMDV was detected by RT-PCR in the 13 epithelium samples tested and successfully isolated from 11 out of the 13. The serotype was identified as FMDV-O in Ag-ELISA for all isolates and conventional RT-PCR VP1 sequencing confirmed serotype O. These results were obtained and transmitted in less than 24h after sample reception by the NRL. Finally, a comparative analysis of the VP1 sequences with the sequences available in the GenBank database showed homology with O-type sequences belonging to the ME-SA topotype Ind-2001d lineage. Overall these results enabled a precise identification of the FMDV strain involved and guided the choice of the appropriate vaccine to stop its spread.

P2 15: A Novel Double Antigen ELISA for the Species Independent Detection of CCHFV Antibodies

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by the Crimean-Congo hemorrhagic fever virus (CCHFV), which is a Bunyavirus belonging to the family Nairoviridae, genus Orthonairovirus. CCHF was first described in the mid-1940s on the Crimean peninsula. The disease occurs in humans sporadically throughout much of Africa, South Asia, and Southern Europe. CCHFV is transmitted to humans by tick bites, crushing of ticks and exposure to blood, tissues or bodily fluids of viremic livestock or infected patients. The virus causes an often fatal hemorrhagic illness in humans, with up to 50% case fatality rate. On the contrary, animals do not develop clinical symptoms upon infection but viremia and CCHFV-specific antibodies can be observed. The detection of anti-CCHFV antibodies in livestock is therefore used to reveal CCHFV infection risk areas. CCHFV-specific antibodies can be detected either by viral neutralization, ELISAs or indirect immunofluorescence assays.

This work presents the development and validation of a novel CCHF double antigen ELISA for the detection of anti-CCHFV nucleoprotein (NP) antibodies. The test requires 30µl of serum to be tested, and results are obtained within 90 minutes. As the ELISA is based on recombinant protein it can be run under standard biosafety conditions.

For assay validation, 106 cattle and 104 small ruminant sera from animals from CCHF endemic regions (origin: Albania, Cameroon, Kosovo, Macedonia, Turkey) served as a CCHFV positive reference serum panel. The CCHF antibody status of the positive reference samples had been previously confirmed by three serological assays (FLI-inhouse ELISA, species adapted VectorBest ELISA and Euroimmune IFA) (ref. 1-3). 402 cattle and 808 small ruminant sera from Germany and France served as negative serum panel, as both countries are considered outside of the CCHFV endemic zone. Moreover, sera from monkeys, camels, rats, ferrets, raccoon dogs, raccoons, foxes, hares, pigs and humans were tested, in order to determine the suitability of this novel ELISA for these species.

All negative reference sera were confirmed by the novel CCHF double antigen ELISA indicating a specificity of 100% (CI 95%: 99.7% - 100%). 195 of 210 positive reference sera were tested positive for CCHFV-specific antibodies which means a sensitivity of 93% (CI 95%: 88.5% - 95.9%).

The novel CCHF double antigen ELISA will soon be commercialized by IDvet.

Acknowledgements
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P2 16: Immune Response in Chickens to Fowl Pox Virus Vaccine and Newcastle Disease Vaccine Co-Administered by Non-Invasive Routes

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Background: It is recognized that in the routine use of vaccines against different diseases, vaccines are often administered at the same time. This practice is beneficial in terms of reducing costs and reducing the number of separate interventions. This study will assess any potential interference between a live Newcastle Disease Vaccine, administered in by eye-drop, and a live Fowl Pox Vaccine, concurrently administered by feather follicle route, to determine the practical viability of co-administration of these two vaccines by non-parenteral routes of administration. This is very important in many developing countries where para-veterinarians and community animal health workers are not legally allowed to administer parenteral products. A commercial prototype Newcastle Disease Vaccine containing the I2 strain was selected based on the fact that this vaccine is widely used in Africa.

Objectives:

- to evaluate if any adverse health reactions occur when a Fowl Pox vaccine is administered concurrently with a Newcastle Disease vaccine in six or three week old chickens
- measurement of the antibody responses to Fowl Pox virus in chickens that were vaccinated concurrently against Fowl Pox and against Newcastle Disease at the age of six or three weeks
- measurement of the antibody responses to Newcastle Disease virus (NDV/APMV-1) in chickens that were vaccinated concurrently against Fowl Pox and against Newcastle Disease at the age of six or three weeks

Design: The study is a partially-blinded, parallel group designed, randomised study on the safety and immunogenicity of Fowl Pox and Newcastle Disease vaccines in chickens. SPF chickens of both sexes and 3 weeks (± 4 days) or 6 weeks (± 4 days) of age at vaccination will be used. After acclimatisation of 14 days the study will be conducted over a period of 22 days in an indoor positive pressure unit and the animals will be housed in groups of three to five. Fowl Pox vaccine only will be administered once to animals in group 1, at 6 weeks of age, on Day 0 by feather-follicle technique. NDV only will be administered once to animals in group 2, at 6 weeks of age, on Day 0 by micropipette eyedropper. Fowl Pox vaccine and ND vaccine will be administered concurrently to animals in groups 3, at 6 weeks of age, and 4, at 3 weeks of age, on Day 0. Administration site observations, measurement of body weights, blood sampling and daily observations will be performed. Death is an endpoint in this study and the animals will be humanely slaughtered and incinerated at the test facility at the end of the study.

Serological assays used for testing the samples: NDV - Haemaglutination inhibition (HI) test, according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2016, Chapter 2.3.14 Newcastle Disease (Infection with Newcastle Disease Virus). FP – ELISA, established according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2016, Chapter 2.3.10 Fowl pox.

Acknowledgements: This study is based on research funded in part by the Bill & Melinda Gates Foundation and the UK Government. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of the Bill & Melinda Gates Foundation or the UK Government. We acknowledge Clinvet, South Africa for the conduct of the *in-vivo* phase and Deltamune, South Africa for the *in-vitro* phase of the study.

P2 17: Identification and isolation of Swinepox Virus from Sick Swines in the Belgorod Region in 2013

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In 2013 in the Belgorod region disease of swine, characterized by development of vesicles, pustules and scabs on the skin of body has been registered.

The scabs from these animals were collected for isolation and identification of causative agent. In order to isolate virus two pigs were inoculated with suspension of scabs from infected animals. virus was administrated subcutaneously in four points in the volume of 0,25 ml. on days 5-6 post infection the vesicles appeared in the points of virus inoculation.

Suspension of vesicles taken from experimentally infected pigs was used for inoculation of continuous cell culture of pig kidney (PK-15). After 7 days post inoculation the cell monolayer was harvested and next passage was performed. In the first two passages, only minor changes in the monolayer of the infected cell culture were noted in comparison with the control. At the third passage, after 3-4 days, a more pronounced manifestation of the cytopathic effect of the virus was noted, characterized by an increase in cell size, rounding, and destruction of the monolayer. On the 5-6 day of cultivation a complete destruction of the monolayer occurred. The culture virus-containing material was used for DNA isolation, amplification and sequencing of viral genes.

In order to identify the isolated virus VLTF-3 gene was amplified using protocol, described by Maria Luiza G. et al. in 2010. Nucleotide sequencing was performed on Genetic Analyzer 3130XL (Applied Biosystems, USA). Obtained nucleotide sequence (426 b.p.) were aligned with the sequences of poxviruses' VLTF-3 gene presented in the GenBank database. A phylogenetic analysis showed that the sequence of the VLTF-3 gene of the isolated virus refers to a cluster of swinepox virus.

The isolated swinepox virus was deposited in the State collection of microorganisms of National research institute of veterinary virology and microbiology of Russia under the accession number 3072 as the «Alekseevskii» strain. VLTF-3 gene of strain «Alekseevskii» has 100 % and 99% nucleotide identity with VLTF-3 gene of strains «17077-099» and «Holambra», respectively.

As a result of our work the virus, caused disease in swines in Belgorod region in 2013 was isolated in cell culture of pig kidney and on pigs follow experimental infection. This pathogen was identified as swinepox virus using sequencing of VLTF-3 gene.

P2 18: Distribution and genetic diversity of Peste des Petits Ruminants virus in Mali

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Peste de Petits Ruminants (PPR) is a highly contagious infectious disease of sheep and goats. The disease is endemic in Africa, Middle East and Asia (Libeau, Diallo et al. 2014). PPR has been classified among diseases that must be notified to the World Organization for Animal Health (OIE). The causal agent of the disease is a virus, peste-des-petitsruminants virus (PPRV). The genome of the PPRV encodes for two non-structural proteins C and V and six structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M) fusion protein (F) hemagglutinin protein (H) and viral RNA-dependant polymerase (L) (Bailey, Banyard et al. 2005). Based on the partial sequences of the N gene, PPRV has been classified into four genetically distinct lineages (I, II, III, and IV) (Banyard et al., 2010). The PPRV circulating in Asian and the Middle East belong to the lineage III and IV. But in Africa, all the four lineages are present. Until now, the PPRV present in West Africa belong to the lineage I and II (Banyard et al., 2010). The lineage II is thought to have replace lineage I in its historical distribution in West Africa (Banyard et al. 2010). In this study, we characterized the PPR virus from five different regions of Mali (Bamako, Ségou, Kayes, Sikasso and Mopti). The samples have been collected in 1999, 2014, 2015, 2016 and 2017. We analyzed the partial N-gene sequence in comparison with other viruses from Africa. The phylogenetic tree that we obtain shows that the samples recently collected in 2017 in Mopti belong to the lineage IV and are very closely related to the lineage IV of Nigeria (Woma et al. 2015). All the other samples belong to the lineage I and II. Our results represent the first confirmations of the persistence of lineage I and of the presence of lineage IV in a region dominated by lineage II. We also sequenced the full genome of the PPR virus of those samples. The phylogeographic and phylogenetic analysis were performed to assess the persistence of the lineage I and II in Mali and the spread of PPRV circulating from Eastern Africa in Mali.

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P2 19: Investigation of pathogenicity of lumpy skin disease virus for sheep

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Lumpy skin disease (LSD) is an economically important viral disease of livestock characterized by fever and nodular lesions on the skin, mucous membranes, and internal organs. LSD is a disease with high morbidity and low mortality rate affecting cattle, water buffalo and wild ruminants of all ages and breeds, but young animals and cows in the peak of lactation are more severely affected.

The aim of this article was investigation of pathogenicity of LSD virus for sheep. In our work the suspension of skin nodules collected from sick cattle in the Republic of North Ossetia-Alania in 2015 was used for experimental challenge. Four lambs in the age of three months were inoculated intravenously and intradermally with this suspension. The virus was administrated intravenously in the volume of 1,5 ml and intradermally in the volume of 0,25 ml in four points on skin of armpits. All animals were daily clinically monitored and nasal swabs and blood samples were collected every three days for the detection of LSD virus genome. Real-Time PCR was carried according to protocol published by T.R. Bowden et al. in 2008 (1).

All lambs showed normal body temperature during the whole period of experiment. On days 10-13 after infection nodules in the points of intradermal inoculation of virus appeared whereas nothing nodules in other parts of body appeared (Figure 1). Lamb number 3 was euthanized on 13 day after infection and samples of spleen, lung, liver and lymph nodes were taken. Testing of these samples by Real-Time PCR showed the presence of LSD virus genome in the samples of liver and lymph nodes. The genome of LSD virus was detected in nasal swabs, collected from lambs numbers 1,2,4 from 20 to 27 days after infection. In the blood samples, collected from these animals, the genome of LSD virus was detected from 9 to 27 days after infection. The LSD virus was isolated in continuous cell culture of sheep kidney from PCR-positive blood samples. It confirmed the presence of virus in the blood of infected sheep.

As a result of the work, we showed that LSD virus isolated from sick cattle is pathogenic for sheep. Experimental infection is characterized by viremia and the appearance of nodules in the points of intradermal inoculation.

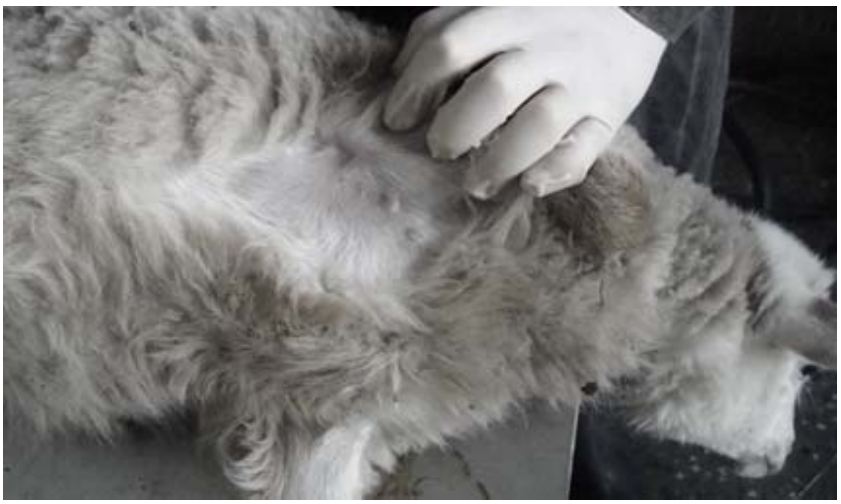

Figure 1: Nodules on the skin of sheep infected with LSD virus

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P2 20: Triplex qPCR assay for simultaneous detection and differentiation of LSDV field strains from LSDV vaccine strains

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Lumpy skin disease is very important disease of cattle which is included in OIE list of notifiable terrestrial and aquatic animal diseases. The etiological agent of a disease is Lumpy skin disease virus. The disease is of great economic importance because it causes great direct and indirect losses to cattle breeding. Severity of the disease depends on the virus strain, the age, race and the immune status of cattle and may range from subclinical infection to death.

LSD virus belongs to the family Poxyirididae, genus Capripoxvirus. Besides LSDV this genus includes sheep pox virus (SPPV) and goat pox virus (GTPV). These viruses are usually species specific for a host and geographic region. They are serologically indistinguishable and cause cross-immune protection and also cross-infection in experimentally induced cases.

Attenuated strains of LSD and SPPV are mainly used successfully as a vaccine candidates in LSD enzootic or in infected areas.

Once the LSD vaccination policy is implemented in some country, it is necessary to be able to differentiate field strains of LSDV from vaccine strain due to the possibility of mild or systemic post-vaccination reactions in vaccinated animals or, especially, in cases where infected cattle were vaccinated in incubation period.

Here we describe triplex DIVA qPCR assay for simultaneous detection of both field and vaccine LSDV strains with FAM labeled probe and specific detection of field strains of LSDV using VIC labeled probe. Assay includes also ROX labeled probe for internal control of amplification.

FAM labeled probe and primers (Bowden et al., 2008) are specific for all Capripoxviruses and the assay was previously validated (Stubbs et al., 2012). Primers and probe labeled with VIC are designed for specific detection of field strains of LSDV (Vidanovic et al, 2016) and do not detect Neethling and SIS vaccine strains nor GTPV or SPPV strains. This assay is proven to detect not only field LSDV strains from Serbia, Bulgaria and FRY Macedonia, but also from Israel, Sudan, Kenya, Ethiopia and Egypt (23 different strains). ROX labeled probe and primers are from commercial kit for DNA extraction control and internal amplification control.

Sensitivity of this multiplex qPCR assay, when used with quality multiplex qPCR master mix, is only slightly reduced when comparing with singleplex reactions.

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Topic 3 - Current challenges inside Europe

P3 2: Recent spread of an old disease: tuberculosis in wild ungulates from southern Spain

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The increasing threat of shared infections among wild and domestic animal populations has renewed the interest in factors leading to the re-emergence of well-known diseases. Disease spread is spatially and temporally non-uniform, and one needs to understand its variation to better evaluate its potential impact and to target control interventions. Animal tuberculosis (TB), a chronic disease caused by infection with the *Mycobacterium tuberculosis* complex (MTC), affects cattle but also Eurasian wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) in Mediterranean Spain.

The aim of this study is to characterize the spatial patterns of TB spread among wild ungulates and to evaluate the temporal evolution of diffusion rates along eleven years (2006-2016).

For this, data is based on a long-term sanitary monitoring of wild ungulates from Doñana National Park, Spain. Inter- and intra-specific models applying trend surface analyses (TSA) by period (T1: 2006-2011 and T2: 2012-2016) have been fitted, validated and used to predict MTC distribution in the study area in order to explore the epidemic spread of this infection. Overall, infection prevalence has increased from 46% to 57.8% in the whole community during the study period. The initial prevalence and the increase through time are higher in wild boar (T1 61.6%; T2 75.1%; n=852), followed by red deer (T1 36.9%; T2 48.7%; n=356). By contrast, prevalence is lower and apparently stable in fallow deer (T1 20.5%; T2 19.3%; n=338). Regarding the geographic pattern of TB diffusion, TSA models demonstrated variability among periods and species, with a generally increasing TB prevalence in the periphery of initial infected areas. However, these patterns differ from what one would expect if the TB spread radially from positive individuals at T1. For instance, a main corridor of diffusion through the humid ecotone closer to the seasonal marshland -according to previous risk factor studies in this Park- is clearly evident among depicted maps. Additionally, spread maps identified several areas with high likelihood of TB diffusion in red deer closely correlated to those observed in T1 for wild boar (Pearson´s r=0.87; $p<0.001$).

