

EPIZONE



13th EPIZONE Annual Meeting „Breaking Walls“

26-28 August 2019, Berlin, Germany
Hosted by the Friedrich-Loeffler-Institut

Thomas C. Mettenleiter, Martin Beer, Franz J. Conraths, Sandra Blome, and Bianca M. Bussmann
August 2019, Greifswald - Insel Riems, Germany

Contents

Welcome	2
Acknowledgment	2
Conference Committees	7
Programme	9
Abstracts	17
Oral Presentations	17
Keynote Lectures	17
Threats at the European Borders	27
Current Challenges inside Europe	39
African Swine Fever	57
Vector-borne Diseases	70
Posters	77
General Information	78
African Swine Fever	79
Animal Health in a changing World	108
Current Challenges inside Europe	144
Threats at the European Borders	177
Vector-borne diseases	192
Notes	213



WELCOME

13th EPIZONE Annual Meeting *“Breaking Walls”*



Dear EPIZONE friends,

We are happy to welcome you to the 13th EPIZONE Annual Meeting in Berlin!

The Friedrich-Loeffler-Institut (FLI) is hosting this meeting and has done its utmost to develop an inspiring conference program, in the lovely venue ‘Kalkscheune’ in the heart of Berlin. The theme of this meeting is “Breaking Walls” referring to the history of the city and as metaphor for the Epizone goal to improve international cooperation within the field of epizootic diseases research and control. Infectious diseases don’t respect walls or borders!

Our organising and scientific committees developed a program with 9 renowned keynote speakers and more than 200 abstracts presented as talks or posters. The focus of the meeting will be on the EPIZONE partner institutes’ recent research in the field of epizootic animal diseases to exchange the latest research information and to establish new contacts and collaborations. The Young EPIZONE group has organised an interesting program for young scientists focussing on issues relevant for junior scientists.

Looking at the current challenges to control important epizootic diseases like African swine fever and bluetongue, the collaborations established by and maintained within EPIZONE are as important as ever. With this 13th annual meeting, we hope to foster them further to which the enjoyable social program certainly also will contribute. We are sure that the lively capital of Germany will be the perfect place to create a stimulating atmosphere of exciting talks, inspiring discussions, and scientific curiosity.

We wish you an interesting and fruitful meeting,

Thomas C. Mettenleiter
President of the Friedrich-Loeffler-Institut

Wim van der Poel
Coordinator of EPIZONE





Thank you!

We are very grateful to the following companies for supporting
the 13th EPIZONE Annual Meeting!

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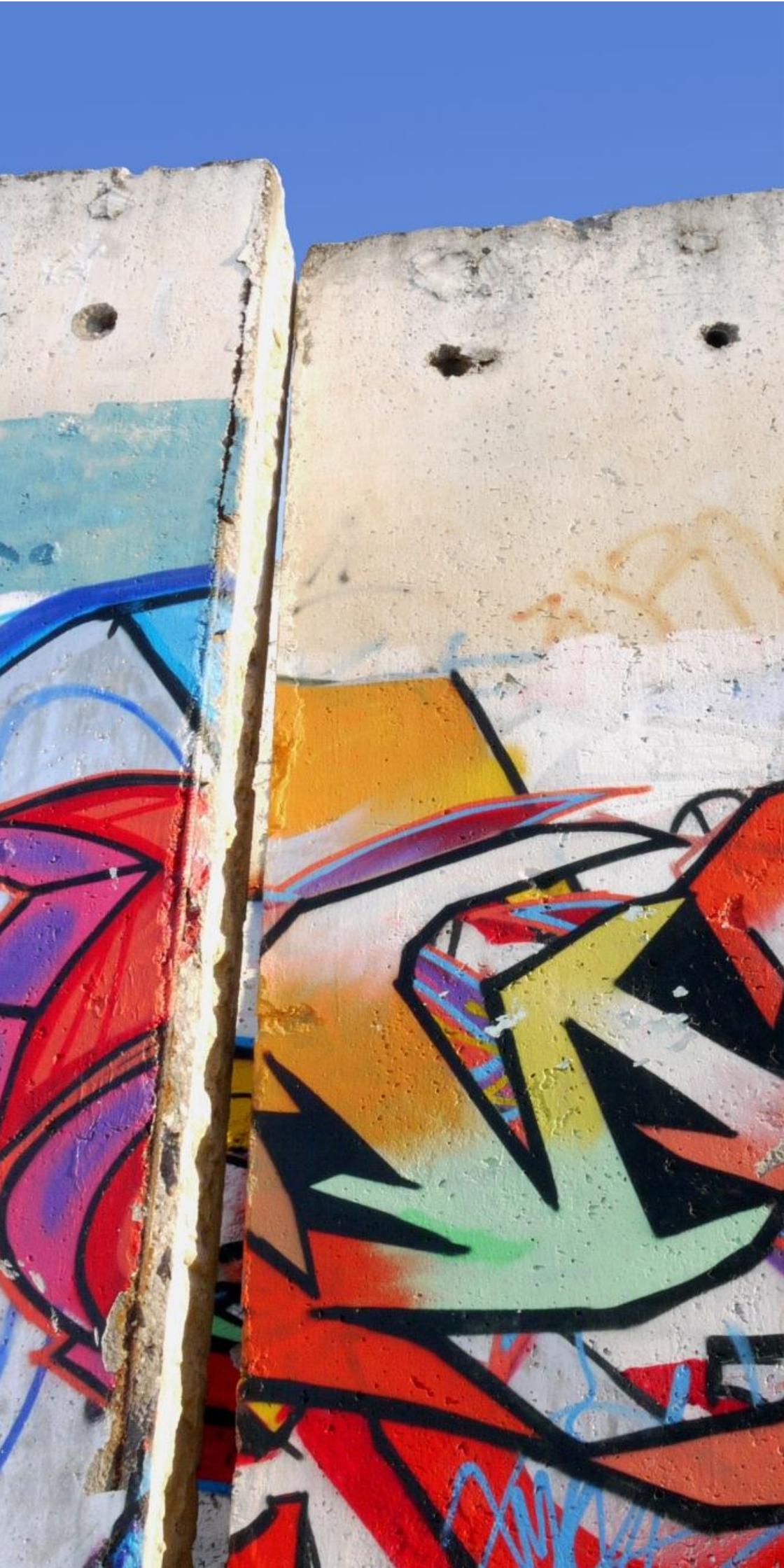
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THANK YOU to everybody else involved in the 13th EPIZONE Annual Meeting

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





Conference Committees

Conference Committees

The 13th EPIZONE Annual Meeting is organized by the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Germany.

Local Organizing Committee

- ◆ Thomas C. Mettenleiter
- ◆ Anne Balkema-Buschmann
- ◆ Martin Beer
- ◆ Sandra Blome
- ◆ Bianca M. Bussmann
- ◆ Franz J. Conraths
- ◆ Anca Dorhoi
- ◆ Martin H. Groschup
- ◆ Carola Sauter-Louis
- ◆ Cornelia Silaghi
- ◆ Annika Graaf (Young EPIZONE)
- ◆ Christina Ries (Young EPIZONE)

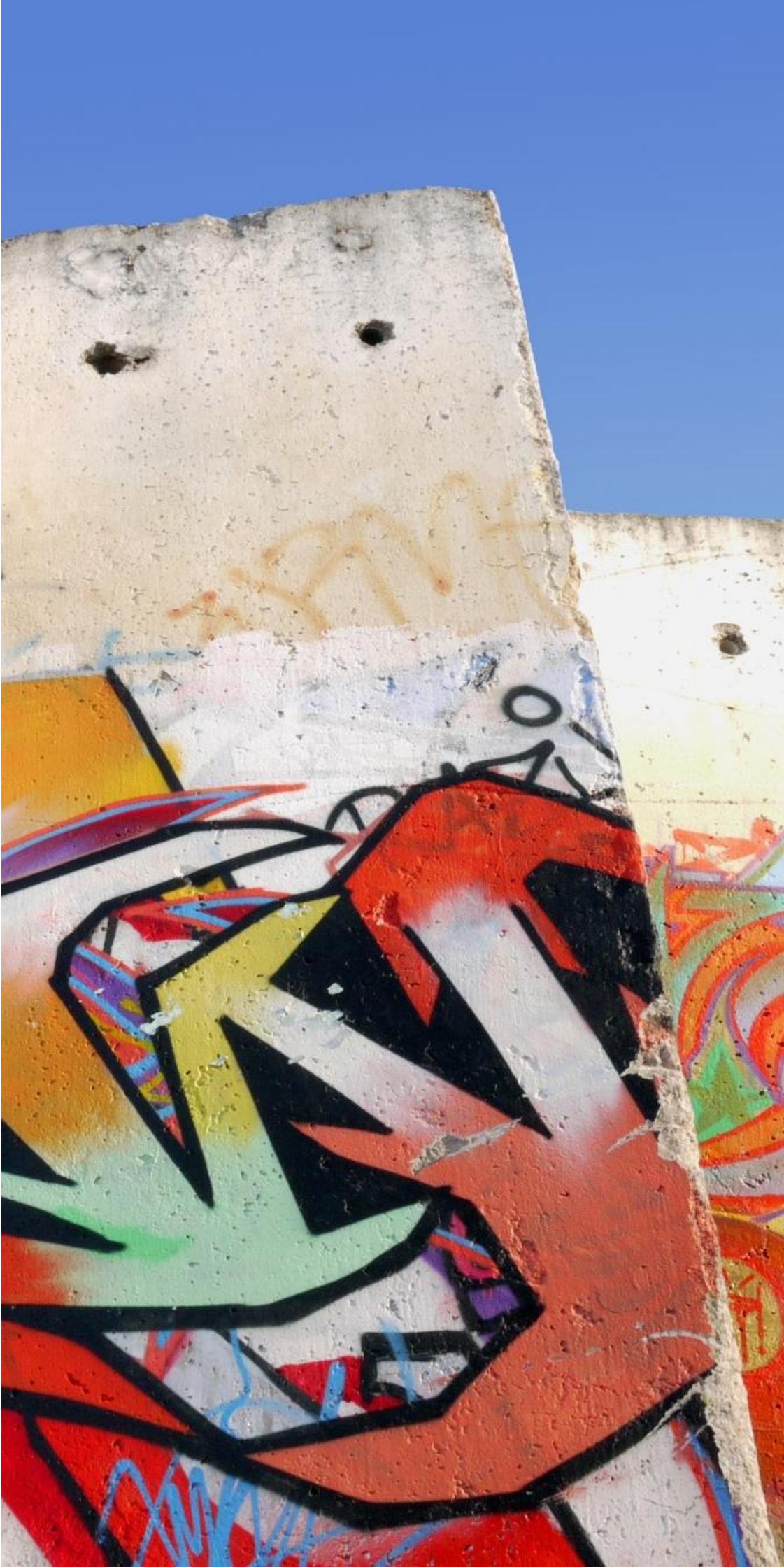
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- ◆ Stéphan Zientara, Maisons-Alfort Laboratory for Animal Health, France
- ◆ Donald King, The Pirbright Institute, United Kingdom
- ◆ Antonio Lavazza, Experimental Zooprophyllactic Institute of Lombardy and Emilia Romagna, Italy
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- ◆ Alejandro Brun, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spain
- ◆ Krzysztof Smietanka, PIWET, National Veterinary Research Institute, Poland
- ◆ Christian Griot, Institut für Virologie und Immunologie, Swiss Confederation
- ◆ Dolores Gavier-Widen, National Veterinary Institute, Sweden
- ◆ Ana Maria Moreno, IZSLer, Italy
- ◆ Linda Dixon, The Pirbright Institute, United Kingdom
- ◆ Liliane Ganges, IRTA, CReSA, Spain
- ◆ Tony Fooks, Animal and Plant Health Agency

EPIZONE Organizing Committee

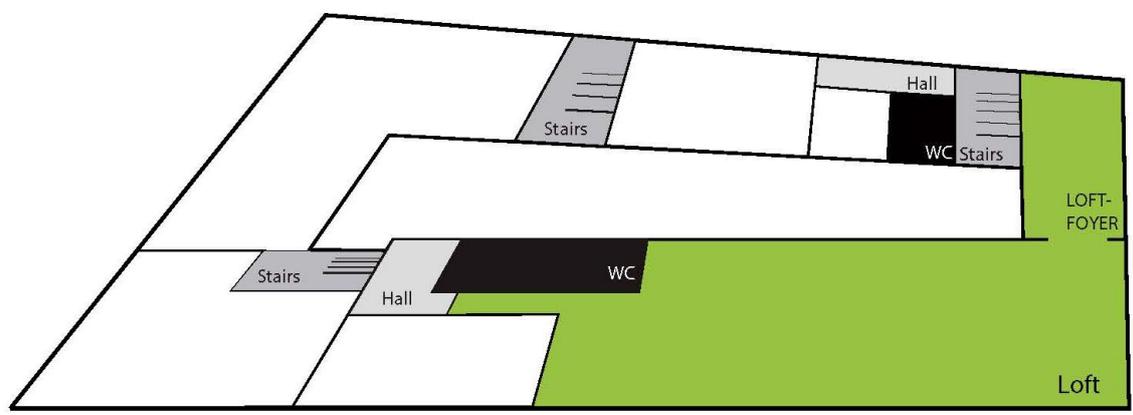
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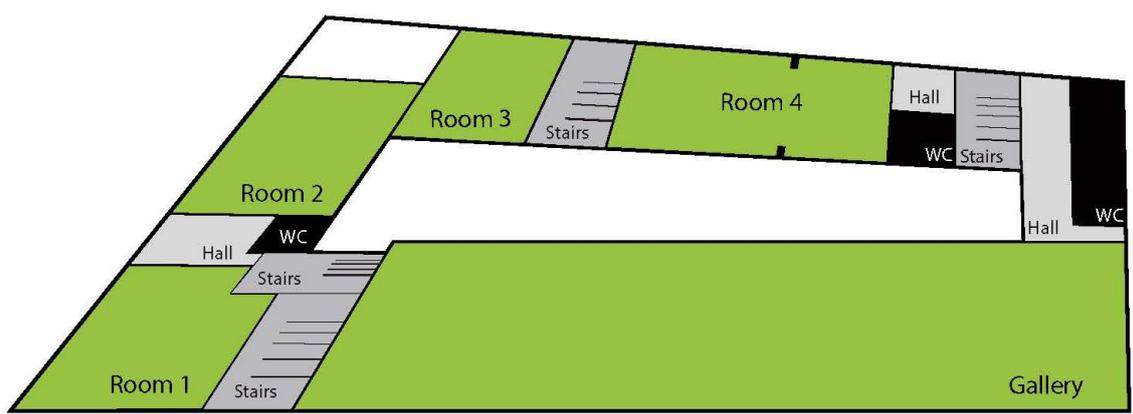


Programme

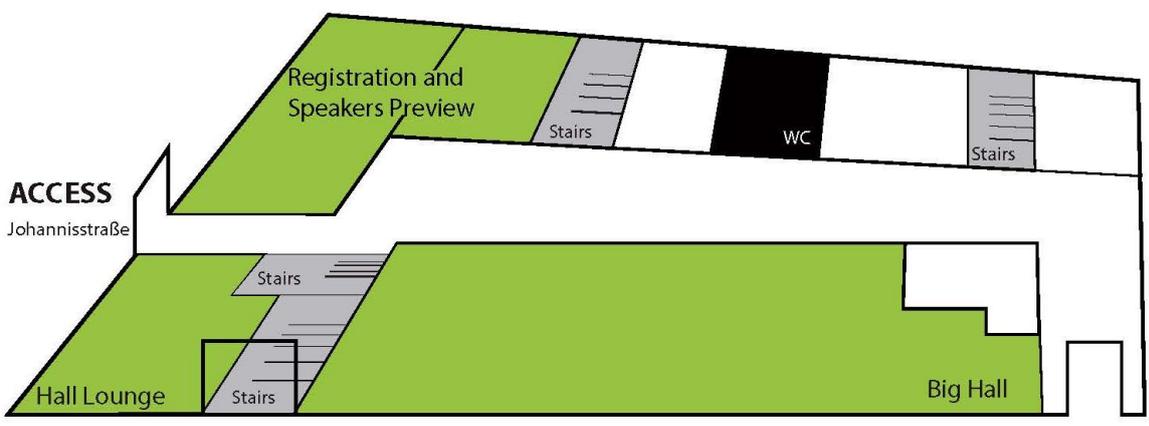
Venue Kalkscheune



2nd floor



1st floor



Ground floor

Monday, 26th August 2019

- From 8⁰⁰ **REGISTRATION** - *Ground floor*
- 9⁰⁰ - 13⁰⁰ **YOUNG EPIZONE** - *Room 4*
WELCOME WORKSHOP
 “Sketchnoting and Storytelling”
- 12³⁰ - 14⁰⁰ **EPIZONE Coordinating Forum & Executive Committee meeting**
Studio
- 13⁰⁰ - 14⁰⁰ **YOUNG EPIZONE Lunch Break** - *Room 3* (restricted meeting, CF&EC members only)
- 14⁰⁰ - 14¹⁵ **WELCOME** to the 13th EPIZONE Annual Meeting - *Gallery*
 By Wim van der Poel and Thomas C. Mettenleiter
- 14¹⁵ - 15⁰⁰ **KEYNOTE LECTURE - Threats at the European borders 1** - *Gallery*
Viral Hemorrhagic fevers - a coming threat for Europe?
 Roger Hewson - Public Health England - Porton Down, United Kingdom
 (Chairperson: Martin H. Groschup)
- 15⁰⁰ - 15⁴⁵ **KEYNOTE LECTURE - Current challenges inside Europe 1** - *Gallery*
Influenza at the domestic - wildlife interface
 Thijs Kuiken - Erasmus MC, The Netherlands
 (Chairperson: Sharon Brookes)
- 15⁴⁵ - 16¹⁵ **COFFEE BREAK** - *Big Hall*
- 16¹⁵ - 17³⁰ **SESSION 1: Threats at the European borders 1** - *Gallery*
 (Chairpersons: Thomas C. Mettenleiter and Stephan Zientara)
- 16¹⁵ **Heterogeneities that contribute to increased Peste des petits ruminants seroprevalence in sheep, goats, and cattle in northern Tanzania**
 Catherine M Herzog - Pennsylvania State University, State College, Pennsylvania, USA
- 16³⁰ **Molecular basis of Peste des Petits Ruminants Virus emergence in Mongolian wildlife**
 Camilla Benfield - Royal Veterinary College, University of London
- SESSION 2: Current challenges inside Europe 1** - *Loft*
 (Chairpersons: Sharon Brookes and Christian Griot)
- Modulation of HPAIV H5N8 infection in naturally LPAIV- exposed Mallards**
 Lorenz Ulrich - Friedrich-Loeffler-Institut, Germany
- The effect of defective viral particles on the course of infection with avian influenza virus in chickens and turkeys**
 Krzysztof Smietanka - National Veterinary Research Institute, Poland

- 16¹⁵ - 17³⁰ **SESSION 1: Threats at the European borders 1 - Gallery** *Continuation*
(Chairpersons: Thomas C. Mettenleiter and Stephan Zientara)
- 16⁴⁵ **Evolution of attenuation and risk of reversal in Peste des Petits Ruminants vaccine strain Nigeria 75/1**
Roger-junior Eloiflin - UMR ASTRE, CIRAD, Montpellier France
- 17⁰⁰ **Development of live attenuated PPR DIVA vaccines using reverse genetics**
Satya Parida - The Pirbright Institute, UK
- 17¹⁵ **An experimental vaccine based on a recombinant MERS-CoV S1-protein induces broad protective immune responses in llama**
Jordi Rodon Aldrufeu - IRTA, Centre de Recerca en Sanitat Animal (CRESA, IRTA-UAB), Spain
- 17⁴⁵ - 18⁴⁵ **WELCOME LECTURE - Gallery**
Diseases and the media; fake news or reality?
Christian Griot - Institute of Virology and Immunology, Switzerland
(Chairperson: Thomas C. Mettenleiter)
- 19⁰⁰ - 21⁰⁰ **WELCOME RECEPTION and POSTER SESSION - Big Hall, Hall Lounge, Rooms 1 & 2**
- 16¹⁵ - 17³⁰ **SESSION 2: Current challenges inside Europe 1 - Loft** *Continuation*
(Chairpersons: Sharon Brookes and Christian Griot)
- 16⁴⁵ **Evolution of highly pathogenic non-H5/H7 virus from a natural H4N2 avian influenza virus with a polybasic cleavage motif**
Elsayed M. Abdelwhab - Friedrich-Loeffler-Institut, Germany
- 17⁰⁰ **Using Sediment, Environmental and Wild Bird Samples as Tools for Avian Influenza Surveillance**
Alexander MP Byrne - Animal and Plant Health Agency, UK
- 17¹⁵ **Connect to Protect: Insights into the Role of Captive Birds in Virus Spread and Reassortment during the Outbreak of Highly Pathogenic Avian Influenza, Germany 2016-2018**
Jacqueline King - Friedrich-Loeffler-Institut, Germany

Tuesday, 27th August 2019

From 8⁰⁰ **REGISTRATION - Ground floor**

9⁰⁰ - 9⁴⁵ **KEYNOTE LECTURE - Threats at the European borders 2 - Gallery**
PPR: a threat or a problem?
Michael Baron - The Pirbright Institute, United Kingdom
(Chairperson: Wim van der Poel)

9⁴⁵ - 10³⁰ **KEYNOTE LECTURE - Current challenges inside Europe 2 - Gallery**
***Besnoitia besnoiti*: An emerging protozoal infection in cattle in Europe**
Philippe Jacquet - Veterinary School of Toulouse, France
(Chairperson: Franz J. Conraths)

10³⁰-11⁰⁰ **COFFEE BREAK** - *Big Hall*

11⁰⁰-12³⁰ **SESSION 3: Threats at the European borders 2** - *Gallery*

(Chairpersons: Karin Darpel and Antonio Lavazza)

11⁰⁰ **A poly-uridine insertion in the 3'-untranslated region found in a classical swine fever low virulent strain modulates virulence**

Miaomiao Wang - OIE Reference Laboratory for classical swine fever, IRTA-CReSA, Spain

11¹⁵ **Immunological mechanisms underlying classical swine fever persistent infection**

José Alejandro Bohórquez - OIE Reference Laboratory for classical swine fever, IRTA-CReSA, Spain

11³⁰ **Gene Signatures Associated with Foot-And-Mouth Disease Virus Infection and Persistence Part II: Proteogenomics Uncovers Critical Elements of Host Response in Bovine Soft Palate Cells**

Florian Pfaff - Friedrich-Loeffler-Institut, Germany

11⁴⁵ **Evaluation of environmental sampling as a low technology method for surveillance of foot-and-mouth disease virus in an endemic area**

Claire Colenutt - The Pirbright Institute, UK

12⁰⁰ **RAPID, ON SITE, DIAGNOSIS OF FMD AND SAFE AND COST-EFFECTIVE SHIPMENT OF SAMPLES**

Aurore Romey - Laboratoire de Santé Animale de Maisons-Alfort, France

12¹⁵ **Studies on pathogenesis of Capripox virus-induced diseases and evaluation of diagnostic tools**

Janika Möller - Friedrich-Loeffler-Institut, Germany

SESSION 4: Current challenges inside Europe 2 - *Loft*

(Chairpersons: Anette Bøtner and Thierry van den Berg)

Polymorphism in the hemagglutinin proteolytic cleavage site of H9N2 affected proteolytic activation and cell-to-cell spread in cell culture and virus excretion in infected poultry

Claudia Blaurock - Friedrich-Loeffler-Institut, Germany

Comparison of experimental infections of mulard ducks with French H5N8 and H5N1 avian influenza viruses

Beatrice Grasland - Anses Laboratory of Ploufragan-Plouzané-Niort, France

ts-mutants of porcine influenza viruses: Pathway to live-attenuated vaccines in pigs?

Annika Graaf - Friedrich-Loeffler-Institut, Germany

Transcriptional response after single and double infection of Vero cells with Mammalian Orthoreovirus and Porcine Epidemic Diarrhea Virus

Marcel Hulst - Wageningen Bioveterinary Research, The Netherlands

GII NOROVIRUS IN SWINE FAECAL SAMPLES IN NORTHERN ITALY: IDENTIFICATION OF TWO DIFFERENT GENOTYPES

Lara Cavicchio - Istituto Zooprofilattico Sperimentale delle Venezie, Italy

Seroprevalences of newly discovered porcine pestiviruses in German pig farms

Anna Michelitsch - Friedrich-Loeffler-Institut, Germany

12³⁰-13³⁰ **LUNCH BREAK** - *Big Hall*

- 13³⁰ - 14¹⁵ **KEYNOTE LECTURE - African Swine Fever 1 - Gallery**
ASF in Sardinia: is eradication so close?
 Alberto Laddomada - Istituto Zooprofilattico Sperimentale della Sardegna, Italy
 (Chairperson: Linda Dixon)
- 14¹⁵ - 15⁰⁰ **KEYNOTE LECTURE - Vector-borne diseases 1 - Gallery**
Ticks and Tick-borne pathogens - always new challenges ahead
 Martin Pfeffer - University Leipzig, Germany
 (Chairperson: Cornelia Silaghi)
- 15⁰⁰ - 16³⁰ **COFFEE BREAK and POSTER SESSION - Big Hall, Hall Lounge, Rooms 1 & 2**
- 16³⁰ - 18⁰⁰ **SESSION 5: African Swine Fever 1 Gallery**
 (Chairpersons: Linda Dixon and Denis Kolbasov)
- 16³⁰ **African swine fever in domestic pigs - Epidemiological insights from Estonia**
 Imbi Nurmoja - Estonian University of Life Sciences, Estonia; Veterinary and Food Laboratory, Estonia
- 16⁴⁵ **Comprehensive approach to control and prevent African Swine Fever in Italy**
 Francesco Feliziani - Istituto Zooprofilattico Sperimentale Umbria e Marche, Italy
- 17⁰⁰ **Smallholders' perceptions on biosecurity and disease control in relation to African swine fever in an endemically infected area in northern Uganda**
 Erika Chenais - National Veterinary Institute, Sweden
- 17¹⁵ **Experimental study of soft tick vector competence for African Swine Fever in Europe**
 Rémi Pereira De Oliveira - CIRAD-INRA, France
- 17³⁰ **Immunopathogenesis and diagnosis of african and classical swine fever virus co-infections in domestic pigs**
 Katarzyna Stępniewska - OIE Reference Laboratory for classical swine fever, Spain; National Veterinary Research Institute, Poland
- SESSION 6: Current challenges inside Europe 3 - Loft**
 (Chairpersons: Wim van der Poel and Martin Beer)
- Genome sequencing of bat coronaviruses obtained from faecal samples using a metagenomics approach**
 Christina M. Lazov - DTU Vet, Technical University of Denmark, Denmark
- Expanding the known distribution of European Bat 1 Lyssaviruses - Investigation of two common Pipistrelle Bats from Baden-Wuerttemberg**
 Sten Calvelage - Friedrich-Loeffler-Institut, Germany
- First cases of CWD in Sweden - perhaps spontaneously occurring?**
 Maria Nöremark - National Veterinary Institute, SVA, Uppsala, Sweden
- Could IgM antibodies be a marker of acute Bb infection in cattle?**
 Loic Comtet - IDvet, France
- Testing of a Pan-virus Metagenomic Array Detection assay (MAD-Vir) using panels of samples from animals experimentally infected with known viruses**
 Miguel Ángel Jiménez-Clavero - INIA-CISA, Valdeolmos, Spain

- 16³⁰ - 18⁰⁰ **SESSION 5: African Swine Fever 1** *Gallery* *Continuation*
(Chairpersons: Linda Dixon and Denis Kolbasov)
- 17⁴⁵ **ASFV evolution in the natural host - a co-evolutionary virus-vector arms race?**
Jan Hendrik Forth - Friedrich-Loeffler-Institut, Germany
- SESSION 6: Current challenges inside Europe 3 - Loft** *Continuation*
(Chairpersons: Wim van der Poel and Martin Beer)
- One Health approach in animal disease management, based on demographic features and socio-economic indicators**
Federica Loi - Osservatorio Epidemiologico Veterinario Regionale, Cagliari, Italy
- 19³⁰ - 22³⁰ **SOCIAL EVENING - Dinner cruise on the river Spree - Pier "Friedrichstraße/Reichstagsufer (a guide to the pier starts at 18⁵⁰ at the Kalkscheune).**

Wednesday, 28th August 2019

- From 8⁰⁰ **REGISTRATION - Ground floor**
- 9⁰⁰ - 9⁴⁵ **KEYNOTE LECTURE - African Swine Fever 2 - Gallery**
Managing African swine fever - the African experience
Marie-Louise Penrith - University of Pretoria, Department of Veterinary Tropical Diseases, South Africa
(Chairperson: Sandra Blome)
- 9⁴⁵ - 10³⁰ **KEYNOTE LECTURE - Vector-borne diseases 2 - Gallery**
Orbiviruses on the move
Karin Darpel - The Pirbright Institute, United Kingdom
(Chairperson: Martin Beer)
- 10³⁰ - 11⁰⁰ **COFFEE BREAK - Big Hall**
- 11⁰⁰ - 12³⁰ **SESSION 7: African Swine Fever 2** *Gallery*
(Chairpersons: Marisa Luisa Arias and Alejandro Brun)
- 11⁰⁰ **Promoter mapping and transcriptome analyses of the African Swine Fever Virus**
Gwenny Cackett - University College London, UK
- SESSION 8: Vector-borne diseases 1** *Loft*
(Chairpersons: Krzysztof Smietanka and Martin Pfeffer)
- Genome manipulation of West Nile and Usutu viruses**
Alessio Lorusso - Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Italy

11 ⁰⁰ - 12 ³⁰	SESSION 7: African Swine Fever 2 <i>Gallery</i> <i>Continuation</i> (Chairpersons: Marisa Luisa Arias and Alejandro Brun)	SESSION 8: Vector-borne diseases 1 <i>Loft</i> <i>Continuation</i> (Chairpersons: Krzysztof Smietanka and Martin Pfeffer)
11 ¹⁵	African swine fever virus Armenia/07 virulent strain controls IFN-β production through cGAS-STING pathway Daniel Pérez-Núñez - CBMSO-CSIC, Spain	Identification of amino acid changes playing a role in RVFV attenuation in mice Belén Borrego - Centro de Investigación en Sanidad Animal, INIA-CISA, Spain
11 ³⁰	Generation of defined gene deletion mutants of African swine fever virus as live vaccine candidates Tonny Kabuuka - Friedrich-Loeffler-Institut, Germany	New clinical pattern of Bluetongue serotype 8 in continental France Stephan Zientara - ANSES Alfort, France
11 ⁴⁵	Vectored vaccines for African swine fever virus: testing potential T-cell antigens delivered via adenovirus and vaccinia vectors Raquel Portugal - The Pirbright Institute, UK	Exploring the immunogenicity and efficacy of bivalent Rift Valley Fever and Bluetongue experimental vaccines Sandra Moreno - Centro de Investigación en Sanidad Animal, INIA-CISA, Spain
12 ⁰⁰	Biological and genetic characteristics of African swine fever virus adapted strain ASFV/ARRIAH/CV-1/30 derived from Odintsovo 02/14 isolate Ali Mazloun - Federal Centre for Animal Health, Russian Federation	Vector competence for Lumpy Skin Disease Virus of laboratory-reared and field-collected insects from Switzerland Anca Ioana Paslaru - University of Zürich and Institute of Virology and Immunology, Mittelhäusern, Switzerland
12 ¹⁵	The African swine fever virus A179L Bcl-2 family member is required for efficient replication in porcine macrophages Linda Dixon - The Pirbright Institute, UK	Risk of transmission of LSDV from experimentally infected cattle to insect vectors Beatriz Sanz Bernardo - The Pirbright Institute, UK
12 ⁴⁵ - 13 ⁰⁰	SUMMARY and FAREWELL - <i>Gallery</i>	



Oral Presentations

Keynote Lectures

Michael Baron

The Pirbright Institute, United Kingdom



Michael Baron worked for 25 years at the Institute for Animal Health (now The Pirbright Institute), where he was a group leader working first on rinderpest virus (RPV), then on peste des petits ruminants virus (PPRV) and Nairobi sheep disease virus. Most of his work was on the detailed molecular and cell biology of these viruses, but he has also worked on novel vaccines and diagnostic systems. He retired from running his own research group in 2015, but continues to be active as TPI's OIE Expert in RPV and PPRV.