This study demonstrates how spatial analytical techniques can lead to estimate patterns of disease diffusion and transmission directionality. These scenarios should be considered for TB control in wildlife where the spatial aspect is crucial for designing cost-effective management operations such as vaccination or culling.

P3 3: Understanding the use of antimicrobial susceptibilitytesting by veterinarians to interpret surveillancedata on resistance: a qualitative study exploringdeterminants and issues in France

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Context: For public health reasons, increasing attention is drawn toward a more prudent and restrictive use of antimicrobials in farm animals. Antimicrobial susceptibility testing (AST) covers dual benefits in the fight against antibiotics resistance: it guides practitioners in their daily prescriptions and it allows surveillance of resistance. A comprehensive understanding of the underpinning drivers, motivators and reasons behind the use of AST would both help to interpret surveillance data of antimicrobials resistance and highlight opportunities or pitfall to increase the use of antibiograms in veterinary medicine. Precisely, the aim of the study was to provide a better understanding of veterinarians' motivations and stakeholders' influences around the choice to ask for AST.

Method: Semi-structured interviews with veterinarians from cattle, swine, poultry and equine sectors were conducted in France. Thematic analysis was used to analyse transcripts.

Results: In total, we surveyed sixty-six French veterinarians. The use of AST in veterinary medicine was multifactorial and varied between sectors: it was quasi-systematic in poultry, frequent in swine and rare in cattle and equine sectors. The decision making to use this test was influenced by relationships between stakeholders, especially veterinarian-customer and veterinarian-laboratory. The new French regulation (2016) restraining the prescription of last resort antibiotics to the use of AST did not increase the use of analysis but it induced a change in prescriptions due to field constraints and time needed for analysis. Veterinarians perceived it as an assistance to promote responsible and prudent use of antibiotics and to foster the use of alternatives.

Discussion: Studies using antimicrobial surveillance data should take into account specify of animal sector before making any comparison: AST results should be stratified at least on species and at best on type of production, because its use varied a lot between animal sectors. Conclusions should also take into account the limits of the surveillance system and veterinary practices. One of the most important factors which could influence a greater use of AST is the ability to get rapid results: there is clearly a need for future innovation. The network density of laboratories contributed to ease the use of analysis in poultry and swine sectors, especially in Northwestern France. To fight antibiotics resistance, veterinarians tackle sanitary event comprehensively, reinforcing their competence in large area of activity. From cultural and social point of view, it introduced a paradigm shift in vet practice: reorienting vet practices around a more global and preventive approach.

Conclusion: Those findings will be useful to help veterinary representative bodies and regulatory authorities in the design of new measures, targeted communication, policy and regulation. Moreover, considering a network to collect AST results as the best tool to monitor antimicrobial resistance, our results are essential to properly use antimicrobial resistance surveillance data. Such cross-cutting and interdisciplinary approach should be applied to other research fields to properly interpret surveillance data.

P3 4: Identification of a recombinant PEDV/SeCoV strains during a molecular surveillance of Italian epidemic waves between 2015 and 2017

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Introduction: Porcine epidemic diarrhea (PED) is an acute and highly contagious enteric disease characterized by severe enteritis, vomiting, watery diarrhea and high mortality among suckling pigs. The PED virus (PEDV) belongs to the *Coronaviridae* family, characterized by a positive-sense singlestranded RNA genome. Coronaviruses (CoVs) are prone to genetic evolution through accumulation of point mutations and homologous recombination.
In the last years, PED had a big economic impact on swine industry in Asia and United States of America (1). In 2014, PEDV has also re-emerged in many European countries; a PEDV strain genetically related to the moderate pathogenic American S-INDEL OH851 was reported in Belgium, Germany, Netherlands, France, Spain, Slovenia, Austria and Portugal (1).
In Italy, strains circulating during 2007-2012 showed a high genetic variability (2). In particular, from 2009 to 2012 a new recombinant coronavirus, called Swine enteric Coronavirus (SeCoV), was present; the genome of this recombinant belongs to another swine Coronavirus, the Transmissible Gastroenteritis Virus (TGEV), but the S-gene derived from PEDV (2). In July 2014, two new Italian cases of PED were detected with high nucleotide identity to PEDV S-INDEL circulating in Europe (2) and at the beginning of 2015 a new severe epidemic wave occurred.
This study reports the detection and genetic characterization of PEDV circulating in Italian epidemic waves during 2015-2017 and the rapid spread of a recently discovered recombinant strain.

Materials and Methods: From January 2015 to May 2017, feces or intestine samples of pig enteritis cases were analyzed by S1 PEDV Real-Time PCR. Most of the samples came from the North of Italy (i.e. the area with the highest density of pig production) and only few from the rest of the country. To identify PEDV variants, S1 gene sequence was obtained from 489 out of 526 PEDV positive samples coming from 408 farms. For the phylogenetic analysis, 213 complete S1 gene sequences were selected, excluding identical and/or partial nucleotide sequences. The tree was constructed by using the neighbor-joining method p-distance model and bootstrap test of 1,000 replicates in MEGA 6. Putative recombination breakpoints were determined using the Recombination Detection Program (RDP) 4.43.

Results and discussion: Since January 2015, PEDV rapidly spread in the high-density pig production area in the North of Italy. Between 2015 and 2017, three peaks of outbreaks occurred during winter months. PEDV-reinfection occurred in 24.3% and 19.7% of previously infected farms during the second and third epidemic wave, respectively.
The S1 phylogenetic tree showed different clusters consistent with the hypothesis that different entry events could have occurred in Italy. S1 gene sequencing of 190 samples (89.2%) showed a high sequence similarity with European S-INDEL strains circulating during 2014-2015 (98.5-100% for nucleotide and 97.4-100% for amino acid sequence). The remaining 23 samples (10.8%) shared a high degree of sequence identity (99.4-100% for nucleotide and 99.1-100% for amino acid sequence) with the PEDV strain SLOreBAS-1, identified in Slovenia during 2015 (KY019623). The S1 gene of Slovene strain and Italian homologous strains contained a fragment (~400 nt) showing high identity with SeCoV. Recombination analysis detected breakpoints at position 240-636, suggesting the occurrence of a recombination event between PEDV and SeCoV. In Italy, the PEDV/SeCoV recombinant strain was detected for the first time in May 2016, but between January and April 2017 it rapidly spread. The PEDV/SeCoV strain has overcome the S-INDEL OH851-like strain, representing the 93.7% of the circulating strains.
The emergence of new PEDV variants with potentially different pathogenic features is of great concern for swine health. A continuous molecular surveillance is important to allow implementation of efficient control measures.

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P3 5: Environmental determinants of the concomitant infection with *Mycobacterium bovis* in cattle andbadgers in France

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Landscape epidemiology analyses how environmental characteristics influence pathogen transmission between hosts of one or several species by inducing constraints on space use by hosts, and/or on pathogen survival in the environment. Here we analysed how environmental variables could be associated with the concomitant infection with *Mycobacterium bovis* in both cattle and badgers, in an area of South-Western France. We defined circular spatial units (500 m and 1000 m radiuses) centred on 113 setts of trapped badgers and including cattle pastures. The characteristics of spatial units where only one species had been found infected were compared with the ones where both cattle and badgers had been found infected. A multivariate logistic model was used to analyse the association between concomitant infection and three groups of variables describing landscape, animal population and terrain features of spatial units. The terrain ruggedness index of pastures and the percentage of sand in their soil were positively associated with the odds of concomitant infection in spatial units. The number of neighbouring badger groups was negatively associated with the odds of concomitant infection (spatial units of 1000 m radius), whereas the number of crop parcels was positively associated with the odds of concomitant infection (spatial units of 500 m radius). These results suggest that terrain features may influence *M. bovis* transmission between badgers and cattle, leading to concomitant infection of both species.

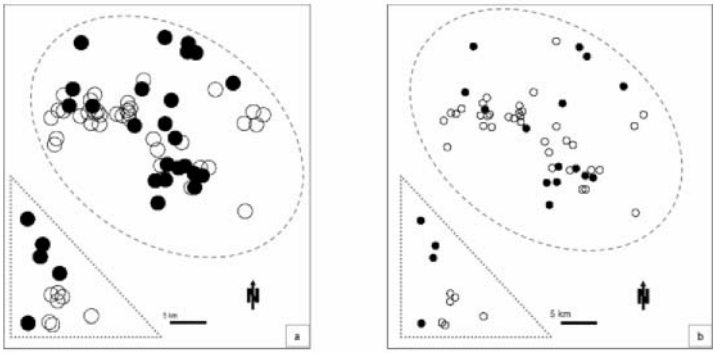

Figure 1: Locations of spatial units (SUs) in the study area (black points: SUs with concomitant infection in both badgers and cattle; white points: SUs without concomitant infection in both badgers and cattle; *a:* 1000 m radius SUs; *b:* 500 m radius SUs; SB0821 areas are surrounded by a dotted line; SB0832 areas are surrounded by a spotted line)

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P3 6: Astrovirus associated with encephalitis in a sheep: indication of cross-species transmission with cattle

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Introduction: astroviruses are best known to cause gastroenteritis in different mammal species. Lately, some strains have been associated with encephalitis in humans, minks, cattle and sheep. We report the identification of such a neurotropic astrovirus in a sheep, named ovine astrovirus CH16 (OvAstV-CH16); interestingly, this virus is genetically almost identical to an astrovirus recently described in neurologically diseased cattle, bovine astrovirus CH15 (BoAstV-CH15).

Material and methods: We submitted three ovine encephalitis samples from our archives to next-generation sequencing (NGS) and a bioinformatics pipeline for virus discovery. We Sanger sequenced the whole genome length of the virus that we obtained in one of these samples, and performed a phylogenetic comparison of the recovered sequence with other known astroviruses. We also tested brain samples of the affected sheep by immunochemistry (IHC) with antibodies aimed at BoAstV-CH15.

Results: One ovine sample that went through next-generation sequencing presented sequences with high similarity to BoAstV-CH15, a virus found recently in cattle with encephalitis. Once the viral sequence was confirmed by Sanger sequencing, a phylogenetic analysis confirmed its close relationship to BoAstV-CH15, as well as to other ruminant neurotropic astroviruses. The samples tested also reacted positively in the IHC.

Discussion: Our study indicates that astroviruses of the same genotype species may cause encephalitis in different species.

Conclusion: Our results confirm a very close relationship of OvAstV-CH16 to BoAstV-CH15, as well as to other astroviruses that were found in association with encephalitis in cattle and sheep.

Acknowledgements

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P3 7: A recombination between two genotype 1 PRRSV modified live vaccines results in a field strain with increased virulence

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Introduction : Porcine Reproductive and Respiratory Syndrome (PRRS) is the most costly disease for swine industry worldwide. This disease is characterized by reproductive failure in sows and respiratory disorders and growth retardation in growing pigs. The causing agent, PRRS virus (PRRSV) is a small enveloped virus with a single-stranded, positive-sense RNA genome belonging to the family *Arteriviridae*. PRRSV can be divided into two major genotypes: type 1 (European strains) and type 2 (North American strains). Modified live vaccines (MLV) of genotype 1 (MLV1) and 2 (MLV2) are widely used throughout the world to control consequences of PRRSV infections. In a French pig farm, with a history of PRRSV infection and where PRRS vaccination was implemented successively with VP-046 BIS and then DV vaccine strains, we isolated in December 2014 an atypical PRRSV strain resulting from a recombination between the two commercial attenuated strains VP-046 BIS and DV. In order to assess the virulence of the recombinant strain, we set-up an *in vivo* assay to compare the clinical, virological and transmission parameters between the recombinant strain and both parental vaccine strains.

Materials and Methods : Forty-two seven-week-old SPF pigs hosted in our BSL3 facilities were allocated into four groups. In groups 1, 2 and 3 (12 piglets/group), 6 piglets were respectively inoculated with DV, VP-046 BIS or the recombinant strain ($10^{4,2}$ TCID50/ml and 2 ml/pig through the intramuscular route). Twenty-four hours after inoculation, 6 non-inoculated contact piglets were added to each inoculated groups. Group 4 (6 piglets) was left uninoculated as a negative control group. All animals were monitored daily for rectal temperature and clinical signs. Blood and nasal swabs were collected twice a week after inoculation to monitor the genome virus load (RT-qPCR) and antibodies production (ELISA). All pigs were euthanized at 36-39 days post-inoculation (dpi). During the necropsy, samples were collected for additional quantification of the viral genome in tissues.

Results: No difference in hyperthermia and clinical signs were detected in the groups 1, 2, 3 compared to the control group 4. PRRS viral load in inoculated piglets of group 3 was 10 to 100 fold higher in serum and 5 to 100 fold higher in nasal swabs in comparison with inoculated piglets from groups 1 and 2. At necropsy, the PRRS viral load in tonsils was higher in group 3 inoculated piglets compared to inoculated animals from groups 1 and 2.
In contact piglets, the first viremic animal was detected as soon as 2 dpi in the recombinant strain group 3 compared to 10 dpi for group 1 and 17 dpi for group 2. Similarly to inoculated animals, the level of viremia of contact piglets from group 3 was 10 to 100 fold higher than for contact piglets from groups 1 and 2. All (6/6) piglets inoculated with the recombinant strain (group 3) had seroconverted at 10 dpi whereas only 2/6 piglets in groups 1 and 2 had seroconverted at the same time. Contact piglets showed the same seroconversion kinetics, shifted in time, than inoculated pigs.

Conclusion : For the first time, a PRRSV recombinant strain, originating from two MLV1, was isolated from the field. Compared to the parental vaccine strains, the recombinant strain was able to replicate at a higher level with a higher shedding level and a faster transmission. These results suggest an increase of virulence of this specific atypical strain resulting from the recombination of 2 attenuated PRRSV vaccine strains. Considering this increase of virulence, measures should be implemented to avoid the recombination of PRRSV vaccine strains under field conditions.

P3 8: Atypical porcine pestivirus in Spain: Retrospective study from 1997-2017

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A novel Pestivirus named atypical porcine pestivirus (APPV) was discovered in the USA in 2015, being also identified in Germany, The Netherlands, Austria and China. APPV has been linked with the development of congenital tremor (CT) in newborn piglets, which increases pre-weaning mortality. This study aimed to determine the circulation of APPV in Spain from 1997 to 2017 and provide new information regarding viral pathogenesis, epidemiology, and the molecular epidemiological link with recently reported APPV strains.
Two-day old piglets (n=10) with moderate-severe CT from a Spanish farm were received. Sera, nasal and rectal swabs and tissue samples were collected. qRT-PCR was performed and a retrospective study to detect APPV RNA was carried out using a serum collection that included samples of 643 pigs, from 1997 to 2017, with their anamnesis data. Furthermore, a phylogenetic analysis was conducted in the positive samples amplifying a fragment of the NS2-3. The phylogenetic tree was constructed using the Maximum Likelihood method implemented in Mega 6 software.
APPV genome was identified with high and moderate RNA loads in different tissues. On the other hand, 90 out of the 643 analyzed samples (13.9%) were positive by the specific qRT-PCR. The first APPV positive serum was dated in 1997 and the last in 2017. Fifty-five out of 162 (33.9%) piglets in the first week of age were APPV positive. However, viremic pigs at older ages were also detected. Phylogenetic analysis from 1615 NS2-3 nucleotides showed at least three well defined APPV clades, grouping high genetic diversity strains from Spanish, Germany and USA.
APPV has been circulating in Spain at least since 1997, being the earliest date of detection of this virus worldwide and suggesting that APPV may be widespread. Data support that CT was related to the presence of APPV in viremic piglets below 1 week of age, in which the viral RNA load was the highest. A high APPV RNA load was detected in lymphoid organs, suggesting that these constitute a target for APPV replication. Thus, a potential immunosuppressive capacity of APPV cannot be underrated. A considerable number of animals between 4 and 14 weeks of age and some one-week old piglets were viremic in the absence of CT, which can act as carriers of the virus. The phylogenetic analysis and the calculated mutation rates shows a high genetic diversity in the APPV strains from Spain, Germany and USA, as well as a close epidemiological relationship of APPV strains in Europe, given that strains from two different clusters are circulating in Spain and Germany simultaneously. The mutation rate (s/s/y) between Spanish strain from 2001 (LT631727, Cluster I) to 2016 (LT631734, outside of cluster I) was also determined, being 5.04×10^{-3} s/s/y. This suggests the possible evolution of APPV in this period, considering that these values are similar to those previously reported for European (2.7×10^{-3} s/s/y) and Caribbean (6.7×10^{-3} s/s/y) CSFV outbreaks.

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P3 9: Surveillance of classical swine fever, blue tongue and rabies in European targeted countries: assessment through expert elicitation

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We implemented a survey in Italy, Switzerland and United-Kingdom to describe the surveillance systems in place targeting CSF, BT and rabies; and we assessed through experts' opinion the characteristics of the surveillance systems according to different criteria.

Experts' opinion was elicited in October-November 2016 using questionnaires sent via e-mail. As a first step the experts were asked to identify the relevant components of the surveillance systems in place in their country. The questionnaire was based on the design tool developed by RISKSUR [1]. The assessment (based on actual knowledge and perception) was based on 12 attributes that were selected taking into consideration those targeted by RISKSUR evaluation tool (EVA) and other evaluation frameworks for surveillance systems [2–7] (timeliness, sensitivity, representativeness, acceptability, flexibility, coverage, costs, effectiveness, efficiency, impact, benefit, and communication/dissemination).

Nine experts from CH (3 experts), UK (3) and IT (3), answered the questionnaires; in each country one expert was selected for each disease. To evaluate the surveillance systems, we favoured a qualitative method (through expert's elicitation) rather than a quantitative approach to gather the information needed.

The objective of a surveillance system is defined considering the previous and current health status of the country and the estimated risk of introduction/spread of the disease. For example, Country 1 and 3 specified the same purpose and objectives with regards CSF surveillance but the implementation differs substantially between the two countries. This highlights the fact that for the same disease and same objectives, the implementation of specific actions is multifactorial and related also to specific contexts, political dynamics, interest and specific concern of countries. A surveillance system should be adapted and tailored to the country needs. Such differences between countries among Europe highlight the fact that, even under the same legislation, countries have flexibility in implementing surveillance system [8]. This is understandable but can raise concerns over the lack of harmonisation of systems in EU and the consequent potential difference in terms of performance within a common trade area.

Only few components are shared among the different studied surveillance systems of each country. For BT, no surveillance component is even common among all 3 countries. However, for rabies, the three countries implement a passive surveillance in wildlife. This component was then highly ranked among the three countries with a median score of 3 (maximum on a 4-point scale).

Only country 1 described the presence of surveillance components related to trade (import and export) for CSF and BT even if outbreaks could have such a huge impact on trade and country health status (mentioned by the experts). The veterinary border control is a key factor to ensure that the live animals and animal products entering to the European Union are safe and meeting the specific import conditions laid down in the Union legislation.

This study provided valuable information about surveillance scenarios for rabies, BT and CSF in IT, CH and UK. The methodology developed here can be used to gather information from other experts and risk managers to update and revise current practices to prevent the introduction and spread of the targeted diseases.

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P3 10: Comparative analysis of different serological and molecular tests for the detection of small ruminant lentiviruses (SRLVs) in Belgian sheep and goats

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Introduction: Small ruminant lentiviruses (SRLVs) belong to the Retroviridae family and are a heterogeneous group of viruses, including Maedi Visna virus (MVV) and caprine arthritis encephalitis virus (CAEV). Although these viruses have a serious impact on small ruminant animal health and cause economic losses, no vaccines or therapies are available. Therefore the control of this disease mostly relies on the early detection of infected animals. ELISAs, agar gel immunodiffusion (AGID) and qPCRs are nowadays often used for SRLV detection. However, due to the high genetic and antigenic heterogeneity of these viruses, no gold standard test is available to determine the SRLV infection status of an animal [1]. Therefore our goal was to compare the concordance of different diagnostic tests and select the most suitable test or combination of tests for SRLV detection in the Belgian sheep and goat population.

Methods: 555 sheep from 87 farms and 396 goats from 76 farms were sampled in the context of a nationwide SRLV seroprevalence study in Belgium. All collected sera were tested in five commercial ELISA kits (Elitest MVV/CAEV (Hyphen), CAEV/MVV total antibody screening test (Idexx), ID screen MVV/CAEV indirect (IDvet), LSIVet ruminant Maedi-Visna/CAEV serum ELISA kit (LSI) and Eradikit SRLV screening test (IN3 diagnostic)) and two commercial AGID tests (AGID-CAEV p28 kit (Idexx) and Maeditect kit (Apha Scientific)). The results of both AGID tests were interpreted together to obtain one final AGID result per animal. For the qPCR based diagnosis, leucocytes pellets were prepared from whole blood samples and two "inhouse" qPCRs for the detection of genotypes A and B strains were used.

Results: In the absence of a gold standard test, the infection status of an animal was determined by considering all 6 serological results. A final status was accorded to an animal if at least four out of 6 serological tests gave the same result. In total, 84.1% of samples had identical results in all tests. 14.6, 0.8 and 0.4 % had 1, 2 or 3 deviating results, respectively. The latter were excluded from the analysis. Unexpectedly, the combination of both AGID tests performed best since 100% sensitivity and specificity were found for both sheep and goat samples. An overall good performance was also found for the ELISAs with some differences between the 5 kits. The highest sensitivities in the sheep study were observed in the Hyphen (98.0%) and IDVet (100%) kits. In goats, IDVet, LSI and Biosellal kits showed a sensitivity of 100%. Where the Idexx ELISA was the least sensitive kit, it showed to have the highest specificity in both sheep (99.6%) and goats (100%). Only the LSI kit had a markedly reduced specificity (92.8% in sheep and 85.7% in goats).

The qPCR showed to be highly specific in both species (100%), but the sensitivity remained suboptimal with 87.5% in sheep and 83.3% in goats. This indicates that our qPCR needs further optimization to include extra strains and to lower the limit of detection.

Conclusion: Most commercial tests showed good sensitivity and specificity for SRLV diagnostics in Belgium. It seems possible to use several combinations of 2 or 3 tests performed in sequence to allow a fast and reliable determination of the correct infection status of animal. These results will be useful to optimize SRLV monitoring and certification in Belgium.

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P3 11: Phylogenetic study and evolution of the Swine Vesicular Disease Virus over 25 years of circulation in Italy

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Swine Vesicular Disease Virus (SVDV) belongs to the genus Enterovirus, family *Picornaviridae*; its genome consists of a positive strand RNA of about 7400 nt codifying for a polyprotein processed, during viral replication, in structural and non-structural proteins by viral proteases.
Although only one serotype has been described, four SVDV distinct antigenic variants have been circulating in Italy from 1966 to 2015, identified by using panels of Monoclonal Antibodies (MAbs). The first variant corresponds to the first virus isolated in 1966 in Italy, the second includes viruses circulated in Europe and Far-East during the '70s, the third includes viruses circulating from 1988 to 1992 only in Italy and the fourth variant persisted from 1992 to 2015.
The aim of this work was to study the molecular evolution of SVD virus in Italy, with focus for viruses of the fourth variant, using complete genome sequences of 191 representative strains.
Two different amplification techniques were used to prepare the samples to be submitted to the Miseq Illumina Instrument: Sequence-Independent Single-Primer Amplification (SISPA) and the production of two amplicons of 3315nt and 5012nt covering all the viral genome length. For each strain, nearly complete sequences of 7335 nt were obtained.
The Bayesian phylogenetic analysis performed on the polyprotein coding region shows a clear separation of the four antigenic variants in four distinct genetic lineages. Within the fourth variant two main sub-lineages can be distinguished, both derived from a unique common ancestor dated back to 1990-91.
The first sub-lineage, hereafter named "Italian sub-lineage A", includes viruses evolved exclusively in Italy from 1995 to 2010. After a first period of co-circulation in both North and South Italy, from 2000 this sub-lineage was maintained in Southern Italy causing almost exclusively sub-clinical forms. However, a new incursion in North Italy, affecting mainly the Lombardy region, occurred in 2006-2007 with two distinct epidemic waves.
Viruses of the second sub-lineage, named "Italian sub-lineage B", are organized in two distinct sub-groups: one includes viruses circulated from 1992 to 1999 mainly in Southern Italy and closely related to isolates detected in Spain and Portugal in 1993 and 1995 respectively; the second sub-group is composed by viral strains detected in Italy from 2004 and more closely related to those found in Portugal in 2003, suggesting a probable reintroduction in Italy following trades with Iberian Peninsula.
SVDV evolution in Italy shows a neutral selection, with a uniform distribution of the synonymous mutations throughout the genome. Differently, non-synonymous mutations occurred with higher frequency in the VP1 capsid protein and in the non-structural proteins 2A and 3A, involved in viral replication. Another event that contributed to SVDV evolution in Italy was the recombination between sub-lineage A and B, which occurred in 2007. A total of 20 recombinant strains have been identified and sequenced, the breakpoint for each of them was calculated by Simplot analysis and resulted within the range of 3761-3821nt position.
All the recombinant strains are likely to originate from a unique recombination event, as suggested by phylogenetic analysis performed on the recombined genome portions.
In conclusion, the fourth antigenic variant lasted in Italy for over 25 years, unlike the previous variants that were temporarily more limited; co-circulation of different genetic sub-lineages occurred along several years, probably promoted by trade relationships with other countries; this enabled a recombination event in 2007 and, after three years of simultaneous circulation of the parental and recombinant viruses, from 2010 only recombinant viruses have been detected.

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P3 12: Phylogenetic analysis of ORF virus, isolated in the Republic of Tuva in 2015

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Contagious ecthyma (ORF, contagious pustular dermatitis or contagious pustular stomatitis) is an infectious disease mainly of sheep and goats, characterized by lesions of the mucous membranes of the mouth, lips, head, breast, and extremities, accompanied by the formation of nodules, papules, vesicles, pustules and crusts with a primary lesion of any part of the body of the animal, most often the skin of the lips and mucosa of the oral cavity. The causative agent is the contagious ecthyma virus (ORF virus), a member of the genus Parapoxvirus in the subfamily Chordopoxvirinae of the family Poxviridae.

The aim of this work was molecular characterization of strain Erzinskiy of ORF virus, isolated from sheep in the Republic of Tuva in 2015. For this purpose, B2L gene was selected for sequencing and phylogenetic analysis because it codes conserved major envelope immunogenic protein. Scabs from infected sheep was used for extraction of viral DNA. B2L gene was amplified in PCR with 3 pairs of primers (ORF F1: cccatc ccc gaa gat gtg; ORF R 1: gcggttcatgaggtcccagg; ORF F2: ggc gggcgtcaactactaca; ORF R2: tcgtccacgatgagcagctt; ORF F3: atggtgccggtgatcaagca; ORF R3: tagcagttggggtcgtgcct). Amplicons were sequenced by the Sanger method using a 3130xl Genetic Analyzer (Applied Biosystems, USA). Obtained sequence was deposited into GenBank under accession number KY652170. This sequence was aligned with complete sequences of B2L gene available in GenBank (https://www.ncbi.nlm.nih.gov) and analyzed using MEGA 7.0 software. Phylogenetic analysis was carried out using Maximum Parsimony method.

Constructed phylogenetic tree showed that strain Erzinskiy belongs to the cluster of ORF virus strains, isolated in India. B2L gene of strain Erzinskiy shared maximum percent identity (98 – 98,4 %) with strains Kodaikanal-1 (KU597728) and Kodaikanal-2 (KU597729).

Thus, the B2L gene of ORF virus, isolated in the Republic of Tuva in 2015, was sequenced and phylogenetic analysis of this strain was carried out.

P3 13: Identification of novel enterotropic astroviruses incattle

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Introduction

Astroviruses are single-stranded positive-sense RNA viruses with a genome size of 6.2-7kb. The genome includes at least three open reading frames (ORF): ORF1a and ORF1ab encode the nonstructural proteins nsp1a and nsp1ab respectively, and ORF2 encodes the capsid protein precursor. In humans and mammalian animals, astroviruses are mainly enterotropic and found either in association with gastroenteritis or subclinical intestinal infection. Recently, our lab has identified a divergent bovine astrovirus (BoAstV-CH13) in brain tissues of cattle with encephalitis and neurological disease. The source of BoAstV-CH13 infection in these animals remains unknown, in particular we do not know whether this virus is shed by subclinical infected cattle via the feces, similarly to what is the case for known enterotropic BoAstV strains.

Methods

We collected 148 bovine stool samples and analyzed them with a pan-astrovirus RT-PCR. Amplicons from 11 RT-PCR positive samples were Sanger-sequenced and the obtained sequences were aligned by BLASTN against the NCBI nucleotide collection database. Consequently, four RT-PCR positive samples were subjected to Next-Generation Sequencing (NGS) for more detailed genotyping. Stool samples were homogenized, filtered (0.22µm), treated with Benzonase and RNA was extracted. RNA libraries were prepared and sequenced using an Illumina HiSeq2500 machine. Between 89,201,550 and 100,111,185 reads per sample were generated and then analyzed using drVM (detect and reconstruct known viral genomes from metagenomes). Contigs matching to sequences of the genus Mamastrovirus were selected.

Results

Amplicon sequences of all 11 RT-PCR positive samples revealed highest similarities to known enterotropic BoAstV and not to BoAstV-CH13. Contigs displaying similarities to Mamastrovirus sequences were found in all four NGS sequenced samples, and ranged from 2,849 to 4,972 nucleotides in size; in one of the samples, two distinct contigs were obtained. Deduced amino acid sequences were aligned to the RefSeq Protein database. The best hits for nsp1a and nsp1ab sequences in all five contigs were to known enterotropic BoAstV, such as BoAstV-B76-2/HK and -B18/HK, with >91% identity. A very similar situation was found for the capsid protein sequence in two contigs, with >92% similarity to BoAstV-B76-2/HK and GX7/CHN. However, in three contigs the capsid protein sequence identity to those of known astroviruses was only 52%, 57% and 72% respectively.

Discussion

The neurotropic BoAstV-CH13 was not found in our study, suggesting that it does not occur at a high frequency in feces of cattle in Switzerland. However, we identified astrovirus sequences with high similarity to known enterotropic BoAstVs in ~7% of feces samples. Three of the putative astrovirus genomes encode divergent capsid proteins, what may indicate recombination events with so far unknown astrovirus genotype species.

P3 14: Neuropathological survey reveals an underestimation of the prevalence of neuroinfectious diseases in cattle livestock in Switzerland

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Neuroinfectious diseases in livestock represent a severe threat to animal health, but their prevalence is not well documented and the etiology of disease oftentimes remains unidentified. The aim of this study was to first generate baseline data on the prevalence of neuroinfectious diseases in cattle in Switzerland by a neuropathological survey and, in a second step, to identify disease associated pathogens. The survey was performed over a one year period using a representative number of brainstem samples (n=1816) from fallen stock cattle. In total 3.7% (n=67) of the animals revealed significant lesions with the most frequent types of lesions being indicative for a viral (n=27) and bacterial (n=19) etiology, respectively. Follow-up diagnostics identified infections with Listeria monocytogenes (n=6), ovine herpesvirus 2 (n=7), bovine astrovirus CH13 (n=2), bovine herpesvirus 6 (n=6), bovine retrovirus CH15 (n=2), posavirus 1 (n=2) and porcine astroviruses (n=2). A retrospective questionnaire-based investigation indicated that clinical signs of neurological disease were observed by the animals' owner in around one third of the cases with lesions, which was estimated to correspond to approximately 100 cases in the fallen stock adult cattle population in Switzerland per year. This estimation is in sharp contrast to the number of cases notified to the authorities and indicates a gap in disease surveillance. A systematic neuropathological examination and follow-up molecular testing of neurological diseased cattle could significantly enhance the efficiency of disease detection for the purpose of prevalence estimation of endemic diseases, the identification of new or re-emerging pathogens and "early warning" of disease outbreaks.

P3 15: Strengthening of scientific excellence of the National Veterinary Research Institute in animal health and food chain safety

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The general objective of the VET-TWIN project is to increase the potential and research capacity of the National Veterinary Research Institute (NVRI) by cooperation with internationally-leading counterparts from Germany (Federal Institute for Risk Assessment-BfR) and Denmark (Technical University of Denmark, National Veterinary Institute; DTU Vet) - to act in an international scientific and research environment as a leading institute in the fields of animal infectious diseases, zoonoses and food chain safety.

The objective defined in such a way is accomplished through a number of activities, in particular through the implementation of the Joint Strategy of Cooperation and Development and by the interaction of scientists at the operational level, as well as through activities helping in the exchange of knowledge and experiences among scientists

The VET-TWIN project fully addresses the objectives of the Horizon 2020 programme. Through activities (trainings, workshops, cascade trainings, summer schools, congress for young scientists) which increase the level of scientific excellence of the institute and its research staff, the project fully implements the specific objective Spreading Excellence and Widening Participation.