PPR: a threat or a problem?

Since its first identification as a disease distinct from the now-eradicated rinderpest, peste des petits ruminants (PPR) has been reported in 64 different countries, and is suspected to be in more. It is clear that, while the available evidence suggests that PPR virus (PPRV) has been around for a long time in some parts of the world, it is also now spreading into new countries, including several on the borders of the EU. PPR is now considered one of the most important diseases affecting small ruminants (sheep/goats), because the impact of the disease falls primarily on low income livestock keepers, and primarily in developing countries.

This spread of PPR has occurred despite the existence of cheap and effective vaccines, and readily available diagnostic tests. This presentation will consider the factors that have led to PPR's seemingly inexorable spread, and to what extent the disease is a serious threat, or a problem that can be managed.

Contact: Michael Baron
michael.baron@pirbright.ac.uk

Karin Darpel

The Pirbright Institute, United Kingdom



Karin Darpel is a veterinarian with more than 10 years' experience in pathogenesis, immunology and virology of *Culicoides*-borne viruses focusing specifically on arboviruses of veterinary importance. Her group at the Pirbright Institute studies the pathogenesis and innate and adaptive immune responses of *Culicoides*-borne orbiviruses in their mammalian host context, mostly focusing on bluetongue virus (BTV). Of specific interests are mechanisms of virus entry and exit across a wide range of natural target cells for the different BTV particle types (for example whole virus, infectious sub viral particles) to identify key mechanisms facilitating transmission and infection initiation. Additional areas of research focus within the Orbivirus Research group include the mammalian immune response towards insect blood-feeding and/or insect saliva and how the skin-vector interface may influence the dissemination and pathogenesis of transmitted viruses. Karin contributes to BSc Veterinary Medicine, MSc and PhD programmes and she is a guest lecturer in Veterinary Virology at the School of Veterinary Medicine, University of Surrey.

Orbiviruses on the move

Orbiviruses such as bluetongue virus (BTV), Epizootic haemorrhagic disease virus (EHDV) and African horsesickness virus (AHSV) are transmitted almost exclusively during blood-feeding of their biological vector *Culicoides* biting midges and often cause severe disease in their respective ruminant or equine hosts. During the last two decades orbiviruses have significantly expanded their geographical distribution with BTV spreading into Northern European countries with no prior history of virus circulation. To facilitate their “move” BTV and other orbiviruses may exploit intriguing strategies to navigate a complex mammalian host- insect vector interface and infect and replicate both within an invertebrate insect as well as a mammalian host. Inflammatory processes of the ruminant skin initiated in response to insect blood-feeding and saliva inoculation may aid in recruiting migratory cell populations that can become infected by BTV. Furthermore *Culicoides* saliva can directly alter BTV infectivity through protease activity that cleaves the outer viral protein VP2 to generate infectious subviral particles (ISVP). Utilising different infectious viral particle types might constitute an additional viral strategy to navigate cell entry and infection across a wide range of divergent host cells. Interestingly bacterial LPS, including LPS present within *Culicoides* saliva, further alters BTV infectivity for certain target cells such as monocytes. To-date at least 27 serotypes of BTV have been detected, with numerous strains per serotype displaying varying ability to infect and replicate within certain *Culicoides* species as well as causing disease within ruminant hosts. This exceptional strain diversity might further constitute a viral strategy to navigate cross-species transmission and new strains are constantly emerging, facilitated by the ability of different BTV strains to exchange dsRNA genome segments during dual infection of the same target cell. Viral genetic factors driving key characteristics such as infection of insect vectors or virulence within certain ruminant host species remain still largely unknown but can be explored comparing reassorted strains of BTV.

Contact: Karin Darpel
karin.darpel@pirbright.ac.uk

Christian Griot

Institute of Virology and Immunology, Switzerland



Christian Griot, by training a veterinarian from the University of Zürich (1982), did his PhD on the subject of calcium metabolism in pigs. After that, he changed early on into the field of virology at the Swiss Federal Vaccine Institute in Basel studying the effect of foot and mouth disease vaccines applied on various localizations in cattle and sheep. In 1989, he moved to Bern and worked at the Institute of Animal Neurology on the pathogenesis of canine distemper virus. His second post doc (1990-1994) was in the laboratory of Neal Nathanson at the School of Medicine of the University of Pennsylvania on the subject of pathogenesis of bunyaviridae. Since 1995, he is the director of the Institute of Virology and Immunology (IVI) in Mithelhäusern and Bern. The IVI serves at the Swiss reference center for highly contagious and other animal viruses. In addition, he holds a master degree in public administration (2005) and is a professor of the Vetsuisse faculty Bern and Zürich. His research interests are emerging diseases and interaction of media with public perceptions during outbreak situations.

Media and diseases; fake news or reality?

The World Health Organization (WHO) and the World Organization for Animal Health (OIE) are warning that infectious diseases are emerging at a rate that has not been seen before. Since the 1970s, more than 40 infectious zoonotic diseases have been newly discovered, including SARS, MERS, Ebola, Hendra, Nipah, avian influenza A H5N1, and others. Despite public health and veterinary public health improvements in the past, human and animal populations remain vulnerable to health threats caused by infectious diseases.

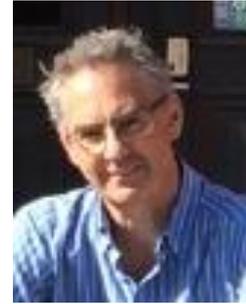
Livestock diseases such as the most recent introduction of notifiable African swine fever into Europe or the previous outbreak of foot and mouth disease in the UK in 2001 clearly show that all involved must stay vigilant for disease introduction at any time and day.

The experience indicates that diseases are of major concern also for the public. The media including social media should add a value to the public perception and initiate an in-depth discussion of the impact of a disease on humans and animals including livestock. Do they really do this? Alternatively, is a “story” of the potential danger of a disease for humans or animals a unique selling point? Selected examples on how media were dealing with recent (emerging) diseases will be presented.

Contact: Christian Griot
christian.griot@ivi.admin.ch

Roger Hewson

Public Health England - Porton Down, United Kingdom



Roger Hewson is the Scientific Leader for Viral Haemorrhagic fevers & Arboviruses at Public Health England - Porton Down and Head of the institutes WHO Collaborating Centre for Virus Research & Reference. His research group - Virology & Pathogenesis, is focused on public health aspects of virology, pathogenesis and new and emerging disease. It includes an in vivo and in vitro capability with highly pathogenic viruses at high containment and a surveillance programme on vector borne and emerging disease with the MoD and key collaborations in the Americas, Africa, Central and East Asia, the Middle East and the Caucuses. Current areas of interest include molecular epidemiology of Crimean-Congo Haemorrhagic Fever viruses, studies on host interactions and disease pathogenesis, collaborative programmes, including capacity building, with partners in endemic countries such as Azerbaijan, Bulgaria, Georgia Kazakhstan, Kosova, Nigeria, Oman, Pakistan, Tajikistan and Turkey. The Virology & Pathogenesis group's remit covers basic diagnostics, genomics and virus isolation for a wide range of viruses including from the families Arenaviridae, Filoviridae, Hantaviridae, Nairoviridae and those in the genera; Alphavirus, Flavivirus, Henipavirus, Orthobunyavirus, Orthopoxvirus and Phlebovirus in the context of public health. We also work on applied translational research involving therapeutics and vaccine interventions.

Viral Hemorrhagic fevers - a coming threat for Europe?

Viral haemorrhagic fevers (VHF) are a diverse group of human clinical syndromes characterised by malaise, fever, vascular permeability, decreased plasma volume, coagulation abnormalities as well as gastrointestinal symptoms. While varying degrees of haemorrhage are observed and generally represent the most severe forms of these diseases, they often result in high fatality rates and are highly infectious posing a serious risk of human to human and community transmission. They are caused by a broad range of zoonotic RNA viruses including over 50 species composed of members of the families: Arenaviridae (e.g. Lassa, Junin, and Lujo virus), Flaviviridae (e.g. yellow fever virus and dengue virus), Filoviridae (e.g. Ebola and Marburg virus), Hantaviridae (e.g. Hantaan virus), Nairoviridae (e.g. Crimean Congo haemorrhagic fever [CCHF]), and Rhabdoviridae (e.g. Bas-Congo). While many of these pathogens have traditionally been thought to be exotic to Europe; one in particular (CCHF) is already endemic in parts of the Europe. Changes in climate, vector distribution, global trade, farming practices, international movement of people and other anthropogenic factors potentially increase the risk that such pathogens could be further spread, introduced and possibly become endemic in some parts of the EU.

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Philippe Jacquet, Professor in Parasitology in the National Veterinary School of Toulouse, is working on bovine besnoitiosis since 2007. Regarding this emerging disease in Europe, his main interests are the understanding of transmission of the parasite, the development of efficient diagnostic tools (including serology and real-time PCR) and the implementation of control programs in collaboration with cattle breeders and veterinarian practitioners. Philippe Jacquet is member of the French Besnoitiosis Committee.

***Besnoitia besnoiti*: An emerging protozoal infection in cattle in Europe**

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Bovine besnoitiosis is caused by the tissue cyst-forming parasite *Besnoitia besnoiti*. Infected cattle may show severe clinical signs leading to cachexia and death. However, most of the infected animals remain totally asymptomatic. Spreading of bovine besnoitiosis in France and in European countries is more likely related to commercial trade of asymptotically infected cattle. Transhumance, extensive breeding and high vectors' densities may have a major impact on local parasite dissemination. *B. besnoiti* bradyzoites could be transmitted by a low number of stable flies (300) provided that an interrupted blood meal on a chronically infected cow is immediately completed on a susceptible host. Therefore, control of stable flies could be a keystone; however, insecticides of the pyrethroids family have shown lack of efficacy in many cattle farms in southwestern France. As neither efficient treatment nor vaccines are available in Europe, the control of bovine besnoitiosis relies on management practices coupled to diagnosis. Some commercial ELISA kits demonstrated high sensitivity and specificity after evaluation on a French serum bank. The remaining available options are: i) to avoid the contamination of besnoitiosis-free farms by serological testing of newly acquired animals and ii) to discard progressively seropositive animals from infected farms.

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Thijs Kuiken

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Thijs Kuiken is Professor of Comparative Pathology at the Department of Viroscience, Erasmus MC, in Rotterdam. He graduated as a veterinarian at Utrecht University in 1988, obtained his Ph.D. in 1998 from the University of Saskatchewan on Newcastle disease virus in cormorants, and qualified as a Diplomate of the American College of Veterinary Pathologists in 2002. He has published over 200 scientific articles, including more than twenty in journals like Science, Nature, Lancet, and PNAS, co-coordinates the EC-funded project Delta-Flu and participates in multiple other international and national projects. He was part of the team that identified the etiological agent of Severe Acute Respiratory Syndrome (SARS), and determined that avian H5N1 influenza virus was highly virulent for cats and other carnivores. Recent achievements of his group include an improved understanding of bat-acquired rabies in order to obtain an earlier diagnosis of rabies in affected people, confirmation that long-distance migratory birds can play a major role in the global spread of avian influenza viruses, and evidence that migratory waterbirds across Eurasia are at risk for substantial numbers of bird deaths during future avian influenza outbreaks. His group's current focus is the pathogenesis of influenza-associated pneumonia and encephalitis, comparison of viral infections between bats and people, and identification of underlying factors for viruses to cross the species barrier from wildlife reservoirs to humans.

Influenza at the interface between domestic animals and wildlife

Although highly pathogenic avian influenza (HPAI) originates in poultry, it has repeatedly spilled over into wild birds in recent years. Between 2014 and 2019, outbreaks of H5 HPAI virus of clade 2.3.4.4 occurred in Europe in all winters except 2015/2016. These outbreaks were similar in that the responsible viruses most likely arrived from the east via autumn migration of wild birds, that viruses spread from wild birds to poultry, and that there were no reports of virus spread to people or other mammals. However, the H5N8 HPAI virus from 2016/2017 caused much higher avian mortality than that from 2014/2015, probably due to increased virulence. Also, more wild raptors than waterbirds died in the second half of the 2017/2018 winter. In conclusion, H5 HPAI viruses of clade 2.3.4.4 have caused high and repeated mortality in both wild birds and poultry in Europe in the last five years, and efforts are needed to stop these viruses from spilling over from poultry to wild birds in the first place. In the meantime, avian influenza surveillance needs to be refined to enable earlier and more efficient detection in both apparently healthy and dead wild birds.

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Alberto Laddomada graduated in veterinary medicine with post-doctoral studies in microbiology and virology. He worked for about fifteen years as a virologist at the Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy. At that time most of his scientific work concerned African swine fever. In 1997 he joined the European Commission's Animal Health Unit in Brussels, where he worked on EU animal disease policy and legislation, including classical and African swine fever and avian influenza. He directed the Animal Health Unit from 2007 until 2015. During those years he led the process of adopting the Commission's proposal for the new EU legislation on animal health which later became Regulation (EU) 2016 / 429 (the "animal health law"). In 2015 he was appointed director general of the Istituto Zooprofilattico Sperimentale della Sardegna and since then he has also been involved in the program to eradicate African swine fever currently ongoing in Sardinia.

ASF in Sardinia: is eradication so close?

African Swine Fever (ASF) has been endemic in Sardinia for more than 40 years. The paramount role of free-ranging pigs (FRPigs) as a very important source of ASF virus in Sardinia has been recently confirmed.

In order to assess the impact of ongoing eradication measures, different disease trends have been evaluated in three areas. Data collected from the "Orgosolo area", where the highest number of FRPigs have been culled and the highest prevalence of ASF was detected, were compared with those gathered in two other areas of Sardinia without FRPigs, "Osilo-Nulvi" and "Goceano".

Furthermore, the role of FRPigs in ASF persistence has been demonstrated comparing the disease trend before and after depopulation action, showing a statistically significant decrease in seroprevalence and virus prevalence in the area in question, not only in FRPigs but also in domestic pigs and the wild boar (WB), related to a lower FRPigs density. Furthermore, the occurrence of FRPigs that scored positive of ASF virus and/or antibodies was associated with a higher probability to observe virus positive WB during the subsequent hunting season (OR = 1.006, CI 95% = 1.002-1.010, p-value = 0.003). Differently from what is currently observed in several European countries, persistence of ASF in the WB seems to be highly correlated with animal density (OR = 1.03, CI 95% = 1.01-1.04, p-value < 0.001).

All data available show a considerable overall decrease in ASF incidence and prevalence in Sardinia in three pig populations concerned (domestic pigs, FRPigs and WB), suggesting that the eradication measures implemented in the last years have been very effective and that ASF virus is on the brink of eradication from the whole island.

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Mary Louise Penrith

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After a career spanning more than 50 years first in zoological and then in veterinary research, I am currently attached to the Department of Veterinary Tropical Diseases of the University of Pretoria, South Africa, as an Extraordinary Professor, providing online postgraduate teaching in management of high impact and emerging animal diseases and advanced One Health for a web-based MSc offered in collaboration with the Institute for Tropical Medicine in Antwerp, Belgium and supervision for MSc and PhD candidates in viral diseases. My involvement in pig diseases and specifically African swine fever started more than 20 years ago in the Pathology Section of the Onderstepoort Veterinary Institute (now ARC-OVR), where I also coordinated a programme for animal health assistance to small-scale farmers. At international level I have provided expert assistance on ASF to the Food & Agriculture Organization of United Nations (FAO) in 10 countries in African and the Caucasus, as well as to the World Organisation for Animal Health (OIE). I am a member of the Guiding Group on Animal Health for the African Union Interafrican Bureau for Animal Resources (AU-IBAR). A former colleague and I also have a small company that provides consulting services on control of transboundary animal diseases and issues of livestock trade at the livestock-wildlife interface.

Managing African Swine Fever - the African Experience

Managing African swine fever in Africa, where it evolved, faces various challenges. Eastern and Southern Africa are home to the ancient sylvatic cycle between warthogs (*Phacochoerus africanus*) and argasid ticks of the *Ornithodoros* complex that live in their burrows. Two other wild African suid genera are, like warthogs, resistant to the pathogenic effects of the ASF virus and can theoretically be reservoirs of the infection. Maintaining separation between domestic and wild pigs has proven highly successful in preventing ASF in biosecure pig farms. Unfortunately the great majority of pigs in Africa are raised in smallholder farms with low biosecurity or traditional free-range systems without any biosecurity. By far the majority of ASF outbreaks are caused by movements of domestic pigs and pork, which has enabled the disease to spread to regions in West, Central and South Africa where the sylvatic cycle is absent. Throughout sub-Saharan Africa maintenance of ASF depends mainly on low biosecurity farming systems and complex informal trading networks in which keeping and trading pigs is a coping mechanism for people who are poor. Managing ASF and if possible eliminating it from domestic pigs depends on creating opportunities to increase the profitability of pig production, thereby creating incentives to modernise the sector and improve biosecurity. This goal is supported by a strategy developed by FAO, AU-IBAR and ILRI, which if implemented should provide a progressive pathway to better control and permit the full potential of pig production to be realised.

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After getting my high school diploma in 1981, I did my duties in civil service as paramedic in Mainz driving ambulance cars (exciting for a young man). Between 1983 and 1989 I studied veterinary medicine in Munich and after graduation did my doctoral thesis there on the “detection of orthopoxvirus DNA using nonradioactive-labelled DNA-probes” (new for a veterinarian). My subsequent Post-Doc time was dedicated to RNA-viruses and here I had my first contacts with arthropods as vectors for pathogens. During this time, I did my board exam in microbiology and I had the chance for a 7-months visit of the CDC in Fort Collins, Colorado, USA (extraordinary beer over there and great environment). However, the easy life at the university (that is a joke) went to an end and I joined the army in the rank of a major to work in an institute dedicated to biological warfare prevention (again a new environment). I finished my habilitation during the six years there and became diplomate of the European College of Veterinary Public Health. Work was on viruses but also on bacteria and toxins. However, six years in the army are more than enough (although I got promoted to Lieutenant-Colonel) and I was very happy when I got the call of the University in Leipzig to become professor for epidemiology at the vet school there. I am there for 10 years now and I like the city and the job. I did my board exam on bacteriology and mycology and the one on epidemiology in the meantime, but dropped out of the ECVPH. My scientific interests however stayed always similar with trying to unravel the epidemiology of (mainly) vector-borne diseases.

Ticks and Tick-borne pathogens - always new challenges ahead

Ticks are blood-sucking arthropods which live on our planet for more than 100 million years the way we know them today. They survived the great extinction period 60 million years ago and they will survive us. They are indeed hard to kill and if you can starve for more than one year, it is almost impossible to die of hunger. But the approx. 700 tick species worldwide are not only fascinating creatures, they are harmful and dangerous. Many pathogens are transmitted during the blood-feeding although transmission modes vary. The biggest impact in public and veterinary public health have broadly distributed, abundant tick species feeding on three different hosts. They have the maximal potential to spread pathogens between animals, and animals and humans. Some Ixodes ticks are among these generalists and the talk will cover aspects of some of the old and new microbes lurking in the midgut or salivary gland of these ticks. The ecology of some tick-borne pathogens will be presented in this context and in terms of future aspects like the changing climate and the international travel.

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Oral presentations

Threats at the European Borders

An experimental vaccine based on a recombinant MERS-CoV S1-protein induces broad protective immune responses in llama

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Middle East respiratory syndrome coronavirus (MERS-CoV) causes severe respiratory disease in humans. Dromedaries are the main source of transmission. Therefore, vaccination of the reservoir host may prevent zoonotic transmission. Llamas are useful surrogates for dromedaries to study MERS-CoV, since they are susceptible to the infection and are able to transmit the virus in a direct-contact model. Here, the humoral immune responses to vaccination against MERS-CoV in llamas were characterized.

Five llamas were intramuscularly vaccinated twice, three weeks apart, with a recombinant S1 protein. Two weeks after the boost, these animals were mixed in the same pen (direct-contact) with three MERS-CoV (Qatar15/2015 strain) inoculated llamas. Sera were collected at immunization points, day of inoculation and weekly after challenge for seroneutralization, ELISA, hemagglutination inhibition and receptor binding inhibition assays. Nasal swabs were collected also during the experiment to assess viral RNA presence and virus titration. Animals were euthanized 3-weeks after infection.

Directly-inoculated llamas shed virus for 2 weeks. After direct-contact, only one out of five vaccinated llamas got high levels of viral RNA in the nasal cavity, but no infectious MERS-CoV was detected in any of the vaccinated animals. Sera from all the S1-vaccinated llamas developed antibodies against the sialic acid binding S1 N-terminal and the receptor binding domains and displayed virus neutralization titers at 3 weeks post immunization. Mucosal neutralizing antibodies were elicited in three vaccinated llamas.

The S1 recombinant protein vaccine induced broad protective immune responses against MERS-CoV, including mucosal neutralizing antibodies, proving its eventual suitability for field trials.

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Molecular basis of Peste des Petits Ruminants Virus emergence in Mongolian wildlife

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Peste des petits ruminants virus (PPRV) is a morbillivirus of small ruminants which threatens 1.7 billion sheep and goats. PPRV prevents sustainable livestock production and drives food insecurity. Consequently FAO has pledged to eradicate PPRV globally by 2030. PPRV can also infect wild ruminants, with significant implications both for PPRV eradication and for biodiversity, as exemplified by the emergence of PPRV in critically endangered saiga antelope in Mongolia in 2016, killing ~60% of the population. Hitherto, the haemagglutinin (H) and fusion (F) genes from PPRV infecting any wild species have not been characterised, although these are known to be critical mediators of morbillivirus host tropism.

This project characterised the genetic changes in H and F from PPRV infecting Mongolian wildlife, and their effects on host cell receptor usage.

Full length H and F amplified by RT-PCR from PPRV-infected saiga, ibex and Goitered gazelle showed several amino acid changes relative to PPRV infecting Mongolian livestock. SLAM-F1, the universal morbillivirus receptor, was also cloned from saiga, which showed 95% amino acid identity to goat SLAM-F1. Structural homology models reveal that several amino acid changes lie at the H-SLAM binding interface. Results of infectivity assays utilising viral pseudotypes will be presented, which address how livestock and wildlife-derived H/F utilise saiga and goat SLAM-F1 as entry receptors.

In summary, we have identified potentially important evolution in H and F genes of PPRV strains infecting endangered Mongolian wildlife, helping to illuminate the ecology and evolution of PPRV and underpin successful PPRV eradication.

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Immunological mechanisms underlying classical swine fever persistent infection

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Classical swine fever virus (CSFV) remains one of the most relevant pathogens affecting swine. The capacity of CSFV to generate viral persistence following infection early after birth has been demonstrated; these animals appear clinically healthy or show unspecific clinical signs despite being permanently viremic and shedding high amounts of virus, in absence of immune response to the virus. Despite its importance, the establishment and maintenance of persistent infection is not fully understood. Aiming to clarify the mechanisms underlying this disease form, three-week-old pigs (n=9) age were infected with a CSFV moderate virulence strain, at a dose proven to generate persistent infection. Animals were sampled weekly during 6 weeks and viral, clinical, pathological and immunological tests were carried out. Cellular markers of immune suppression were evaluated by flow cytometry with the purpose to relate their presence with CSFV persistent infection. 44.4% of animals were CSFV persistently infected, 33.3% died and 22.2% developed specific antibody response. This is the first report of CSFV capacity to confer postnatal persistent infection in pigs infected at 3 weeks after birth, an age in which the weaning could be carried out in some swine production systems. Remarkably, markers related to the immune exhaustion phenomenon were detected in CSFV persistently infected pigs. This marker was associated with the CSFV persistence capacity in the host and should be the subject of further studies.

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Evaluation of environmental sampling as a low technology method for surveillance of foot-and-mouth disease virus in an endemic area

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Environmental sampling enables disease surveillance beyond regular investigation of clinical cases, extending available data on the circulation of a pathogen relevant to a specific area. Environmental contamination by foot-and-mouth disease virus (FMDV) in excretions and secretions from infected individuals promotes transmission, but also provides an opportunity for non-invasive sample collection, facilitating diagnostic and surveillance purposes.

Electrostatic dust cloths were used to collect environmental swabs at sites in the Kathmandu Valley, Nepal, which is endemic for FMD. Twelve sites with reported outbreaks of FMDV were visited and sampled between November 2016 and March 2018. Goat markets were also sampled, representing sampling of a population of animals that FMDV is known to circulate in, but is not always clinically evident.

FMDV RNA was detected by rRT-PCR in environmental samples from premises with animals at all stages of clinical disease, from uninfected, suspected preclinical, clinical and recovering cattle. VP1 sequence data was generated from a select number of samples, enabling determination of viral serotype. Goat market samples were tested for both FMDV and Peste des petit ruminants virus (PPRV), with a small proportion of samples positive for each virus.

Development of methods that can reliably detect FMDV RNA in the environment is significant, as this extends the toolbox available for surveillance of FMDV and other livestock diseases. Development of low-tech, straightforward surveillance methods can support robust responses to disease outbreaks in animal populations; improving capability for the rapid detection of outbreaks and providing additional data to assess virus circulation in specific areas.

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Evolution of attenuation and risk of reversal in Peste des Petits Ruminants vaccine strain Nigeria 75/1

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Peste des Petits Ruminants (PPR) is a highly infectious disease caused by a virus of the Morbillivirus genus. The current PPR eradication effort rely mainly on the implementation of massive vaccination campaigns. One of the most widely used PPR vaccine is the Nigeria 75/1 strain obtained after attenuation by 75 serial passages of the wild type isolate in cell cultures. Here we use high throughput deep sequencing on historical passages that led to the Nigeria 75/1 strain, to follow the evolution of PPRV attenuation and assess risk of reversal in different cell types. Comparison of the consensus sequences for the wild type and vaccine strain showed that only 18 fixed mutations separate the two strains. At the earliest attenuation passage at our disposal (passage 47), 12 out of the 18 mutations were already present with 100% frequency. Low frequency variants were identified along the genome for all passages. Sequencing of passages posterior to the vaccine strain showed evidence of genetic drift during cell passages, especially in cell expressing the SLAM receptor targeted by PPRV. However, 15 out of 18 mutations related to attenuation remained fixed in the population. In vitro experiments suggest that one mutation in the leader region of the PPRV genome affects virus replication. Our study suggest that only few mutations can have a serious impact on the pathogenicity of PPRV. Risk of reversal of PPRV strain Nigeria 75/1 after serial passages in cell cultures seems low but limiting the number of passages during vaccine production is recommended.

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Heterogeneities that contribute to increased Peste des petits ruminants seroprevalence in sheep, goats, and cattle in northern Tanzania

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Peste des petits ruminants virus (PPRV) causes a contagious disease of high morbidity and mortality in sheep and goats and has been shown to elicit seroconversion in cattle. PPRV threatens 80% of the global small ruminant population of nearly 2 billion animals. Using a large serosurvey (n=7538) from 20 villages in Tanzania, we investigated PPRV age-seroprevalence and household survey data to determine risk factors and specific management practices for increased PPRV circulation, to explore spatial variation in the force of infection at multiple scales, and to identify the age cohort(s) responsible for PPRV transmission among sheep, goats, and cattle. We used generalized linear mixed models within a catalytic framework to calculate the force of infection (FOI, per capita infection rate of susceptible hosts) and reproductive numbers using both an age constant and piecewise constant model. We identified specific management risk factors for PPRV seroconversion such as confinement and grazing practices, seasonal camp attendance, animal introductions, and herd size. We found the overall observed seroprevalence was 21.1%. The dentition-based age group with the highest FOI for sheep and goats was 1.5-3 years of age, and 3.5-4.5 years of age for cattle. Pastoral management systems had higher FOI and a wider range of ages with a higher FOI than agropastoral systems. Insights from this investigation of specific management practices will lead to improved control strategies through feasible changes in non-vaccination practices, geographical areas and host species to target, and discovery of additional ecological mechanisms driving PPRV seroconversion.

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Studies on pathogenesis of Capripox virus-induced diseases and evaluation of diagnostic tools

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Lumpy Skin Disease virus (LSDV), Sheeppox virus (SPPV) and Goatpox virus (GTPV) form the genus Capripox virus of the Poxviridae family. Besides high fever and ocular and nasal discharge, clinical symptoms like emaciation, lesions of the skin, temporary reduction in milk production, and sometimes death can be observed after infection with Capripox viruses. For their control, only live attenuated vaccines are commercially available, which are currently not authorized in the European Union. Additionally, use of these live vaccines leads to the loss of the Capripox virus-free status in infected countries.

We performed animal trials to analyze the pathogenesis of LSDV (field and vaccine strain), SPPV and GTPV in their respective target species. Interestingly, inoculation with a Macedonian LSDV field strain and an Indian GTPV strain led to severe clinical symptoms in cattle and goats, respectively. In contrast, only mild clinical reaction could be observed after inoculation with the attenuated LSDV vaccine strain “Neethling”, whereas sheep inoculated with a Russian SPPV strain did not show any clinical reaction at all. These findings correlate with the analyses of viral genome load in EDTA-blood, serum, nasal and oral swabs. Animals with severe clinical course showed higher viral genome load in all tested matrices. Additionally, seroconversion could be detected in most animals, except the SPPV Russia-inoculated sheep.

Based on the samples obtained from the performed animal trials, we validated different molecular and serological diagnostic tools, amongst other things our newly developed DIVA real-time qPCR assays for differentiation between LSDV field and vaccine strains.

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Development of live attenuated PPR DIVA vaccines using reverse genetics

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Across the Africa, Middle East and Asia, Peste des petits ruminants virus (PPRV), places a huge disease burden on agriculture, in particular affecting small ruminant production. The recent outbreaks in Northern Africa, European part of Turkey and Bulgaria represent a significant threat to mainland of Europe, as a source of disease spread. Although two safe and efficacious live attenuated vaccines are available for PPR control, current serological tests do not enable to differentiate between naturally infected and vaccinated animals (DIVA). The vaccinated animals develop a full range of immune responses to viral proteins and therefore cannot be distinguished serologically from those that have recovered from natural infection. This is posing a serious problem for the sero-surveillance programs. Further, during the latter stages of any eradication programme ongoing vaccination is only possible if the vaccine used is fully DIVA compliant. Using reverse genetics we have developed two DIVA vaccines (two existing established vaccine strains; Sungri 96 and Nigeria/75/1) and two recombinant ELISAs that can differentiate between vaccinated and infected animals. As a proof of principle both DIVA vaccines have been evaluated in goats in pilot studies and the animals were fully protected similar to the parent vaccines upon virulent PPR virus challenge. Further, we can differentiate infected from vaccinated animals by two ELISAs using two recombinant proteins. As these DIVA vaccines and associated ELISAs can differentiate between infected and vaccinated animals that facilitate the sero-monitoring process and speed up the implementation of global PPR eradication through vaccination.