The Implementation of the project fits directly in with the specific challenge of Twinning. By creating a partnership between the aforementioned entities, it is possible to cooperate and exchange knowledge on an international level. Furthermore, the involvement of two scientific centers of excellent repute in the international research community to the VET-TWIN project, prevent the crowding out effect for an institution from a country with a lower Composite indicator of Research Excellence.

PIWet

BfR Bundesinstitut für Risikobewertung

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POSTERS
Topic 4 - African swine fever

P4 1: Optimization of proteasomal processing enhances the immunogenicity of a DNA vaccine encoding African swine fever virus CD8$^+$ T-cell epitopes

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Live attenuated viruses have allowed demonstrating the key role that CD8$^+$ T-cells play in protection against ASF virus (ASFV)[1]. However, safety issues have hampered their use in the field so far. Thus, developing safer subunit vaccines against ASFV is mandatory. Taking advantage of CD8+ T-cell epitopes from proteins A, B and C*, previously identified in the lab using the virulent E75 ASFV strain, two different DNA-vaccine formulations were designed, in this occasion based on the sequence of Georgia07, ASFV strain currently circulating in Europe: i) a cocktail of three plasmids encoding each one a different full-length protein (A, B or C) fused to ubiquitin (Ub) to optimize their delivery to the proteasome[2], and ii) a multi-epitope DNA construct encoding theoretically predicted SLA-I hot spots for A, B and C proteins (including the already characterized peptides), spaced by optimal proteasomal cleavage sites and with Ub as a leader sequence (pCMV-UbmeABC), aiming to optimize the processing of the proteasome driven antigens[3]. A positive control group was immunized with synthetic peptides formulated in Freund´s adjuvant, including both the CD8$^+$ T-cell already characterized epitopes and the theoretically predicted ones. Two additional groups with 3 animals each were inoculated with either Freund´s adjuvant or empty pCMV-Ub plasmid as controls.

Specific CD8$^+$ T-cell responses were only detected after the boost. When stimulating with the predicted peptides mapping in the hot spots, pigs immunized with the multi-epitope construct showed a much higher number of peptide-specific IFNγ-secreting cells than the animals immunized with the plasmid cocktail.

Unexpectedly, most of the recognized peptides mapped in a single domain of protein C from Georgia07, opening the door to future challenge experiments.

In conclusion, we show that optimization of proteasomal processing enhances the immunogenicity of a DNA vaccine encoding ASFV CD8$^+$ T-cell epitopes, confirming the importance of vector design for successful DNA vaccine performance. This knowledge might be extrapolated in the future for other CD8$^+$ T cell determinants to be characterized and most probably to any delivery vector chosen by our partners from Boehringer Ingelheim.

**Boehringer Ingelheim confidential agreement and conflict of interest avoid using the correct nomenclature for A, B and C ASFV-proteins.*

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P4 2: Field evaluation of a new rapid kit test to African Swine Fever antigen detection

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African swine fever (ASF) is a viral and highly contagious infectious disease of domestic and wild pigs, causing a wide range of syndromes from mild disease to lethal hemorrhagic fever frequently characterized by high mortality [1, 2]. In Europe ASF was introduced first in Portugal (1957) and subsequently in other countries as Spain, but finally eradicated. Nowadays, the disease is endemic in Sub-saharan countries and in Sardinia (Italy) [3, 4], causing serious economic and social damage. In Sardinia, the disease has been present since 1978 with a specific epidemiological situation influenced by different factors as the illegally free range pigs in wild boars (WB) territories. The implementation of Public Health programs has proved to be essential for controlling the disease. Since no treatment or vaccine is currently available to prevent ASF, the use of a field test on both wild boar (WB) and illegally bred pigs can be a valuable tool to support the new disease eradication's program. As has been previously demonstrated by the same authors, the use of the field test would also have important economic benefits [5]. After the validation of antibodies field rapid kit test [6], the aim of this work is the evaluation of the new antigen Pen-Side (INGENASA ©) (PS) kit test on field. This study was completed using samples of WB hunted during the 2016/2017 season (Fig. 1); 73 animals were tested with PS and Real Time PCR. The concordance of each test was evaluated using Kappa Coefficient (k) and the final sensibility and specificity were calculated using the OIE real-time PCR as gold standard. Although sensitivity and specificity of tests carried out in the field are reduced (Se. 69.88%; Sp. 96.76%), this results are in agreement with those found in laboratory validation conducted by Sastre et al., 2016 [7]. Those results are represented with agreement chart (Fig. 2), a valid alternative to the receiver-operating characteristics (ROC) curve for diagnostic tests, as explained by Bangdiwala (2008) [8]. In conclusion, we believe it's necessary to study in deep the use of both rapid field tests (antibody and antigen) and evaluate their use as screening test, in association with laboratory tests, as valuable emergency management method during an outbreak, for early diagnosis and virus detection.

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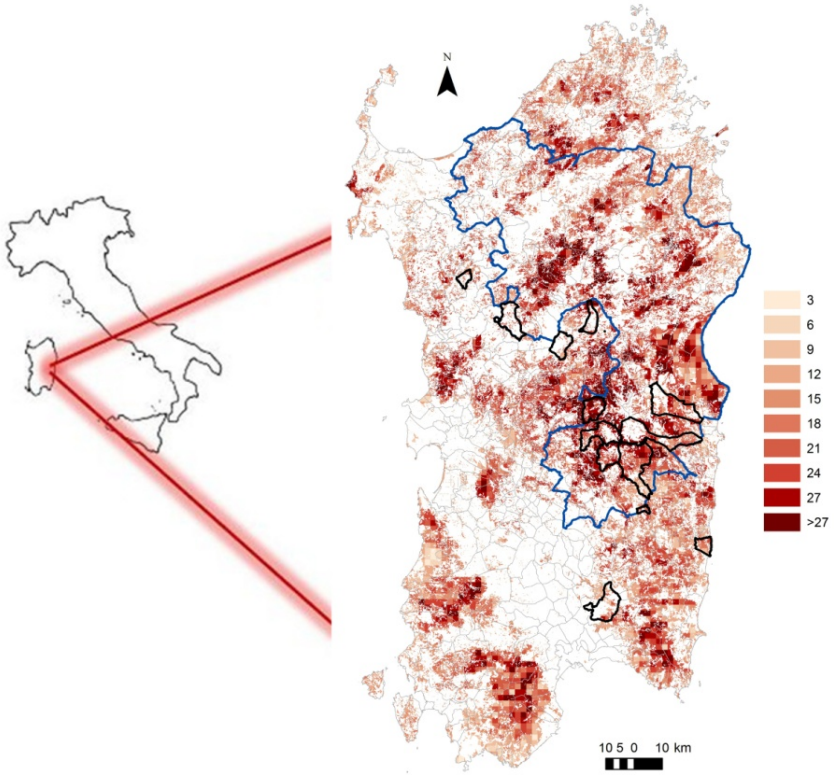

Figure 1: Wild boar density (number of animals) into the Island is showed in different red-grade colors; in blue outline are delaine the WB infected area and in black the area from which the samples were collected.

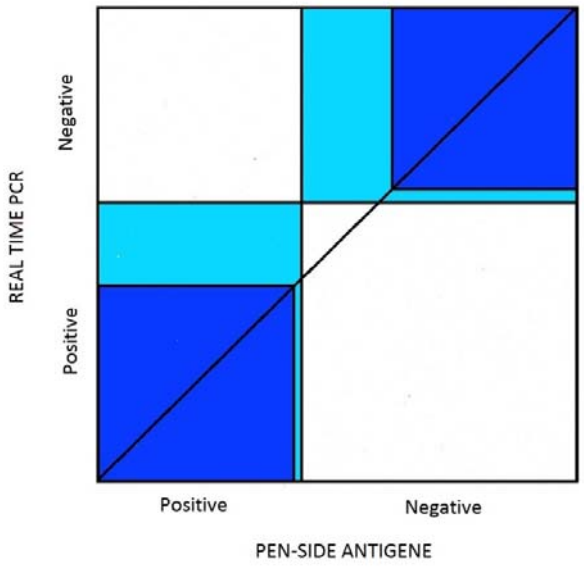

Figure 2: Final results of diagnostic PS test presented by means of agreement chart.

P4 3: Report of a simulation exercise regarding a suspect case of African Swine Fever (ASF)

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Outbreaks of transboundary animal disease, such as ASF swine fever, have a great impact on the social and economic situation of a country and region. To front this evidence European Union obliges the Member States to draw up a contingency plan specifying the national measures to be implemented in the case of outbreaks of a notifiable disease. In order to check the implementation level of contingency plans, the Member States should provide animal disease outbreak exercises.

A simulation exercise was held to verify the preparedness of Italian Veterinary Service to front an emergency situation created by a suspect of Swine Fever. The purpose of the exercise was to ensure the effectiveness of national contingency plan in case of suspect of the diseases.

The scenario provided the occurrence of swine fever symptomatology in a swine fattening farm. The local public veterinary service, supported by the Istituto Zooprofilattico Sperimentale of Umbria and Marche, was kept busy in clinical and post mortem investigations to verify the consistence of the suspect; samples of organs and blood were collected and sent for laboratory confirmation.

Fig. 1-2: the veterinary inspectors adopted biosecurity measures before to begin the inspection

The suspect ASF case was notified to the competent authorities and the measures to prevent the eventual spread of the disease were prescribed to the farmer; an epidemiological inquiry was set up. On the basis of these information several hypothesis about the trace back and the trace on of infection were.

The exercise was stopped after the notification of laboratory confirmation.

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P4 4: Soft tick studies in Ukraine: A cooperative effort to elucidate ecology, distribution, and role in African Swine Fever epidemiology for *Ornithodoros* ticks in Eastern Europe and neighboring regions

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African Swine Fever (ASF) is an arboviral disease of suids that currently constitutes one of the major challenges for the pig production industry in Eastern Europe, including several EU member states. After its initial introduction into the Caucasus in 2007, ASF has been spreading westwards through domestic and wild pig populations and eventually reached Poland and the Baltic states in 2014 (1). African Swine Fever Virus (ASFV), the causative agent of the disease, has a complex epidemiology with several transmission cycles that in addition to domestic pig populations may involve wildlife hosts and (or) tick vectors. Certain soft tick species of the genus *Ornithodoros* have been implicated as biological vectors and arthropod reservoir hosts of ASFV in East Africa and in the Iberian Peninsula, contributing to ASF persistence in the affected territories. Therefore, occurrence of *Ornithodoros* ticks in a given area is usually considered as a risk factor for the disease endemicity (2). However, due to the lack of information regarding current distribution and ecology of the ticks in Eastern Europe and neighboring regions, their potential role for ASF persistence remains to be fully assessed.

Through the international collaboration and multidisciplinary approach, our research group works on bridging this knowledge gap. Thus, we confirmed presence of the potential ASF vector, *O. verrucosus*, in Ukraine, which is affected by ASF since 2014 (3). Currently, efforts to assess the complete species distribution range are being made. The presence/absence data generated through our surveys represent a useful information which might be utilized for ecological modelling that will further increase understanding of soft tick distributional patterns in the region. Another stride towards elucidating the role of local *Ornithodoros* species in ASF epidemiology is the establishment of a laboratory colony of *O. verrucosus* at the NSC IECVM in Kharkiv. This accomplishment allowed resuming soft tick biology research in Ukraine, and enabled efforts to conduct ASFV vector competence experiments with Global African Swine Fever Research Alliance partners.

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P4 5: CRISPR/Cas9-mediated inhibition of pseudorabies virus and African swine fever virus replication in cell culture

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During the last years, specific RNA-mediated prokaryotic defense systems have been converted into powerful tools for genome editing in eukaryotes. This also opens new possibilities for prevention and control of virus infections, i.e. by facilitating targeted viral gene deletions or mutations during development of attenuated live vaccines. On the other hand, resistant host organisms can be generated, either by knocking out cellular virus receptor genes or by constitutive expression of antiviral RNAs by the host. In the present study, we utilized the widely used CRISPR (clustered regularly interspaced short palindromic repeats) – Cas9 nuclease system from *Streptococcus pyogenes* for targeting two swine pathogens: pseudorabies virus (PrV, *Suid herpesvirus 1*) and *African swine fever virus* (ASFV). To this end, permissive rabbit kidney (RK13, for PrV) or wild boar lung (WSL, for ASFV) cell lines were modified by stable transfection with plasmids containing neomycin or puromycin resistance genes, together with expression cassettes for Cas9 and guide RNAs with virus gene-specific target sequences of 20 nucleotides. The target sequences were chosen within conserved regions of essential open reading frames, encoding the major capsid protein (UL19), and the major immediate-early regulatory protein IE180 of PrV, or the major capsid protein p72 (B646L), DNA polymerase (G1211R), an inner envelope protein (E199L), or the secreted phosphoprotein p30 (CP204L) of ASFV. Due to targeted Cas9 cleavage of the virus genome, plating efficiencies of reporter protein (GFP or DsRed)-labeled PrV and ASFV mutants were significantly (> 100fold) reduced in many of the obtained cell clones compared to the parental lines. However, frequently escape virus mutants arose, which were no longer recognized after point mutations in the target sequences, and which led to obviously tolerated amino acid substitutions or deletions in the affected proteins. This problem could be widely overcome by the generation of cell lines which simultaneously expressed two to four different guide RNAs targeting one or more viral gene loci, where productive virus replication was almost completely abolished. Almost complete suppression of ASFV replication was also observed in WSL cells expressing a single guide RNA against the viral p30 gene, indicating that in this case the targeted region (codons 72 to 77) was crucial for protein function. The specificity of this effect could be demonstrated by using a natural ASFV isolate possessing four base exchanges (resulting in only one amino acid substitution) within the target sequence, which was not inhibited in the corresponding Cas9-expressing cell lines. Growth of most of the various CRISPR/Cas cell lines was not affected, and transgene expression as well as virus inhibition proved to be stable over many (> 20) passages. Thus, it appears conceivable that Cas9 nuclease and PRV- or ASFV gene-specific guide RNAs can be also permanently expressed in transgenic pigs to make them resistant against the respective pathogens.

P4 6: Molecular genetic study of ASF virus isolated from Samara and Irkutsk Regions, Russia in 2017

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African swine fever is a high contagious viral disease affecting pigs. This disease is a global threat for pig breeding, exacerbated by high virulence and persistence of the virus in the environment and lack of an efficient vaccine. Outbreaks of the disease are regularly registered worldwide and cause a significant damage for domestic pig and wild boar populations.

In March 2017, the outbreaks of African swine fever occurred in disease free Samara (Volga) and Irkutsk (Syberia) regions, Russia. Distance between these outbreaks was more than 4000 km. The disease led to loss of 92 pigs in Samara and 40 pigs in Irkutsk regions. The outbreaks were timely localized and eliminated. The infection presumably entered Samara region from a neighboring Saratov region with wild boars. The source of ASFV to Irkutsk outbreaks is still unknown.

In order to perform genotyping of the virus and assess the risks, we have sequenced several genes and assessed similarity with known isolates identified in Russia and surrounding countries in previous years.

We obtained DNA sequences for B646L (p72), E183L (p54), D117L (p17), B602L genes and intergenic site I73R-I329L of this isolate. Analysis of B646L (p72), E183L (p54), D117L (p17), and B602L genes showed that these isolates belongs to ASF genotype II; their sequences were identical to sequences of the appropriate genes of Georgia 2007/1 isolate, which is considered to be the parent for all isolates identified in the Russian Federation so far. The intergenic site I73R-I329 of Samara isolate comprises GGAATATATA tandem repeat (GII-CVRI-UGRII), originally described in 2012 and characteristic of the younger isolates, in particular Ukr12/Zapo (Gallardo et al.). However, ASFV from Irkutsk regions does not have tandem repeat insertion and belongs to GII-CVRI-IGRI variant. Therefore, these isolates are closely related to isolates previously identified in European part of Russia but have different IGRs. This funding may suggest about different sources of infection in those regions.