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Gene Signatures Associated with Foot-And-Mouth Disease Virus Infection and Persistence Part II: Proteogenomics Uncovers Critical Elements of Host Response in Bovine Soft Palate Cells

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Foot-and-mouth disease (FMD) is the most devastating disease of cloven-hoofed livestock, with a crippling economic burden in endemic areas and immense costs associated with outbreaks in free countries. Foot-and-mouth disease virus (FMDV), a picornavirus, will spread rapidly in naïve populations, reaching morbidity rates of up to 100% in cattle. Even after recovery, over 50% of cattle remain subclinically infected and infectious virus can be recovered from the nasopharynx. The transcriptional alterations of the nasopharynx and soft palate (SP) during FMDV infection and persistence in cattle are not well understood. Here, an air-liquid interface multilayer model of bovine SP cells was used to study the hosts transcriptional signatures during acute (24 hours post infection - HPI) and persistent FMDV infection (28 days post infection - DPI). Therefore, whole-transcriptome libraries were sequenced with the Ion S5XL. Furthermore, expression levels of selected genes were confirmed by RT-qPCR and protein mass spectrometry.

During the acute phase viral RNA and protein was detectable in large quantities and in response hundreds of interferon-stimulated genes (ISG) were overexpressed, mediating antiviral activity and apoptosis. Although the number of pro-apoptotic ISGs and the extent of their regulation decreased during persistence, some ISGs with antiviral activity were still highly expressed at that stage. This indicates a long-lasting but ultimately ineffective stimulation of ISGs (MX1, OAS2, IFIH1) during FMDV persistence. Furthermore, downregulation of relevant genes (ANKRD1, NCAM1, collagens) suggests an interference with the extracellular matrix that may contribute to the skewed virus-host equilibrium in soft palate epithelial cells.

“Gene Signatures Associated with Foot-And-Mouth Disease Virus Infection and Persistence Part I: Persistent FMDV in Long-Term Multilayer Cultures of Soft Palate Cells” is presented as poster (Topic: Threats at the European Borders).

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RAPID, ON SITE, DIAGNOSIS OF FMD AND SAFE AND COST-EFFECTIVE SHIPMENT OF SAMPLES

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Foot-and-mouth disease virus (FMDV) causes one of the most economically devastating animal diseases affecting artiodactyls. Identification of circulating strains is important for efficient FMD control. However, shipping requirements and high cost of submission of suspected positives samples to reference laboratories remain a major obstacle. A cost-effective and safe method for shipment of samples from FMD-suspected cases, based on the inactivation of FMDV on lateral flow devices (LFDs, immunodetection strips) has been developed and validated in the laboratory. This method allows detection and typing of FMDV by RT-PCR and virus rescue using RNA transfection (Romey et al. 2017). The present study aims to further evaluate this protocol on freshly collected clinical samples through collaborations with endemic countries in order to test performance and safety of the entire process directly in the field.

Epithelium or vesicular fluid samples have been collected from suspect clinical cases of FMD in Nigeria, Turkey and Pakistan and have been tested in the field using LFDs. The selected positive inactivated (or not) LFDs have been submitted to reference laboratories (France, Denmark) for molecular detection and virus rescue. Safety of this inactivation protocol has been confirmed directly in Nigerian and Turkish laboratories. Transfections are currently being optimized to ensure virus rescue from RNA genomes recovered from inactivated LFDs.

This study will contribute to demonstrate that using LFDs is a safe way for room-temperature, dry-transport of inactivated FMDV samples from endemic areas. It will substantially decrease the shipping cost thus increasing field sample submission.

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A poly-uridine insertion in the 3'-untranslated region found in a classical swine fever low virulent strain modulates virulence

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The aim of the present study was to assess the possible role for virulence in piglets of a unique uninterrupted 36-uridine (poly-U) sequence found in the 3'-untranslated region (3' UTR) of the low virulent CSFV Pinar de Rio (PdR) strain isolated from an endemic country. We constructed a functional cDNA clone of CSFV PdR harboring the original poly-U sequence in the 3'UTR (pPdR-36U). All other CSFV isolates sequenced to date carry typically 4 or 5 uridines at this position. Accordingly, the poly-U sequence of pPdR-36U was replaced with the standard 5 uridines (pPdR-5U). Two groups of 5-day-old piglets (n=20) were infected with the cDNA-derived viruses vPdR-36U and vPdR-5U, respectively. Ten contact piglets were added to each group and disease progression, virus replication and immune responses were monitored during 5 weeks. The vPdR-5U virus was clearly more virulent than the original vPdR-36U virus, with more severe disease, higher mortality and significantly higher viral loads in serum and body secretions. The two viruses were transmitted to all contact piglets. 90% of the piglets infected with vPdR-36U seroconverted after 2 to 3 weeks of infection. Conversely, only one piglet infected with vPdR-5U developed detectable antibody response. These latter piglets showed only transient IFN- α responses in the serum after one week of infection while the vPdR-36U-infected piglets showed sustained IFN- α levels during the two first weeks of infection. The 3'UTR poly-U insertion acquired by the PdR isolate in the field reduces viral virulence and activates the innate and humoral immune responses without affecting viral transmission

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Oral presentations

Current Challenges inside Europe

Evolution of highly pathogenic non-H5/H7 virus from a natural H4N2 avian influenza virus with a polybasic cleavage motif

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Highly pathogenic (HP) avian influenza viruses (AIVs) have an intravenous pathogenicity index (IVPI) ≥ 1.2 , or are H5 and H7 viruses with a polybasic cleavage site (pCS) in the hemagglutinin (HA). Few non-H5/H7 viruses fulfill the criteria of HPAIVs; nevertheless, it remains unknown why these viruses did not spread in poultry. In 2012, an avirulent H4N2 virus with a pCS was isolated from quails in California. Here, the evolution of HPAIV H4N2 after mutations in the pCS, reassortment with HPAIVs H5N1 or H7N7, and passaging in chicken eggs (designated EP20) was investigated. Replication and chicken-to-chicken transmission of H4N2 were compromised by increasing the basic amino acids in the pCS, but restored after reassortment with HPAIV H5N1, although not with H7N7. The NA, NS and NP gene segments of HPAIV H5N1 together contributed to the high virulence of H4N2 virus. Moreover, EP20 exhibited high virulence and possessed 10 mutations in five segments, which, interestingly, were found as minor variants also in the wild-type virus population. Thus, H4N2 can evolve to HPAIV; however, the lower fitness of H4N2 with pCS and the need for specific gene constellations to exhibit high virulence are potential factors why non-H5/H7 viruses did not yet evolve to HPAIV.

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Polymorphism in the hemagglutinin proteolytic cleavage site of H9N2 affected proteolytic activation and cell-to-cell spread in cell culture and virus excretion in infected poultry

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Avian influenza viruses (AIV), members of the family Orthomyxoviridae, are enveloped RNA viruses with segmented genome. AIV specify 16 hemagglutinin (HA) and 9 neuraminidase subtypes. AIV H9N2 is generally low pathogenic (LP) in poultry causing mild or no clinical signs; however, morbidity and mortality increased due to co-infection with other bacteria and viruses. H9N2 infect mammals including humans. The HA plays important roles in interspecies transmission, virulence and immune-evasion. Since 2012, H9N2 viruses are common in poultry in Germany and polymorphism in the hemagglutinin proteolytic cleavage site (HACS), a major virulence determinant of AIV in poultry was observed. Using reverse genetics, recombinant H9N2 viruses carrying single mutations in the HACS, mimicking the field viruses, were successfully rescued and propagated. In vitro, mutations in the HACS increased cell-to-cell spread. Proteolytic activation of the HA was trypsin-dependent, a typical feature of LPAIV. In chickens, mutations in the HACS affected virus shedding in infected birds without impact on virulence or transmission after oculonasal or intravenous infection. In turkeys, HACS mutants exhibited variable virulence and excreted from inoculated and contact turkeys. Together, mutations in the HACS of H9N2 contributed to virus fitness in vitro and in vivo, particularly in turkeys.

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Using Sediment, Environmental and Wild Bird Samples as Tools for Avian Influenza Surveillance

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Multiple global clade 2.3.4.4 H5Nx highly pathogenic avian influenza viruses (HPAIVs) have occurred since 2014, typified by circulation in wild birds and poultry outbreaks. Surveillance in the UK for such threatening avian influenza viruses (AIVs) that have the potential to cause significant, has included a risk-based strategy for wild birds, whereby clinical samples from carcasses are laboratory screened for H5Nx AIVs. Assessment involves initial testing by generic real-time reverse transcriptase PCRs (RRT-PCR), followed by a H5-subtype-specific RRT-PCR, viral RNA sequencing and virus isolation for non-negative samples. However, AIVs are mainly excreted from wild birds via faeces, therefore analysis of environmental samples containing faecal matter may complement testing of wild bird carcasses. The results generated from testing environmental samples collected from studies of experimentally-infected poultry, as well as AIV outbreaks will be described. A pilot study to collect environmental samples from wetland sites where AIV-positive wild birds have been detected previously, has also begun via the Horizon 2020 Delta-Flu project. We have also investigated using a genomics-based approach (targeted resequencing) to detect and characterise AIVs from sediment samples collected during the 2014/2015 H5N2 clade 2.3.4.4 HPAIV outbreak in the Fraser Valley, Canada. Of the 300 sediment samples collected, 20.6% were positive for AIV, which included 13 haemagglutinin and 9 neuraminidase subtypes. It is anticipated that data generated from testing environmental samples may supplement the information that is obtained from wild bird samples, regarding the AIVs that are present in the wild bird environment.

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Expanding the known distribution of European Bat 1 Lyssaviruses - Investigation of two common Pipistrelle Bats from Baden-Wuerttemberg

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Lyssaviruses have their ancestral reservoir in bats (Chiroptera), and several lyssavirus species circulate in bat populations across Europe, sustaining the potential of a rabies infection for humans and animals. These lyssaviruses are associated with specific bat species representing their main reservoir, although spillover events to other bat species have been reported. The vast majority of cases in Europe were identified as European bat 1 lyssavirus (EBLV-1), isolated from Serotine bats (*Eptesicus serotinus*). Here, we describe the detection of EBLV-1 in two common pipistrelle bats (*Pipistrellus pipistrellus*) found in Baden-Wuerttemberg, Germany. The brain material was initially tested negative for lyssaviruses using the fluorescent antibody test (FAT). However, subsequent screening with a panlyssavirus RT-qPCR assay identified both samples as lyssavirus positive and further investigations were conducted via next-generation sequencing (NGS) methods. Since the original sample material was substantially degraded, adaptations of the library preparation workflow were necessary. These improved the genome coverage to up to 99 %. As a result, both lyssaviruses were identified as EBLV-1, revealing not only a rare case of an EBLV-1 infection in the unusual spillover host *P. pipistrellus*, but also confirming the occurrence of EBLV-1 in Baden-Wuerttemberg for the first time. Phylogenetic analyses showcase the close relationship to previously investigated German EBLV-1 samples from *E. serotinus* within sublineage EBLV-1a, thus suggesting spill-overs from Serotine bats.

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GII NOROVIRUS IN SWINE FAECAL SAMPLES IN NORTHERN ITALY: IDENTIFICATION OF TWO DIFFERENT GENOTYPES

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Noroviruses (NoVs) are one of the major causative agents of non-bacterial gastroenteritis in humans all over the world. NoVs, belonging to Caliciviridae, are classified into seven genogroups, from GI to GVII, which are further subdivided into 40 genotypes. In naturally infected swine, the main genogroup identified is GII (21 genotypes), which can infect both swine and humans. Aim of this study was to assess the presence and distribution of NoVs in pigs in North Eastern Italy, through an active surveillance based on the collection of faeces and sera at slaughterhouse and farms between 2017 and 2019. In total 310 faeces samples and 1,161 serum samples, collected from 153 different farms, were analyzed. The serological investigation showed presence of antibodies against GII NoVs in the swine population analyzed. The virological data showed the circulation of NoVs belonging to two different genotypes: GII.11 and GII.18. The phylogenetic analysis revealed the existence of an extensive genetic diversity among the Italian NoVs. Within each genotype, distinct genetic clusters were identified. The co-circulation of two genotypes characterized by a high degree of genetic variability poses questions regarding the origin and evolution pathways of swine NoVs in North East Italy. So far, this is the first study reporting the identification of GII.18 in the swine population in Italy and Europe. Continuous monitoring of NoVs in the swine and animal reservoir will add information on the distribution of GII and its genetic characteristics aiding the understanding on the origin and evolution of NoVs.

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Could IgM antibodies be a marker of acute Bb infection in cattle?

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Besnoitia besnoiti (Bb) is the cause of bovine besnoitiosis. Serological tools such as ELISA, western blot or IFAT play an essential role for diagnosis. In ELISAs, IgG Ab are detected 5 to 6 weeks post-infection. To date, there is no Bb tests with good diagnostic performance during the febrile/acute phase of infection: Bb detection by qPCR on whole blood in the early stages of infection is unconstant, making differential diagnosis difficult.

This study presents the first results of Bb IgM ELISA prototypes (indirect or IMAC) with samples from experimental infection or field samples.

2 bovine (A and B) were inoculated with *B. besnoiti* at 1×10^4 and 2×10^7 tachyzoites per mL, respectively, by intradermic and intravenous ways, and bled at different days post inoculation (dpi). Only one IgM ELISA allowed IgM detection from 11 to 42 dpi (A) and from 7 to 42 dpi (B), whereas IgG were detected as of day 32 (A) to 21 (B).

59 cattle samples from a high prevalence infected herd were tested in parallel by qPCR, IgM and IgG ELISAs. 8/59 samples, qPCR positive, were IgM positive whereas they were IgG-negative.

To conclude, experimental results confirm that IgM antibodies are present in Bb infection. If their persistence could be quite short (around 4 weeks), they seem to appear in the early stages of infection and could be detected 4 weeks before IgG. Further studies are needed to see if Bb IgM Ab ELISA could be used as an early detection tool for Besnoitiosis.

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ts-mutants of porcine influenza viruses: Pathway to live-attenuated vaccines in pigs?

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Due to the wide spread of swine influenza A virus (swIAV) and the associated respiratory diseases, influenza infections in pigs have considerable pathogenic and economic losses. Over the past decades in Europe, four pathogenic lineages (H1N1av, H1N2hu, H3N2, H1N1pdm/2009) emerged, some of them reassortant viruses between avian and human influenza viruses. New options to prevent respiratory symptoms in pigs by developing live vaccine candidates against infections with these four lineages are being explored. In this study, the focus is on the classical vaccine concept of temperature-sensitive (ts) live attenuated influenza vaccines (ts-LAIV), which has yet to be evaluated in pigs. Recent field isolates of each of the four lineages have been cold-adapted (ca) through serial passaging at decreasing incubation temperature in MDCK-II and swine testicle cells. Full genome sequencing revealed multiple mutations in several gene segments compared to parental viruses. Growth kinetics established the ts-ca phenotype of the serially passaged viruses. The att phenotype will be assessed by experimental infections of mice and of the final target species pig. As swIAV have been critically involved in the development of at least the most recent human influenza pandemic virus, zoonotic potential of the vaccine virus candidates will be tested in the ferret as a human influenza model species.

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Comparison of experimental infections of mulard ducks with French H5N8 and H5N1 avian influenza viruses

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France was stricken twice by highly pathogenic (HP) H5 avian influenza viruses (AIV), first in winter 2015-2016 and then in next winter 2016-2017. In the first epizooty, several HP H5 AIV viruses with same H5 gene were circulating in flocks of mulard ducks like H5N1, H5N2, H5N9 viruses. In the second epizooty, several introductions of HP H5N8 probably from avian wild birds were characterized. The aim of this study was to compare the viral shedding and transmission after experimental infections of mulard ducks using HP H5N1 and H5N8 AIV viruses isolated in France in 2015 and 2016 respectively.

Each of eight mulard ducks of 6 weeks of age received 10⁶EID₅₀ in eyes and were in contact one day after inoculation with four naive contact mulard ducks. Clinical signs as well as respiratory and cloacal shedding were monitored for 18 days after inoculation. The experimental trials were carried out in Anses animal facilities according European regulation on animal experimentation. The main differences between the two strains were the duration of the shedding (4 to 10 days for HP H5N1 and 7 to 15 for HP H5N8 AIV) and the route of shedding (mainly cloacal for H5N1 and respiratory for H5N8).

In conclusion, the results of the experimental infections were in agreement with natural infections in herds with both H5 HP AIV isolates. These infection models can be used further to evaluate virulence factors or vaccine protection experimentally.

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Transcriptional response after single and double infection of Vero cells with Mammalian Orthoreovirus and Porcine Epidemic Diarrhea Virus

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Mammalian Orthoreovirus 3 (MRV3) was first detected in fecal samples of diarrheic pigs infected with Porcine Epidemic Diarrhea Virus (PEDV) during the 2013-2015 outbreak of PEDV in the USA. Since then, screening of fecal samples from diarrheic pigs in Europe showed a relatively high prevalence of MRVs in fecal samples, especially in samples obtained from farms affected by PEDV. Considering the economic impact of PEDV infection for pig production, a study on the effect of co-existence of MRV with PEDV would provide insight whether a synergistic effect between MRV and PEDV exists and contributes to induction of diarrhea in pigs. Recent studies indicated that both PEDV and MRV affect the expression of antiviral proteins in infected cells by suppressing (PEDV) or stimulation (MRV) of the activity of the transcription regulator “interferon-regulatory factor 1”. To elucidate if the response in cells to infection with MRV or PEDV enhance or repress replication of the other virus, the transcriptional response in Vero cells was recorded. Cells were infected with PEDV alone, MRV alone, and simultaneously with both viruses, and gene expression was measured early after infection (4-6h) using NGS-RNAseq. Measured quantities of mRNA's were extracted from NGS files and corresponding sets of differential expressed genes were analysed using functional bioinformatics programs to detect biological processes in the Vero cells specifically induced by MRV or PEDV alone, or only after simultaneous infection. Results of this study will be presented and discussed in relation to the co-existence of MRV in PEDV infected swine.

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Testing of a Pan-virus Metagenomic Array Detection assay (MAD-Vir) using panels of samples from animals experimentally infected with known viruses

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Statens Serum Institute (SSI) has developed a metagenomics PanVirus microarray with clinical sensitivity that can simultaneously identify all known human and animal virus sequences present in GenBank in 2018. This system is currently under evaluation for its operability by a consortium funded by the EU H2020 OHEJP (JRP04 MAD-VIR). The technology has been transferred by SSI to three European laboratories including INIA-CISA. The detection range of the PanVirus microarray was initially evaluated with a sample panel (n=16) including blood, serum, feathers, faeces, and several organs, obtained from mice, pigs, sheep and partridges, experimentally infected, at INIA-CISA animal facilities, with 8 different virus species (WNV, ASFV, CSFV, PTV, PPRV, BTV, USUV and BAGV) belonging to 6 genera of 5 virus families.

The array succeeded to identify the expected virus in most samples from all animal species examined. Indeed, the array identified correctly (at the virus species level) the recently described porcine teschovirus 12 (PTV12), not included in the array design. However, it failed to identify the virus involved in three samples, either because of a very low RNA content (Usutu) or nucleotide divergence with WNV probes designed (Koutango and Malaysian WNV lineages). This gap and others that may arise in further experiments will be addressed in the next version of the assay.

The wide range of testing and the one-health perspective ensure that the next upgraded microarray prototype, to be released soon, will be a useful tool in sanitary emergencies whose origin is unknown.

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Connect to Protect: Insights into the Role of Captive Birds in Virus Spread and Reassortment during the Outbreak of Highly Pathogenic Avian Influenza, Germany 2016-2018

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During the winter of 2016-2017, highly pathogenic avian influenza viruses (HPAIV) of subtype H5 clade 2.3.4.4b caused the most severe epizootic outbreak among wild birds and poultry ever reported in Europe. The HPAI H5N8, H5N5 and more recently H5N6 viruses, circulating until summer 2018 in Germany, led to devastating (economic) losses in both domestic gallinaceous poultry, waterfowl holdings and aquatic wild bird populations.

Although the already evaluated viruses from this outbreak were of low zoonotic potential, frequent reassortant events of this clade constantly lead to the possibility of the emergence of new viruses with different characteristics, increased pathological findings or increased zoonotic propensity. The unprecedented variety of genetic variants within this clade points to the need for fast and accurate analyses of viral genomes as a basis for investigating incursion routes, exploring possible spread and detecting connections between outbreaks. We herein present a sequenced-based machine-learning approach that combines routine sequencing of AIV samples with a bioinformatics workflow including, but not limited to, network analyses, phylogenetic classification and reassortment detection of HPAIV H5Nx subtypes from the respective outbreak.

We applied the workflow to samples from 68 outbreaks from German commercial holdings, including 24 outbreaks from small holders and sequenced at least one sample per outbreak, confirming known and searching for possible new reassortants. We were able to gain detailed insights into the role of captive birds in virus spread and reassortment events during the outbreak, also permitting the identification of connections within/between local outbreaks.

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Genome sequencing of bat coronaviruses obtained from faecal samples using a metagenomics approach

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Coronaviruses are enveloped positive-sense, single stranded, RNA viruses with the largest known genomes for RNA viruses of around 27-31 kb. Many new coronavirus species have been discovered as a result of sequencing studies. In particular, wild bat species are under investigation worldwide to discover the diversity of their viruses and identify potential emerging pathogens that are harboured by these hosts.

We have previously shown that distinct alphacoronaviruses are present in five different species of bats in Denmark with a prevalence of around 20 % (Lazov et al., 2018).

In the present study, RNA was extracted from selected samples of bat faeces from Denmark and subjected to DNA depletion, random PCR amplification, library preparation and next generation sequencing on an Illumina MiSeq machine. The reads were trimmed and sequences were assembled de-novo using META-SPAdes for metagenomic data in Geneious. Coronavirus contigs were identified and the reads were mapped back to these resulting in identification of 8.000 to 460.000 coronavirus reads per sample. Consensus sequences were prepared, aligned and annotated.

Using this approach, it has been possible to assemble six full-length coronavirus genomes from three different bat species. These sequences will be made publicly available and thus contribute to the knowledge of bat coronavirus diversity.

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One Health approach in animal disease management, based on demographic features and socio-economic indicators

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A better understanding of the role of social factors in the efficacy of animal disease control measures is increasingly being felt necessary worldwide. Many risk analysis studies have been conducted to assess the role of various factors in the development of animal diseases; however, very few have accounted for the role of social factors. The aim of this work is to bridge this gap, with the main hypothesis that different socio-economic factors could be valid indicators for the occurrence and persistence of several animal diseases. A socio-economic analysis was performed using demographic characteristics of the farmers and data from 44 social indicators released by the Italian National Institute of Statistics (ISTAT) database. African swine fever (ASF) and other animal diseases and zoonoses that are endemic in Sardinia were considered, such as cistic echinococcosis (CE), contagious agalactia (CA), trichinellosis, West Nile disease (WND), and bluetongue (BT). Seven different negative binomial regression models were fitted using the number of cases between 2011-2017. Associations between ISTAT indicators and animal diseases have been studied in depth and showed statistically significant results, but with varying effects. The present work highlights the role of a few socio-economic factors as indicators to identify macro-areas or municipalities at the greatest risk of farm animal-related diseases. A specific application on ASF management based on social factor has been implemented. These results may help understand the social context in which these diseases may occur and guide the design of specific risk management measures that go beyond well-known veterinary measures.

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Seroprevalences of newly discovered porcine pestiviruses in German pig farms

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During the last years, several novel porcine pestiviruses were discovered. Linked to actual disease outbreaks in pig farms, Pestivirus F (syn. Bungowannah virus) and Pestivirus K (syn. atypical porcine pestivirus) pose a serious threat to commercial pig production. Pestivirus F was isolated in the Australian region Bungowannah during an outbreak of sudden death in young pigs, but has never been detected outside the initial outbreak farm complex. Pestivirus K on the other hand was found in multiple countries worldwide and is also causing problems in young pigs in the form of congenital tremor. To assess the distribution in German pig farms, a total of 1,115 samples originating from 122 farms located in seven federal states collected during the years 2009/10 and 2018 were serologically investigated. While antibodies against Pestivirus F were detected by a traditional in-house indirect immunofluorescence test against the culture-grown virus isolate, Pestivirus K-specific antibodies were evaluated by a newly developed test system utilizing a chimeric construct of Pestivirus A (syn. bovine viral diarrhea virus 1) containing the E1 and E2 encoding sequences of Pestivirus K. None of the tested sera showed antibodies against Pestivirus F, further confirming the initial outbreak as a restricted event. Pestivirus K proved to be constantly present throughout Germany, since positive sera were found in all seven federal states at both time points. Although fluctuation on state level did occur, the overall seroprevalence remained stable over the years, as it is to be expected for a virus lacking any form of restrictive measures

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First cases of CWD in Sweden - perhaps spontaneously occurring?

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The first cases of Chronic Wasting Disease (CWD) in Sweden have been detected in two free ranging moose (*Alces alces*) in Norrbotten county. Both cases were in 16-year-old female moose which were euthanised after being observed emaciated and with behavioural changes.

Samples from the moose were found positive and confirmed for CWD at the National Veterinary Institute (SVA) within the frame of the ongoing CWD surveillance programme. Further confirmatory analyses have been performed at the Norwegian Veterinary Institute. The Swedish cases shows similar features with cases of atypical CWD (Nor16CWD) previously detected in Norwegian moose, such as both cases being confirmed in older moose and only brain and brainstem were positive with no detectable prions in lymph-node. Further analysis will show the degree of similarity with Norwegian and Finish cases.

One hypothesis raised in Norway, based on absence of prions in lymph nodes and low detected prevalence, is that these atypical cases in old animals may be spontaneously occurring. Increased sampling in the Norrbotten area will contribute with epidemiological data indicating if these cases in older moose are contagious or not. Sixteen years of age is approaching the expected maximum life length of moose in the region where a single moose above 20 years of age was identified, while all other were younger. The hunting management in the region have resulted in a high proportion of old females and age data will be an important factor in the increased surveillance efforts in the region.

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The effect of defective viral particles on the course of infection with avian influenza virus in chickens and turkeys

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Defective interfering particles (DIPs) of influenza viruses contain highly deleted forms of genome segments and are propagated at the expense of fully infectious virus. Due to their interfering and immunostimulatory properties DIPs are considered as potential antiviral drugs and vaccine components. To study the influence of DIPs on the clinical outcome of infection with avian influenza virus (AIV) in poultry, experiments were performed in chickens and turkeys using two isolates of low pathogenic H7N7 AIV with different levels of DIPs (DIP(+)) or DIP(-)). Five birds were inoculated with either isolate and were co-housed with five direct and five indirect contact birds. Clinical observation was carried out for 14 days. At specified time-points oropharyngeal and cloacal swabs were collected and analysed using RT-qPCR.

No clinical signs, mortality or transmission to contact birds was observed in chickens infected with DIP(+) or DIP(-). Turkeys infected with DIP(+) showed slight apathy but no mortality. The virus was transmitted efficiently only to direct contact birds. In contrast, turkeys from the DIP(-) group showed clinical signs of infection (apathy, lower water and food intake, respiratory system disorders). Mortality was observed in inoculated (3/5), direct (4/5) and indirect (2/5) contact group. High level of shedding and seroconversion was found in all turkeys. The results suggest that the presence of DIPs in the inoculum mitigates the clinical course of infection and prevents mortality and aerosol transmission.

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Modulation of HPAIV H5N8 infection in naturally LPAIV - exposed Mallards

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The 2016-2018 highly pathogenic avian influenza virus (HPAIV) H5Nx clade 2.3.4.4b outbreak was the most severe ever reported for Germany with unprecedented mortality rates.

To determine the role of wild birds in clade 2.3.4.4b HPAIV dynamics after prior field exposure to low pathogenic AIV, mallards were infected with clade 2.3.4.4b H5N8 (heterologous infection) and rechallenged 21 days after infection (homologous re-infection). Prior to the experiment these mallards were kept as sentinels for field AIV circulating among wild birds. Regular testing revealed infection with four low pathogenic AIV subtypes of these mallards over time. Susceptibility of the mallards towards the 2016 H5N8 after AIV pre-exposure, viral shedding and transmission to seronegative contact ducklings were investigated.

Upon heterologous infection the mallards, although one died 2 dpi, did not develop prominent clinical disease, but viral shedding was sufficient to infect three out of four contact animals fatally. The surviving contact duck showed protracted viral titer increase and symptoms. Infectious virus could be isolated from several water samples.

After the homologous rechallenge of surviving ducks, neither clinical disease nor virus shedding was observed for directly inoculated mallards nor newly cohoused contact ducklings.

In our study, we simulated a HPAIV H5N8 outbreak under semi-natural conditions with AIV pre-exposed waterfowl. We postulate that AIV pre-exposed mallards might become a key-player and Trojan horse in clade 2.3.4.4b HPAIV dynamics, as our infection-model showed that the mallards were clinically protected, but still shed virus efficiently.

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Oral Presentations

African Swine Fever

Promoter mapping and transcriptome analyses of the African Swine Fever Virus

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The African Swine Fever Virus (ASFV) is a nucleocytoplasmic large DNA virus that causes lethal and incurable haemorrhagic fever in pigs. ASFV carries out RNA transcription and modification independently of host cell machinery. ASFV encodes an 8-subunit RNA polymerase (RNAP), a poly-A polymerase, and mRNA capping enzyme. Strikingly, the ASFV transcription system, encompassing ASFV-RNAP and general transcription factors, is reminiscent of the RNAP-II-like system. Interestingly, ASFV utilises a mix Vaccinia-like viral transcription factors along with divergent homologs of the basal factors TATA-binding protein (TBP) and transcription factor II B (TFIIB) for transcription initiation. However, the underlying molecular mechanisms of ASFV-RNAP and its factors are unknown, as is the promoter architecture and DNA motifs including TATA-boxes and B-recognition elements that could serve as binding sites for TBP and TFIIB, respectively.

We have applied a combination of deep sequencing techniques including CAGE and RNA-seq to determine (i) the global transcription start site (TSS) map and (ii) the transcriptome of the ASFV BA71 strain. We could assign primary TSS's to 151 ASFV-BA71V genes with high confidence, enabling correct annotations for ORFs with alternative start codons, investigation of ASFV RNA 5' untranslated regions, while also predicting novel genes and antisense RNA. Importantly, we have determined the promoter DNA consensus elements by sequence analyses of the regions surrounding the TSS, uncovering conserved motifs upstream of early and late genes. We characterised the transcriptome of infected Vero cells at 5h (early) and 16h (late) post-infection, which indicated 74 genes were significantly differentially expressed.

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Smallholders' perceptions on biosecurity and disease control in relation to African swine fever in an endemically infected area in northern Uganda

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This study investigated how increased understanding of knowledge of ASF epidemiology and control in low income settings can be transferred into successfully implemented biosecurity interventions on farm and community level. Structured interviews with 200 randomly selected, pig-keeping households in northern Uganda were undertaken three times. Perceptions related to general biosecurity, hypothetical control interventions, and attitudes towards pig farming were investigated by measuring the agreement to statements using a Likert scale.