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P4 7: Expression of African swine fever virus proteins by a pseudorabies virus vector

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African swine fever virus (ASFV) is the hitherto only member of the family *Asfarviridae,* and causes a severe disease (ASF) of domestic pigs and wild boar with fatality rates of nearly 100 %. ASFV is endemic in major parts of Sub-Saharan Africa as well as in Sardinia, and since the introduction of a highly virulent strain in 2007 to Transcaucasia, the virus spread to Russia, Belarus, Ukraine, the Baltic countries and eastern Poland. ASFV is a complex virus with a dsDNA genome of approximately 180 kbp, which mainly replicates in the cytoplasm of porcine cells of the monocyte/macrophage lineage, but also in arthropod vectors (soft ticks). Due to specifics of ASFV biology targeted mutagenesis of the virus genome is still difficult, and up to now no safe and reliable vaccines are available.
As an alternative to attenuated ASFV mutants we selected an established live vaccine virus against Aujeszky's disease of pigs as a vector for the expression of probably immunogenic ASFV proteins. To this end, the genome of pseudorabies virus (*Suid herpesvirus 1*) vaccine strain Bartha (PrV-Ba) has been cloned as a bacterial artificial chromosome by insertion of a mini-F plasmid and a GFP reporter cassette at the non-essential glycoprotein G gene locus. Using the CRISPR/Cas9 system, these foreign sequences were then substituted by codon-optimized expression cassettes for the proteins pE199L, p22 (KP177R), CD2v (EP402R) or p30 (CP204L) of ASFV Armenia (genotype II). *In vitro* studies revealed that these insertions did not significantly affect replication of PrV-Ba, and that all ASFV proteins were abundantly expressed in cells infected with the PrV recombinants. To evaluate immunogenicity and protective efficacy, three groups of four 7 week-old pigs were intramuscularly immunized twice at three-week intervals with mixtures of three (pE199L, p22 and CD2v), or four (pE199L, p22, CD2v and p30) of the PrV recombinants, or with unmodified PrV-Ba. Western blot, immunofluorescence and ELISA tests demonstrated that all vaccinated pigs developed PrV-specific antibodies, and antibodies against ASFV pE199L and p30 were detectable in all animals immunized with the respective PrV recombinants. Interestingly, p22-specific antibodies were mostly found in pigs which received the three-recombinant mix, and antibodies against CD2v were not detectable in either group. First flow cytometric analyses of peripheral blood did not reveal significant changes of B-cell subpopulations, but a general increase of $CD8^+$ T-cells was observed in all vaccinated groups within the first days after oronasal homologous challenge with ASFV Armenia, which was applied three weeks after boost vaccination. However, despite of the abundant expression of the tested ASFV proteins and the observed ASFV-specific immune responses, all vaccinated pigs developed high fever and other typical symptoms of ASF, and had to be euthanized eight to ten days after challenge. Moreover, real-time PCR analyses of pharyngeal swab samples and blood did not indicate a significant reduction of challenge virus shedding compared to PrV-Ba infected or non-vaccinated control animals. Thus, the chosen ASFV proteins are obviously not relevant or not sufficient for induction of protection. Future studies will show whether other ASFV proteins are more efficacious, and whether co-expression of selected porcine cytokines by the PrV vector might improve the protective immune response.

P4 8: Do antibodies play a role in partial protection of pigs inoculated with an attenuated ASFV isolate, OURT88/3, against the highly virulent Georgia 2007/1 isolate?

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Since the introduction of African swine fever to Georgia in 2007, the infection has spread to neighbouring countries including the Russian Federation and eastern countries of the European Union. The absence of vaccine available against ASFV limits the options for control.

We have previously demonstrated that immunisation with an attenuated ASFV strain, OURT88/3, from genotype I can protect pigs against related genotype I isolates and also against a genotype X isolate from Uganda (1). Complete genome sequencing indicated that the Georgia 2007/1 isolate sequence is more closely related to the OURT88/3 isolate sequence than the genotype X isolates (2). We therefore tested whether the immunisation of pigs with the OURT88/3 isolate could protect pigs against challenge with the Georgia 2007/1 isolate.

Three groups of 8 pigs were housed separately. Pigs of group 1 were immunised intra-muscularly with 10e4 $TCID_{50}$/pig of the ASFV attenuated isolate OURT88/3 and boosted 3 weeks later with the same dose of OURT88/3. Pigs of group 2 were also primed with OURT88/3 at 10e3 $TCID_{50}$/pig then boosted 3 weeks after with the closely related virulent isolate OURT88/1 (10e4 $TCID_{50}$/pig). Pigs of group 3 were not immunised (negative control group). Three weeks later, pigs of the 3 groups were challenged with the ASFV Georgia 2007/1 isolate at 10e4 $TCID_{50}$/pig. The results showed that 50% of the immune pigs challenged with Georgia 2007/1 isolate survived. Several of the immunised pigs challenged with Georgia 2007/1 isolate developed clinical signs earlier than the control non-immune group. This indicates an immune enhancement of disease might have occurred. On the other hand, no virus in blood or spleen was detected by qPCR at the end of experiment in the immune pigs that survived from challenge. This suggests that OURT88/3 immunisation could induce protective immunity against Georgian isolate.

The role of antibodies in protection or in enhancement of the disease needs further investigation. We have therefore developed and assessed different protocols to quantify a neutralizing activity versus an increase in virus multiplication, in presence of immune/non immune sera. The results will be presented and discussed.

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P4 9: Semi-qualitative model to African Swine Fever risk evaluation in Sardinia

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African swine fever (ASF) is a viral disease caused by a DNA virus (ASFV), family Asfarviridae. ASF first description dates back to 1920 in Kenya, where it caused a hemorrhagic febrile disease with 100% mortality in domestic pigs [1].
ASF has been endemic in Sardinia since 1978. Historically, areas at higher risk are located in some inner parts of this island, where domestic pigs are still illegally kept in semi-wild conditions, living in contact with the local wild boar population, thus creating perfect conditions for disease endemicity [2, 3]. Currently, an eradication plan is ongoing, that is based on a comprehensive strategy adapted to the local situation. The plan provides *inter alia* for disease control measures supplementary to those laid down in the national and european legislation. These measures are carried out by official veterinarians on the whole island in a coordinated and progressive manner, starting from areas of lower risk. One of plan's fundaments is the classification of pigfarms as "controlled" or "certified", based on clinical, structural and biosecurity characteristics. In order to be defined as "controlled", pigs in the farm in question have to be clinically checked and tested for ASF with negative results. In order to be "certified", a number of additional requirements have to be complied with, including detailed procedures for animal identification and registration and stringent animal welfare and biosecurity standards, in accordance with appropriate guidelines. The "certified" status of a farm may bring to financial incentives for the owner. The plan also provides for a strong action against illegal farms and pigmeat marketing channels.
A proper geographical risk assessment is of major importance for the success of the plan, as it allows the identification of areas where human resources devoted to official controls should be allocated as a priority and more intensive eradication measures should be implemented. In this context, a semi-qualitative analysis based on data gathered in 2015-2016 was performed in order to assign a risk level to each of the 377 municipalities of Sardinia, which took into account: a) pigfarmer's compliance with the plan's requirements and the farm's status, b) the detection of disease outbreaks in domestic pig farms and cases in the wild boar; c) the detection of seropositive animals; d) the disease trend during the previous years e) the level of compliance with the official controls' calendar established in the eradication plan; f) the sighting of illegal pigs, g) the estimated number of wild boars. The results of this study, expressed as risk score and classified in 5 risk levels, are shown in the map (fig. 1). The actions to be carried out in the three years are set according to the territory risk assessment based on municipality.

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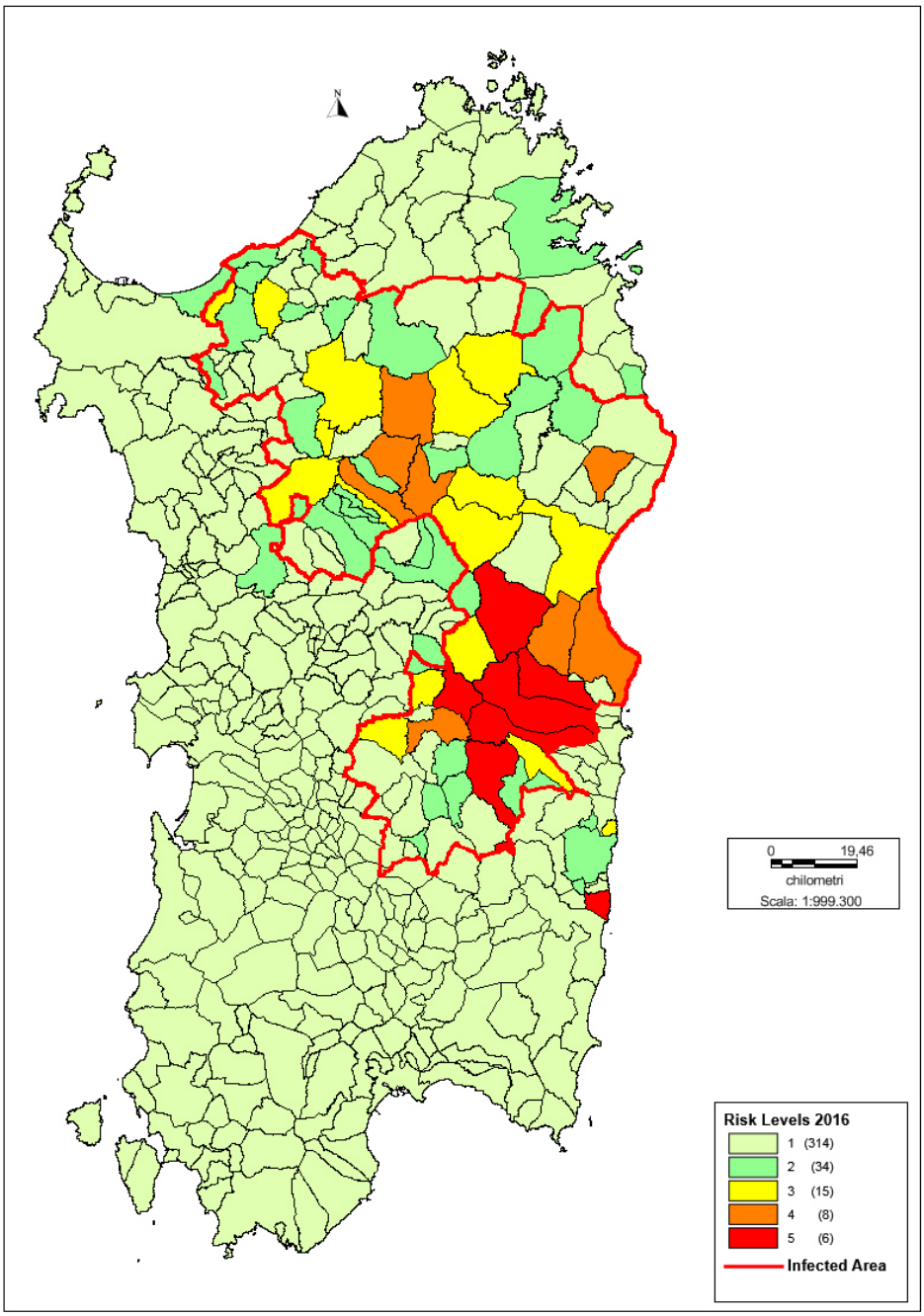
0 19,46
chilometri
Scala: 1:999.300
Risk Levels 2016
1 (314)
2 (34)
3 (15)
4 (8)
5 (6)
Infected Area

P4 11: Real Time Cell Analysis for Measuring African Swine Fever CPE

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African swine fever (ASF) is an acute viral hemorrhagic disease of domestic swine with mortality rates approaching 100%. African swine fever is caused by a large, icosahedral DNA virus, African swine fever virus (ASFV) that is the only member of the *Asfarviridae* family.

Even though porcine monocytes/macrophages are the only target cells for ASF virus in vivo, ASFV isolates might growth in different permanent cell lines (i.e. COS-1, CV-1, PK, Vero) and can produce plaques and cytopathic effects (CPE). Determining virus titer by CPE is time consuming process and shows no kinetics of virus replication.

Here, we used Real Time Cell Analysis (RTCA) system Xcelligence (ACEA Inc. USA) to kinetically characterize ASFV-induced cytopathic effect in COS-1. The gene deleted (ΔCongo-CD2v, DSwCongoCD2v-Lectin) and attenuated ASFV strains (KK262) have been used. Apoptosis activator (Cycloheximide, 10 µg/ml) was used as a control of cell death. The growth, proliferation, adhesion kinetics and onset of CPE of COS-1 cells were determined using an electrical impedance assay via cell index (CI) recordings.

Based on growth curve and CI values a time point at 24 hours after seeding the cells was set for virus inoculation. The ΔCongo-CD2v strain showed significant decrease of CI values at 4 hours post infection and till 72 hours. Similar kinetics was recorded for Cycloheximide treated COS-1 cells. Nevertheless, KK262 and DSwCongoCD2v-Lectin did not show any CPE and CI values were like mock infected cells although the virus replicates successfully.

Thus, the presented type of RTCA-based kinetic comparison can be used for assessing the relative fitness/virulence of ASF virus isolates/strains. This method was therefore suitable to measure the course of ASFV-depended CPE induction in COS-1 cell line and replace subjective visual scoring by microscopy.

Acknowledgements
We would like to thank Kirill Bliznetsov, Alamed for help with the experiments. Financial support for these studies was provided by the Russian Science Foundation (grant 16-16-00090).

P4 12: Interaction of ASFV strains of diverse virulence with monocyte-derived dendritic cells in different activation status

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African swine fever virus (ASFV) is a devastating disease of domestic pigs and wild boar. Its aetiological agent primarily infects cells of the myeloid lineage and despite the central role of dendritic cells (DCs) in induction of immunity, few studies analysed their interaction with the virus. Due to the infrequency of DCs in the circulation and lymphoid organs, myeloid DCs can be generated *in vitro* by monocytes, through incubation with GM-CSF and IL-4. Our study aimed to conduct a detailed characterization of the interaction between moDCs and ASFV strains of different virulence. MoDCs were left untreated or matured with TNF-alpha and/or IFN-alpha. Cells were infected with an avirulent (BA71V) or a low virulent (NH/P68) or a Sardinian virulent (22653/14) strains of ASFV, alongside mock-infected controls. ASFV infection and its effect on moDC phenotype and function were analyzed with flow cytometry, ELISA and RT-PCR. All the isolates infected moDCs, but differences were observed with IFN-alpha /TNF-alpha activated moDCs. Using an MOI of 1, lower levels of late protein p72 but not early protein p30 were observed in BA71V-infected cells pretreated with IFN-alpha, but not TNF-alpha, whereas IFN-alpha/TNF-alpha treatment increased p72 expression in all the virulent isolates, independently on the genotype. NH/P68 was sensitive to IFN-alpha only using an MOI 0.01. This low virulent isolate replicate more efficiently than 22653/14 in moDCs. Ongoing experiments investigating the effect of ASFV strains of diverse virulence on surface markers expression and cytokine response by moDCs will be presented. We hope that the data generated by this study will aid our understanding of the immunomodulation of host cell responses by ASFV.

Acknowledgements
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P4 13: Whole-genome next generation sequencing of African swine fever virus in blood samples obtained from experimentally infected pigs

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Introduction

African swine fever (ASF) is a severe viral haemorrhagic disease of domestic pigs and wild boar, which has been spreading throughout regions in the Baltic countries and Poland since 2014[1]. The disease is caused by African swine fever virus (ASFV), a large (170-193 kbp) double-stranded DNA virus within the *Asfarviridae* family[2].

Due to the large size of the genome and the rather slow evolution of DNA viruses, routine genome fragment analyses for partial sequencing have shown a low resolution power with regards to detailed analyses of variation among isolates[3]. Hence, information from larger genome fragments or whole-genomes are needed in order to look more into the molecular basis of virus evolution and pathogenesis. Whole-genome next generation sequencing (WG-NGS) might be a tool to achieve a higher resolution power and could provide a better understanding of e.g. virus-host interactions and molecular evolution of the virus.

In the present study, WG-NGS was applied to obtain whole-genome ASFV sequences from blood samples obtained from infected pigs.

Materials and Methods

EDTA-stabilized blood was obtained from domestic pigs experimentally infected with a Polish ASFV isolate. Within 12 hours after obtaining the blood samples, erythrocytes were separated from the mononuclear cells and plasma fraction of the blood using a Lymphoprep™ density gradient medium (STEMCELL™ Technologies). DNA was extracted from the erythrocytes-enriched samples on a MagNA Pure 96 system (Roche) and 13 ASFV-positive samples were sequenced on a MiSeq platform (Illumina). The raw sequence data was analyzed and mapped to the complete genome of ASFV Georgia 2007/1 (accession number FR682468) in CLC Genomics Workbench (QIAGEN).

Results

When the 13 samples were analyzed individually the number of total reads ranged from 0.8-5.6 million reads (average 2.5±1.6 million reads) and 1574-58324 reads (average 17803±16506 reads) were mapped to the complete genome of ASFV Georgia 2007/1. This constituted 0.1-1.5 percent of the total reads. From some of the best samples almost complete genomes could be obtained with an average sequencing depth up to 46.

When the raw reads from the 13 samples were analyzed together, 3.7 million total reads were obtained. Of these 253045 (0.69 %) were mapped to the Georgian complete ASFV genome, yielding a depth of 200 and a consensus sequence of 189390 nucleotides, constituting a complete genome.

It is contemplated, that regions in which variation is observed between the obtained sequences and the Georgian reference genome will be further evaluated in order to obtain more knowledge about variation within and between infected pigs.

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P4 14: Effect of Tetherin (BST2) restriction factor on African swine fever virus infection

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Tetherin (BST2) is an antiviral protein induced by type I interferon. The antiviral activity of tetherin resides mainly in its ability to restrict the release of enveloped viruses. 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 AP-1 during ASFV infection. The repercussion of this interaction on infectivity is still unknown. Tetherin is located at the plasma membrane and in intracellular compartments being described to bind to AP-1 complex, which furthermore seems to be responsible of intracellular tetherin distribution. We hypothesize that tetherin may possess anti-viral activity against ASFV and that ASFV could harbor anti-tetherin resistance during virulent strains infection through the control of AP-1/CD2v -dependent mechanism.

Our results showed that both virulent (E70) and attenuated (NH/P68) ASFV strains induced tetherin expression in swine macrophage at 16hpi and an accumulation of intracellular levels of tetherin was observed in ASFV-infected cells. However, tetherin downregulation was detected from cell surface of both COS and swine macrophages after the infection. Interestingly, we found that BST-2 down regulation was stronger after virulent E70 strain infection compared to the attenuated NH/P68 strain, indicating a strain-specific viral mechanism to control tetherin function at the cell surface. In addition, a decrease of viral egress from COS cells overexpressing HA-BST2 in the cell surface, affecting particularly to E70 strain but not to the NH/P68, was observed. These results suggest different viral mechanisms to evade tetherin function by virulent vs attenuated ASFV strains. On the other hand, we found that BST2 and AP-1 co-localized in uninfected swine macrophages, an event that increased during both E70 and NHV/P68 infection. This enhancement in the co-localization between both proteins might reflect a virus-induced hijacking of tetherin by AP-1 to prevent BST2 expression at the cell surface.

In connection to this, in AP-1 silenced COS cells viral egress of E70 decreased, indicating a connection between AP1, virulent viral egress and BST2. This effect was not observed in AP-1-silenced cells after infection with NH/P68 strain, reinforcing the hypothesis that cellular mechanisms preventing viral egress may affect differently to ASFV strains. Such kind of differences may add light on the factors and mechanisms determining virulence vs attenuation in the context of BST2.

In resume, our model suggests that BST2 performs its anti-viral function on the cell surface and is regulated by the ASFV infection. Hence, the intracellular localization of BST-2 during the infection is a key factor for its anti-viral role, which may be modulated by AP-1, explaining at least partly the role of the complex in ASFV restriction. Currently, the viral factor(s) from virulent and attenuated strains putatively involve in this mechanism are under investigation.

P4 15: Results of the Ornithodoros genus research, in Sardinian pig farms.

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African swine fever (ASF) is a viral disease caused by DNA virus (ASFV), which mainly replicates in the cytoplasm and belongs to the Asfarviridae family, Asphavirus genus. ASF has been present in Sardinia since 1978 [1], causing serious problems for industrial pig sector and authorities, in spite of the numerous interventions aimed to its eradication [2]. Nowadays, the disease has been reported in both domestic pigs and wild boars, mostly seropositive and some virological findings. It has been well documented that the soft tick of the *Ornitodoros* genus (*O. Erraticus*) plays an important role in ASF epidemiology in main regions, including the Iberian peninsula [3,4,5]. The last project targeted to the research of the *Ornitodoros* findings is dates back to the 1982 [6]. This Sardinia control programme was focused on the field research of ticks on the Island that are capable of maintaining the disease. Although research has been performer in 357 holdings in 20 different districts in the province of Nuoro, none *Ornitodoros* was found. Inasmuch as the studies that sought to expand the knowledge about the epidemiological role of this insect in Sardinia were few and old, our current research has tried to fill some gaps about the arthropod Ornithodoros sp. absence/presence in Sardinia region. The research was performed using the Dragging techniques and/or CO_2 traps (Dry-ice or CO^2 cylinders with constant and long-lasting release) (Figure 1 and 2), around shelter pigs area, carry out in 22 different municipality, show in the map (Figure 3). Overall, 454 arthropods were found, of which 420 ticks, 27 louses and 7 mites. All of those insects were tested with real-time PCR, 30 of them gave positive results. No one *Ornitodoros* specimen were found during the samples. Other itinerant research within the regional territory led to the finding of the *Ornithodoros marittimus* at the municipality of Teulada (Cagliari province). This species, the only soft patch found in the territory of Sardinia, is not bibliographically associated with ASF's spread and transmission [7]. Those results confirmed what previously demonstrated about the role of this insect vector in ASF's epidemiology. Thus, further research projects using currently techniques has to be encouraged in order to study in deep the knowledge of arthropod's role.

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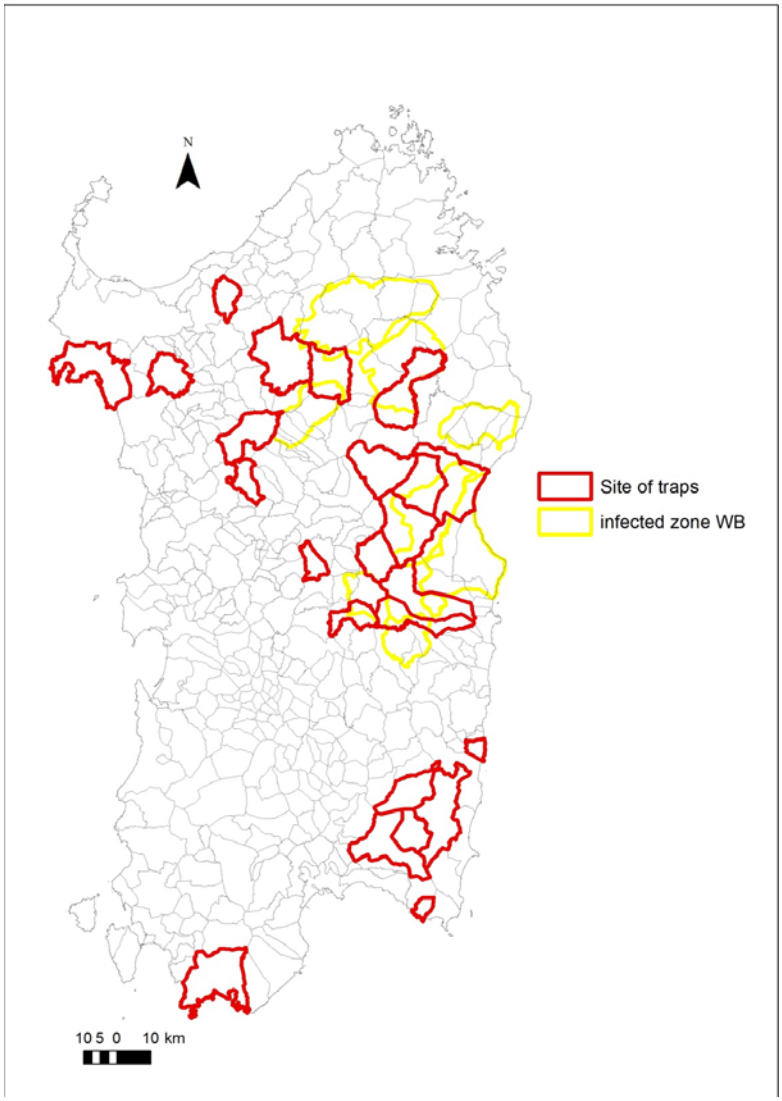
Figure 1. Dry-ice trap

Figure 2. CO_2 trap.

Figure 3. Sardinian map showing the regional sites (red line) were the *Ornithodoros'* research was performed. In yellow the wild boars infected area.



P4 16: Comparative analysis of three African Swine Fever Virus immunogenes (A238L, I329L and DP71L).

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African swine fever (ASF) is a hemorrhagic and frequently lethal disease of domestic pigs and wild boars caused by complex DNA-virus. ASF virus is a macrophage-tropic virus and can manipulate both an innate and adaptive immune response modulating the macrophage functions. Currently several proteins of ASF virus, which inhibit host immune response were identified. Among these proteins 5EL (A238L), K11L (I329L) and I14L (DP71L) were chosen for comparative analysis in order to understand ASFV genetic variability of immunomodulatory proteins. Identification of the genetic features of these proteins might help to discover new mechanisms of virus evasion and virus-host interactions.

In order to determine phylogenetic relationships based on immunogene loci between ASFV strains from different geographical locations, we sequenced three immunogenes (A238L, I329L and DP71L) of 15 ASF attenuated and virulent strains belonging to 7 serogroups (SGs) (VNIIVViM virus collection).

Phylogenetic analysis of A238L, I329L and DP71L gene showed that the available isolates clustered in 5 main clades. The Clade 1 included ASFV isolates of West-African origin, belonging to Genotype I. The Clade 2 consisted of the Russian Federation ASFV isolates (SG8, Genotype II). The Clade 3 comprised ASF virus isolates from South Africa (SG2; Genotypes I and VI). The Clade 4 contained ASFV strains from South-Eastern Africa belonging to Genotype V (SG3 and 7). The Clade 5 included the strains and isolates from the Tanzania and Kenya predominantly Genotype X.

Interestingly, that 5 analyzed East-African strains (Genotype X) were distinct from the others ASF virus genomes based on the number of I14L amino acids. In these ASF strains DP71L gene formed by fusion of the two families of 110 genes 13L and 14L that makes them unique among ASFV genomes. Similar funding was reported by Bishop et al. recently.

Conclusion: the A238L, I329L and DP71L genes are valuable genetic markers for studying ASFV genetic variability. Phylogenetic analysis based on the sequencing of I329L, A238L and DP71L genes shows that ASF virus isolates from the Russian Federation are identical to the parental strain Georgia_2007/1. East – African ASFV isolates have unique genetic signatures in DP71 gene.

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P4 17: Whole genome analysis of twelve African swine fever isolates originating from ASF cases and outbreaks in Poland

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African swine fever virus (ASFV) developed different molecular mechanisms to evade host immune response including alteration of interferon production by multigene family protein (MGF505-2R), inhibion of NF-κB and nuclear activating factor in T-cells by the A238L protein, or modulation of host defense by CD2v lectin-like protein encoded by EP402R and EP153R genes. We have previously shown on the basis of analysis of 67 ASFV isolates that nucleotide sequence derived from their A238L and EP153R regions showed 100% identity. Minor but remarkable genetic diversity was found within EP402R and MGF505-2R genes suggesting slow but consistent molecular evolution of ASFV and the important role of these genes in modulation of interferon I production or hemadsorption phenomenon.
These results are only partial and show the virus variability is below 0.8% per analyzed genomic region. Therefore the aim of this study was in-depth genomic analysis of the selected ASFV isolates from the territory of Poland. The current issue is the circulation of ASFV in wild boar population but still the virus pose a threat for pig production in Poland. Therefore, it would be reasonable to find the possible molecular markers to trace the virus and to determine its origin and variability. The presented study was conducted using next-generation sequencing method (NGS) to compare 12 representative ASFV isolates originating from ASF outbreaks in pigs and cases in wild boars to find the possible molecular markers for identification of their origin and confirmation of ASFV molecular evolution. The details from NGS analysis will be further presented.

Acknowledgements

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P4 18: Survival of African swine fever Polish isolates in artificially contaminated soil, leaf litter and water in different environmental conditions.

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African swine fever (ASF) poses a threat to East-European pig production since of 10 years. In Poland, the disease was first reported in 2014, up to now 23 outbreaks in pigs and 306 cases in wild boars were confirmed, and this number is increasing rapidly. The carcasses of infected wild boars are the main virus source among susceptible animals population in Eastern Europe and Baltic states. The recent studies related to ASFV survivability and stability was performed using isolates belonging to variety of genotypes, whereas viral strains currently circulating in Poland fall into genotype II. The aim of presented study is the survivability investigation of Polish ASFV field isolates using artificially contaminated fomites like soil, litter and water obtained from ASF affected, simulating natural environmental conditions. The study may aid to evaluate the possibility of virus spread via different ways of transmission.
The first step of this study was performed using tissue samples originating both from infected domestic pigs and wild boars collected in Polish ASFV National Reference Laboratory (NRL) between 2014 and 2017. The infectious material was propagated in pulmonary alveolar macrophages (PAMs). The medium from inoculated cultures was subjected to DNA extraction (QIAamp DNA Mini Kit, QIAGEN). The resulted DNA samples were tested to detect ASFV genome using UPL real-time PCR. ASFV replication was proved by hemadsorption assay (HAD). Virus titers were estimated by endpoint dilutions using HAD test and reached from $10^{6.32}$ to $10^{7.57}$ HAD units per ml. DNA originating from the obtained 12 isolates was sequenced using Next Generation Sequencing (NGS) to select the most representative strain, which was applied to artificial contamination of soil, leaf litter and water samples. Each fomite sample was contaminated with $10^{6.12}$ HAD units of ASFV, and incubated in different temperature conditions ranging from -20 to +37°C in relative humidity between 5 and 90%. The samples were collected after various time spans and processed by DNA extraction and virus titration. The results of this study will be further presented.

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11th EPIZONE Annual Meeting
Crossing Barriers
Paris, France
POSTERS
Topic 5 - Animal influenza viruses

P5 1: Comparison of adjuvant technologies for avian influenza vaccines

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Water-in-oil adjuvants induce a strong long-term humoral immune response and are widely used for the formulation of inactivated poultry vaccines globally, for broiler, layer and breeder chickens. Other technologies such as double emulsion and aluminium hydroxide adjuvants are also available for poultry vaccination. In this study we compared water in mineral oil (W/O), water in mineral oil in water (W/O/W) and aluminium hydroxide adjuvant technologies for avian influenza (AI) vaccination. We could demonstrate that selection of adapted water-in-oil adjuvants improve vaccine efficacy in poultry compared to other technologies.

Experimental AI vaccines based on W/O adjuvant Montanide™ ISA 71 VG (ISA 71), standard W/O adjuvant, double emulsion W/O/W adjuvant and aluminium hydroxide adjuvant were compared in a chicken trial. All vaccines contained the same strain (H5N1 Re-5) and the same antigenic load. Safety and efficacy profile of the adjuvanted vaccines was assessed in 7 days old broiler chickens (30/group). Chickens were injected at D0 with 0.5ml of experimental AI vaccines. Blood samples were taken from non-challenged chickens at weeks 1, 2, 3, 4, 5, 6 post vaccination for antibody titration by haemagglutination inhibition assay. 2 weeks after vaccination, 10 chickens of each group were also challenged by intranasal inoculation of 10^5 ELD_{50} of highly pathogenic AI virus subtype H5 (strain A/Duck/FuJian/31/2007). Viral shedding and mortality was assessed during 14 days after infection.

Both water in oil based vaccines induced significantly higher antibody levels than other formulations in chickens. After AI challenge, the ISA 71 formulation was the only vaccine able to induce a full protection against pathogenic challenge (no mortality and no viral shedding for 14 days after challenge).

We have shown that the selection of adapted water in oil adjuvant allows the formulation of safe and efficient avian influenza vaccines that confer higher protection and longer-lasting antibody titers in chicken than other adjuvant technologies.

P5 2: Avian Influenza H5N8 in Russia

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The introduction of the influenza virus subtype H5N8 in the territory of the Russian Federation was recorded in 2016 in the Republic of Tyva on the Lake Ubsu-Nur. The Russian Federation has reported more than 20 outbreaks of avian influenza in poultry. Migrating birds are regarded as a transboundary vector of influenza virus distribution. Risk factors for outbreaks include an inadequate level of biological protection for poultry farms, which is manifested in the absence of a clear zoning of production facilities, and the availability of conditions for the nesting of wild birds on their territory. It is established that the intersection of routes on-farm traffic and movement of people, lack of vaccination of the susceptible livestock, dense inter-farm connections between poultry enterprises and private farms contribute to the rapid spread of avian influenza. In addition, we believe, that high tourist activity in a number of regions of the country (routes of the Golden Ring of Russia) can contribute to the transboundary drift of the influenza virus subtype H5N8 from the countries of South-East Asia. The grounds for suspicion of an disease outbreak was the death of poultry, which 5 days before diagnosis amounted to more than 13.8%. The dynamics of pathoanatomical changes characteristic of subtype H5N8 is shown, manifesting as lesions of mucous and serous membranes of the digestive and ovarian organs, an increase in the spleen and a violation of the permeability of the vascular bed. Laboratory studies have shown that influenza virus subtype H5N8 has high pathogenicity, i.e., the intravenous pathogenicity index is 2.85.