Respondents generally conveyed positivism towards pig farming, biosecurity, and the potential of biosecurity for preventing ASF outbreaks. These positive attitudes, as well as the will to invest in biosecurity, were reduced in households that had experienced ASF outbreaks. Among the control interventions change of boots before entering the pig stable was highly accepted and seasonal adaptation of pig rearing times accepted on medium level. Statements on preventive sales of healthy pigs in connection with outbreaks and on buying pork products from slaughter operations receiving ASF-contact pigs received low acceptance, increasing, however, for households that had experienced ASF outbreaks. Consumption of pork from ASF infected pigs was generally not accepted.

Communicating information regarding the ASF not being zoonotic, and how the virus is neutralized will be important for increasing acceptance and enhancing implementation for the hypothetical control interventions preventive sales, safe slaughter, and consumption of processed and safe pork. Likewise, participatory development to adopt any control interventions to the local context on community level will be necessary for successful implementation.

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The African swine fever virus A179L Bcl-2 family member is required for efficient replication in porcine macrophages

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The African swine fever virus encoded anti-apoptotic Bcl-2 family member A179L binds to pro-apoptotic members of the cellular Bcl-2 family thus exerting an anti-apoptotic effect and additionally binds to beclin-1 thus inhibiting autophagy. Structural analysis and binding dynamics of A179L bound to BH3 domain peptides revealed A179L is the first anti-apoptotic Bcl-2 family member to bind all core death inducing mammalian Bcl-2 proteins. Additionally we demonstrated that A179L inhibits stress-induced apoptosis mediated through the ATF4/CHOP pathway. We demonstrated that deletion of A179L gene from the genome of a tissue-culture adapted ASFV strain BA71V did not reduce ability of the virus to replicate in Vero cells. In contrast deletion of the A179L gene from the genome of the virulent ASFV isolate Benin97/1 dramatically reduced the ability to replicate in primary macrophages. Activation of the pathways targeted by A179L in macrophages infected with the Benin A179L deletion mutant is under investigation to determine which is particularly important for virus replication in macrophages.

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Comprehensive approach to control and prevent African Swine Fever in Italy

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African Swine Fever (ASF) is a threatening disease because its spread may result in severe economic consequences in the swine sector. In the past several incursions were reported in mainland Italy (1967-69-83), all the outbreaks were efficiently controlled and eradicated; however, the disease has been endemic in the island of Sardinia as from 1978.

As from early 2015, strict cooperation between national and regional authorities has led to enhanced measures to eradicate ASF from Sardinia, with very promising results. A so-called "Unità di Progetto" was established to ensure full synergy between all public bodies concerned, aimed at the implementation of the new measures established to tackle the specific risk factors that made it possible the persistence and the spread of the ASF virus.

The most effective measure to eradicate ASF virus was the active culling of almost 4 000 illegal free ranging pigs that were still kept in the historical endemic area.

At a national level, it is not possible to disregard the risk of ASF introduction from the foreign countries. The persistence of the infection in certain eastern European countries (with which Italy has strong links), as well as the occurrence of the disease in new areas (e.g. Belgium), leads to an increased alert level. In particular, the human factor has been taken into consideration as a possible vector for the virus.

For these reasons, the Health Ministry introduced a comprehensive plan with the aim to increase the preparedness and the awareness to confront the ASF risk.

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ASFV evolution in the natural host - a co-evolutionary virus-vector arms race?

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African swine fever (ASF) is a devastating disease of swine with global economic impact. Despite almost 100 years of intensive research and its occurrence on four continents, very little is known about the causative agent, African swine fever virus (ASFV). In particular, questions about viral evolution and why ASFV is still the only known member of its genus (Asfivirus) and family (Asfarviridae) remain to be answered.

In this study, we used next-generation-sequencing for the identification and characterisation of numerous endogenous ASFV-like (EASFL)-elements in the genome of *O. moubata*, the natural tick vector of ASFV. We showed the existence of EASFL-elements in all soft ticks of the *O. moubata* complex including recent and 100 year-old *O. porcinus* field ticks from Africa. Through orthologous dating and molecular clock analysis we were able to show that the integration into the tick genome occurred at least 1.46 million years ago. Further experiments, including tick infection experiments and small RNA sequencing, indicated that the newly discovered EASFL-elements most likely protect the tick from ASFV-infection through RNA interference, and that this protection might be dependent on the geographical origin of the tick and the virus. Therefore, our data suggest the existence of a “co-evolutionary arms race” between ASFV and its natural tick vector. We believe that this research on the EASFL-elements is pivotal to a better understanding of the evolutionary dynamics and genetic variability of ASFV and the role of endogenous viral elements in the arms race between viruses and their hosts.

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Generation of defined gene deletion mutants of African swine fever virus as live vaccine candidates

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African swine fever virus (ASFV) is a large DNA virus representing the sole member of family Asfarviridae, which causes a fatal disease of domestic pigs and wild boar. ASFV is endemic in 28 African countries, and 24 different genotypes have been described. Few of them were temporally introduced to Europe, and since 2007 a highly virulent genotype II virus spread over many Eurasian countries. Up to now, no vaccines are available, but attenuated ASFV mutants are the most promising candidates, since surviving pigs are usually protected against challenge with similar virus strains.

To develop putative live or single cycle vaccines we started to generate gene deletion mutants of an Armenian genotype II-, and a Kenyan genotype IX isolate lacking known nonessential genes (encoding TK, dUTPase, CD2v, 9GL), presumably essential genes (p12, pA104R), or genes with widely unknown functions (pK145R, p285L, p22). Recombination plasmids were prepared by cloning of ASFV genome fragments, and substitution of the desired open reading frames by reporter cassettes for fluorescent proteins (GFP, DsRed), or human CD4. After transfection with these plasmids, a permissive wild boar lung cell line (WSL) was infected with ASFV, and the mutants raised by homologous recombination were selected utilizing the fluorescence of infected cells, or antibody-mediated cell sorting. To enable the purification of ASFV mutants lacking essential genes, stable WSL cell lines expressing the respective virus proteins were prepared, and the CRISPR/Cas9 system was used to enhance mutagenesis. The in vitro replication properties of successfully purified ASFV recombinants are currently investigated.

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Biological and genetic characteristics of African swine fever virus adapted strain ASFV/ARRIAH/CV-1/30 derived from Odintsovo 02/14 isolate

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African Swine Fever (ASF) is an acute, contagious and one of the most fatal diseases of domestic swine. Up till today, there is no effective vaccine against it. However, attenuated strains of ASFV as a candidate for vaccine development is still one of the most promising approaches.

ASFV isolate Odintsovo 02/14 had a fatality rate of 85,7% when used to infect pigs. It was later used for adaptation in CV-1 continuous cell line. The adapted virus from the 20th passage was used for swine bioassay, fatality rate decreased to 37,5%, all surviving pigs were protected from challenge by 1000 hemadsorbing doses (HAD) of homologous virulent virus Arm 07.

Adapted virus from 30th passage was also used for bioassay. In this experiment we used two groups of pigs, healthy and weak. In the group of healthy animals, all animals survived, while in the second group only 50% survived. Thus, the common viral fatality rate reached 16,7%. Despite the fact that all pigs that survived in the experiment were protected from challenge by 1000 HAD of Arm 07, further attenuation of such viral variant is required.

The whole genome of the adapted virus from the 30th passage was sequenced, and consequently compared to the original isolate Odintsovo 02/14 and to the reference strain Georgia 2007/1. Comparison showed point mutations in 4 genes (M1249L, NP419L, E199L и I196L), and a huge deletion in the left variable region of the genome of 2 945 base pairs, located between 180975-183920 base pairs of Georgia 2007/1.

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African swine fever in domestic pigs - Epidemiological insights from Estonia

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African swine fever (ASF) was first detected in Estonia in September 2014, and the first ten months the virus circulated only in wild boar population. The first ASF outbreak in domestic pig farm was confirmed in July 2015. From 2014 to 2017, the virus spread through the wild boar population of the entire country leaving only some islands free of the infection. Within the same period, 27 domestic pig farms were affected.

In the present study, we retrospectively analyzed the epidemiology of ASF in all domestic pig outbreak farms in Estonia. The study summarizes the results of the outbreak investigations and describes the course of the disease in herds. Furthermore, it includes statistical analysis, as well as description of clinical manifestation of the disease and identification the possible origin of the virus.

The results of the study suggest that the presence of ASF virus in wild boar populations is the main risk for farms to become infected. The spread of the virus within farms has been slow and the contagiousness of the virus has been relatively low. Clinical manifestation of the disease has been unspecific and the first clinical symptoms mild in most of affected herds. Herd-level mortality was reported as being generally low and often limited to a single pen or unit.

Following the study results, it can be hypothesised that in most of the farms, the virus was probably introduced via indirect transmission routes resulting from a lack of biosecurity, sometimes due to minor errors in biosecurity procedures.

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Experimental study of soft tick vector competence for African Swine Fever in Europe

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African swine fever (ASF) is a lethal viral disease that has spread to Europe and Asia. Infected tick bite is one of the known routes of ASF virus (ASFV) transmission. Some species in the *Ornithodoros* genus are competent vector for ASFV. However, their role in transmission and maintenance of ASFV currently circulating in Europe remains to be determined.

Three *Ornithodoros* species were selected for transmissions trials: *O. moubata* (known vector in Africa), *O. erraticus* (reported vector in Portugal) and *O. verrucosus* (suspected vector in Ukraine). Four ASFV strains were selected (Liv13/33, OurT88/1, Georgia2007/1 and Ukr12/Zapo) and matched to tick species, which yielded five pairs for vector competence testing: *O. moubata*-Liv13/33 (OmL), *O. moubata*-Georgia2007/1 (OmG), *O. erraticus*-OurT88/1 (OeO), *O. erraticus*-Georgia2007/1 (OeG) and *O. verrucosus*-Zapo (OvZ). Tick infection was attempted by feeding on viraemic pigs then re-feeding two to eight months later on healthy pigs to test for virus transmission ability. Only *O. moubata* was able to transmit ASFV. For other pairs, ticks were crushed and the homogenate of supernatant was injected in naïve pigs. All inoculated pigs developed clinical signs and viremia within a week, confirming ASFV was infectious in these tick-virus pairs.

For the first time, the vectorial competence of *O. erraticus* and *O. verrucosus* for European strains was tested under experimental conditions and showed the ability of these both ticks to keep the virus infectious after feeding on viraemic pig. These experiments reinforce the importance of studying underlying mechanisms of soft tick vector competence for ASFV.

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African swine fever virus Armenia/07 virulent strain controls IFN- β production through cGAS-STING pathway

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African swine fever virus (ASFV) is a complex, cytoplasmic dsDNA virus, currently expanding throughout the world. Currently circulating virulent genotype II Armenia/07-like viruses causes fatal disease in pigs and wild boar, whereas attenuated strains induce infections with varying levels of chronic illness. Sensing cytosolic dsDNA, mainly by the key DNA sensor cGAS, leads to the synthesis of type I interferons, and involves signaling through STING, TBK1 and IRF3. STING phosphorylates and translocates from the ER to the Golgi and to the perinuclear region, connecting the cytosolic detection of DNA to the TBK1-IRF3 signaling pathway.

We demonstrate that attenuated NH/P68, but not virulent Armenia/07, activates the cGAS-STING-IRF3 cascade very early during infection, inducing STING phosphorylation and trafficking through a mechanism involving cGAMP. Both TBK1 and IRF3 are subsequently activated, and in response to this, high level of IFN- β production was produced during NH/P68 infection; in contrast, Armenia/07 infection generated induced IFN- β levels below those of uninfected cells. Our results show that virulent Armenia/07 ASFV controls DNA sensing at the cGAS-STING pathway, but these mechanisms are not at play when porcine macrophages are infected with the attenuated NH/P68 ASFV.

These findings show for the first time the involvement of the cGAS-STING-IRF3 route in ASFV infection, where leading to IFN- β production or inhibition was found after infection by attenuated or virulent ASFV strains, respectively. Our results reinforce the idea that ASFV virulence vs. attenuation may be a phenomenon grounded in ASFV-mediated innate immune modulation where the cGAS-STING pathway might play an important role.

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Vectored vaccines for African swine fever virus: testing potential T-cell antigens delivered via adenovirus and vaccinia vectors

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African swine fever virus (ASFV) is a lethal haemorrhagic disease of domestic pigs that is threatening global food security and for which there is no licensed vaccine. Studies with attenuated viruses have shown that cellular immunity plays a pivotal role in protection against virulent ASFV. In an attempt to identify T-cell antigens, we previously screened responses of lymphocytes from pigs immunized with pools of plasmids and recombinant vaccinia viruses expressing ASFV genes. Promising genes were cloned in adenovirus and modified vaccinia Ankara (MVA) and used in pools in immunization experiments. A pool of eight viral genes was identified that protected pigs from severe disease after challenge with virulent ASFV when delivered by adenovirus-MVA prime-boost vaccination. With the aim of further reducing the number of genes in such a potential vectored vaccine, different sub-pools of the eight genes were tested. Interestingly, two of the most immunogenic viral proteins were not present in the pool that conferred highest protection, suggesting these viral proteins are not important for protective immune responses. We also observed that heterologous prime and boost with adenovirus and MVA had no advantage over prime and boost with adenovirus alone, relevant for reducing the complexity of a future vectored vaccine. Further in-depth analysis of specific cellular and humoral immune responses to the different proteins will allow us to narrow down viral antigens that may confer protection, and gain insight on immune responses that may correlate with protection.

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Immunopathogenesis and diagnosis of african and classical swine fever virus co-infections in domestic pigs

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African swine fever (ASF) and Classical swine fever (CSF) are highly contagious viral diseases of swine. A highly virulent ASF virus (ASFV) has spread from Sub-Saharan Africa to Eastern Europe and recently has been reported also in Belgium. Likewise, in Asia, ASFV has been detected in China, Vietnam and Korea. CSF remains endemic in countries of Central and South America, Asia and Eastern Europe, some of them are also endemic for ASF. The aim of the study was to establish parameters related to CSF virus (CSFV) replication and the immune response during subclinical infection in pigs. On the other hand, to evaluate pathogenesis and immune response in domestic pigs after infection with a moderately virulent ASFV strain. Furthermore, to study the pathogenesis, diagnostic and immune response after CSFV and ASFV co-infection in domestic pigs. Twenty 9-days old piglets were infected with the CSFV strain that reproduced the CSF subclinical disease. After 4 weeks animals with subclinical or persistent form of CSF were inoculated with a previously characterized ASFV moderately virulent strain. Ten animals were used as CSF or ASF infection controls, respectively. Immunological and virological parameters will be presented, as well as the optimization of the differential ASF and CSF diagnosis. Previous results showed that in CSFV subclinically infected wild boars, the ASFV infection could be a trigger factor for the acute and haemorrhagic disease progression in CSFV subclinically infected animals. The virological and immunological factors associated with the haemorrhagic disease development will be discussed.

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Oral Presentations

Vector-borne Diseases

Identification of amino acid changes playing a role in RVFV attenuation in mice

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Rift Valley fever (RVF) is an emerging zoonotic bunyaviral disease relevant for both animal and human health. The virus (RVFV) is transmitted by mosquito bites or by contact with material from infected animals. Live attenuated vaccines have been shown to be effective to control the disease in animals but their use is still limited due to the risks associated to their residual virulence.

In a work aimed to analyze the effect of the mutagenic drug favipiravir on RVFV in cell culture, we selected a mutant virus highly attenuated in IFNAR(-/-) mice but still immunogenic in immunocompetent mice. Whole genome sequencing of this virus revealed a total of 42 nucleotide changes spanning the three genomic segments, leading to 23 amino acid substitutions in the viral proteins. Some of these changes are strong candidates to be contributing to viral attenuation, in particular those located within the catalytic core of the viral RNA polymerase as well as those in the NSs protein, considered to be the main virulence factor of RVFV.

In order to elucidate the potential role that these amino acid substitutions may play in viral attenuation, we have used a reverse genetics system to generate rRVFVs carrying selected single mutations and have analyzed their infectivity in mice. The identification of changes that could render attenuated RVFV variants yet retaining the ability to induce protective immune responses would open new strategies to improve the safety of RVFV live attenuated vaccines.

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Genome manipulation of 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. Recent data indicate that WNV outbreaks have increased in number and that clinical neuro-invasive USUV infections in humans may have been underestimated. Although it is well known that the antigenic characteristics of flaviviruses are mainly determined by their envelope (E) protein structures, virulence factors of USUV, unlike WNV, are still poorly investigated. Therefore, in this study by using the ISA (infectious-subgenomic-amplicons)-based reverse genetics method, recombinant wild-type (r-wt) and chimeric WNV/USUV viruses were generated. While r-wt USUV was not rescued from transfected BSR cells, r-wt WNV was successfully rescued as well as two chimeric viruses including r-WNVE-USUV and r-USUV5' UTR-WNV. r-WNVE-USUV is formed by the WNV genome backbone and the E of USUV whereas r-USUV5' UTR-WNV has the 5'UTR of WNV in the USUV backbone. Confirmation of viral growth was provided by immunofluorescence whereas genome stability was investigated by sequencing each virus after serial propagation (up to the 10th passage) in VERO cells. Rescued viruses were tested by neutralization using anti-WNV and anti-USUV hyperimmune sera and viral kinetics in vitro has been evaluated. r-WNVE-USUV was neutralized only by the USUV antiserum whereas r-wt WNV and r-USUV5' UTR-WNV were neutralized, as expected, by WNV and USUV antisera, respectively. In vitro growth analysis of rescued viruses did not differ from parental viruses. In vivo studies in mice to confirm (r-wt WNV) and explore (r-WNVE-USUV and r-USUV5' UTR-WNV) the biological properties of rescued viruses are currently on-going.

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Exploring the immunogenicity and efficacy of bivalent Rift Valley Fever and Bluetongue experimental vaccines

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Climate and globalization can be major drivers for the spread of arboviral diseases. Bluetongue (BT), a ruminant arboviral disease, became endemic in Europe since its first introduction from Africa and, nowadays, can only be controlled by vaccination. In the context of accelerated global warming other arboviral diseases of ruminants, such as Rift Valley fever (RVF), could follow similar introductory patterns, with unpredictable consequences for both animal and public health. Preventive vaccination measures when risk of disease outbreak is high could help to ameliorate such consequences but these measures should bring additional benefits. Bivalent vaccines could provide such incentives when deploying a vaccine against an endemic disease that prevents the spread of other emerging pathogen. In this work we explored two strategies for developing dual BT and RVF vaccines. Firstly, by the use of MVA recombinant virus expressing both RVFV and BTV vaccine antigens and, secondly, by generating chimeric RVF viruses encoding BTV antigens. When assayed in mice these vaccines were able to elicit specific immune responses against the heterologous antigens, demonstrating the induction of immunity and protection after challenge. Both vaccine strategies were also assayed in natural hosts (sheep) and evaluated in terms of immune responses induction and efficacy upon challenge.

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Vector competence for Lumpy Skin Disease Virus of laboratory-reared and field-collected insects from Switzerland

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Lumpy skin disease (LSD) is an OIE listed viral disorder of cattle following severe infections leading to high economic losses. The occurrence of LSD was restricted to the African continent. In 2015, the first European outbreak occurred in Greece, whereas countries from the Balkan region (Serbia, Kosovo, Bulgaria, Romania, Albania, Montenegro and North Macedonia) were affected in 2016 resulting in large economic losses. Due to intensive vaccination campaigns, only Greece and North Macedonia were reporting LSD cases in 2017. In 2018, Turkey, Russia and Georgia reported cases. Although the role of arthropods in the transmission of LSDV is unclear, *Stomoxys calcitrans* is considered a potential vector.

In this talk, we are presenting preliminary data of a project, funded by the Swiss Federal Food Safety and Veterinary Office (FSVO grant nr. 1.18.d), investigating the transmission efficiency (mechanical, biological) for LSDV of field-collected and laboratory-reared mosquitoes (*Aedes japonicus*, *Aedes aegypti*, *Culex pipiens*), *Culicoides* spp. (*Culicoides nubeculosus*, *Obsoletus* and *Pulicaris* group species) and flies (*Stomoxys calcitrans*). Arthropods were fed on LSDV-spiked blood, incubated under realistic fluctuating climatic conditions, and body parts (heads and abdomens) screened for viral DNA by qPCR at different time intervals post infection.

Viral DNA was detected in *S. calcitrans* up to 72 (Ct 26-34, abdomens and Ct 27-34, heads) hrs post oral infection whereas *C. nubeculosus* were still found positive (Ct 27-34, heads) after 7 days post infection. Transmission efficiency was investigated by recovering of viral DNA on cotton pads soaked in blood and FTA cards.

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Risk of transmission of LSDV from experimentally infected cattle to insect vectors

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Lumpy skin disease (LSD) is a neglected tropical viral disease of cattle, characterised by numerous cutaneous lesions disseminated throughout the body. Historically endemic to the African continent, in the last decade the distribution of the disease has widened and it has become a threat to Europe following the outbreaks of LSD in the Middle East and Eastern Europe.

LSD virus (LSDV) is a Capripoxvirus transmitted by insect vectors. Experimental and epidemiological studies have indicated a role for the stable fly (*Stomoxys calcitrans*) and the mosquito *Aedes aegypti*. Nevertheless, the relative importance of these vector species and others is unclear. A study was designed to explore the risk of transmission of LSDV from cattle to four different vector species: *Aedes aegypti*, *Culex quinquefasciatus*, *Stomoxys calcitrans* and *Culicoides nubeculosus*. Cattle were challenged with LSDV to produce a bovine experimental model used as a natural source of LSDV to the potential vectors. Cattle samples were taken to quantify LSDV in different tissues and characterise the disease. All insect species were allowed to feed on LSDV-challenged cattle at regular intervals and incubated for up to eight days. This data was then used to model the dynamics of LSDV infection and transmission. All four species were able to acquire and maintain LSDV for up to eight days post feeding, and the risk of transmission from bovine donor to insect was dependent on the severity of the disease. These results will help on the development of targeted control measures to prevent LSDV transmission.

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New clinical pattern of Bluetongue serotype 8 in continental France

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France is infected with two Bluetongue serotypes, BTV-4 and BTV-8. Since mid-December 2018, cases of calves born blind, puny and dying in a few days or weeks have been reported in different departments in continental France. The brains of these calves showed hydranencephaly lesions. Since January 2019, the number of reported cases has increased considerably. According to analyses carried out in departmental laboratories (positive PCR on calf blood samples) and in the Maisons-Alfort animal health laboratory of the NRL-ANSES (BTV National reference laboratory) (positive PCR in the blood and calves' spleen), it is very likely these clinical signs are attributable to bluetongue virus serotype 8 (BTV-8). Indeed, the NRL tested brains of these calves which proved to be negative for the Schmallenberg virus, another possible cause of this type of clinical manifestation. The detection of PCR-positive calves, less than one week old and born during the vector inactivity season, suggests that they were infected in utero. It appears that in infected farms, 2% to 15% of newborn calves were affected by these clinical manifestations. Investigations are under way to study this phenomenon more precisely, in partnership with local services. As of March 2019, the NRL has received and analyzed 418 blood samples from such calves (94% were serotype 8 RT-PCR positive).

After a brief description of BTV emergence in France, this paper will describe the new clinical and epidemiological pattern of BTV-8 in France and the molecular characterization of four BTV-8 isolates obtained from “dummy” calves.

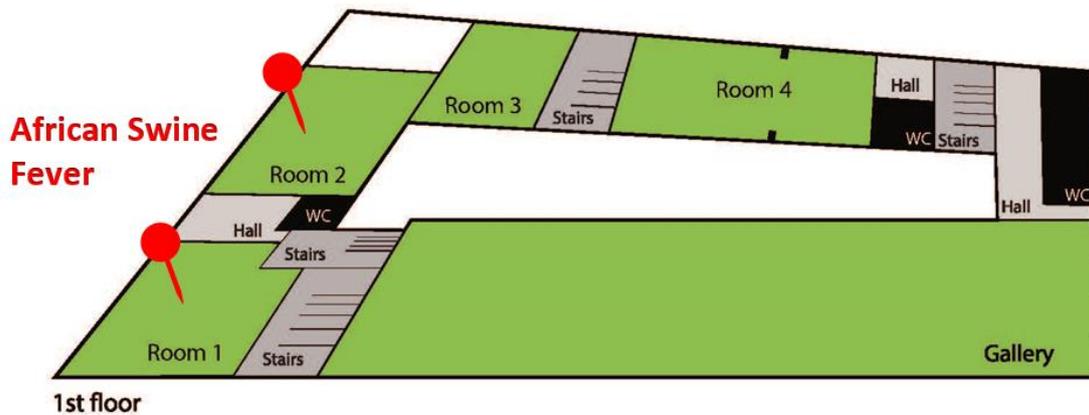
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Posters

Floor plan



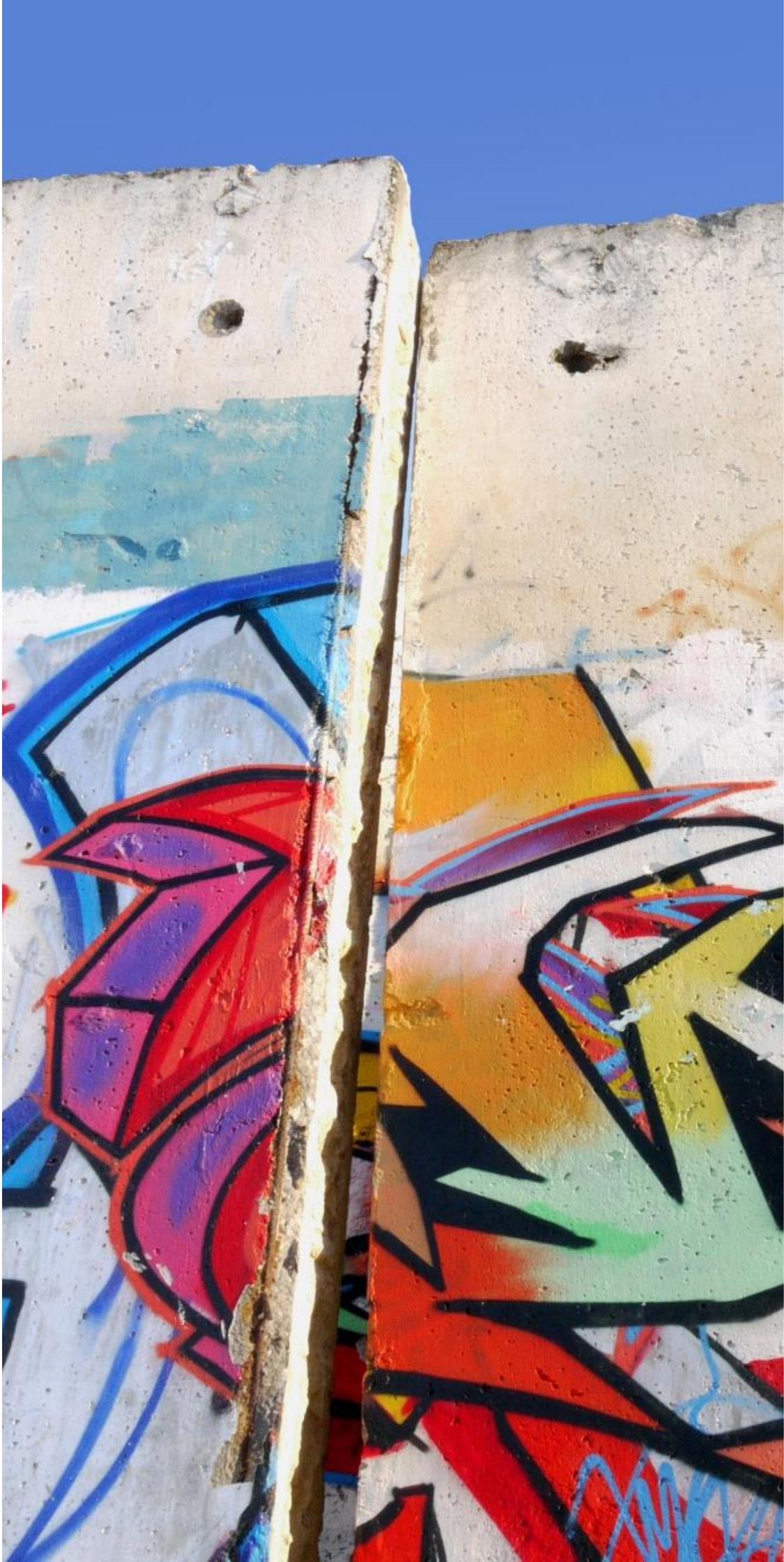
Poster Sessions

Poster sessions are scheduled for Monday, August 26th, 19⁰⁰ - 21⁰⁰ and Tuesday, August 27th, 15⁰⁰ - 16³⁰.

Poster presenters should be available to answer questions on their poster:

- ◆ Monday, 20⁰⁰ - 21⁰⁰ (Threats at the European Borders, Current Challenges inside Europe, Animal Health in a changing World)
- ◆ Tuesday, 15³⁰ - 16³⁰ (African Swine Fever, Vector Borne Diseases).





Posters

African Swine Fever

P17 - Development of a regression model for the determination of risk factors for the occurrence of predators at wild boar carcasses

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To obtain a broader insight into transmission patterns of African swine fever (ASF) among wild boars, we investigated scavenging activities on wild boar carcasses. To this end, thirty-two wild boar carcasses were exposed at nine sites in Greifswald, northeast Germany, from October 2015 until October 2016. The carcasses were monitored using wildlife cameras with motion sensing. One of the main issue of this study was to evaluate the number of visits of scavengers to the wild boar carcasses and the respective risk factors that might affect scavengers' activity behaviour. Since more than one carcass was exposed at each site, we had to deal with clustered data. Moreover, longitudinal data arose, since multiple observations were made on the same carcass over time. The dependent outcome variable 'number of visits' was measured repeatedly at several time points for each carcass. Similar study designs are becoming increasingly common in many clinical or biological settings. It is therefore of general interest to develop a statistical workflow for analyzing the resulting data.

We analyzed the data by fitting a generalized linear mixed model for count data. To identify the best fitting model, we tested for several parameters, e.g. distribution and overdispersion and finally selected a generalized linear mixed model for count data (negative binomial model) with fixed and random effect parameters.

A total of 5,828 visits of 22 species were counted at the exposure sites. Significant effects were found for the taxonomic group (birds vs. mammals), carcass type, site visibility and time since exposure.

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P12 - Inhibition of African swine fever virus replication by CRISPR/Cas9-mediated knockout of viral and cellular genes in wild boar lung (WSL) cells

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African swine fever virus (ASFV) is a large and complex double-stranded DNA virus causing a lethal hemorrhagic disease of domestic pigs and wild boar that is currently spreading throughout Europe and Asia. Presently, no approved vaccines exist to prevent this devastating disease, breeding of resistant pigs would be a promising alternative. To this end, we used the CRISPR/Cas9 system to target single (Hübner et al., 2018, Sci Rep 8, 1449) or multiple essential ASFV genes in a permissive wild boar lung cell line (WSL). Although stable expression of virus-specific single guide RNAs (sgRNAs) and Cas9 nuclease efficiently inhibited ASFV replication in vitro, lifelong presence of corresponding transgenes in swine might be problematic. Therefore, we also started a host genome-wide CRISPR/Cas9 knockout screen with a lentivirus-based library of more than 83,000 sgRNAs targeting all known protein encoding genes of swine. After transduction the WSL cells were infected with different ASFV strains, including virus from the current epidemic. Deep sequencing analysis of the sgRNA populations in surviving cells is underway. The target genes of enriched sgRNAs will be checked for mutations, and reproducibly affected genes or gene regions will be verified by stable transformation of naïve WSL cells with individual CRISPR/Cas9 plasmids, followed by ASFV infection. This might lead to identification of virus receptors, or host factors involved in pathways critical for ASFV replication, but unnecessary for cell viability. These factors might be suitable targets for antiviral therapy or deleted in genetically modified swine to make them resistant to ASFV infection

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P27 - Assessment of the suitability of different deterrents to prevent contact of wild boar with potentially ASF - positive carcasses

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In the course of the African Swine Fever (ASF) epidemic in Europe it became evident that the epidemic is maintained by an epidemiological cycle unknown before - the habitat cycle. Wild boar get infected through contact with infectious carcasses, favoured by the high tenacity of ASF-Virus. Therefore, one of the most important measures in ASF control is considered the search for and timely removal of potentially infectious carcasses. If immediate removal is not possible e.g. for logistic reasons, deterring wild boar from the carcasses might be an option.