P5 3: Genotyping of French highly pathogenic H5N8 during 2016-2017

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During winter 2016-2017, Europe was affected by a highly pathogenic H5N8 avian influenza virus mainly detected in wild birds, and then rapidly in poultry farms. The virus was detected in many European countries (including Poland, Germany, Switzerland, the Netherlands, Sweden, Croatia, Hungary etc..). The presence of this virus was first confirmed in France on 26 November 2016 on decoy ducks in the Pas-de-Calais departement. It was detected in Southwestern France quickly thenafter (mainly in duck farms). Analysis of the H5 gene sequences obtained indicates that the virus isolates belonged to clade 2.3.4.4. The same clade had been already detected in several European countries during the winter 2014-2015 and also contains H5N8 and H5N6 viruses that have been circulating in Asia for several years. Unlike the various H5 HP viruses that circulated only in Southwestern France during the 2015-2016 episode, the 2016-2017 virus appears to be more pathogenic in palmipeds and has spread to Europe. Complete sequencing of seven H5N8 viruses, followed by phylogenetic analysis of their 8 genomic segments suggests that at least two different introductions took place in France (Table 1). These results are consistent with those obtained at the European level, which describe two H5N8 genetic lines, one spreading mainly along the Baltic and the other in Central Europe. Moreover, the comparison of the sequences of the different French isolates seems to indicate that reassortments with other avian influenza viruses and affecting the PB2, PA and NP segments may have taken place. The comparison with H5N8 virus sequences found elsewhere in Europe, as well as the completion of full-genome sequencing in several other French H5N8 viruses (ongoing) will allow to determine whether these reassortment events occurred before or after the introduction of the viruses in France. Such a spread of different genogroups and multiple simultaneous introductions via wild avifauna had already been observed in 2006 with the highly pathogenic H5N1 virus that belonged to clade 2.2 [1].

		North and west of France			South-west of France			
	Virus	161104	161585	161323	161108	161587	170100	170347
Virus genes	PB2							
	PB1							
	PA							
	HA							
	NP							
	NA							
	M							
	NS							

Table 1: Representation of the highly pathogenic avian influenza H5N8 virus genotypes characterized in France. For each viral segment, each color corresponds to a different phylogenetic group

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P5 4: Poultry vector vaccines: innovative serological and molecular assays fordiagnosis, vaccination monitoring and DIVA testing for avian Influenza A

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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). The present study focus on Avian Influenza.

Inactivated vaccines, as well as vector vaccines are used to protect animals against AI. For many years, inactivated vaccines based on circulating hemagglutinin or neuraminidase were developed to protect flocks, and some non-vaccinated birds were used as sentinels to monitor field infections. Furthermore, at the end of 1990's, another strategy were tested with vaccines using the same hemagglutinin that the circulating strain, but with a different neuraminidase. This strategy allowed to differentiate infected and vaccinated birds with serological tools. However, because of the limited efficacy of these classical vaccines, the use of recombinant HVT-AI vector vaccines has become widespread. HVT-AIV vector vaccines, which based on a H5 protein, induce immunity in day old birds, and its effectiveness is not affected by maternal antibodies. To monitor vaccination uptake 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.). Finally, IDvet had also developed a range of molecular tools for the detection of Avian Influenza pathogens.

Results obtained with AI serological tests are presented. Validated by European reference laboratories, the H5 competitive ELISA shows high sensitivity and is able to detect new circulation clades of H5N8. The indirect ELISA specifically detects antibodies to the virus nucleoprotein. It may be used to monitor killed vaccines in chickens and turkeys, and thanks to its very high sensitivity, to monitor disease in SPF animals. Finally, use together, the iELISA may be used in combination the H5 competitive ELISA to detect natural infection in animals vaccinated with rHVT-H5 vaccine alone.

P5 5: The *in vitro* propagation of highly pathogenic avian influenza H5N1A/chicken/France/150169a/2015 isconsistent with its predicted tropism for avian species

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Avian influenza A viruses (AI) are a major threat to both animal and public health due to their possible high pathogenicity for birds and their potential to infect humans, either directly or after reassortment with other influenza viruses adapted to humans. Different host restriction determinants of influenza viruses have been described in the literature, for example: (i) cell receptor-specificity of the viral hemagglutinin; (ii) temperature sensitivity of viral replication related to primary infection sites: upper respiratory tract (33°C) for human influenza viruses and respiratory (37°C) and digestive tracts (40°C) for avian influenza viruses.

Since 1997, several highly pathogenic (HP) H5N1 avian viruses directly transmitted from poultry to humans caused numerous humans deaths in various countries. These viruses had a considerable economic impact on the poultry market with a high mortality rate and massive preventive culling. Since 2003, the virus has become enzootic in East Asia and Egypt where cases of infections are still reported. Global surveillance and study of AI H5 viruses are essential to improve knowledge on persistence, transmission, and evolution of these viruses. During the 2015/2016 winter, a HP-IA outbreak occurred in Southwestern France. Different HP-H5 subtypes circulated in poultry farms, including a HP-H5N1: A/chicken/France/150169a/2015. Genomic analysis of this virus indicates that it does not possess the set of determinants known to promote the transmission of avian viruses to humans, with respect to the molecular determinants of species specificity and virulence described in the literature [1]. However, a number of determinants promoting replication and/or interfering with antiviral responses in mammals are present as in other contemporary H5-AI viruses. In order to assess the absence of zoonotic potential of this HP-H5N1 150169a virus, its temperature sensitivity was analyzed through the plaque phenotype at 33, 37 and 40°C and the multiplication and genomic replication kinetics at 33 and 37°C on mammalian cells (MDCK, Madin-Darby Canine Kidney cells). Receptor specificity was also studied using a receptor binding assay with synthetic sialylglycopolymers (ELISA).

Plaques of the HP-H5N1 150169a virus were significantly smaller at 33°C as compared to 37°C and 40°C, as observed for the LP (low pathogenic) H5N1 avian virus used as control. The HP-H5N1 150169a virus titers were slightly lower but not significantly different at 33°C as compared with 37°C, which can be explained by the plaque counting difficulty at 33°C due to reduced plaque size. For the multiplication kinetics carried out at a high multiplicity of infection, a delay was observed between 8 and 12 hours post-infection at 33°C as compared to 37°C: the viral titre at 33°C represented 15% to 20% of the titre at 37°C. The maximum titre was reached at 24 hours post-infection and was reduced 33°C as compared to 37°C. Analysis of genomic replication kinetics using quantitative PCR is under progress. Concerning the receptor specificity of the HP-H5N1 150169a virus, the receptor binding assay showed that the virus preferentially recognizes avian receptor as other avian viruses used as controls in this test. Our results suggest that HP-H5N1 A/chicken/France/150169a/2015 has a classical avian phenotype in vitro in accordance with the initial in silico predictions based on genomic markers. Further analyses are under way to consolidate these first results.

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P5 6: Determining the pathogenicity and immunosuppression effect on chickens of two H9N2 clones originated from a same isolate

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H9N2 of the G1-lineage is a low pathogenic avian influenza virus (LPAI) which can cause significant economic losses in poultry industry via drop in egg production and increases in mortality among commercial domestic poultry and wild birds.

In chickens, H9N2-G1 viruses display variable morbidity and mortality. Coinfection or environmental factors can play a role on disease-outcome in the field, such as increased pathogenicity by the presence of secondary virus or bacteria. In addition to external conditions, the differences in outcome of the disease might also be due to the internal factors: such as intrinsic replication properties of the virus; host immune responses via cytokine and interferon production.

To evaluate these intrinsic effects on disease outcome, two H9N2 viral clones, purified from the same viral isolate, but on either chicken embryo fibroblast (CEF) or Madin-Darby canine kidney (MDCK) cells, were characterized for their pathogenicity, viral replication in target organs, ability to induce early immune response and immunosuppressive effect following oculo/nasal infection of 4-week-old SPF chickens.

The higher pathogenicity of MDCK clone in combination with the strong induction of pro-inflammatory cytokines in contrast to the reduced pathogenicity of CEF clone coinciding with the high level of antiviral cytokines suggest a causal link between pathogenicity and early immune response. Additionally, a longer immunosuppression was observed after chickens' infection with MDCK clone. Full genome sequencing revealed some molecular differences between the two clones. The role of each mutation or combination on the different virulence profiles of both viral H9N2 clones will be investigated by using reverse genetic and in vitro as well as in vivo model.

P5 7: Lack of protection conferred in Muscovy ducks by four avian influenza H5 vaccines, against highly pathogenic avian influenza H5N9 strain A/duck/France/150236b/2015

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From November 2015 to August 2016, numerous outbreaks of highly pathogenic (HP) avian influenza viruses (AIV) were detected in poultry farms in south-western France: several neuraminidase subtypes of H5 HP viruses were involved (subtype H5N9 HPAIV was the most frequently characterized) and most infection cases were detected in duck flocks with no symptoms. Sequence data showed that these different H5 HP viruses present in France were not directly related to the A/goose/Guandong/1/1996 (H5N1) lineage viruses but were related for their H5 sequences to contemporary low pathogenic H5 viruses circulating in Europe. The objective of the study was to assess the clinical and virological protection conferred in SPF Muscovy ducks by various avian influenza vaccines against recently isolated H5 HPAIV in south-western France.

Two heterologous inactivated vaccines (Vac1 and Vac2, as single injection at 3 weeks of age) and two recombinant vector vaccines expressing heterologous H5 hemagglutinins (Vac3 as a single 1-day-old injection, and Vac4 as a 1-day-old prime vaccination with a 15-day-old Vac2 boost injection) were used in 4 groups of 8 ducks housed in a BSL3 animal facility. Depending on the vaccine used, the H5 specific antibody response detected by ELISA 4 weeks after vaccination was inconstant, whereas the NP antibody response (specific for influenza A viruses) was detectable in all subjects as early as 2 weeks after injection of the inactivated vaccines.

The ducks were then challenged, 32 days after the prime vaccinations, by intra-ocular instillation of the H5N9 HP A/duck/France/150236b/2015 virus strain (10^6 DIE_{50} per subject). None of the vaccinated or unvaccinated control ducks showed clinical signs during the 15 days of observation following the challenge. A statistically significant reduction in the amount of excreted virus (measured by real-time RT-PCR) was observed only 1 day after challenge for cloacal excretion in Vac1- and Vac2-vaccinated ducks, and only 3 days after challenge for oropharyngeal excretion in Vac1-vaccinated ducks. However, this reduction remained limited at the beginning of infection, of low intensity and did not reduce the peak of viral excretion. These four vaccination protocols tested in Muscovy ducks showed no practical relevance as control or prophylactic tools against the H5 HPAIV characterized in France in 2015.

P5 8: Influenza D virus pathogenesis in mice

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A novel genus within the *Orthomyxoviridae* family, was identified in recent studies in the US and France, named Influenza D virus (IDV). Bovine have been proposed to be the primary host. Two main viral lineages (D/swine/Oklahoma-like and D/bovine/Oklahoma-like) have been described in the literature, both of them circulating in cattle in the USA. Experimental infections were so far performed in swine, ferret, calf and guinea pig, in order to study IDV pathogenesis.

We developed a murine experimental model to ease the study of IDV pathogenesis and immune response.

Three mice lines (C57BL/6, BALB/c et DBA/2) were inoculated intranasaly with 10^3 $TCID_{50}$ of D/bovine/Nebraska/9-5/2012 (D/bovine/Oklahoma-like). No clinical signs and weight loss were observed. The viral replication occurred in the upper respiratory tract of the three mice lines with a peak 6 days post-infection, especially in nasal turbinates. DBA/2 and BALB/c mice were more sensitive to IDV than C57BL/6 mice.

Then, DBA/2 mice were inoculated with 10^5 $TCID_{50}$ of D/bovine/France/5920/2014 (D/swine/Oklahoma-like). Absence of clinical signs and weight loss were confirmed. The viral replication was observed in the respiratory tract (nasal turbinates, trachea and lungs) of infected mice, with a peak at 4 days post-infection. Moreover, the virus was also detected in the liver 2 days post-infection, suggesting viremia. Mild lesions were only observed in nasal turbinates at 6 days post-infection, with infiltration of immune and plasma cells, cells degeneration and loss of cilia. All infected mice seroconverted by 14 days post infection (HI titers ranging from 80 to 320).

DBA/2 mice will also be inoculated with another four distinct IDV strains: D/bovine/Mississippi/C00158N/2015, D/bovine/Mississippi/C00046N/2014, D/bovine/Mississippi/C00148N/2014 (D/swine/Oklahoma-like) and D/bovine/Mississippi/C00013N/2014 (D/bovine/Oklahoma-like), in order to compare their pathogenesis and tissue tropism.

Our DBA/2 mouse model is susceptible to IDV infection. It allows for the study of IDV replication and fitness, before selected viruses may be inoculated on calves.

Acknowledgements
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P5 9: Evaluation of avian influenza vaccination program including Vectormune-AI vaccine (rHVT-H5) and AI virus-like particles to protect chickens against a recent HPH5N8 virus.

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A reassortant variant of the highly pathogenic avian influenza (HPAI) H5N8 (clade 2.3.4.4) virus, emerged in Asia in 2010 and causing numerous outbreaks in South-Korea, Japan and the South of China, reached the European continent in November 2014 for the first time. Again in 2016 an evolved variant HP H5N8 was re-introduced into the Europe via infected wild bird to provoke an unprecedented epizootic in Europe, hitting many poultry holdings as well as captive and wild bird species in more than 30 European countries (OIE website), inducing high mortality and morbidity in galliformes birds.
In the context of the recent H5N8 outbreaks among commercial fowls in France, more than 4 million birds have been slaughtered in attempt to block the spread of H5 HPAIV. Poultry vaccination as alternative of massive culling is more and more considered by the competent authorities. An urgent need for efficient AI poultry vaccination is required. The recombinant turkey herpesvirus vector vaccine expressing the H5 gene from a clade 2.2 H5N1 strain (rHVT-H5(AI)), has already demonstrated high efficacy against Asian HP H5N1 strains of various clades (1, 2.2, 2.2.1, 2.2.1.1, 2.1.3, 2.3.2.1), and has been used in H5N1- endemic countries such as Egypt and Bangladesh. Its protective efficacy combined with a significant reduction of viral excretion against the 2014 EU H5N8 strain was also reported at 4 weeks of age in SPF chickens. In this aim, an AI vaccination program including rHVT-H5(AI) vaccination at day-old and a boost vaccination at 3 weeks with a recombinant virus-like particle expressing the H5 gene was investigated in SPF chickens. To evaluate its efficacy against the recent HPH5N8 strain, a challenge experiment was set up at 6 weeks post vaccination with a HPH5N8 (2017) strain isolated from a chicken from a small backyard poultry holding. Morbidity and mortality were followed daily in unvaccinated and vaccinated groups, as well as viral shedding by oropharyngeal and cloacal routes at 2, 5 and 9 days post-infection. Serological monitoring after vaccination and challenge was also carried out. The results will be presented and discussed here*.
* At the time of the writing of the abstract, this study was in preparation of execution.

P5 10: Towards a better understanding of the pathogenesis and transmission of Influenza D virus

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The novel influenza D virus of the *Orthomyxoviridae* family was found spread among swine and ruminants on four continents so far, confirming a worldwide distribution among several mammalian species. The respiratory tropism of IDV is doubtless yet very little is known about the biological features of this virus. To understand the physiopathological, virological, immunological and clinical aspects in addition to the aerosol transmission potential of IDV, an experimental infection was carried out. Sixteen calves were distributed into two separate pens; inoculated (n=8) and indirect contact (n=3) groups in a pen (4 meters apart), and non-infected (n=5, in a distinct pen) group. The French strain D/bovine/France/5920/2014 was intranasally nebulized through a mask (10^7 $TCID_{50}$ per calf) and was able to induce mild to moderate respiratory signs (Figure). Despite its longer persistence in the nasal cavities, the virus successfully replicated in both upper and lower respiratory tracts. Moreover, IDV also showed its ability to transmit via aerosol droplets. In fact, IDV genome was detected in air samples at several time points post inoculation and was able to infect a calf in the indirect contact group. Macroscopically, lesions indicating pneumonia were observed in the lungs of calves euthanized 8 days post infection. Histological modifications of several organs and tissues were also assessed. We are now further evaluating the host immune response throughout the infection looking at seroconversion, T-cell proliferation, and transcriptomic analysis of bronchoalveolar lavages.