A study to identify suitable deterrents is carried out on five sites in a forest next to the city of Greifswald, Germany. The study sites (four test and one control site) are standardised as far as possible. The carcasses (as an entity of attraction to the wild boar) are simulated by baiting automats (offering maize). Each site is monitored by two wildlife cameras. Baiting areas (2 X 4 m) are located within a rectangle of slender posts connected by a wire, the latter serving as a frame to carry the deterrents to be tested. Some preliminary results concerning the effectivity of different physical and chemical deterrents will be presented.

It has to be borne in mind that a temporary effect of deterrents may be sufficient since an eventual removal and proper disposal of the carcass has to be an imperative in ASF control.

Successful candidates of deterrents need to be tested on carcasses.

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P23 - African Swine Fever: lessons to learn from past eradication experiences

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Considering the absence of an efficient vaccination strategy, the efforts to control African Swine Fever (ASF) should be based on prevention, early detection, prompt reaction. Moreover, appropriate surveillance able to early detect the disease both in domestic and in wild animals, and the implementation of consolidated contingency plans are the best ways to front the occurrence of the infection. The main purpose of this study was understand throughout history, the lessons to be learned.

A systematic review was conducted through PRISMA flow chart approach. Query was defined for searching surveillance and control strategies applied by worldwide countries for ASF eradication. Papers describing the experiences collected in Belgium, Brazil, Cuba, mainland Italy, Portugal and Spain were included because having eradicated the ASF. History proved that eradication was possible in different epidemiological and cultural contexts. Classical surveillance strategies like active and passive surveillance both at farm and slaughterhouse levels, targeted surveillance in at risk areas together with conventional biosafety and sanitary measures led to eradication also in countries where the epidemiological tick's role was demonstrated.

Historical surveillance data analysis was useful to highlight that eradication was possible between the fifties and the nineties of the twentieth century when technological tools either were not available or less performing compared to current time, furthermore to emphasize that data of good quality both on surveillance and on animal population are a crucial factor to plan effective surveillance and to target proper control and intervention strategies for ASF eradication when established in a country.

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P25 - Infectivity of African swine fever virus after drying and heat inactivation on crops

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African swine fever (ASF), a viral disease that can cause a hemorrhagic fever like illness with exceptionally high lethality in pigs, has now conquered three continents and is threatening to spread further. Despite its limited host range, and non-existent zoonotic potential, its socio-economic impact is extremely high and many stakeholders are involved. Recently, the role of feed, water, and bedding has been discussed for disease transmission and it was demonstrated that infection can be induced under certain circumstances by liquids or contaminated feed.

To mitigate the risk of disease introduction by these routes, heat treatment of source materials could be an option. Here, we tested the effect of moderate heat on the survival/inactivation of ASF virus (ASFV) contaminating different crops.

In detail, 20g of wheat, barley, rye, triticale, corn and pea were contaminated with 900µl of infectious blood with a titer of 10⁶ HAD₅₀/ml. After 2h of drying at room temperature, mimicking a transport process, samples were incubated 1h at different temperatures between 40°C and 75°C for heat inactivation. Crop samples were washed with 5ml of cell culture medium by shaking and vortexing. The washing solution was analysed by real-time PCR and haemadsorption test.

First results indicated that while viral genome was detected in all contaminated samples, no viable virus could be recovered already after drying for 2h at room temperature. Possible reasons are under investigation therefore additional tests with protein-stabilized virus will be conducted.

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P10 - Challenges of ASFV whole-genome sequencing

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African swine fever (ASF) is a highly pathogenic viral disease of swine. Currently spreading in Europe and Asia, tremendous efforts are undertaken to characterize the whole genomes of the circulating African swine fever virus (ASFV) strains with special regard to virus evolution and molecular epidemiology. The viral genome, a linear molecule of double stranded DNA with a length of 170-194 kbp, harbors numerous homopolymer and repeat regions as well as inverted terminal repeats at both ends of the genome. Therefore, whole-genome sequencing using modern high-throughput sequencing (HTS) platforms is still challenging. Furthermore, different HTS platforms have unique advantages and disadvantages, e.g. in regard to error probability (especially in homopolymer regions) and read length, resulting in assembly artefacts that need to be evaluated carefully to obtain complete high-quality viral genome sequences. Here, we compared and combined different sequencing approaches, namely Ion Torrent S5, Illumina MiSeq and Oxford Nanopore's MinION for the generation of ASFV whole genome sequences. Furthermore, we employed myBaits®-based in-solution capture (target enrichment) prior to sequencing and sequenced from different sample types including organ tissue and cell culture supernatant. Altogether, we present challenges in sample preparation, sequencing and data analysis along with an optimized protocol for the generation of high quality ASFV whole genome sequences that has been employed for the generation of different ASFV whole-genome sequences from Europe. As an overall result, we were able to generate high-quality whole ASFV genomes which have the character of reference standards for future ASFV sequence comparison and phylogeny.

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P14 - Development and Validation of a new real time PCR

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African Swine Fever Virus (ASFV) is a notifiable, highly contagious disease becoming a serious threat worldwide. As there is still no vaccine or treatment available, monitoring and controlling of the disease is of outmost importance. In order to improve diagnostics, we developed and validated a reliable, sensitive and specific duplex real-time PCR kit.

VetMAX™ ASFV Detection kit composes of a duplex rtPCR including internal control. This kit is validated according to the French standard (NF U 47-600-2). For extraction of viral DNA two internal magnetic beads extraction methods were validated. In order to demonstrate the sensitivity and specificity of the kit, different internal and field studies (INIA, Spain; CVI, Netherlands; Germany) were carried out.

The limit of detection (LoD) was 16 genome copies per PCR reaction. The experimental LoD was 5E+03 copies per ml in serum and 1E+04 copies per ml in blood.

Test results of the 100 ASFV positive samples coming from Africa and Europe showed 100% sensitivity with all tested sample materials (blood, serum and tissue samples). By testing 1600 negative samples coming from ASFV free region a diagnostic specificity of 100% was demonstrated. Additionally, all tested samples positive for different pathogens scored negative in the ASFV specific assay.

In conclusion, VetMAX™ ASFV Detection kit fulfills all the validation criteria of PCR characteristics and complete method required by the French standard. The kit helps enable control of the spread of disease and monitors circulating virus outbreaks.

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P19 - No real perspectives of vaccine development against African swine fever? Biosecurity measures, disinfection as alternatives to prevent spread of the disease

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Due to the lack of effective vaccines against African swine fever (ASF), the disease might be controlled by stamping out of infected pigs and maintaining strict biosecurity measures. Therefore, the effective disinfection plays a crucial role in combat against ASF. Several active compounds are generally approved as capable to inactivate enveloped viruses, including ASFV. These active agents are commercially available disinfectants which differs in concentration and formulation. In the study four disinfectants were selected to represent the main groups of chemical compounds comprising of: sodium hypochlorite, oxidising compounds, glutaraldehyde and quaternary ammonium compounds. All of disinfectants were examined in three different concentrations and two different soiling environments. BA71v strain has been propagated in VERO cells. After 3 days of incubation (37°C, 5% CO₂), the cytopathic effect was observed. If the difference between titre of the tested disinfectant and the virus control was equal or higher than 4 log₁₀ the result has been considered as positive.

Only two out of selected disinfectants were considered as effective against ASFV. One, based on sodium hypochlorite in 1% and 0.5% concentration, the second, based on potassium peroxymonosulfate in 1% concentration. The other two, proved to be either cytotoxic or ineffective against ASFV. Selection of a proper disinfectant along with the proper concentration, the contact time and the influence of soiling level, should be taken into account by pig producers in order to guarantee the effectiveness against ASFV.

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P1 - MODULATION OF MACROPHAGE SUBSETS BY AFRICAN SWINE FEVER VIRUS STRAINS OF DIVERSE VIRULENCE

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African swine fever (ASF) is a devastating disease for which there is no vaccine. The aetiological ASF virus (ASFV) has a predilection for cells of the myeloid lineage and macrophages are its main target. Polarized activation allows macrophages to perform diverse functions in immune responses, which can determine whether these responses contribute to tissue repair or destruction. However, little is known about the interaction of ASFV with polarized macrophages. This study focused on the *in vitro* interactions of porcine monocyte-derived un-activated (moMΦ), classically (moM1), alternatively (moM2), and IFN-alpha activated macrophages with a virulent (22653/14) and an attenuated (NH/P68) ASFV strain. Attenuated ASFV NH/P68 presented a reduced ability to infect both moM1 and IFN-alpha activated moMΦ compared to 22653/14. Both viruses grew efficiently in all macrophage subsets, with only a delayed kinetic in moM1. Infection with NH/P68 but not 22653/14 resulted in a reduced expression of MHC class I in all macrophage subsets and higher levels of IL-1alpha, IL1-beta and IL-18 were released by NH/P68-infected moM1 compared to 22653/14 or mock-infected control. Neither strain affected moM1 polarization and similar or higher levels of IL-1alpha, IL-1beta, IL-6, IL-12, TNF-alpha were observed in ASFV-infected moMΦ compared to mock-infected moMΦ in response to LPS and IFN-gamma stimulation. Overall, differences observed between these ASFV strains suggested that virulent ASFV strains have developed mechanisms to covertly replicate in all macrophage subsets, and impairment of macrophage responses might affect the development of a protective immune response. Information generated in this study might underpin vaccine development effort.

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P11 - Whole-blood transcriptional signatures of heterologous protection by BA71DCD2 vaccination in pigs

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African swine fever (ASF) is a devastating pig disease causing huge economic losses to agricultural sector. While a relatively large body of knowledge on viral interference with host immune responses is generated from in vitro studies, little is known from in vivo studies, particularly mechanism of protection by some vaccine candidates. This study aims to identify whole-blood transcriptional signatures of heterologous protection by BA71DCD2 vaccination against challenge with virulent ASF virus.

An experimental vaccination/challenge in pigs was performed in BSL3 animal facilities at CRESA/IRTA, Barcelona, Spain. RNA was extracted from 60 whole blood samples that were collected from 10 pigs in two groups (vaccination/challenge vs control/challenge) over 6 time points. Paired-end sequencing of 60 libraries in one flowcell was performed on a NovaSeq by SNP&SEQ technology platform in Uppsala, Sweden. Reads were mapped to pig genome by HISAT2, abundance was estimated by StrinTie and differentiation was computed by Ballgown. In addition, qRT-PCR was used to verify expression of selected genes.

Whole blood RNA-Seq data analysis revealed predominant upregulation of immune response and programmed cell death in the control pigs 4 days post challenge with virulent Georgia strain, whereas vaccinated pigs seemed lack of such clear modulations instead of showing up slight changes in metabolism etc. Quantitative PCR further identifies upregulation of granzymes. The results suggest that BA71DCD2 vaccination, among other mechanisms, may promote cytotoxicity to eliminate infected cells and reduce excessive immune responses upon challenge with virulent virus, therefore achieving heterologous protection.

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P26 - African swine fever and hard ticks - the potential mechanical vector in virus transmission between wild boars?

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During the last 10 years, African swine fever (ASF) has turn into the most challenging threat for pig production in Europe and Asia. ASFV efficiently replicates in *Ornithodoros* spp. soft ticks, but does not in hard ticks which are widely distributed in Europe and are represented mostly by *Ixodes ricinus* and *Dermacentor reticulatus* species. Since the transmission of ASF between wild boars is mainly mediated by a contact with infected wild boar carcass or direct contact between infected and susceptible animals, other less important mechanical vectors including hard ticks are still uncovered.

Several hard ticks (n=25) identified as *D. reticulatus*, were collected from the field in Mazowieckie voivodeship, where the high density of ASFV-infected wild boars is present. Fifteen ticks were collected from fresh wild boar carcasses, out of which 11 were fully engorged with blood, the remaining 4 were adults but not yet engorged ones. The other 10 adult ticks were collected from clothes of the people walking in the forest. Real-time PCR confirmed the presence of ASFV DNA in total in 18/25 ticks, as follows: engorged ticks (11/11), adult ticks collected from wild boars (3/4) and collected from clothes (4/10). Viral isolation on PBMC was performed using selected (n=10) PCR-confirmed samples, the specific hemadsorption pattern was observed in 8/10 ticks.

This is the first evidence for viable ASFV presence in hard ticks collected from the field; therefore *D. reticulatus* may present a relevant source of virus and play a minor role in ASFV transmission among wild boars.

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P8 - African swine fever virus MGF110: retrospective genetic screening of the Russian isolates 2012-2018

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Multigene families (MGF100, 110, 360, 505, 530) of African swine fever virus (ASFV) is a “hotspot” genome region inclined to rapid changes over virus evolution. The MGF110 metamorphosis has been recently noticed in ASFV field isolates. Large deletion of MGF110 in ASFV isolate Estonia/2014 has been identified. Surprisingly, 12 out of 14 MGF110 genes were absent in ASFV Estonia/2014. Such a big deleterious mutation drastically has changed virus virulence and phenotype. Similar, but minor deleterious changes have been also identified in Sardinian ASFV isolate.

Here, we screened 39 ASFV isolates from different regions across the Russian Federation isolated between 2012-2018 with the aim to identify any genetic changes in MGF 110 region.

The MGF110 region of ASFV isolates from domestic pigs and wild boars was sequenced using standard Sanger sequencing approach using overlapping primer set. The sequences were aligned and compared with the ASFV Georgia 2007/1"(FR682468.1). Several single nucleotide polymorphisms were identified (C7620T, G7746C, A7651T, A7204C, T7207A, A8936G). No gene deletions were revealed. Overall identify across MGF110 region of ASFV isolates from the Russian Federation originated between 2012-2018 approached 99%. The data suggest relative stability of ASFV MGF110 region in Russian isolates from 2012-2018.

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P9 - Expression of ASFV CD2v protein fused with the porcine IgG Fc fragment

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The African swine fever virus (ASFV) is a causative agent of the dangerous disease of pigs causing detrimental economic losses for the affected countries. The spread of ASFV in the Russian Federation and Eastern European countries indicates the ineffectiveness of existing control measures and reinforces the need studying virus biology and disease pathogenesis.

ASFV surface antigens are important targets for host immune system and play the pivotal role in immune evasion mechanisms. ASFV CD2v protein has been identified as one of the serotype-specific proteins which carries out B- and T-cell epitopes.

The aim of our work is to generate the functional domains of the CD2 protein, fused with the Fc-fragment of porcine immunoglobulin G.

We expressed the extracellular part of the CD2v protein, which is capable to trigger of B- and T-cell response. The extracellular part of the CD2v protein (positions 17- 204 AA) was used to obtain the chimeric molecules fused with the Fc-domain of porcine immunoglobulin G at the N- and C-terminus.

The transient expression of recombinant proteins in CHO-S cells showed that the molecules are consist of two parts: CD2v antigen and the Fc-domain of porcine immunoglobulin G. Chimeric proteins CD2v-Fc and Fc-CD2v had molecular weight 120 kDa and retained the properties of their precursors-glycoprotein CD2v and porcine immunoglobulin G.

Further study will reveal the functional characteristics of the chimeric molecules CD2v-Fc and Fc-CD2v and prove the validity of increased representation of the ASFV CD2v protein.

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P6 - The integration vector for development of the chimeric African swine fever virus containing genes EP402R and EP153R of strains belonging to seroimmunotypes III and IV

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African swine fever (ASF) is a contagious, septic viral disease of pigs, characterized by fever, toxicosis, hemorrhages, and high mortality rate. The viral protein CD2v (EP402R) and a C-type lectin protein (EP153R) are directly associated with a haemadsorption phenomenon. Genotyping on the basis of the genetic locus encoding these two proteins also showed a concurrence with the grouping of ASF virus (ASFV) strains based on their seroimmunotypes.

The purpose of this project is investigation of the role of the CD2v and C-type lectin proteins of the ASFV in the induction of the type-specific immunological protection.

We designed an integration vector carrying the “shoulders” of homologous recombination, flanking the region of thymidine kinase (K196R) of the attenuated ASFV strain FK-32/135 (seroimmunotype IV), the selective chimeric “eGFP-P2A-PuroR” gene under the control of the CP204L (p30) gene promoter, as well as the genetic locus of transcriptionally active genes EP402R and EP153R of the ASFV strain MK-200 (seroimmunotype III).

This integration vector (pASFV_TK_eGFP-P2A-PuroR[EP153R-EP402R_MK-200]) will be used for generation of the chimeric ASFV strain FK-32/135 carrying the genes EP402R and EP153R of its own (seroimmunotype IV), and those from the attenuated strain MK-200 (seroimmunotypes III). The serotype specificity of the recombinant ASFV will be investigated in vitro by the haemadsorption inhibition assay (HADIA) and in vivo by bioassay (evaluation of protection of pigs immunized with recombinant ASFV followed by challenge with the corresponding virulent strains).

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P2 - The Large White Babraham pig line as a model to study immunity against African swine fever virus

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Since the introduction of African swine fever (ASF) to Georgia in 2007 the disease has spread across Eurasia and now poses a serious threat to global food security. Control of the disease is limited by the lack of an effective vaccine, although attenuated strains can protect against virulent ASF viruses (ASFV) experimentally. Understanding the immune response generated after inoculation with these attenuated strains will help steer development of both rationally attenuated live virus vaccines and subunit vaccines against ASFV.

Babraham pigs are an inbred line of animals derived from the Large White (Yorkshire) breed that were developed in the 1970s. SNP analysis has revealed that Babraham pigs are approximately 85% homozygous across the whole genome and furthermore are essentially homozygous across the MHC locus. The animals are sufficiently inbred to permit adoptive transfer experiment and so represent a powerful tool to study the immune response to ASFV.

We characterised both the cellular and humoral immune response in Babraham pigs after inoculation with the OUR T88/3 non-haemadsorbing strain of ASFV and subsequent challenge with the virulent OUR T88/1 strain. OUR T88/3 induced robust cellular immune responses as measured by ELISpot and flow cytometry and antibody responses as measured by ELISA. Strikingly, variable responses were observed after challenge with approximately 50% of the animals surviving and the survivors appearing to have stronger antibody responses. Our data suggests that the Babrahams present a useful model for studying the role of the antibody response in protection against ASFV.

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P7 - Expression of African swine fever virus proteins in a live-attenuated pseudorabies virus vector

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African swine fever virus (ASFV) causes a lethal disease of domestic pigs and wild boar. It is endemic in Sub-Saharan Africa, and currently spreading throughout Europe and Asia. Up to now, no vaccines are available, and little is known about immunogenicity of the predicted 150-160 different ASFV proteins.

Therefore, we expressed several of them in the pseudorabies virus vaccine strain Bartha (PrV-Ba), using an efficient mutagenesis system based on CRISPR/Cas9-supported rescue of a defective, GFP-expressing plasmid clone (Hübner et al., 2018, *J Virol Methods* 262:38-47). Transgenes were inserted at the nonessential glycoprotein G locus (US4) of PrV-Ba. Optionally the upstream protein kinase gene US3 was additionally deleted to improve attenuation. Protein expression under control of the strong CAG promoter was optimized by codon adaptation. Up to now, 12 ASFV gene products, including membrane (p12, p22, p54, pE199L, p285L, CD2v), capsid (p72), other structural (p11.5, p30, pA104R), as well as abundant nonstructural (pK145R, pB602L) proteins were individually expressed. Since pB602L is a chaperone required for proper processing of p72, both proteins, spaced by the “self-cleaving” teschovirus 2A peptide, were also co-expressed.

The mutations did not inhibit PrV replication in cell culture, and abundant transgene expression was demonstrated using monospecific antisera. In immunized pigs, the tested p30, p22 and pE199L mutants induced ASFV-specific antibodies. Thus, our PrV-based vector system allows the analysis of immune responses to single or multiple ASFV proteins in the context of a nonlethal virus infection of swine, and might provide a platform for vaccine development.

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P3 - Biological characterization of the Belgian ASFV isolate BELGIUM/01 in European wild boar

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African swine fever (AF) is one of the most important infectious diseases in domestic pigs and wild boar. Over the last decade, the disease has spread to several European and Asian countries and is now one of the major threats to profitable pig production world-wide. One of the more recently affected countries is Belgium. To date, only wild boar (n=809 confirmed positive cases as of May 30th 2019) are affected in a rather defined area in the Luxembourg region close to France, Luxembourg, and Germany.

To produce standardized reference materials for routine diagnosis and ring trials, and to characterize the virus in the main host, the Belgian strain BELGIUM/01 will be tested in four sub-adult wild boar (two male, two female). To this means $10^{4.5}$ hemadsorbing units will be oronasally inoculated. Upon inoculation, clinical signs will be monitored using a harmonized score systems, and samples will be checked for virus and antibodies employing routine diagnostic methods (PCR, virus isolation, antibody ELISA, lateral flow assays, and indirect immunoperoxidase testing). Results of the ongoing study will be presented and discussed.

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P18 - African Swine Fever Virus Fragments Sequence Analysis In Samples Collected From Wild Boars And Domestic Pigs

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The ASFV genome is a linear double-stranded DNA molecule that ranges in length between isolates from about 170 to 193 kbp. ASFV encodes for between 151 and 167 open reading frames (ORFs) and the differences in genome length and gene number are largely due to gain or loss of ORFs from the multigene families (MGF) encoded by the virus. Sequence analysis of virus genomes have established that central region is conserved but large length variations occur at the terminal ends, particularly within 40kbp of the left end of the genome, but also within 15kbp from right end of genome. ASFV genotyping is based on the analysis of three regions located at the conserved central area of the ASFV genome : sequencing of C-terminal end of the gene B646L encoding protein p72; sequencing of the central variable region within B602L-gene (CVR); sequencing of intergenic region between I73R and I329L genes in African swine fever virus and characterized by the presence of TRS.

For ASFV-positive clinical samples (spleen, kidney, lung, bone marrow, blood) collected in 2014-2016 years from infected wild boars and domestic pigs initial genetic characterization was performed by using standardized genotyping procedures on virus DNA extracted directly from homogenized tissues bone marrow and from blood samples. We compared the nucleotide sequences obtained from PCRs with those of Caucasus 2007 previously described representative isolates. We used Clustal Omega (<http://www.clustal.org/>) to perform multiple sequence alignments.

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P24 - African swine fever: Decomposition of wild boar carcasses

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When African swine fever is introduced into a wild boar population, it is crucial to determine the time of death of the first specimens found in the field as precisely as possible to estimate (i) the time point of disease introduction and (ii) the size of the affected area. We describe the decomposition process of eight carcasses exposed in cages: (1) wild boar vs. domestic pig, and (2) wild boar in different microenvironments (sunlight, shade, water, buried).

The opening of the abdomen occurred in the wild boar later than in the domestic pig. While only bones and desiccated skin were left from the domestic pig after twelve months, one and a half years later, a large proportion of a hard and crumbly substance remained from wild boar. In addition, the species community and development of necrophagous insects differed between the wild and domestic pig.

The persistence time of wild boar carcasses may vary substantially, depending on different factors including carcass size, microenvironment, season and insect activity. Wild boar seem to decompose slower than domestic pigs, maybe due to their hard and thick skin. This type of skin may retain moisture for a longer time and might slow down the rate at which maggots metabolize carcass material. Sunlight seems to accelerate the decomposition, while standing water may slow it down. The larger the carcass, the more environmental factors seem to lose relative importance.

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P16 - Diagnostic tools for the surveillance and control of African swine fever in domestic pigs and wild boar

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Numerous outbreaks of African swine fever virus (ASFV) have been reported in the last months, in wild boar and domestic pigs in Europe, Africa and Asia.

Since there is no vaccine available, diagnostic tools are essential for the control of the disease. Here, we present 3 different tools: a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and a lateral flow assay (LFA) for antigen detection; and one indirect ELISA (iELISA) for the detection of ASF-specific antibodies.

INgezim ASF CROM Ag and INgezim PPA DAS have been developed and evaluated for the detection of the ASFV in serum and blood samples. A cohort of negative blood samples from different origins have been assayed, as well as negative samples spiked with different concentrations of the native viral protein p72 or the inactivated virus. On the other hand, to determine the presence of antibodies in serum and blood samples, a new prototype of iELISA has been developed. Seven hundred and fifty four negative and 254 positive samples of domestic pigs and wild boar have been assayed.

The DAS-ELISA and ASF CROM Ag showed a specificity of 100% and 99.7% respectively, and sensitivity of 69 and 61% respectively for acute forms of the disease. Regarding the evaluation of the indirect ELISA, specificity was 100% and sensitivity 87.8%. Further external evaluation needs to be performed.

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P13 - More control and confidence in detecting African Swine Fever Virus in difficult sample material

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African Swine Fever (ASF) is one of the most severe diseases affecting wild boars and domestic pigs worldwide. The new virotype ASFV 2.0 PCR Kit from INDICAL comes with a double control strategy that offers a higher level of confidence in the interpretation of qPCR results, especially when testing difficult sample material. This triplex qPCR assay includes an endogenous internal control, ensuring sample presence and quality; and an exogenous internal control added to the lysis buffer, making sure that the extraction procedure was successful.

The performance of the virotype ASFV 2.0 PCR Kit was tested using in-vitro ASFV-DNA and DNA extracted from 245 positive and 223 negative samples, provided by several EURLs. Column- and bead-based extraction kits from INIDICAL, QIAGEN and Roche were used for the extraction of ASFV-DNA from blood, tissue and swab samples.

Testing a titration series of in-vitro ASFV-DNA showed the detection limit to be five copies of ASFV-DNA.

The assay demonstrated a high sensitivity and a specificity of 100%. No cross-reactivity was detected with porcine viral pathogens such as CSFV, PRRSV, SIV or PCV2. Testing the intra- and inter-assay variance showed excellent repeatability and reproducibility.

In combination with the IndiSpin Pathogen or IndiMag Pathogen extraction kits, INDICAL provides complete workflow solutions for accurate ASFV detection.

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P28 - Is the African swine fever epidemic in Estonian and Latvian wild boar subsiding? Epidemiological analyses of surveillance data

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In Estonia and Latvia, African Swine Fever (ASF) has been circulating since 2014. The control of ASF in wild boar has proven to be difficult. However, recent surveillance data from these two countries suggest an increase of exclusively ASF-seropositive and a decrease of PCR-positive wild boar. Following recent findings, it can be hypothesized that in areas, where the number of seropositive, but PCR-negative, animals dominates, the incidence of ASF infections is decreasing. When we investigated the course of the ASF epidemic, we tested the hypothesis that the ASF epidemic might be subsiding in Estonian and Latvian wild boar.

ASF surveillance data of wild boar, obtained from the CSF/ASF wild boar surveillance database of the European Union (<https://surv-wildboar.eu>), were used to estimate and compare the prevalences of ASF virus-positive and ASF-seropositive wild boar in defined regions between 2014 and 2019. Statistical analyses were performed using the software package R (<http://www.r-project.org>).

In both countries, an increase of wild boar that were ASF-seropositive, but PCR-negative was detected. This increase was first observed in areas that had been affected at the start of the epidemic in 2014, but later also in regions where ASF occurred only within the last 2-3 years. Simultaneously, the prevalence of PCR-positive wild boar decreased over time. The results of our study may indicate that ASF in wild boar has started to subside in Estonia and Latvia. However, it remains to be seen, if and where new ASF cases will occur in the future.

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P20 - African swine fever emergency management within an area of the Sassari ASL: measures adopted in the domestic and wild population for disease control purposes

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In the year 2013 a recrudescence of the ASF virus was registered in north Sardinia. The affected area accounted for of 591 sq km including 17 Municipalities, 35 were the outbreaks recorded in the period May-July 34 of which in only one Municipality (Nulvi). Soon after the emergency in domestic pigs and epidemic was signaled in the wild boars population. The Animal Health Service promptly sat up a strategy to control and extinguish the outbreaks within 24-48 hours. To expedite the operations some outbreaks were detected based only on clinical evidence, then a timely verification of the clinical and biosecurity conditions of the pig farms in the reference area was assessed. Repopulation in the extinguished outbreaks was forbidden, and the hunting was allowed only by derogation . Following the outbreaks as a result during the subsequent hunting season, 10 serological positivities and 11 virus positivities were recorded. All the control activities on pig farms and hunting were therefore very much intensified with following results: in 2014 the outbreaks recorded were reduced to 5, then to 3 in 2015 and finally no outbreak was signaled from 2016 to date. The non-compliance of farms with the regarding biosecurity measures has decreased from 34.81% in 2015 to 12.24% in 2018. The virus prevalence in hunted wild boars has declined from 0.03% to 0% during the three hunting seasons following the epidemic. Seroprevalence has had a mitigation from 0.03% to 0.01% today, however, affected animals are over 30 months old.

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P21 - African swine fever: passive surveillance activity in wild boar within three Wildlife Protection Areas

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In Sardinia, wild boar hunting is carried out from November to January. Samples of blood and spleen are performed on all the hunted wild boars to research ASF. Except for this period, very little information is available, particularly for wildlife protection areas (WPA) where hunting is always forbidden, in fact these areas could be considered a haven for plague-positive boars.

The purpose of this work is to illustrate the results of the passive surveillance activities performed by the Sassari ASSSL within three WPAs: Monte Pisanu, Foresta Anela, and Foresta Fiorentini, in whose vicinity virological positive wild boars were found during the last hunting season.

The area of each WPA (1200 hectares) was covered on foot by 80 employees of the Forest Service. In 6 days samples of faeces and of wild boar bones and two carcasses were collected. The discovery of mandibles has been decisive in determining the age of some animals, many bone findings have been found to be rather remote because lacking of bone marrow and therefore not suitable for laboratory tests. Virological positivity was found in two carcasses in the WPA of the Municipality of Bultei, This finding confirm a recent viral circulation signaled by two virus positive boars killed in the vicinity of this WPA during the last hunting season.

These results show how crucial are the passive surveillance activities carried out in wild boar within the WPA in order to verify the real epidemiological situation regarding African Swine Fever virus in protected areas.

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P22 - Extraordinary African Swine Fever Plan (ASF) in Sardinia: operational protocol and checklist criteria during official controls

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The African Swine Fever Extraordinary Program aims to eradicate the disease from the Regional territory of Sardinia. Since 2015 official controls are based on a risk map which splits the municipalities into 5 bands. The risk map takes into account the pig farms health qualifications and the areas where viral circulation in the wild boar was assessed, these areas are called "infected area of the wild". In the named infected areas, an enhanced biosecurity is required to prevent the contact between the domestic and the wild.

The objective of this work is to illustrate the methods by which the veterinary official control are structured and the requisites wanted by pig farms in order to acquire the highest health qualification named "PSA Certified farm".

Checks include a clinical visits, blood sampling, verification of compliance about pigs registration, animal welfare standards, biosecurity requirements, reproductive parameters and finally the disposal methods regarding porcine by-products.

Following the official control the attributed qualifications are "controlled farm" when the outcome of the inspection is unfavorable but there is at least clinical and serological compliance; "certified farm" when the outcome is favorable for all the parameters considered.

All the collected data are included in a computerized regional information system (Sisar-VET PSA) which shows that in the Sassari ASSL (north Sardinia) the percentage of non-conformities range from the 39.72% in 2015 to 21.8% in 2018.

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P15 - Evaluation of nasal swabs in surveillance programs of African Swine Fever in wild boar

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African swine fever (ASF) is one of the most important infectious diseases in domestic pigs and wild boar. Reaching Georgia in 2007, the ASF virus spread since then to several European and Asian countries and became a major threat for the pig production industries. On 12 September 2018 two wild boars were declared ASF positive in Belgium, contaminated by an unknown source. Since then nearly 3000 wild boar found dead or shot were analyzed. Because of the high risk for ASF virus spread, sampling the spleen was carried out under high security conditions. To facilitate sampling on found dead animals, we investigated if buccal and nasal swabs have the same sensitivity to detect ASF positive animals as spleen samples. For our analysis, buccal and nasal swabs together with spleen were sampled on the same animals and analyzed for ASFV by real-time PCR. After comparing X buccal and nasal swabs with the spleen, it became clear that the sensitivity of buccal swabs was insufficient in contrast to nasal swabs. We compared 183 nasal swabs and spleens from the same animal and obtained 100% concordant results. From these results we can conclude that nasal swabs can be used in surveillance programs for detection of ASF on found dead animals.

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P5 - The first animal trial with highly virulent African swine fever isolate from Poland. Clinical course and post-mortem lesions analysis

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African swine fever (ASF) still remains an unresolved problem around the world. Early detection of the disease seems to be crucial and currently is the only way to prevent spreading of ASF. In order to increase the effectiveness of combating ASF, the National Reference Laboratory in Poland performed a pilot study on ASFV pathogenesis in pigs after infection with highly virulent Polish ASFV isolate. The study presents a part of the project on elaboration of CRISPR/Cas9 system for the construction of recombinant ASFV strain.

Eight 5-week old piglets were infected with Pol18_28298_0111 isolated in Chelm region in 2018. Nasal infection has been conducted using 1000 HAU of ASFV. Firstly, infection caused acute form of the disease with no typical clinical signs. High fever (over 41°C) was diagnosed in two animals at 5th day post infection (dpi), fever was correlated with viremia, deaths were recorded without any other clinical signs at 6th dpi. Post-mortem examination revealed dark-chocolate coloured, enlarged spleen and high amount of exudate in abdominal cavity. Secondly, the subacute form of ASF was observed in remaining animals with joint swelling (one pig) and mild diarrhea (two pigs). Characteristic for ASF post-mortem lesions (i.e. petechiae in kidneys) were noticed after 14 dpi. Virus caused nearly 90% mortality at 22nd dpi, the shortest incubation period was estimated at 5 days, the longest at 16 days. No significant clinical signs and non-characteristic post mortem lesions makes ASF infection extremely difficult to diagnose during daily control of pigs within the holding.

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P4 - African swine fever in a Bulgarian backyard farm - a case report

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African swine fever (ASF) is one of the most threatening diseases for the pig farming sector worldwide. As an effective vaccine is lacking, strict application of control measures is the only way to fight the disease in both industrial farms and backyard holdings. With generally low biosecurity standards, the latter are at particular risk for disease introduction and offer challenging conditions for disease control. In the presented case report, we describe the overall course of an ASF outbreak in a Bulgarian backyard farm and the implemented control measures. Farm facilities and available data have been investigated to estimate the possible source, spread and time point of virus introduction. Contact to contaminated fomites entering the stable via human activities was regarded to be the most likely introduction route. The slow disease spread within the farm contributes to the hypothesis of a moderate contagiousity. As no further ASF outbreaks have been detected in domestic pig farms in the region, it could be demonstrated that successful disease control in small-scale farms can be reached.

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Posters

Animal Health in a changing World

P11 - Bacteriological and serological study of *Brucella* spp. and *Salmonella* spp. in high-risk regions of Azerbaijan

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Brucellosis remains the widespread zoonotic disease worldwide. Central Veterinary Laboratory implement serosurveillance to detect prevalence of brucellosis annually. *Salmonella* recognized as a dangerous zoonotic pathogen for Azerbaijan. Both *Brucella* spp. and *Salmonella* spp. cause animal abortion and fever that results in economic losses for the country. There are 14 brucellosis cases and 6 salmonellosis cases among small ruminants caused 10 abortions in Azerbaijan in 2011-2016. The aim of investigation is to study brucellosis and salmonellosis among small ruminants as co-infections.

In the framework of our investigation the milk, blood samples and abortion materials were collected from unvaccinated small ruminants by the local veterinarians from the high-risk regions of Azerbaijan. The samples were transferred to the Regional Testing Laboratory for processing. The milk samples were examined for *Brucella* spp. using Milk Ring Test. Blood samples were processed to detect antibodies against *Brucella* using the Rose-Bengal Test and confirmed by ELISA. The suspensions of abortion materials were cultured on *Brucella* Selective Agar to detect *Brucella* spp. and Xylose-Lysine-Tergitol 4 Agar to identify *Salmonella* spp.

Totally 90 milk, 270 blood samples and 10 aborted fetuses were collected from unvaccinated sheep. The 14 milk samples and 14 blood samples were positive for *Brucella* spp. *Brucella* spp. cultures were isolated from 4 aborted fetuses. *Salmonella* spp. were isolated from 6 aborted fetuses. *Brucella* spp. and *Salmonella* spp. were isolated from 4 animals.

It is recommended to study all collected aborted fetuses for both brucellosis and salmonellosis to get more data and analyze these infections as co-infections.

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P9 - Two A/H1N1pdm cases in turkey breeders in Northeastern Italy

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Since the 2009 pandemic, several international reports have described A/H1N1pdm virus infection of human origin in turkeys. The transmission is reported solely in turkey breeders, during artificial insemination.

Two cases have been reported in turkey breeder in Northeastern Italy, the first occurring in 2011 and the second in April 2019.

In both cases, the flock showed no clinical signs except for a sudden drop in egg production, which led to the prompt reporting of suspected notifiable disease for official sampling.

The National Reference Laboratory for Avian Influenza confirmed the presence of an A/H1N1pdm infection by detailed laboratory investigations including Haemagglutination Inhibition test, Real-Time RT-PCR, RT-PCR and nucleotide sequencing of the hemagglutinin gene.

Both cases occurred in the first weeks of the production cycle (in the third week in the first case and throughout the seventh in the 2019 case) and led to a drop in egg production of respectively 69.5 % and 46%.

Though such high decrease was temporary, egg production did not return to normal levels, remaining respectively 34 % and 16 % below the standard production curve.

Since the H1N1pdm human to turkey transmission occurs through artificial insemination, the major poultry breeder companies recommend seasonal influenza vaccination for their workers.

Considering that turkeys are refractory to this virus infection through aerosol and oral transmission routes, the awareness of biosecurity measures and the strict application of good working practices on the farm remain the key factors in order to prevent severe economic losses at holding level.

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P33 - Comparative analysis of the immune response induced by BoHV-4-based vector versus DNA vaccination

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In the recent years, Bovine herpes virus 4 (BoHV-4) has been considered as an attractive gene delivery viral vector, mainly for vaccination purposes in the veterinary field.

In the present study, a new infectious clone of BoHV-4 genome, clone as a bacterial artificial chromosome (BoHV-4-BAC) to express truncated form of glycoprotein D (tgD) of Bovine herpesvirus type 1 (BoHV-4-tgD Δ TK) was developed. BoHV-4-tgD Δ TK immunogenicity was compared with that a DNA vector expressing the same antigen (pC-tgD) in BALB/c mice model. After mice immunization, splenocyte proliferation assay, cytotoxicity assay, virus neutralization assay, antibody isotyping and cytokine analysis were conducted. Mice from both immunized groups developed significant humoral and cellular immune response toward BoHV-1 tgD. In almost all the assays, BoHV-4-tgD Δ TK group showed a better immune responses respect to pCDNA-tgD. It can be stated that BoHV-4 based viral vector is a better immunization system respect to DNA immunization, at least in this specific experimental setting.

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P28 - Main infectious agents found in bulls and boars semen : Impact on semen quality and diagnostic tools

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A variety of pathogenic contaminants might be isolated from semen, processed for artificial insemination programs: virus, bacteria and parasites .The growth in use artificial insemination has increased the risk of quick and widespread transmission of venereally transmissible pathogens .

The aim of this paper are as follows to provide information:

- On the main infectious agents transmitted by bulls and boars semen
- On the effects of infectious agents on the quality of the semen (poor motility, abnormal form, damaged acrosomes, sperm agglutination ...)
- On the diagnostics means to identify infectious agents in the semen

Different viruses have been detected in porcine and bull semen. Some of them are on the list of the world organization for animal health (OIE 2018 chapter 4.6).Other viruses are not in the list but may also isolated and cause disease and major economic losses to the industry. The period during which virus can be detected in the semen using different test and the impact of these virus on semen quality is shown based on the data from LNCR.

Regarding the bacteria isolated in the semen, we distinguish the recognized pathogenic bacteria such as leptopires and the bacteria following a contamination during the collection of the semen .The effect of such contamination have been reported to highly impair sperm quality, like sperm motility, morphology, and acrosome and membrane integrity, and have repercussions on the recipient sow and the reproductive performance.

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P17 - CHARACTERIZING FOOT-AND-MOUTH DISEASE VIRUS IN CLINICAL SAMPLES USING NANOPORE SEQUENCING

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Foot-and-mouth disease virus (FMDV) is the aetiological agent of foot-and-mouth disease (FMD), a globally important livestock disease affecting cloven-hoofed animals. Outbreaks of FMD cause large production losses, particularly in endemic regions such as Asia and Africa. Oxford Nanopore's MinION portable sequencer is able to generate long reads and produce real time data which can be used for strain identification, outbreak tracing and vaccine selection. The cost and size of the sequencer lends itself to insitu diagnosis and has been deployed for use during outbreaks such as Ebola and Zika. We investigated the suitability of the MinION to characterize FMDV in clinical samples and its potential use in FMD control programmes.

A single two-step FMDV universal PCR was used to amplify the capsid region of three FMDV serotypes: A, O and Asia1 from cell culture adapted viruses (n=3) and clinical samples (tongue epithelium (n=5) and oral swabs (n=12)). Amplicons were visualised by gel electrophoresis, quantified using a Qubit v3 fluorometer and prepared for MinION and Sanger sequencing. Sequences were assembled and compared using BioEdit.

Sufficient DNA for MinION sequencing was achieved from all cell culture adapted viruses, all epithelium and 50% of the oral swabs tested. Following sequencing, the consensus sequences from the MinION and Sanger were compared to determine sequence accuracy. This preliminary study provides information useful to assess nanopore sequencing as a tool for use with clinical samples. Future research could investigate the suitability of this pipeline on portable PCR platforms for use in endemic regions.

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P13 - Implementing Innovative Methods of Preventing Infectious Diseases using Geographic Information Systems (GIS) in Georgian Public Health

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Tularemia, Zoonotic disease distributed across the northern hemisphere in Europe and Central Asia, has also found in country of Georgia over the seventy years. Using GIS, we studied impact of precipitation on the distribution of Tularemia positive cases among the vectors in Georgia. For conducting GIS innovative analyze, layer of precipitation shapefile and imagery file from ArcGIS online were used, conjunction with Global Positioning System data of Tularemia positive samples of ticks. Our study indicated that, out of 1457 tested samples, collected during the fieldwork, only 27 (1.85%) ticks were positive on Tularemia, which were identified in only 4 districts of Georgia. Standard Deviation for Positive cases 2.7; mean 6.75; min 4 and max 10 obtained of statistical operations from the "Statistics" summary of attribute table of positive cases shapefile. Analyze showed that only four different types of positive ticks were found. *Hyalomma marginatum* were distributed in the extreme east of the country where precipitation is 300-500 mm; *Dermacentor marginatus* and *Haemaphysalis otophia* were places in a more central area of the country where precipitation varies from 600 to 1200 mm, and *Haemaphysalis parva* were spread more to the west, where the precipitation ranges 800-1000 mm. To conclude, the research has shown that precipitation may affect distribution on tularemia among the vectors, because the same type of Tularemia positive ticks are distributed in a specific geographic area, and to determine how specific ticks are spread on a particular precipitation level, additional research are recommended.

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P4 - The IDScreen® *Besnoitia* indirect 2.0 serum ELISA perfectly correlates with confirmatory techniques

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Bovine besnoitiosis (Bb), considered by EFSA as an emerging disease in Europe, cause important economical consequences. Serology is used to identify infected animals and prevent the introduction of infected animals into disease-free herds. We present validation data for the ID Screen® *Besnoitia* Indirect 2.0 ELISA, detecting anti-B. besnoiti antibodies in bovine serum samples.

Specificity (Sp) was evaluated with 814 cattle sera collected in 2011 from French bovine herds where Besnoitiosis had never been reported. All samples were found negative, giving a Sp of 100% (99,5 - 100,0%).

To verify the absence of cross-reactions with other apicomplexan protozoa, 15 *T. gondii* and *N. caninum* seropositive samples were tested: all were found negative for Bb, indicating the excellent exclusivity of the ELISA.

Sensitivity (Se) was assessed with samples confirmed by another serological method (ELISA or Western-Blot (WB)). 105/107 were found positive, giving a Se of 98.1% (95,6 - 100%).

In a independent study (ENVT, Toulouse; Departmental Laboratory, Ariège; France), 520 cattle samples (195 positive, 325 negative), previously characterized by WB were tested. The overall agreement was 99,2%/kappa= 0.984 [0.968 - 1]). With respect to the WB, the Sp was 99,7% (99,1 - 100,0%), and the Se 98,5% (96,8 - 100,0%).

The IDScreen® ELISA shows excellent sensitivity and specificity and an excellent agreement with the Western-Blot technique. Thanks to its high performance, the positive and negative predictive values remain high regardless of the prevalence of the tested herd, making the test a reliable method to prevent disease spread.

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P5 - FIRST EVIDENCE OF INTERFERON-GAMMA RESPONSE IN BESNOITIA BESNOITI NATURALLY INFECTED ANIMALS

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Besnoitia besnoitii (Bb) is an apicomplexan parasite causing bovine besnoitiosis. Cell mediated immune response (CMI) play an important role in Bb related parasites, such as *Toxoplasma gondii* (Tg) and *Neospora caninum* (Nc). In besnoitiosis, CMI may play a major role as a T-cell response was already described.

Here, the CMI to Bb was investigated by IGRA (Interferon Gamma -IFNg- Release Assay) in naturally infected animals. Heparinized whole blood and serum were taken from 54 cattle from a high prevalence infected herd, and were stimulated with Bb and Nc semi-purified antigens -specific and non-specific antigens- and controls (PBS, mitogen). IFNg in stimulated plasmas was detected using ID Screen® Ruminant IFN-g ELISA. Antibody (Ab) response against Bb, Tg and Nc were evaluated using IDvet's ELISAs.

For some animals, a specific interferon gamma secretion in response to Bb antigens was observed whereas the Nc extracts did not generate any IFNg response. 40/44 were Ab and IGRA positive, and 9 were Ab and IFNg negative. Only one animal was Ab positive and IGRA negative. Interestingly, 4 animals were Ab negative but IGRA positive; 1 was at the threshold, 2 of them were giving moderate signal and 1 a very strong response.

Bb seem to elicit a strong CMI response, globally correlated to the Ab response ($k=0.725$; [0.495-0.955]). However, the discrepant results require further research and raise the question to what extent the humoral response might reflect the disease protection status. IGRA tests can be used to investigate the CMI to Bb.

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P6 - A new indirect ELISA for the detection of *Besnoitia besnoiti* antibodies in individual and bulk milk samples

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Bovine besnoitiosis (Bb), considered by EFSA as an emerging disease in Europe, cause important economical consequence. Antibody detection is currently performed by different techniques on plasma or serum samples. This poster presents validation data for a commercially-available ELISA allowing for the detection of Bb antibodies in individual or bulk milks.

Paired individual blood and milk samples from 805 cattle were tested with the ID Screen® Bb milk and serum ELISAs. The kappa value was very high $k=0.920$ [0.886-0.948] demonstrating that Bb antibodies can be efficiently detected in milk.

Sensitivity was assessed with 333 paired samples containing at least one clinical case confirmed by serum ELISA or Western Blot. All animals found positive with the serum ELISA were positive with the milk ELISA, even weak positive serum samples.

Specificity (Sp) was evaluated through the analysis of 263 individual milk samples collected from 4 herds in France and Belgium with no history of bovine besnoitiosis. Measured Sp: 100% (99,6-100,0%).

To verify the absence of cross-reactions with other apicomplexan protozoa, 90 milk samples from an *N. caninum*-infected French herd (abortions; 50% seroprevalence) were tested; all samples were found negative, suggesting an excellent exclusivity.

This study demonstrates the possibility of detecting Bb antibodies by ELISA from milk, and an excellent agreement with the serum ELISA. Bulk milk ELISA results from 12 herds exhibiting different seroprevalences were also tested. The kit is able to detect low to moderate prevalences, giving the possibility of bulk milk testing for Bb herd monitoring/surveillance.

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P10 - Molecular and Pathological Study of Maedi Disease in Awassi Sheep in Jordan

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Maedi, also called Ovine Progressive Pneumonia (OPP), is a chronic respiratory disease of sheep caused by a slow lentivirus. It can be a trade limiting disease, resulting in very significant economic losses. We have observed lungs tissues which had lesions compatible to Maedi in 6 months old lambs, without having clinical signs of the disease. In this study, we report the seroprevalence of Maedi using commercially available ELISA kit, and pathological lesions associated with the disease, using histopathological examinations. Out of 130 sheep blood samples tested we found 43 samples (33%) were positive. Out of 117 lungs of sheep examined, around and older than six months old, collected from slaughterhouses in Northern and Middle of Jordan, 17 lungs had concurrently the three characteristic pathological lesions; lymphoid nodules in the parenchyma of the lungs, peribronchial lymphoid hyperplasia, and smooth muscle hyperplasia. We considered these animals positive for Maedi. In addition, we found 38 lungs had independent lymphoid nodules, 27 had lymphoid nodules and peribronchial lymphoid hyperplasia, and 18 had lymphoid nodules and smooth muscles hyperplasia without peribronchial hyperplasia. These were considered strongly suspected for Maedi disease. We tested six tissues from the 17 tissues considered positive, using conventional PCR, and were positive for Maedi. The PCR product will be sequenced and results will be compared to other Maedi lentiviruses in the region. These results demonstrate not only the Maedi lentivirus is circulating in Jordanian Awassi sheep flocks, but also found in young lambs and inducing pathological lesions in the lungs.

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P1 - Development of a multiplex assay for antibody detection in serum against pathogens affecting ruminants

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In many cases, distinct pathogens of domestic and wild ruminants induce similar symptoms rendering it difficult to precisely identify the causative agent during an epizootic outbreak. This is the case of viruses such as Crimean-Congo haemorrhagic fever virus, Rift Valley fever virus and Schmallenberg virus, which belong to the Bunyavirales order, bluetongue virus, a reovirus, or the bacteria *Mycobacterium bovis*. These pathogens, which can co-circulate in Middle East, Africa and Europe, cause an important economic burden and have an impact on the health of both humans and animals. Thus, there is a need for development of multiplex assays that will allow the simultaneous detection of antibodies against these pathogens in a single serum sample.

In the present work, a multiplex bead-based microarray was developed for the detection of antibodies against the above-mentioned pathogens in different species of ruminants.

Immunogenic antigens of each pathogen were produced recombinantly and used to coat different carboxylated magnetic bead regions (Luminex) to develop a multiplex bead-based microarray.

Initially, the assay performance was evaluated using a limited number of positive and negative experimental sera from cows and sheep infected or vaccinated with these pathogens. To assess the specificity of the test around 100 negative field sera from cows, sheep, goats and horses were analysed. Although more samples need to be tested to validate the assay, the preliminary results showed good values of sensitivity and specificity, demonstrating its utility as a surveillance diagnostic tool in countries where these pathogens could co-circulate.

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P22 - Impact of infection with Influenza A H1N1 on the faecal microbiome of weaned piglets

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One aim of the collaborative project "Kolnfekt" is the establishment of the pig as an animal model for research into infectious diseases of humans. In this context, the systemic effect of a respiratory infection with Influenza A H1N1pdm09 was analyzed. In the present study, we analyzed the composition of the faecal microbiome as a proxy for the gut microbiome, which has a significant impact on the health status of humans and animals. Here, the focus was on bacteria and viruses, but also parasites were analyzed.

On all days all of the trial, infected swine were clinically inapparent and the highest virus shedding was observed four days post infection (dpi). Samples for microbiome analyses were taken before and 4, 21, and 31 dpi. Shotgun DNA libraries prepared from RNA of these samples were sequenced using Ion Torrent-sequencing and the resulting raw data were taxonomically classified using the software pipeline RIEMS.

While the virome compositions were nearly unchanged throughout the trial, significant changes regarding the bacterial portions of the microbiomes were detected 4 dpi. On d 21 pi, the microbiomes resumed to the base microbiome. The most marked changes at 4 dpi were observed for the Prevotellaceae (increase) and Enterobacteriaceae (decrease).

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P7 - Elaboration and validation of IAC-PCR method for detection of myxoma virus in clinical samples

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Background

Myxomatosis is a highly infectious rabbit disease caused by myxoma virus (MYXV). A diagnosis of myxomatosis is usually based on clinical signs, however an identification of the amyxomatous form of the disease requires laboratory testing. The aim of the study was an elaboration of IAC-PCR assay for detection of MYXV DNA in clinical samples.

Methods

The PCR primers targeting a highly conserved region of the M071L MYXV gene fragment were designed using the nucleotide sequences of Polish, vaccine and wild-type virus strains. Additionally, an internal amplification control (IAC) was constructed and incorporated into the assay. The optimal concentration of particular PCR ingredients in the reaction mixture was determined along with an assessment of the method robustness. Validation of IAC-PCR encompassed the following parameters: the limit of detection (LOD), analytical and diagnostic specificity (ASp, DSp), sensitivity (ASe, DSe) of the assay, repeatability, and intra-laboratory reproducibility. The confidence intervals for the estimated DSe and DSp values were assessed using an exact Clopper-Pearson method.

Results

The assay LOD was established at 2 TCID₅₀ of the virus particles/0.2 ml of tissue homogenate with a 100% capacity to detect different MYXV strains (ASp). The method was characterized by good DSp of 0.955 (0.839-0.999 CI) and DSe of 0.976 (0.914-1.00 CI). It was repeatable, reproducible and robust under changing reaction conditions.

Conclusion

The developed IAC-PCR can be used in diagnostic and epidemiological studies of rabbit myxomatosis.

Acknowledgement

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P20 - Routine sample homogenization and robotic nucleic acids is well suitable for metagenomic diagnostics

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Sample homogenization and nucleic acids extraction are two common steps in both molecular and metagenomic diagnostics. The objective of this study was to align metagenomics with routine diagnostic workflow by evaluating possible effects of automated nucleic acids extraction method on metagenomics.

Eight aliquots (4x2 replicates) were prepared from a swine fecal sample and homogenized followed by manual and automated nucleic acid extraction, MiSeq sequencing and data analysis. The whole process was repeated after one week.

The two MiSeq runs produced 18.8 and 17.7 million reads. The reads mapped to a known virus species were 0.05% of total reads. Porcine bocavirus is the only virus found in all manual and automated DNA extracted libraries, and it was the only mammalian virus in the four robotic DNA extracted libraries with a similar number of normalized reads. The number of viruses identified in manual RNA extracted libraries (MR) was 14 (run #1) and 15 (run#2), and in robotic RNA extracted libraries (RR) was 20 (run #1) and 22 (run#2). The median normalized reads per million of sequenced reads were 8.1 (run#1) and 12.75 (run#2) for the libraries MR, and 8.35 (run#1) and 11.8 (run#2) for the libraries RR. While a similar median number of reads was obtained from libraries regardless of extraction method, a higher number of identified RNA viruses was found in the libraries from robotic extraction of total nucleic acids. The results suggest the automated extraction method is well suitable for metagenomic diagnostics.

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P30 - Variation analysis of Swine influenza virus (SwIV) H1N1 sequences in vaccinated and non-vaccinated pigs

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Viral evolution is a phenomenon that plays a role in adaptation, host range, virulence and emerging of new variants due to point mutation, recombination and gene rearrangement. SwIV is a negative 8-segmented RNA virus with a high rate of genetic variations. Nowadays, vaccination against SwIV is based on inactivated viruses that allow controlling the disease but do not avoid virus replication. Therefore, vaccination can play an important role in the process of viral evolution, especially when vaccination cannot provide sterilizing immunity. Hence, the principal aim of our study was to analyze and compare influenza genome variation in non-vaccinated and vaccinated piglets experimentally infected with SwIV.

For this purpose, two groups of 8 piglets each were used. Group A was i.m. immunized with a commercial vaccine and Group B was inoculated with PBS. Three weeks after the second immunization, animals were challenged with 10⁶ TCID₅₀ A/swine/Spain/01/2010 (H1N1) through two administration routes (intranasal and endotracheal). Serial sacrifices were carried out on days 2, 5 and 9 after challenge. Lung, broncho-alveolar lavage fluid and nasal swab samples were collected. Viral RNA was extracted, quantified by RT-qPCR and subjected to NGS. We detected SwIV in all samples on days 2 and 5 post inoculation (dpi). Higher Viral titers were detected at 2 dpi, specially in control animals and lung samples. Preliminary results show point mutations, but no significant differences between groups were detected. Understanding the dynamics of SwIV evolution would be crucial for the design of new vaccines.

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P14 - Propagation of the possibilities for the study of zoonotic infections for the use of GIS and cloud technologies

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Active research in detection of source and reservoir of pathogens, including caused zoonoses demand more effective tools to track the ways of spreading them.

Our previous international experience in the study of viral animal diseases and their prevention and prophylaxes as well as calculations of biorisks, should be used in zoonotic research too.

To predict the spread of dangerous pathogens we created predictive mathematical models in combination with GIS and cloud technologies to be implemented for predicting outbreaks of epizootic and zoonoses diseases. These technologies may contain large amounts of heterogeneous data, including different pollution, climate change indicators, speed trends affecting the viability of pathogens and their distribution.

Thus, using various tools of calculation and prediction biorisks (emergence and spread of dangerous diseases viral pathogens) may create preventive strategies reduce biorisks both humane and veterinary medicine as well as in the environmental field.

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P35 - Animals-in-a-dish - Functional Immortalized Animal Cell Systems

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A major limitation of current research is the shortage of functional cells. Especially cell systems for veterinary research are not available in sufficient numbers. To overcome this shortage, cell immortalization is an attractive alternative. This can be achieved upon expression of immortalizing genes. However, this process of establishing novel immortalized cell lines is unpredictable and cumbersome.

We developed a novel defined immortalization regimen based on a gene library allowing the efficient and reproducible establishment of novel cell lines (Lipps et al., 2018). This regimen was employed to establish cell lines from primary cells (e.g. endothelial cells, astrocytes, smooth muscle cells, chondrocytes, fibroblasts, lung and intestinal epithelial cells) from 9 different species within two to three months.

The resulting cell lines are immortalized as they can be cultivated for more than 100 cumulative population doublings, show a robust proliferation and can be frozen/thawed without any viability loss. Functional characterization of the resulting cell lines demonstrated that the established cell lines retained the expression of cell type specific marker proteins as well as their specific functions (examples include epithelial cells - barrier formation and differentiation to organotypic epithelium; osteoblasts - mini bone formation; endothelial cells - angiogenesis; smooth muscle cells - contraction; mesenchymal stem cells - differentiation into various cell types). These phenotypes were stable throughout the whole cultivation period.

We envision this immortalization approach to provide cell systems for veterinary research in sufficient numbers for any species. Thereby facilitating novel drug development approaches, infection studies and/or vaccine production.

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P23 - Laridae as a reservoir of gamma- and deltacoronaviruses

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Avian coronaviruses (AvCoV) are widespread pathogens causing significant economic losses in poultry industry and recently the search of their source have increased. Herein we report the most recent data on CoV's prevalence in Laridae in territory of Poland. Furthermore, we have attempted to sequence the full genome of selected CoVs. We tested cloacal swabs originated from live birds, collected between 2009-2017. Each sample was screened by modified nested RT-PCR assay targeting polymerase gene of all CoV's. Full genome sequencing for detected CoV's was performed using NGS approach. Out of 786 tested samples, 49 (6,2%) were CoV-positive and included five species: black-headed gull, common gull, herring gull and common tern. Thirty-four of CoV-positives (69,4%) were identified as gammaCoV's and 15 (30,6%) as deltaCoV's. Phylogeny of detected gammaCoV's showed high similarity to gull's CoVs from Russian Commander-Island and Finland and also to CoV's identified in Australian ruddy turnstone. Similarly, Polish deltaCoV's grouped with strains from gulls sampled in Finland. Additionally, we sequenced the whole genome of deltaCoV detected in black-headed gull. The genome size of analyzed strain was 26102 nucleotides. The phylogeny based on the complete genome showed its close similarity to deltaCoV's detected recently in falcon and pigeon in Hong Kong. Our findings demonstrate that Laridae are common host for gamma- and deltaCoV's. Moreover, high homology of polymerase gene of detected CoV's and those from Australia and Northern Asia suggests that gulls could be an important vehicle for CoV's genes flow and virus dissemination.

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P3 - Development of diagnostic test system based on a recombinant nucleocapsid viral protein of Peste des petits ruminants virus

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Peste des petits ruminants (PPR) is a highly contagious acute or subacute viral disease of sheep and goats. When performing diagnostic and monitoring serological studies, a technological and sensitive method of enzyme-linked immunoassay (ELISA) is preferred. This work was aimed at studying the characteristics of the components of ELISA experimental test systems for PPR-diagnosis based on a recombinant nucleocapsid (N) protein in indirect-ELISA using a protein A peroxidase conjugate or in competitive-ELISA using a peroxidase IgG conjugate obtained from polyclonal rabbit sera against the nucleocapsid protein. We have received a clone of E.coli pET32a/N/10 that contains a plasmid with a protein N gene fragment of 1530 b.p. and expresses a virus-specific major polypeptide 70 kDa. An opportunity of constructing a PPR diagnostic test system using the indirect-ELISA in which a protein A peroxidase conjugate was used in order to detect the PPR-specific antibody bound to the antigen was demonstrated in the experiments with sera taken from convalescents goats. The antibody from the blood serum of rabbits immunized with a purified recombinant N-protein was shown to react basically with the same epitopes as the antibody from the PPR-positive goat serum. The sera of PPR-vaccinated goats with neutralization titers $\geq 1:64$ and pigs immunized with a purified PPRV were positive in the competitive-ELISA in which the purified recombinant N-protein and the IgG-based peroxidase conjugate isolated from a rabbit N-protein specific serum were used.

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P24 - Intestinal parasites of Polish bats

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Background: Bats are important reservoirs of many pathogens of veterinary and medical relevance lots with zoonotic potential. There is a wide knowledge on the viral and bacterial pathogens of bats, however, data on their intestinal parasites particularly concerning European bats are limited. The main goal of the study was the estimation of prevalence of intestinal parasites in bat samples collected for the last decade in different regions of Poland.

Methods: Fifty two fecal samples taken from the distal part of the intestine were tested using the centrifugal flotation method. Each faeces sample was examined 3 times. Identified parasite eggs (parasite developmental forms), as well as fragments or entire mature parasite forms were analysed with cellSens software (Olympus).