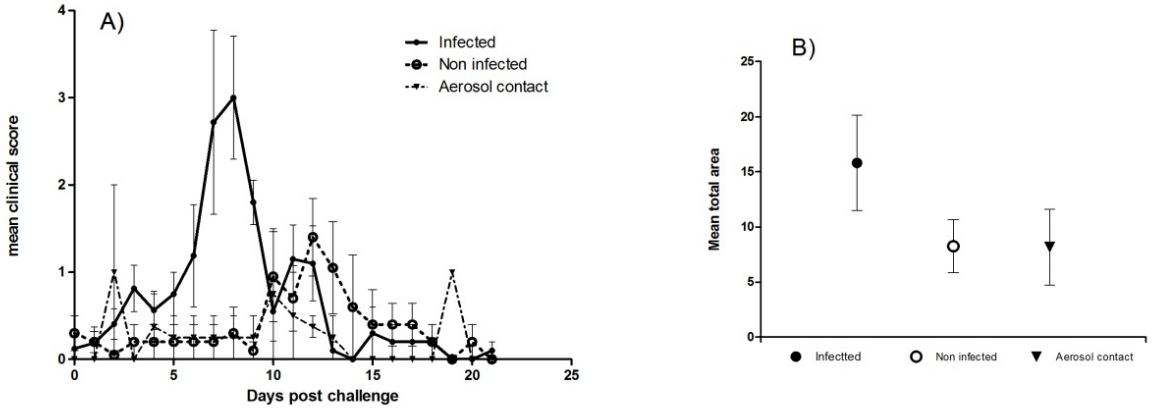

Figure: Mean clinical scores and mean accumulated clinical scores (area under curve) of infected (●), non-infected (❍) calves and indirect contact calves (▼). Significant differences (Two-way Anova with Bonferroni corrections) were observed between infected and other groups for days 7 to 9 post challenge.

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P5 11: Assessment of Influenza D virus in swine: first serological evidence for exposure of breeding sows in France

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Recent studies have identified a new genus within the *Orthomyxoviridae* family, named Influenzavirus D. During the last years, influenza D virus (IDV) has been isolated from cattle diagnosed with bovine respiratory disease complex in the USA, France, China, Italy and Japan, and bovine are hypothesized to represent the reservoir to IDV. However, IDV was also isolated from pigs exhibiting influenza-like symptoms in the USA and in Italy, and it was shown to replicate efficiently and transmit by direct contact in pigs, guinea pigs and ferrets, suggesting that humans could also be infected. In order to assess the emergence threat associated with IDV and to provide additional information about host range and diversity of the emerging pathogen, we started to investigate IDV circulation among pigs in France.

SPF pigs were inoculated with a bovine IDV in order to isolate an IDV strain that replicated in swine and to provide antisera. After propagation on ST cells, a swine IDV was further inoculated to SPF pigs which were hosted in direct contact to naive pigs. Whereas no clinical signs were observed neither in inoculated nor in contact pigs, IDV was isolated in nasal secretions taken from all animals. The isolates (obtained after one, two or three passages in pigs, respectively) were submitted to whole genome sequencing to assess genomic modifications that could relate to virus adaptation to the species. A specific hemagglutination inhibition assay was developed using swine IDV as an antigen and antiserum as a positive control. Sera from naïve SPF pigs were used as negative controls. The positivity threshold has been set to HI titer of 20. A duplex real-time RT-PCR amplifying the IDV PB1 gene as the target and the β-actin gene as an internal control was developed for virological diagnosis in clinical samples.

First, serological tests were conducted on 1627 archived sera collected from 76 different herds with respiratory disorders, located in Brittany, the highest pig populated area in France. Among them, 1048 sera were obtained from breeding sows sampled from January 2014 to June 2015 in 35 farrow-to-finish herds (30 sows per herd). Within this bank, 31 sera (2,9%) originating from six herds (17.2%) contained IDV-specific antibodies. In four of them, only 1 or 2 sows tested positive, with HI titers of 20. However, in two herds (A and B), 22/30 (73.3%) and 4/30 (13.3%) sera tested positive, respectively, with HI titers ranging from 20 to 160. A second bank comprised 300 sera taken from November 2013 to February 2014 on fattening pigs 16 or 22 week old (15 pigs per batch) and bred in 10 herds. All of them tested negative. In a third bank, 279 sera were obtained between 2012 and 2016 from growing pigs of different ages (from post-weaning to slaughtering) and reared in 31 farrow-to-finish herds. These animals, negative towards influenza A virus (IAV), also tested negative in IDV HI test. In a second step, a new sampling plan was implemented in herds A and B where sows were clearly detected IDV seropositive in 2014-2015. In March 2017, 2/15 sows from herd A and 1/30 sow from herd B tested positive with HI titers of 20 to 80. The 30 fattening pigs sampled in each herd were seronegative. Finally, 112 nasal swabs taken from 2015 to April 2017 on growing pigs with acute respiratory syndrome (26 herds, 3 to 10 samples per herd) and previously found IAV M gene negative, tested negative in IDV RT-PCR.

Altogether, these investigations provide the first serological evidence that breeding sows have been exposed to the virus in Brittany. Whereas IDV circulation was not highlighted among growing pigs to date, these preliminary results ask questions about IDV prevalence in this population and its potential contribution to the porcine respiratory disease complex.

P5 12: Avian influenza: epidemiological situation of HPAI H5 in Italy, 2016-2017

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Since October 2016, Highly Pathogenic (HP) Avian Influenza (AI) viruses subtype H5N8, H5N5 and H5N6 have been circulating in several European Countries. Cases have been confirmed both in wild bird population and in domestic poultry (both rural and industrial). Due to the rapid spread of the disease throughout Europe, on 9 November, the Italian Ministry of Health issued a provision to enhance biosecurity measures at national level, with particular focus on poultry farms located in areas at high risk of AI introduction.

On 29 December 2016, a first case of HPAI subtype H5N5 was confirmed in an Eurasian wigeon (*Anas penelope*) found dead in proximity of a wetland in Friuli-Venezia Giulia (North-eastern Italy). On 5 and 10 January, in the same area, passive surveillance allowed to detect two other HPAI viruses of the H5N8 and H5N5 subtypes respectively in an Eurasian wigeon (*Anas penelope*) and a gadwall (*Anas strepera*). In February, a further case was identified in a common shelduck (*Tadorna tadorna*) in the bordering Veneto region.

After the first detections of H5 HPAI viruses in wild waterfowl, the infection also emerged in industrial poultry: on 21 January 2017 a H5N8 HPAI virus was confirmed in a fattening turkey farm in Veneto. Between 23 January and 12 April, the H5N8 HPAI virus was detected in seven fattening turkey farms, two laying hen farms, and five backyards. All of the premises were located in regions of North Italy (Emilia-Romagna, Friuli-Venezia Giulia, Lombardy, Piedmont, Veneto), in areas characterised by the presence of wetlands visited by migratory and resident wild birds, and where the large majority of national poultry production is concentrated. On 30 April 2017, the H5N8 HPAI virus was isolated in a mute swan (*Cygnus olor*) found dead in Piedmont. On 30 May 2017, after about one month, a new HPAI H5N8 outbreak was confirmed in a fattening turkey farm in Lombardy. A dead juvenile grey heron (*Ardea cinerea*) collected in close proximity to the sheds of the last H5N8 outbreak, tested positive to AI virus of the H5 subtype.

All the viruses isolated in wild and domestic birds, belonged to the clade 2.3.4.4, group B, and were closely related to the H5N8 strains circulating in Europe, Russia, Mongolia, India and China, in 2016-2017. Genetic diversity was observed in the samples analysed, pointing out that the Italian viruses belong to four different genotypes (H5N5, H5N8-A/wild duck/Poland/82A/2016-like, H5N8-A/painted stork/India/10CA03/2016-like and H5N8-A/mute swan/Croatia/70/2016-like), likely generated through multiple reassortant events. Viruses with high genetic similarity were identified in multiple cases in domestic poultry and wild birds. However, no epidemiological connections were found to corroborate the hypothesis of a direct between-farm transmission, suggesting the possibility of separated introductions from the wild reservoir. This indicates that while phylogeny may provide paramount support in reconstructing the disease dynamics, it may require further integration with other epidemiological tools to attempt understanding how the disease is actually circulating. Moreover, the recurrence of H5N8 HPAI viruses, also during non-migratory periods highlights the need of considering new surveillance strategies that could provide a clearer picture of the ecology of HPAI viruses in wild bird population.

11th EPIZONE Annual Meeting
Crossing Barriers
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POSTERS
Overview

Topic 1: Arthropod-borne diseases (arboviruses)

P1 1 Use of feathers for detection of West Nile Virus and Bagaza Virus in surveillance programs

P1 2 Bulk-tank milk sampling to assess the circulation of Bluetongue in a free area: the experience of northeast Italy

P1 3 Vaccination of cattle with BTVPUR® containing BTV-4 provides full protection for one year

P1 4 Serological status for BTV-8 in French cattle prior to the 2015 re-emergence

P1 5 Rate of introduction of 36 vector-borne disease agents in the European Union

P1 6 Demonstration of full protection of cattle against BTV-2 challenge provided by a BTVPUR AlSap® vaccine

P1 7 Monitoring of ZIKA virus in mosquitoes in Sardinia, Italy, 2016

P1 8 Concurrent Infection oF BLUETONGUE (BT) and peste des petits ruminants (PPR) in Awassi Sheep in Jordan

P1 9 Mapping protein-protein interactions between tick-borne flaviviruses and their different mammalian hosts

P1 10 Isolation and molecular characterization of bluetongue virus in sheep and goats in the States of São Paulo and Rio De Janeiro – Brazil

P1 11 Mosquito-borne pathogen surveillance in Germany: first results from the years 2015 and 2016

P1 12 First serological evidence of BTV-1, BTV-2 and BTV-3 in south-eastern Brazil

P1 13 Estimation of spread of bluetongue virus serotype 1 in sheep at the end of the 2014 epidemic in Umbria, sheep at the end of the 2014 epidemic in Umbria,central-Italy

P1 14 Evaluation of the surveillance strategy used to detect WNF infection in sentinel equines during 2016 in Bulgaria

P1 15 Surveillance instruments in the Netherlands – Do we detect BTV crossing our borders?

P1 16 DISA vaccines for Bluetongue: An overview of a novel vaccine approach

Topic 2: Threats at the European borders

P2 1 Molecular epidemiology of foot and mouth disease virus (FMDV) in Chad

P2 2 Two-plasmid system to increase the rescue efficiency of paramyxoviruses by reverse genetics: the example of rescuing newcastle disease virus

P2 3 BVDV Transmission Between Countries By Imported Animals

P2 4 A simple method to evaluate the number of doses to include in a bank of vaccines. The case of Lumpy Skin in France

P2 5 Validation of a new ELISA for the detection of LSDV antibodies

P2 6 Abundance and seasonality of biting midges at a continental scale in Europe

P2 7 Simple, quick and cost-efficient: a universal RT-PCR and sequencing strategy for genomic characterisation of foot-and-mouth disease viruses

P2 8 Detection of *Chlamydia trachomatis* in samples from sheep with an enzootic abortion.

P2 9 Genealogy of bovine coronavirus in Brazilian herd of catlle

P2 10 Vaccination of goats with PPR-VAC confers a full protection against a PPR virulent challenge

P2 11 Validation of commercial real-time RT-PCR kits for rapid and specific diagnosis of classical swine fever virus, following the guidelines of the French AFNOR standards NFU47-600

P2 12 Lumpy skin disease detection and control in serbia in 2016

P2 13 Preliminary serology and virology data after vaccination of animals with Neethling strain of LSDV

P2 14 Animal health crisis in the indian ocean: foot and mouth disease virus in mauritius and rodrigues in 2016

P2 15 A novel double antigen elisa for the species independent detection of cchfv antibodies

P2 16 Immune response in chickens to fowl pox virus vaccine and newcastle disease vaccine co-administered by non-invasive routes

P2 17 Identification and isolation of swinepox virus from sick swines in the Belgorod Region in 2013

P2 18 Distribution and genetic diversity of Peste des Petits Ruminants virus in Mali

P2 19 Investigation of pathogenicity of lumpy skin disease virus for sheep

P2 20 Triplex qPCR assay for simultaneous detection and differentiation of LSDV field strains from LSDV vaccine strains

Topic 3: Current challenges inside Europe

P3 2 Recent spread of an old disease: tuberculosis in wild ungulates from southern Spain

P3 3 Understanding the use of antimicrobial susceptibility testing by veterinarians to interpret surveillance data on resistance: a qualitative study exploring determinants and issues in France

P3 4 Identification of a recombinant PEDV/SeCoV strains during a molecular surveillance of Italian epidemic waves between 2015 and 2017

P3 5 Environmental determinants of the concomitant infection with Mycobacterium bovisin cattle and badgers in France

P3 6 Astrovirus associated with encephalitis in a sheep: indication of cross-species transmission with cattle

P3 7 A recombination between two genotype 1 PRRSV modified live vaccines results in a field strain with increased virulence

P3 8 Atypical porcine pestivirus in Spain: retrospective study from 1997-2017

P3 9 Surveillance of classical swine fever, blue tongue and rabies in European targeted countries: assessment through expert elicitation

P3 10 Comparative analysis of different serological and molecular tests for the detection of small ruminant lentiviruses (SRLVs) in Belgian sheep and goats

P3 11 Phylogenetic study and evolution of the Swine Vesicular Disease Virus over 25 years of circulation in Italy

P3 12 Phylogenetic analysis of ORF virus, isolated in the Republic of Tuva in 2015

P3 13 Identification of novel enterotropic astroviruses in astroviruses in cattle

P3 14 Neuropathological survey reveals an underestimation of the prevalence of neuroinfectious diseases in cattle livestock in Switzerland

P3 14 Strengthening of scientific excellence of the National Veterinary Research Institute in animal health and food chain safety

Topic 4: African swine fever

P4 1 Optimization of proteasomal processing enhances the immunogenicity of a DNA vaccine encoding African swine fever virus $CD8^+$ T-cell epitopes

P4 2 Field evaluation of a new rapid kit test to African Swine Fever antigen detection

P4 3 Report of a simulation exercise regarding a suspect case of African Swine Fever (ASF)

P4 4 Soft tick studies in Ukraine: A cooperative effort to elucidate ecology, distribution, and role in African Swine Fever epidemiology for *Ornithodoros* ticks in Eastern Europe and neighboring regions

P4 5 CRISPR/Cas9-mediated inhibition of pseudorabies virus and African swine fever virus replication in cell culture

P4 6 Molecular genetic study of ASF virus isolated from Samara and Irkutsk Regions, Russia in 2017

P4 7 Expression of African swine fever virus proteins by a pseudorabies virus vector

P4 8 Do antibodies play a role in partial protection of pigs inoculated with an attenuated ASFV isolate, OURT88/3, against the highly virulent Georgia 2007/1 isolate?

P4 9 Semi-qualitative model to African Swine Fever risk evaluation in Sardinia

P4 11 Real time cell analysis for measuring African Swine Fever CPE

P4 12 Interaction of ASFV strains of diverse virulence with monocyte-derived dendritic cells in different activation status

P4 13 Whole-genome next generation sequencing of African swine fever virus in blood samples obtained from experimentally infected pigs

P4 14 Effect of Tetherin (BST2) restriction factor on African swine fever virus infection

P4 15 Results of the Ornithodoros genus research, in Sardinian pig farms.

P4 16 Comparative analysis of three African Swine Fever Virus immunogenes (A238L, I329L and DP71L).

P4 17 Whole genome analysis of twelve African swine fever isolates originating from ASF cases and outbreaks in Poland

P4 18 Survival of African swine fever Polish isolates in artificially contaminated soil, leaf litter and water in different environmental conditions

Topic 5: Animal influenza viruses

P5 1 Comparison of adjuvant technologies for avian influenza vaccines

P5 2 Avian influenza H5N8 in Russia

P5 3 Genotyping of French highly pathogenic H5N8 during 2016-2017

P5 4 Poultry vector vaccines: innovative serological and molecular assays for diagnosis, vaccination monitoring and DIVA testing for avian Influenza A

P5 5 The *in vitro* propagation of highly pathogenic avian influenza H5N1A/chicken/France/150169a/2015 is consistent with its predicted tropism for avian species

P5 6 Determining the pathogenicity and immunosuppression effect on chickens of two H9N2 clones originated from a same isolate

P5 7 Lack of protection conferred in Muscovy ducks by four avian influenza H5 vaccines, against highly pathogenic avian influenza H5N9 strain A/duck/France/150236b/2015

P5 8 Influenza D virus pathogenesis in mice

P5 9 Evaluation of avian influenza vaccination program including Vectormune-AI vaccine (rHVT -H5) and AI virus-like particles to protect chickens against a recent HPH5N8 virus.

P5 10 Towards a better understanding of the pathogenesis and transmission of Influenza D virus

P5 11 Assessment of Influenza D virus in swine: first serological evidence for exposure of breeding sows in France

P5 12 Avian influenza: epidemiological situation of HPAI H5 in Italy, 2016-2017

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