Results: The study revealed the presence of developmental forms or mature parasites in 18 out of 52 examined samples (34.61%). Tapeworm eggs of the genus *Hymenolepis* were identified in 9 samples, and trematode eggs were found in 14 samples whereas nematode eggs of the genus *Trichuris* spp./*Capillaria* spp. were found in 2 bat intestines. Additionally, during the study mature forms of trematodes were detected in 2 samples, while in 1 sample fragments of nematodes and trematodes were identified. The vast majority of parasites were detected in *E. serotina* bats (tapeworms, nematodes and trematodes) and *Nyctalus noctula* bats (nematodes and trematodes). Single cases of parasites were detected in *Pipistrellus* bats: *P. pipistrellus* (*Hymenolepis* spp.) and *P. nathusii* (trematodes).

Conclusions: The Polish bats are the reservoir of tapeworms, nematodes and trematodes.

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P25 - The first case of vaccine-induced rabies in Poland

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The study was funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education (No. 05-1/KNOW2/2015).

Background: Introduction of oral rabies vaccination (ORV) of foxes was a milestone in the field of controlling and elimination of rabies in fox population. Many European countries achieved rabies free status after the introduction of ORV. Oral vaccines contain attenuated rabies virus that may have residual pathogenicity and may lead to disease in target as well as non-target animals. A few vaccine-induced rabies cases were reported in some European countries. This study describes the first case of vaccine induced rabies in Poland.

Material and Methods: Rabies antigen was detected using Fluorescent Antibody Test (FAT) in the brain smears of fox found dead in the village Skrzyńka (Małopolska) on the end of December 2017. Three ORV campaigns were carried out in 2017 in Małopolska with Lysvulpen oral vaccines baits. Rapid Tissue Cell Infectious Test (RTCIT) was applied to confirm FAT results. Viral RNA detected using RT-PCR was subjected for Sanger sequencing. Simultaneously, PCR-RFLP was applied for the differentiation of vaccine and field rabies virus strains.

Results: Rabies antigen in brain smears was detected with two conjugates Sifin (Germany) and Bio-Rad (France) while heavy infectious virus was detected in the first passage of RTCIT. PCR-RFLP study revealed the vaccine induced rabies that was confirmed by Sanger sequencing. Phylogenetic analysis performed on 570 bp of the N gene showed the highest homology to rabies virus isolated from different batches of Lyssvulpen.

Conclusions: This is the first report of vaccine-induced rabies in Polish fox confirming the residual pathogenicity of attenuated rabies vaccines strains.

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P27 - Identification and molecular characterization of RNA viruses in fecal samples of diarrheic pigs in the Philippines

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Diarrhea poses a paramount concern for the swine industry in many countries. In the Philippines, Porcine Epidemic Diarrhea virus and Transmissible Gastroenteritis are considered by animal health authorities as primary agents of diarrhea in pigs. However, sequencing studies have identified additional viruses from the gut of diarrheic pigs. In this study, the fecal viral composition of healthy and diarrheic pigs from commercial and backyard farms in the country were characterized using high-throughput sequencing to understand the complexity of the porcine gut virome and the pathogenesis of pig diarrhea. RNA was extracted from the fecal samples of 30 diarrheic and 25 healthy pigs and pooled according to concentration. Pools were sequenced using the NovaSeq platform, subsequent viral metagenomic bioinformatics analysis was performed, and scaffolds were identified using BLAST. Although, co-infection of RNA viruses belonging to the viral genera Rotavirus, Mamastrovirus, Sapelovirus, Sapovirus, Posavirus, Enterovirus, Kobuvirus, and Teschovirus was observed in both diarrheic and healthy pigs, higher viral coverage and a more prominent viral diversity were associated with the diarrheic pig samples. The whole genome and near-full genome sequences obtained were used to generate phylogenetic trees to illustrate their relationship with previously reported viral sequences. The results provide the first description and molecular characterization of the complex enteric RNA virome in Philippine diarrheic and healthy pigs. Impact of these viruses on the overall health of the pigs is yet under-examined and understanding the potential role of each in the development of diarrhea in pigs would merit further biological studies.

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P8 - Indirect ELISA for antibodies to Camelpox virus based on a p32 recombinant protein

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Camelpox is a highly contagious disease of camels with zoonotic potential, it is caused by a host-specific Camelpox virus (CMLV) member of the genus Orthopoxvirus and closely related with Variola virus. The diagnosis can be based on clinical signs in affected animals, however the common signs with other infectious diseases demand camelpox to be differentiated using diagnostic tests. Laboratory confirmation relies mainly on molecular techniques; serological methods, not suitable for primary diagnosis, are useful in secondary testing and retrospective epidemiological studies, in addition to sero-surveillance. ELISA assays for antibodies detection, based on inactivated virus used as coating antigen, have been described; however viral antigen is difficult and expensive to produce, besides biosecurity issues. The aim of this study, therefore, was to develop a convenient and cost-effective indirect ELISA based on the recombinant p32 protein, one of the most immunogenic protein of the Orthopoxvirus. The p32 extracellular domain was produced in *E.coli* and purified in native condition. Its reactivity in indirect ELISA was evaluated with camel sera, namely two experimental camelpox sera and 300 field samples. A monoclonal antibody reacting with ruminant IgG, known to recognize camel antibodies, and home-made polyclonal anti-camel immunoglobulins, both conjugated with peroxidase, were used as tracer. The Optical Density values observed with the 300 field sera were distributed in two populations, with 27% producing values ranging from 0.6 up to 3.5, thus proving to react against the recombinant antigen. These preliminary results provide the conditions for the optimization of a functional antibody-detection ELISA.

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P18 - The Transcriptional Landscape of Marek's Disease Virus in Primary Chicken B Cells Reveals Novel Splice Variants and Genes

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Marek's disease virus (MDV) is an oncogenic alphaherpesvirus that infects chickens and poses a serious threat to poultry health. It is the causative agent of Marek's disease, a deadly lymphomatous condition that can lead to a mortality of up to 100% in unvaccinated birds. In infected animals, MDV replicates in B cells in various lymphoid organs. MDVs coding capacity and viral transcriptome in these primary target cells are currently unknown. Here we used primary chicken B cells in combination with RNAseq to uncover the transcriptional landscape of a very virulent field virus and an attenuated vaccine. Our data confirmed the expression of known genes and identified a novel spliced MDV gene in the unique short region of the genome. Furthermore, we observed genomic regions with extensive splicing events that potentially result in coding and non-coding RNA transcripts. Some of these splicing isoforms could also be confirmed by mass spectrometry and RT-PCR. In addition, the associated transcriptional motifs are highly conserved and closely resembled those of the host transcriptional machinery. Taken together, our data allows a comprehensive re-annotation of the MDV genome with novel genes, splice variants and motifs that could be targeted in further research on MDV replication and tumorigenesis.

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P16 - Applying blockchain technology to secure anti-rabies vaccination certificates during cross-border controls, to prevent counterfeiting and increase trust among countries with different rabies status regulations.

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Introduction

According to EU Regulations 576/2013 and 577/2013, non-commercial movements of domestic carnivores from EU or Third countries must be authorized by a Certificate issued by a EU approved laboratory and stating the satisfactory vaccination status of the pet animal. Customs officers normally check whether such certificate is in compliance with EU current regulations.

However, a Health Certificate may be easily counterfeited by any unknown person and such an occurrence is fairly frequent. In light of this, the EU Commission recommends certifying officers that “satisfactory results for the rabies titration test should not be certified unless the authenticity of the laboratory report has been verified”.

M&M

The digitized certificate is sent to a blockchain-based IT infrastructure. Blockchain is an open, distributed ledger able to efficiently record transactions between two parties in a verifiable and permanent way. Such technology stems from a shared-node infrastructure which uses cryptography, distributed computing and game theory techniques.

Through a QR-Code printed on the paper certificate, customs officers can access the original digital certificate once this has been validated and secured by the blockchain system. By comparing the digital certificate with the paper document it is possible to verify its authenticity.

Conclusions

Blockchain technology can solve the existing problem of verifying the validity, integrity and reliability of an international certificate which reports the animal's immune status, and its implementation avoids any risk of counterfeiting, which strengthens and increases the trust among

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P32 - Poultry vaccines: innovative serological assays for diagnosis and vaccination monitoring for avian Influenza A

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Introduction

Influenza viruses belong to the family Orthomyxoviridae and infect a variety of human and animal hosts. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further divided into subtypes based on their Hemagglutinin (H) and Neuraminidase (N) antigens. Eighteen H antigens (H1 to H18) and eleven N antigens (N1 to N11) have been isolated. Most avian influenza viruses (H1 to 18 subtypes) are low pathogenic, such as H9, whereas some subtypes containing H5 and H7 are associated with highly pathogenic forms. For many years, specific vaccines based on circulating hemagglutinin or neuraminidase were developed to protect flocks against Influenza. Serological techniques are commonly used for disease monitoring. ELISA testing is an efficient and cost-effective method for the analysis of large numbers of samples, particularly in comparison with the Hemagglutination Inhibition Test (HI).

Materials and methods

IDvet has developed new tools to monitor vaccination uptake for H5, H7 and H9 AI: ID Screen® Influenza H5, H7 and H9 Indirect. IDvet's kits are quantitative tests and highly correlate with HI tests (homologous strain). They are the only commercial ELISAs able to detect H5, H7 or H9-specific antibodies for diagnosis and monitoring of vaccination (conventional and recombinant vaccines).

Results and discussion

The following presentation summarizes the validation data obtained for Indirect Avian Influenza diagnostic tools developed by IDvet.

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P15 - The existence probability of highly virulent *Yersinia pseudotuberculosis* strains in nature

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Cytotoxic necrotic factor *Yersinia pseudotuberculosis* (CNFY) is a 114 kDa protein that is similar to CNF 1, prevalent among pathogenic and saprophytic *Escherichia coli* isolates. Rho family small GTP-binding proteins (GTPases) are inactivated in eukaryotic cells that have been treated with CNFY, which induced the development of giant multinucleated cells in them. In 2002 the CNF *Y. pseudotuberculosis* of YPIII strain (USA) was first described. The *cnfY* gene sequence analysis of *Y. pseudotuberculosis* strains isolated in Russia detected 2 alleles. Most virulent *Y. pseudotuberculosis* isolates contain a gene that has significant deletions and substitutions. Few strains carry a gene variant encoding active CNFY protein. Therefore, we have a question: «Is a virulent *Y. pseudotuberculosis* strain containing a full-sized cytotoxin gene and producing an active CNFY toxin living in nature?» To answer this question, we treated HEp-2 cells with CNFY toxin and virulent bacteria together in vitro. As a result, we observed both multinucleate in eukaryotic cells and the formation of pronounced filopodia and lamellapodies on the cell surface, this indicates activation of Cdc42 and Rac GTPases in HEp-2. Activation of these proteins, as shown in previous studies on CNF 1 *E. coli*, leads to apoptosis of eukaryotic cells. Also known that the CNF *Y. pseudotuberculosis* toxin activates only the RhoA protein without the activation of other GTPases. The effect obtained in our studies suggests that virulent strains producing active CNFY are highly virulent bacteria. Possible causes of CNFY inactivation in virulent *Y. pseudotuberculosis* strains are discussed.

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P36 - Development of a novel multiplex real-time PCR for the detection and differentiation of SADS-CoV, an emerging swine coronavirus

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A coronavirus variant known as Swine Acute Diarrhea Syndrome Coronavirus (SADS-CoV) has emerged in China where the first clinical signs were observed in late December 2016. In 2017, an outbreak in several Guangzhou farms was associated with fatal acute diarrhea in neonatal piglets. Here we report the development of a new real-time PCR assay for SADS-CoV that will be multiplexed with two other swine coronaviruses.

There are only a few SADS sequences available on Genbank. These sequences were downloaded and imported into the Sequencher 5.4.5 Software. The software was used to assemble the sequences into a contig. The sequences were visually inspected for conserved region. For Corona Viruses mainly the Nucleocapsid (N) and RNA-dependent RNA polymerase (RdRp) gene regions seem to be highly conserved. For the new SADS assay, the N region was chosen. An In Vitro Transcript (IVT) was created.

The IVT is then tested with the primer, probes and a chosen One-Step RT-PCR kit on the ABI 7500 Real-Time PCR Instrument. The results show the slope of -3.321280 and R2 value of 0.984144 and demonstrate that the chosen design of the rRT-PCR SADS assay is efficient. The analytical specificity of the SADS-CoV PCR assay was evaluated against a panel of enteric swine corona viruses at SDSU to ensure that the test specifically amplifies SADS-CoV viruses and does not cross-react with closely related non-SADS swine coronaviruses. The stand-alone SADS-CoV and multiplex PEDV-SADS-CoV-PDCoV-IC rRT-PCR was tested with collaborative partners, with a collection of swine coronaviruses including SADS-CoV in China.

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P12 - Qualitative risk assessment of foot-and-mouth disease introduction into Saratov Region, Russian Federation

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Foot-and-mouth disease (FMD) is a highly contagious infectious disease causing huge economic impact and affecting cloven-hoofed animals including more than 70 species.

Some outbreaks of FMD were registered in 10 countries, including Russian Federation in the period January 1 - February 22, 2019.

In order to analyze the risk of introduction and distribution FMD in Saratov Region a qualitative risk assessment analysis was applied. We used software ESRI ArcGIS 10.6.1, according the existing methods of cartographic analysis.

Three categories of probability were used: low - an event is possible in some cases, medium - an event is possible, high - an event is possible in many cases.

Three factors for introduction of distribution of FMD Saratov Region (in case of introduction) were used:

- 1) The presence of unregistered outbreaks in neighboring territories in low risk.
- 2) Contact between susceptible animals during grazing in low risk.
- 3) Economic relationships with potentially contaminated regions in high risk.

Three factors for evaluation of distribution of FMD Saratov Region (in case of introduction) were used:

- 1) Distribution of FMD among susceptible animals in low risk.
- 2) Survival of the pathogen in environmental conditions of the Saratov region is low.
- 3) Role of wild animals is low.

Based on the factors considered, it can be concluded that the risk of the development of epizootic situation in Saratov Region for FMD is low.

General risk assessment for introduction and distribution of FMD in the territory of Saratov Region is low.

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P21 - Characterization of genetic variability of equine influenza virus using next-generation sequencing

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The influenza virus, like other RNA viruses, exhibits high evolutionary dynamics. Molecular mechanisms responsible for virus variability include mutations and reassortments, and in rare cases recombination. Due to the low fidelity and lack of proofreading function of viral RNA polymerase, a single host may be infected with viral quasi-species, a population of closely related genetic variants of the virus. Next-generation sequencing gives opportunity to examine the genetic diversity of the viral population. The goal of our study was to investigate genetic variants of equine influenza virus induced during passaging. RNA extracted from nasal swabs and from the second and the fifth passage of A/equi/Pulawy/2005 (H3N8) in embryonated chicken eggs were analyzed. Full length sequences of all eight viral RNA segments were obtained. In total, 50 variants were identified, from 3 to 11 per RNA segment. The most variants were noticed for segment I (PB2), segment II (PB1) and segment IV (HA) - 11, 9 and 9 variants respectively. Variants induced as well as disappearing during passages were observed, including variants in which changes did not show a constant tendency. Of the 50 variants observed, 29 induce a change in the amino acid, 20 are synonymous, and one variant is in the non-coding region of the sequence. Considering that influenza vaccines are typically produced by growing the target viruses in chicken eggs, seems to be important to study the effect of passages on virus variability.

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P19 - Changes in host gene expression following CAstV infection of chickens

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Astroviruses are known to cause enteritis not only in humans but also in various animal species such as chickens, turkeys, sheep, cattle, swine, dogs, cats, mice and others. In poultry they have caused enteritis combined with growth depression and higher mortality but their presence was also described in healthy flocks. Chicken astrovirus (CAstV) was recently indicated as the factor of the “white chicks” condition associated not only with increased embryo/chick mortality but also with weakness and white plumage of hatched chicks.

To increase our understanding of the CAstV pathogenesis, we investigated the response of chickens to this virus. Real-time quantitative PCR was performed on RNA samples from chicken spleens, which were collected at 4 days post-infection. The expression patterns of six cytokines (IFN α , IFN β , IFN γ , IL1 β , IL6, IL10) were investigated. The results showed that infection with CAstV induced significantly higher levels of the antiviral cytokine IFN- β and proinflammatory cytokine IL1 β . IFN- γ transcript levels in virus infected group were statistically lower from the PBS treated control group. However, the expression patterns of the other cytokines that were tested did not show any obvious trends or statistically significant differences between infected and uninfected chicken spleens.

The present study is the first which follow cytokines expression of chicken spleens in response to CAstV infection. However, we need further studies to understand the molecular mechanisms underlying CAstV infection and pathogenesis.

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P2 - The lab in-your-pocket - qPCR results whenever, wherever needed

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Detecting disease outbreaks like African Swine Fever (ASF) or Avian Influenza (AI) need reliable, rapid molecular diagnostic tools for use away from centralized laboratories in veterinary clinics and even on farms. INDICAL is partnering with Biomeme, Inc. to develop a novel point-of-need (PON) PCR system. A small portable thermocycler enables multiplex real-time detection of up to 27 targets from a single sample. Alternatively, 9 samples can be tested for up to 3 targets per run. New extraction devices and shelf-stable PCR reagents eliminate the need for cold chain. The qPCR test results are analyzed and displayed in real-time on a smartphone using an intuitive software.

INDICAL's new PON PCR solution was compared to standard lab workflows. DNA and RNA samples from ASFV and AIV were analyzed using virotype ASFV PCR Kit and virotype Influenza A RT-PCR Kit on commonly used real-time thermocyclers and on the new PON PCR thermocycler. Furthermore, new lyophilized PCR reagents were tested and results compared to the performance of laboratory assays.

ASFV-positive DNA and AIV-positive RNA showed better Ct values on INDICAL's portable qPCR thermocycler. Combining lyophilized reagents with a modified primer/probe mix from the virotype kits produced comparable results on the new PON platform.

INDICAL's portable qPCR thermocycler and its smartphone-based analysis achieved comparable or better results when compared to the standard molecular laboratory equipment.

New extraction cartridges are currently in validation for veterinary specimen. Recent data suggest that even difficult viruses such as FMDV can be reliably extracted in the field.

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P26 - Retrospective studies on the bat species structure submitted for diagnosis of rabies in Poland

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Background: Accurate identification of bat species is essential to determine in which species rabies is most diagnosed and therefore possess a threat to humans. Dead bats may only be identified by a method based on morphological characteristics but very often bats are sent for testing in a state of major body damage or decomposition, which makes it completely unusable for morphological identification.

The main goal of the study was to determine the species of bats submitted for rabies diagnosis using molecular methods.

Methods: The wing membrane were taken from bats sent for rabies diagnosis. Total DNA isolated from 434 bat samples was subjected to fragment cytochrome b gene amplification. The amplicon of 900 nt was subsequently sequenced and compared with the reference sequence using BLAST software to determine bat species.

Results: Analysis of the mitochondrial DNA cytochrome b gene fragment allowed for the identification of 20 bat species. Among all identified bat species the highest percentage was found in Serotine bat (*Eptesicus serotinus*) - 26.5% followed by Noctule (*Nyctalus noctula*) - 16.4% and Common Pipistrelle (*Pipistrellus pipistrellus*) - 11.5%.

Conclusions: Identification of bat species using molecular method is useful in investigation specimens which are damaged and morphological identification is not possible. The most frequent bat species submitted in the frame of passive surveillance of rabies in Poland were Serotine, Noctule and Common Pipistrelle, respectively. In relation to rabies, the highest incidence of rabies cases were diagnosed in Serotine.

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P31 - CO-ADMINISTRATION OF FOWL POX AND NEWCASTLE DISEASE VACCINES BY NON-INVASIVE ROUTES TO SMALLHOLDER CHICKEN IN HANANG DISTRICT OF THE MANYARA REGION OF TANZANIA

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The aim of this study was to demonstrate that the concurrent administration of commercial Fowl Pox (FP) and Newcastle Disease (ND) vaccines given by non-invasive routes is safe and elicits immunity, indicated by local (for FP) or serological (for ND) immune reactions in chicken in extensive smallholder settings in Tanzania. This is important in most developing countries where para-veterinarians and community animal health workers are not legally allowed to administer parenteral products.

This study followed a cluster-randomised controlled design. 1173 chickens from 242 households in seven villages in Hanang District, Tanzania were enrolled. On Day 0, Group 1 (n=237) was vaccinated with FP-Vaccine (feather-follicle method), Group 2 (n=234) with ND-Vaccine (eye-drop), Group 3 (n=240) with both vaccines (ND: eye-drop, FP: feather-follicle), and Group 4 (n=220) with both vaccines (ND: eye-drop, FP: wing-web). Group 5 (n=242) served as unvaccinated control group. On Days 0 and 21 blood samples were collected and used for ND HN-protein antibody titre determination by haemagglutination inhibition test. The study duration for each household was 21 days.

Immunisation after Fowl Pox vaccination was shown in all FP vaccinated groups by the development of “takes” (Group 1: 96% (95% CI: 93; 98); Group 3: 94% (95% CI: 90; 96); Group 4: 96% (95% CI: 93; 98)). Immunity to Newcastle Disease on Day 21 was shown in all ND vaccinated groups (birds with protective antibody titres in Group 2: 88% (95% CI: 81; 92); Group 3: 87% (81; 92); Group 4: 89% (83; 94)). Statistical analysis is ongoing.

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P29 - Vaccination derived maternal antibodies can prevent postnatal classical swine fever virus persistence in suckling pigs

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Classical swine fever (CSF) is one of the most devastating diseases for the pig industry worldwide. While vaccination is banned in the EU, mandatory vaccination is carried out in endemic countries. Recently, the phenomenon of postnatal persistence has been described to impact on vaccination efficiency. Postnatal persistence refers to a disease course that can be induced in piglets in the early hours and days of their lives. In this study, it was tested the hypothesis that solid vaccination of the breeding sows, and thus transfer of maternal antibodies (MDA), can protect against induction of postnatal persistence.

Piglets from vaccinated sows with either C-strain or Suvaxyn®CSF Marker vaccine, as well as piglets from naïve sows were inoculated with the CSFV Catalonia strain 24 hours after birth. Upon weaning, the surviving piglets were vaccinated with the respective vaccine and challenged 28 days post vaccination with a CSFV moderate virulence strain from Germany ("Rösrath"). Clinical and pathological scoring was performed along with routine serological and virological methods.

In a nutshell, all naïve piglets developed postnatal persistence and showed tremendous viral genome loads and no detectable antibodies. These animals did not seroconvert upon active immunization. In contrast, MDA positive piglets were completely protected against clinical infection. Moreover, these animals showed significantly lower genome loads and active antibody production. None of the MDA positive animals developed long-term persistence. No clinical signs were observed upon challenge.

It was confirmed that solid vaccination of breeding sows can prevent the establishment of postnatal persistence in the offspring.

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Posters

Current Challenges inside Europe

P12 - In silico analysis of the genomic variability and phylogenetic relatedness of *Clostridium perfringens*

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The Gram-positive anaerobic spore forming bacterium *Clostridium* (C.) *perfringens* can cause various diseases in different hosts. With the aim to investigate its genomic diversity, publicly available genome sequence data of 76 C. *perfringens* strains from diverse ecological, geographical and temporal niches were analyzed. Data analysis included 30 complete genomes which were composed of one circular chromosome (2.9 to 3.5Mbp) and up to six extrachromosomal elements. A substantial degree of genomic variability was detected in respect to episome content, chromosome size and mobile elements. Insertion sequences could be identified in all genomes, but were particularly frequent in certain genomes. Multiple alignments of complete genomes displayed a considerable degree of conservation in the order of genes in each chromosome except for three genomes. All analyzed 76 C. *perfringens* strains (including also non-closed genomes) were divided into three phylogroups. Phylogroup I contains human food poisoning strains with chromosomally localized enterotoxin gene (*cpe*) and a Darmbrand strain. This phylogroup is characterized by a significant enrichment in mobile elements, relative small genome size and marked loss of chromosomal genes. These genomic traits differ evidently from those of the other two phylogroups and suggest that these strains have been evolving to adapt to a particular habitat where they cause human foodborne disease.

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P27 - Reassortment with H9N2 or NS1-mutations increased virulence of avian-influenza-H5N8 2.3.4.4 in mice but compromised virus replication, virulence and/or transmission in chickens

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Avian influenza viruses (AIV) infect a wide range of birds and mammals. The panzootic H5N8 clade 2.3.4.4 and H9N2 are widespread in poultry. Although both H5N8 and H9N2 exhibit only low virulence in mammals, there is a risk for evolution of potentially zoonotic H5N8 viruses after mutations or reassortment of H5N8 and H9N2 viruses. Here, the virulence of H5N8 after reassortment with H9N2 or carrying mutations in the NS1 was studied in mice and chickens. In mice, reassortment with H9N2-PB2 and to lesser extent PA or NS increased the virulence of H5N8 in mice as indicated by rapid onset of mortality and increased body weight loss. In chickens, reassortment with H9N2 reduced virus virulence, replication and/or transmission with a prominent role for the NS segment in chicken-to-chicken transmission. Likewise, mutations in the NS1 of H5N8 increased virulence of the virus in mice; however, it reduced virus replication and transmission in chickens. Together, reassortment with H9N2 or mutations in NS1 may result in H5N8 viruses with higher virulence in mammals but compromised virus fitness in chickens. This study is important for zoonotic risk assessment of the widespread H5N8 and H9N2.

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P26 - Detection of a new genetic cluster of Influenza D virus in Italian cattle

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Influenza D virus (IDV) has been increasingly reported all over the world. Cattle are considered the major viral reservoir. Based on the HEF gene, three main genetic and antigenic clusters were identified: D/OK worldwide distributed, D/660 detected only in the USA, and D/Japan in Japan.

Up to 2017, all the Italian IDV isolates belonged to D/OK genetic cluster.

We recently performed a surveillance for IDV from 560 respiratory outbreaks in 451 bovine farms in Northern Italy. From January 2018 to May 2019 we examined 666 samples (424 nasal swabs, 242 lung tissues). Sixty-four nasal swabs and 7 lung tissues from 61 farms were positive for IDV (30/451 - 6.6%- in 2018 and 31/140 -22%-in 2019).

Whole genome sequence was obtained, by NGS sequencing, from 28 samples. Unexpectedly, phylogenetic analysis showed the presence of 11 strains belonging to the D/660 cluster, previously unreported in Europe. The earliest D/660 -strain was collected in March 2018 from cattle imported from France. All the remaining D/660 strains were isolated from November 2018 to March 2019 from 6 milk and from 5 meat producing farms. Moreover we detected one viral strain with a reassortant genetic pattern (PB2, PB1, P42, HE and NP segments in D/OK cluster whilst P3 and NS segments in D/660 cluster). These results confirm the circulation of IDV in Italian cattle population and highlight the need of monitoring the developing of spread of this influenza virus, in order to get more information about the epidemiology of Italian IDV viruses.

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P11 - *Mycobacterium caprae* in officially tuberculosis free territory

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Veneto Region (north-eastern Italy) has been declared officially free from bovine tuberculosis (bTB) in 2008 (Decision 2008/404/EC), although sporadic cases have been detected since then. In officially tuberculosis free (OTF) territories, Directive 64/432/ECC defines the measures for the introduction of animals, and to maintain the OTF status allows limiting the surveillance to post-mortem inspection at slaughter. In 2015 and 2017 two outbreaks of *M. caprae* bTB were detected in Veneto, the earlier in a dairy farm (cubicle/free stall system), and the later in a suckler cow farm (pasture-based system). Official screening was conducted by single intradermal skin test (SIT), which resulted in a higher positivity (35%) in the former outbreak compared to the latter one (0,3%). In the second outbreak the gamma-interferon test was also applied, resulting positive in 95% of the tested animals. Typical bTB lesions were found in 35,8% of animals in 2015 and 54,9% in 2017. The bacteriological culture confirmed similar positivity percentages. Two different genotypes of SB0418 spoligotype were identified. The information collected on animal movements allowed to trace back the origin of infected animals from cattle farms located in other OTF territories. Practical constraint in performing the SIT in the pasture-based system may have reduced test sensitivity in the second outbreak. Rather, gamma-interferon test can be a more reliable diagnostic tool, as all animals can be tested once. Moreover, given that OTF territories are allowed to perform only post-mortem surveillance, conducting a risk assessment to quantify the risk of introduction of bTB is deemed pivotal.

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P1 - Molecular characterization of EIAV retroviral strains using targeted sequence enrichment and next generation sequencing

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Equine infectious anemia virus (EIAV) is one of the most divergent members of the lentivirus subfamily of retroviruses. EIAV often leads to apparent clinical disease within a week to a month. Acute disease episodes are generally characterized by fever, anemia, thrombocytopenia and anorexia. EIAV diagnosis is essentially based on detection of antibodies directed against viral epitopes using the agar gel immunodiffusion assay (AGID) but seroconversion of horses can take several months. Therefore, the development of a rapid and efficient molecular diagnosis assay independent from seroconversion is of critical importance. The high mutation rate and the limited number of sequences available for this virus complicate the use of molecular diagnosis. Different methods have been used but involve cloning of overlapping fragments or amplification of viral particles in cell lines, leading to several mutations into the viral genome prior to sequencing. In the present study, we used the SureSelect target enrichment system with Illumina Next Generation Sequencing to characterize the proviral DNA of Equine Infectious Anemia Virus (EIAV). This sensitive approach allows a direct sequencing of the EIAV whole genome without cloning or amplification steps. We obtained the complete sequences of from 50 different viral strains of European origin and analyzed their phylogenetic relationship and genetic variability by comparison with genome sequences from different parts of the world. These results provide new insight into the genetic variability of European EIAV strains, for which obtaining complete genome sequences is of great interest for molecular diagnosis.

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P16 - The challenge of infectious bronchitis virus vaccine: recombinant IBV with modified ADP-ribose-1''-monophosphatase domain may provide a potential new vaccine platform

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The gammacoronavirus infectious bronchitis virus (IBV) poses a serious challenge for vaccination due to the risk of reversion to virulence of traditional egg-attenuated vaccines and the emergence of escape mutants. The IBV non-structural protein (nsp) 3, is a multifunctional protein containing several putative domains, including an ADP-ribose-1''-monophosphatase (ADRP) domain conserved among coronaviruses. Inactivation of the ADRP domain in alpha- and betacoronaviruses does not affect viral replication *in vitro* but is associated with reduced pathogenicity *in vivo*, altered interferon response and cytokine profiles in the host. Therefore, recombinant viruses lacking ADRP functions have been proposed as ideal candidates for live attenuated vaccines. Using reverse genetics, a recombinant IBV (rIBV) was generated in the backbone of the pathogenic M41-K strain containing a mutation in the ADRP domain catalytic core, known to abolish ADRP function. The ADRP-defective rIBV was characterised *in vitro* and *in vivo*; the rIBV displayed a distinctive plaque phenotype and, conversely to previous ADRP-defective coronaviruses, viral replication was slightly reduced *in vitro*. No reversion of the mutation occurred after serial passages of the virus in primary avian cell culture, nor in *ex vivo* tracheal organ cultures utilised as a surrogate for *in vivo* stability testing. Pathogenicity experiments conducted *in vivo* resulted in a reduction in clinical signs in comparison to M41-K-infected birds, and tracheal ciliary activity, a marker for pathogenicity, was comparable to mock infected birds. These data support the role of ADRP as a pathogenic determinant and demonstrate the potential of ADRP-defective rIBVs as promising, stably-attenuated candidate vaccines.

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P28 - Simultaneous detection and differentiation of Avian Influenza A Virus subtypes H5, H7 and H9 in one PCR run

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Avian Influenza A outbreaks have resulted in the loss of millions of domestic birds worldwide in recent years, making sensitive and specific screening a high priority. In addition, reliable diagnostic subtyping is essential to identify the notifiable Avian Influenza Virus (AIV) subtypes H5 and H7 and the non-notifiable but still economically important H9. The newly developed virotype Influenza A H5/H7/H9 RT-PCR Kit is intended for the highly reliable and fast detection and differentiation of those specific AIV subtypes.

Using known AIV-positive samples originated from different outbreaks and AIV-negative samples from domestic and wild birds, we validated the sensitivity and specificity of this new Multiplex RT-qPCR for AIV.

The performance of the assay was tested with in-vitro RNA representing currently circulating strains. A heterologous internal amplification RNA control is included.

High sensitivity and specificity were confirmed for the new virotype Influenza A H5/H7/H9 RT-PCR Kit, with no cross-reactivity for other AIV subtypes or avian pathogens, including Newcastle Disease Virus and Mycoplasma. Furthermore, the kit was shown to reliably identify H5 and H7 RNA to a limit of 1-10 copies and H9 to a limit of 10-100 copies. Intra- and inter-assay variance testing showed excellent reproducibility.

In combination with the proven performance of the virotype Influenza A RT-PCR Kit for AIV screening, INDICAL provides a comprehensive solution for accurate AIV detection and subtyping of AIV H5, H7 and H9.

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P5 - 3D Imaging of Rabies Virus Infections in Solvent-Cleared Brains

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Pathogenicity of virus infections in the infected animals depends on complex molecular and cellular interactions. However, functional analyses of virus-infected tissues to understand crucial pathomechanisms of complex tissues at subcellular resolution remains challenging, since conventional histology usually requires thin sectioning of samples for light microscopy.

We adopted the state-of-the-art deep tissue imaging technique uDISCO (ultimate 3D imaging of solvent-cleared organs) for comparative investigation of the cell tropism of different rabies viruses (RABV) in mouse brains. By high-resolution confocal laser scan imaging of virus and host factors in 1 mm thick brain slices, three-dimensional insights in infected brains at cellular and subcellular resolution are provided. Moreover, differences in the cell tropism of highly virulent street RABV and lab strains were dissected by quantitative 3D image stack analysis.

Specifically, comparison of two dog and fox street RABV with lab strains CVS-11 and highly attenuated SAD L16 revealed that all viruses infected neurons independent of the degree of attenuation. However, in contrast to the lab strains, which are known to abortively infect astrocytes, the street viruses led to strong virus protein expression in 6% to 9% of non-neuronal GFAP-positive astrocytes. These observations led to model in which the ability to establish robust infection in astrocytes is a major factor in street virus pathogenesis, which is most likely determined by different levels of interference with astrocyte-related cytokine release.

Overall, the results demonstrate the power of 3D immunofluorescence imaging of solvent-cleared organs in infection biology and dissection of viral pathogenesis.

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P19 - RHDV2 in Poland - differentiation of viral strains based on the genome analysis of isolates from 2016-2018

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Introduction. The first Polish RHDV2 (GI.2) was identified between 2016-2017 and infections were confirmed during 2018. The studies present the molecular epidemiology of independently introduced RHD2 strains in Poland. **Materials and methods.** Viral RNA was isolated from livers taken from rabbits died in RHD outbreaks. Real-time RT-PCR was used viral genome detection. RT-PCR kit was used to amplify the overlapping fragments of the full genome of RHDV. The nucleotide sequences of four RHDV strains detected in 2018 were aligned with native RHDV2 from 2016-2017 and reference sequences representing classic RHDV, non-pathogenic RCV as well as non-recombinants and recombinants RHDV2 isolates. **Results.** Nucleotide sequence similarity among the three most distanced RHDV2 isolates was from 92.5% to 98 % in complete genome, 91-98% in NSP region and 95-99% in the VP60 gene. The sequence homology between RHDV2 strains from 2018 and 2016-2017 was 88% in the entire genome, 85% in NSP and 96% in the VP60. Phylogenetic analysis of VP60 confirmed that RHDV strains from 2018 belong to RHDV type 2. However, the nucleotide analysis of NSP region revealed the differences between an older and new native RHDV2 strains. The Polish RHDV2 isolates from 2016-2017 cluster together with RHDV2 recombinants identified in Portugal, while all native strains from 2018 belong to the non-recombinants. **Conclusions.** Based on phylogenetic characterization of RHDV2 strains detected in Poland between 2016-2018 and the chronology of their emergence it can be concluded that both groups were introduced independently.

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P7 - A library preparation optimized for metagenomics of RNA viruses

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Our understanding on the structure and role of viral communities in animal hosts has not yet reached the knowledge level attained on the bacteriome. This situation is, among others, probably due to technical constraints in adapting metagenomics, a main tool in bacteriome studies, to the characterisation of viral communities. Technical developments have been achieved for most steps of viral metagenomics. However, there is yet a key step that has received little attention, that of library preparation. This situation is surprising because developments in library preparation have largely facilitated bacteriome studies. Here, we present a library preparation optimized for metagenomics of RNA viruses from animals. The library has been designed to provide advantages found in metabarcoding libraries used in bacteriome studies, while allowing shotgun sequencing as required in viral metagenomics. We have validated a full metagenomics approach incorporating our library preparation and using mock viral communities. We have further tested our approach in two pilot studies with field-caught insects, all vectors of viral diseases. Our approach provided a fold increase in virus-like sequences compared to other studies, and nearly-full genomes from new virus species. Moreover, our results suggested conserved trends in virome composition in a mosquito species, and thus the existence of deterministic forces shaping its virome. Finally, the sensitivity of our approach was relatively good when compared to a commercial diagnostic PCR for the detection of an arbovirus in field-caught vectors. Our approach could facilitate studies on viral communities from animals and the democratisation of metagenomics in virus ecology.

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P31 - Experimental model for HPH5Nx emergence in poultry flock

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At the end of the 20th century, a paradigm breaking highly pathogenic H5 virus emerged, able to infect wild water birds. As a consequence, the highly pathogenic virus can be introduced directly into poultry flocks without the need for adaptation from low to high pathogenicity. Wild migration of HP H5 infected birds resulted in the globalization of these viruses. Since its emergence, the H5N1 goose/Guangdong parent strain evolved, by genetic drift and shift, into a large number of different clades and subclades. Since 2010, different subtypes of the clade 2.3.4.4 viruses appeared in Europe, such as H5N1, H5N6 and H5N8 strains. These strains share the same hemagglutinin (HA) but differ by their Neuraminidase (NA) as a result of reassortment with locally circulating avian influenza viruses.

The possibility of direct HPAI introduction into poultry flocks via wild birds, instead of emergence by genetic drift of a LPAI-H5 virus, combined with the changing pathogenicity and epidemiology, needs a re-thinking of how to better monitor and protect our poultry flocks. In this context, this project aims to develop an experimental model of punctual introduction of HPH5Nx into naïve poultry flocks of different species. This experimental model should allow the Belgian national reference lab to easily adapt to changing epidemiological situations and to extend its expertise in the protection of the poultry sector.

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P3 - DEVELOPMENT OF DIAGNOSTIC MOLECULAR TOOLS FOR SWINE MAMMALIAN ORTHOREOVIRUSES

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Mammalian Orthoreoviruses (MRVs) are double-stranded segmented RNA viruses belonging to the Reoviridae family, genus Orthoreovirus that can cause asymptomatic, respiratory, or gastroenteric infections. MRVs have a wide geographical distribution and host range; they have been detected in pigs, bats and humans. MRVs are divided in 3 different serotypes (MRV1-3) according to their serological properties correlated to the S1 gene. In literature, several cases of genetic reassortment have been reported between swine, human and bat MRVs, suggesting a potential relationship between these different species. Absence of data on the MRVs distribution in the pig and bat populations and the limited number of genetic sequences available hampers the formulation of any hypothesis on the epidemiological links between pigs, bat and human. Molecular tools available were designed to identify MRV sequences dated back to 70s therefore, they are inadequate to identify all currently circulating strains. In order to implement robust surveillance activities and shed light on distribution of MRV in swine and bats, it is crucial to develop and validate up-to-date molecular tools for screening and characterization swine MRV strains. The most promising methods available in literature were tested and in addition all MRV sequences available were analysed to develop Real time RT PCR methods for screening and typing MRV swine strains. In order to verify the performance of the selected methods, they were evaluated using a series of different Italian and Dutch MRV strains of swine origin.

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P17 - DETECTION OF PORCINE CIRCOVIRUS-3 IN STILLBORN PIGLETS WITH MULTI-SYSTEMIC INFLAMMATION

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An increase in stillborn piglets in 2018 in a single herd over a four-month period was investigated. Some stillborn piglets had arthrogryposis, and there was a concurrent increase in congenital tremor (CT) in gilt litters plus a few cases of neurological disease developing postnatally in pigs.

Post-mortem examinations were undertaken by APHA on four stillborn piglets, three pre-weaned pigs with neurological signs and four with CT, all from the same herd. Histopathology, immunohistochemistry (IHC) and molecular assays were performed.

Non-suppurative angiocentric inflammation was observed in a range of tissues in the stillborn and tremor-affected piglets likely reflecting in utero systemic viral infection. Immunohistochemistry and/or PCR was negative for PCV-2 and PRRSV. PCV-3 was detected by a microarray in the stillborn and postnatal tremor-affected piglets and confirmed by qPCR with low Ct values. Brain and spinal cord were tissues consistently having highest viral loads. PCV-3 was detected by qPCR but with high Ct values in the CT-piglets. Only APPV was detected in CT piglets in the microarray in which there was no multisystemic inflammation and lesions typical of CT type A2 in brains and spinal cords. APPV was also detected in tissues of postnatal tremor-affected piglets by qPCR.

Since the first detection in 2016, PCV-3 has been associated with respiratory disease, reproductive failure, neurological disease, or PDNS-like lesions, either alone or in mixed infections. The findings here complement previous reports and suggest a need for wider surveillance for PCV-3 in cases of stillbirth and neurological disease in young piglets.

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P13 - Enhancing Biosafety an Biosecurity in Ukraine

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Biological safety and security are of particular importance in the context of globalization and the emergence of new global threats and risks. Infectious diseases may pose serious threats to modern societies, especially if rapidly spreading and indirectly affecting the quality of life. Moreover, climate changes may additionally enhance endemic transmissions and pathogen spread.

Re-emerging zoonotic diseases include glanders, brucellosis, anthrax as well as Crimean-Congo Hemorrhagic Fever (CCHF), infections which were relevant in Ukraine in the past. Human and animal infections with these agents have been detected sporadically in recent years. Therefore, the G7 Global Partnership Program, with the assistance of the Friedrich-Loeffler-Institute and the donor assistance of the German Ministry of Foreign Affairs, implements the second project in Ukraine for the years 2018-2019 entitled "Introduction of efficient biosecurity procedures to deal with proliferation-critical, highly pathogenic pathogens for humans and animals in Ukraine" by strengthening diagnostic capabilities for the identification of critical pathogens and by improving biosafety and biosecurity measures implemented by them. Ukrainian partners were trained in new serological and molecular diagnostic methods for glanders, brucellosis, anthrax and CCHF. The implementation of all project activities increases the biosafety/biosecurity not only in Ukraine but also worldwide.

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P20 - Consultant Laboratory for Rabbit Haemorrhagic Disease (RHD)-Virus

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Laboratory-based diagnostics of infectious diseases is an essential tool for surveillance and disease control. A special focus lies on quality assessment of diagnostic methods and test systems. In the field of reportable and especially of notifiable animal diseases, the respective National Reference Laboratories play a key role in benchmarking of tests and veterinary laboratories.

The “German Society of Veterinary Medicine“ (DVG) has therefore designated consultant laboratories for a number of important animal diseases caused by infectious agents (<https://www.dvg.net>).

The Consultant Laboratory (CL) for RHDV promotes harmonization of laboratory testing, coordinates and optimizes RHDV diagnosis on a national level, develops and validates new diagnostic systems, provides reference materials, characterizes virus isolates from Germany and partner countries, and acts as consultant for authorities and veterinarians. The CL offers diagnostic support for official and academic institutions and training of laboratory personnel.

The “new or variant” RHDV (called RHDV-2 or RHDVb) emerged in France in 2010 in wild and farmed rabbits (Dalton et al., 2012; Le Gall-Reculé et al., 2013) and rapidly spread in Europe as well as in 2015 in Australia. Since 2015, dissemination of RHDV-2 has been observed in Germany. The FLI conducts differentiation of Caliciviruses (RHDV, RHDV-2, and European brown hare syndrome virus (EBHV)) to collect data for a thorough characterization of field isolates. In close cooperation with the regional state laboratories, distribution of the epizootic new RHDV variant is monitored. A German inter-laboratory comparison test was performed in 2019 and the results will be presented.

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P2 - Phylogenetic analysis of Polish isolates of infectious pancreatic necrosis virus from freshwater fish

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Infectious pancreatic necrosis virus belongs to genus Aquabirnavirus family Birnaviridae. It seems to be the most widespread virus in freshwater aquaculture and is important in global freshwater aquaculture. The genome of IPNV consists of two double stranded RNA segments. Classification based on the similarities of VP2 gene demonstrated that aquatic birnavirus is clustered into 6 genogroups. Genogroup 1 consists of isolates from USA and the Jasper strains. Genogroup 2 contains viruses from Europe and Asia. Genogroup 3 is represented by Canadian isolates and strain Tellina. Genogroup 4 comprises the type strains of the Canadian isolates. All members of European and Asian isolates form Genogroup 5. Genogroup 6 is represented by He strain. New investigations say about new Genogroup 7, which consists of Japanese isolates.

The aim of this study was to genetically analyse IPN viruses occurring in Polish fish farms from 2001 till 2017 year. To achieve this - 1377 bp fragment of VP2 gene was sequenced and analysed.

Fish samples originating from freshwater fish were examined for the presence of IPN virus using isolation on cell cultures, Real Time RT-PCR and RT-PCR method. VP2 gene was amplified and sequenced. Obtained sequences were analysed and assembled into one consensus by Geneious software. A phylogenetic tree was generated by the Neighbour Joining method and the MEGA6 software tool. For the phylogenetic analysis Polish isolates of IPNV were compared with sequences available in GenBank. All of tested Polish isolates belong to Genogroup 5 - same as European Sp isolates.

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P22 - Genetic characterization of E2 gene of bovine viral diarrhoea virus in Poland

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Bovine viral diarrhoea and mucosal disease virus (BVDV-MD) belongs to the family of Flaviviridae of the Pestivirus genus. BVDV has a worldwide distribution and is one of the main causes of economic losses in cattle breeding. Pestiviruses are highly variable. Accumulation of single mutations leads to creation of new variants of the virus. E2 glycoprotein is the main structural component of the virion, has the function of binding to the cellular receptor and is the main target of neutralizing antibodies. At the same time E2 encoding fragment has the highest genetic variability in the BVDV genome.

Samples positive in PCR for 5'UTR were used in this study. Total RNA was extracted using TRI Reagent. For standard RT-PCR six primers were used to amplify E2 region. The PCR products were sequenced and analysed by MEGA, BioEdit, DNASP6, SNAP, NetNGlyc1.0.

We obtained 14 sequences of good quality. The percentage of sequence identity in E2 region was lower compared to other regions of the genome. The dS/dN ratio for the whole E2 fragment was 0.143 which indicates a negative selection. We identified the positive selection in positions 88-99 and 163-174. Four glycosylation sites have been identified. One extra glycosylation site in position 25aa was found in two strains from the same herd. First analysis of E2 sequences of Polish BVDV isolates provided helpful information for designing effective vaccines and BVDV control strategies. In their immunogenic N-terminal part, the sequence identity between vaccine and field strains was low - 55.5%.

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P23 - No significant changes in the distribution of subtypes of bovine viral diarrhoea virus in Poland

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Bovine viral diarrhoea and mucosal disease virus belongs to the Flaviviridae family and together with classical swine fever virus and border disease virus forms the Pestivirus genus. The genome of BVDV consists of ssRNA(+) with a length of 12.3 kb. The virus is highly variable. There are currently two species: BVDV-1 and BVDV-2. Diseases caused by them manifest by various mainly mild, clinical symptoms. However, the infection causes immunosuppression which may increase the risk of superinfection. Previous studies have shown the presence of 5 subtypes (1b, 1d, 1f, 1g, 2a) circulating in Poland. The aim of this study was to type currently circulating BVD viruses in dairy herds.

A total of 9290 serum, tissue homogenate, ear notch and semen samples were collected in years 2015-2018. Total RNA was extracted using TRI Reagent. Reverse transcription-polymerase chain reaction (RT-PCR) was carried out using commercial reagents. cDNA was amplified using primers pair specific for 5'UTR and Npro region. Sequenced PCR products were analysed by MEGA 5 and BioEdit.

Positive results for BVDV were obtained for 63 samples from 30 farms. All strains belonged to BVDV-1 species and subtypes related to 1f, 1b, 1g, 1d and 1e. The highest number of isolates was identified in Wielkopolskie and Lubelskie provinces. Sequence similarity between various subtypes ranged from 81-91% in 5'UTR and 76.5-85.4% in Npro region. Number of subtypes is stable however distribution of subtypes undergoes slight fluctuation. Such studies may improve the efficiency of vaccines used and the reliability of diagnostics.

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P16 - Epidemiological patterns of Aujeszky's Disease virus in wild boars in Italy

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The aim of this study was to evaluate the spatio-temporal trend of ADV infections in the wild boar population in Italy based on PRV genome and gE antibody detection. We considered three different ecological areas (1, 2 and 3) representative of the whole country located in Alps North (Lombardia Region), Apennines Centre (Romagna and Marche) and Apennines South (Campania).

Wild boar genital swabs and blood as well as data such as sex, age and location of shooting will be collected in the three areas during the hunting seasons 2016-2019. One hundred and fifty-two samples were collected in area 1, 107 in area 2 and 107 in area 3. PRV DNA was determined by specific gB gene real-time PCR. Partial sequencing of the gC gene was also performed on PCR positive samples. Virus isolation was attempted through inoculation in PK15 cells. PCR positive samples were detected in only areas 1 (5,92%) and 3 (5,6%). Antibody detection showed 13,3% of positivity in area 1, 51% in area 2 and 17,75 in area 3. Phylogenetic analysis showed a clear distinction between sequences originated from wild boars and domestic pigs. In particular, the epidemiological situation was characterized by the presence of two typical Italian clades 1 (Wild boars) and 2 (pigs) supported by new patterns of aa deletions/insertions. These results show that AD infection is widespread in Central Italy as evidenced by high seroprevalence level. PCR positive samples were instead detected only in the other two areas where seroprevalence values are lower.

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P8 - ASSESSMENT of the EPIDEMIOLOGICAL SITUATION of PRIMARY TUBERCULOSIS in AZERBAIJAN for 2013-2017

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Azerbaijan has passed a long way and achieved significant successes in tuberculosis control however this disease remains one of the main health problems of the country. The purpose of this study is a comparative analysis and an assessment of the tuberculosis epidemic situation for 2013 - 2017.

The assessment was carried out by retrospective analysis of data from annual reports using Electronic Integrated Disease Surveillance System (EIDSS), which allows tracking epidemiological surveillance data. Entering data of tuberculosis patients to EIDSS directly by the staff of dispensaries contributes to the urgent notification of epidemiologists. Estimation of the number of cases was performed based on studies' results including assessment of disease cases recorded using various sources. The tuberculosis cases for the study period were the following: in 2013-4528; in 2014-4384; in 2015- 3989; in 2016-3793; in 2017-3871. A slight increase of cases number from 2016 to 2017 (2.05%) is associated with improvement in diagnosis due to equipping 5 more regional laboratories (in addition to 2) with modern equipment for molecular genetic research (GeneXpert MTB/RIF), as well as with organization of training courses for laboratory technicians. The proportion of children and adolescents among the number of patients in 2013 was 11% (502); 2014-9.2% (402); 2015-9.3% (369); 2016-9.5% (361); 2017-9.6% (373).The mortality rate for 5 years averaged to 3.8.

Overall, the country has made significant progress in diagnosis, prevention and treatment of tuberculosis and plans to expand the laboratory network across other regions.

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P4 - Genetic typing of rotaviruses group A in domestic pigs and human patients

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Rotavirus group A (RVA) is the most important member of genus Reoviridae, as a major agent of acute gastroenteritis in a broad range of young animals and children under the age 5. Due to a high genetic diversity of RVA strains, genotyping based on sequence differences in RNA segment encoding VP7 and VP4 proteins is useful tool for identification of strain origin. The aim of this work was the genetic typing of RVA isolates originating from pigs and human patients in Slovakia focusing on potential genetic relatedness between human and porcine RVA detected in Central Europe.

A total collection of 78 rectal swabs originating from domestic pigs and 30 stool samples from human patients were collected. The whole VP7 (G genotypes) and partial VP4 (P genotypes) ORFs was amplified by RT-PCR. A genetic variability was higher inside porcine sequences, where four G genotypes (G3, G4, G5, G11) and two P genotypes (P[6], P[13]) were detected. Human RVA strains were represented by two G genotypes (G1, G3) and one P genotype (P[8]), but they were almost identical inside genotype. Phylogenetic analysis showed high sequence identity (94.5-96.5%) of some Slovakian porcine sequences with Hungarian human sequences in both G and P genotypes. Results of this work contributed to the epidemiological map of rotavirus infection in Europe. Supported by project No APVV-15-0415.

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P15 - INTERNATIONAL COLLABORATION ON ZOOSES RISK MANAGEMENT ON THE FARM

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Animal husbandry is a high-risk subsector of agriculture as it involves exposures to potentially hazardous animals, materials and machinery. Zoonoses are defined as infectious diseases transmitted from animals to humans and vice versa, and they are considered as one of the main occupational hazards in animal husbandry. An international network ‘Safety Culture and Risk Management in Agriculture (SACURIMA, COST Action 16123)’ was established to evaluate health and safety programs, advance risk management knowledge, improve statistics on injuries and illnesses, and publish obtained results. With the aim to identify emerging zoonoses and to define preventive and control measures for occupational zoonotic hazards, a Workshop “Safety and Risk Management tools adapted to Zoonoses risks” was held in Novi Sad in March 2019. Q-fever, leptospirosis, Hepatitis E, West Nile virus, brucellosis, avian and swine influenza and mycobacterial diseases were recognized as important zoonotic hazards for farm workers. The main risk factors include contact of workers with infected animals (saliva, blood, feces); material and products from infected animals; contaminated aerosols, dust, water and infected vectors involved in transmission of zoonoses. In Serbia, the most common zoonosis among livestock owners is Q-fever with 332 human cases reported between 2007 and 2016. The conclusions of the Workshop were that safe and clean environment, regular health monitoring, education, use of protective equipment, vector and pest control strategies and biosecurity measures are the most important preventive and control measures. The participants initiated work on multidisciplinary approach involving veterinarians, physicians and public health officials to meet these requirements.

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P29 - NA Stalk deletion decreased virulence and transmission of H5N8 and H7N7 avian influenza viruses in chickens

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Avian influenza viruses (AIV), members of the RNA family Orthomyxoviridae, infect a wide range of wild and terrestrial birds. In wild birds, the natural reservoir, AIV are thought to be in a static evolution; however, the transmission of AIV to poultry is accompanied by mutations in all genes. Neuraminidase (NA) is a surface glycoprotein and varies into 9 subtypes (N1-N9). It is formed of stalk and head domains and mediates virus release from infected cells. Since 2000, deletions in the NA stalk domain (NA_{del}) have been observed, mainly in H5N1 viruses in poultry compared to AIV in wild birds. Previous studies showed that NA_{del} increased virus adaptation and/or virulence in chickens. Conversely, the prevalence of NA_{del} is less frequently observed in non-N1 subtypes. Here, using reverse genetics, H5N8 and H7N7 AIV were successfully cloned and two recombinant viruses carrying NA-N8 with 21-amino acid-deletion or NA-N7 with 19-amino acid-insertion were generated, respectively. All viruses replicated at similar levels in mammalian and avian cell culture. The NA_{del} in H5N8 slightly decreased virulence of the virus in chickens. Conversely, the extension of the NA-stalk domain of the LPAIV H7N7 increased chicken-to-chicken transmission as indicated by the amount of virus excretion and seroconversion. Together, these findings suggest that, unlike N1-NA_{del}, the long NA-stalk domain increased virulence and transmission of H7N7 and H5N8 AIV in chickens.

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P30 - Virulence Determinants of a German Avian Influenza Virus Isolate Subtype H7N7 in different poultry species

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Avian influenza viruses (AIVs) are members of the RNA family Orthomyxoviridae. According to the antigenic variation of the hemagglutinin (HA) and neuraminidase (NA) there are 16 HA and 9 NA subtypes. Low pathogenic AIVs (LPAIV) carry a monobasic HA cleavage site (CS) and are activated by tissue-restricted trypsin-like proteases causing mild clinical signs, if any. Some H5 and H7 viruses can exhibit high pathogenicity (HP) by the acquisition of a polybasic CS (pCS) after circulation of LPAIVs in poultry leading to recognition by ubiquitous furin-like proteases and up to 100% mortality. In 2015, LP and HP H7N7 viruses were isolated from the same chicken farm in Germany. Here, virulence determinants of these viruses in chickens, turkeys and ducks were studied. LP H7N7 was avirulent in all birds. In chickens, insertion of the pCS in LP (designated LP-poly) was the main virulence determinant in primarily inoculated chickens and the HP-NS segment was necessary for chicken-to-chicken transmission. In turkeys, LP-poly was significantly less virulent than in chickens and HP-NS or HP-M segments were essential for high virulence and transmission. Moreover, all viruses carrying a polybasic CS showed exclusive endothelial tropism in chickens but not in turkeys. Furthermore, Muscovy and Mallard ducks excreted high amount of viruses with moderate or no clinical signs. Together, virulence determinants of AIV vary in different bird species, in addition to the pCS, mutations in other gene segments are necessary for high virulence and transmission.

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P9 - Cross-validation of generic risk assessment tools for animal disease incursion

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In recent years, generic risk assessment (RA) tools have been developed that can evaluate the incursion risk of multiple animal diseases via multiple introduction pathways. In the G-RAID project (Generic approaches for Risk Assessment of Infectious animal Disease introduction) seven of these tools were compared and contrasted. Furthermore, the opportunities for cross-validation were explored by using all tools to assess the incursion risk of African swine fever (ASF) for the Netherlands and Finland.

Three scenarios were considered: A) actual disease situation in Europe in 2017, B) scenario A + ten cases of ASF in wild boars in Germany, and C) scenario B + one outbreak of ASF on a pig farm in Germany.

All tools agreed that in scenario A, the ASF incursion risk of the pathway “trade in live animals” was higher for the Netherlands than for Finland and that the risk of the “wild boar” pathway was the same or higher for Finland. All tools identified an increased risk for the Netherlands in scenarios B and C, compared to scenario A, with no increased risk for Finland. When comparing the importance of the introduction pathways, most tools agreed that the pathway “import of animal products” had the highest risk for Finland in all scenarios; for the Netherlands the results of the tools were a bit more diverse. With the generic RA tools agreeing upon the main conclusions on the ASF risk for the countries and scenarios considered, the cross-validation contributed to the credibility of their results.

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P6 - Association between the host CXCL16 gene variants and the occurrence of persistent infections of EAV in Polish Hucul horses

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Background: Equine arteritis virus (EAV) is an important causative agent of respiratory and reproductive diseases of equids. The virus is maintained in the population through persistently infected stallions shedding virus in their semen for years. The aims of the study was to monitor the spread of EAV among the stallions in the selected Hucul horses stud, determine the variability of circulating EAVs, and identify allelic variants of CXCL16 genes of the host, associated with susceptibility to development of persistent infections.

Methods: For the study 35 semen samples from 11 breeding stallions were collected between December 2010 and May 2013 on a 6-monthly basis and tested for the presence of EAV. Genomic sequences of viruses from four selected stallions was determined using next generation sequencing. Finally, sequences of CXCL16 gene of all tested stallions were analysed.

Results: The four EAV genomes were homologous, with high (94.89% to 99.57%) identity to each other. A number (140 to 310) of single nucleotide variable positions were identified within EAV sequences from persistently infected stallions, mostly in regions encoding non-structural protein 2 and surface glycoproteins (GP2, GP3, GP4, GP5). Four stallions possessed susceptible CXCL16 genotype, at least three of which were persistently infected. All EAV negative stallions had resistant CXCL16 genotype.

Conclusions: Results of this study show that intra-host diversity of EAV sequences is concentrated at specific sites of the genome.

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P21 - MOLECULAR SURVEY AND PHYLOGENETIC ANALYSIS OF ATYPICAL PORCINE PESTIVIRUS (APPV) IN SWINE AND WILD BOAR IN NORTH ITALY

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Background

Atypical porcine pestivirus (APPV) is a newly recognized member of the Flaviviridae family. Subsequent studies have pointed that APPV is widely distributed in domestic pigs in Europe and Asia. In this study, a virological survey was performed in North Italy to investigate the presence of APPV genome.

Methods

In the 2016-2018 period, 360 pig fetuses detected in pig herds from Lombardy region was analyzed to identify any infectious causes of abortion. In addition, 430 blood samples of wild boar, killed during 2017-2018 hunting season, were examined.

All the samples were screened using a real-time RT-PCR targeting the NS5B gene.

Phylogenetic analysis of NS3 region of positive samples was carried out.

Results

Two positive pig fetuses, in Brescia and in Mantova province respectively, were detected. The pig fetus originating from a farm in the Brescia province was also PCV-3 positive.

Out of the 430 blood samples of wild boar examined, three were APPV positive. The sequences obtained clustered with the corresponding NS3 fragments of some previously identified APPVs in Europe.

Conclusion

Based on the results of the phylogenetic analysis performed, the strains of both pigs and wild boars are correlated with previously identified strains in Spain and Germany.

It remains to be clarified whether the co-infection of the PCV-3 with APPV may have had a synergic effect in infected piglets.

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P10 - Investigation of Chlamydia spp in poultry and humans in Northwest Italy

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Avian Chlamydiosis is a re-emerging zoonotic infection in poultry in Europe and poses a serious health risk for the sector workers exposed. Moreover, two new chlamydial species have been introduced in 2014, *C. avium* and *C. gallinacea*, the zoonotic potential of which is still under investigation.

Here we present the first outcomes of a research project aimed to estimate Chlamydia spp. prevalence and the associated professional health risks in the poultry sector and in Wildlife Rescue Center of Cuneo Province in Piedmont.

Cloacal swabs were collected in 69 poultry farms: 58 from chicken, 5 from mixed poultry, two from duck, two from pigeon and one each from turkey and geese farms.

Initially, the samples were analyzed by using Real Time PCR method for the research of Chlamydia spp. Subsequently, species characterization was performed for *C. psittaci*, *C. abortus*, *C. avium*, *C. gallinacea*.

The results obtained so far show that the highest prevalence of Chlamydia spp. infection was found in chicken (9/58) and mixed poultry farms (4/5). Species characterization revealed that *C. gallinacea* was the only species detected in chickens (9/58), while *C. psittaci* was only found in ducks and pigeons. Neither *C. avium* nor *C. abortus* were detected in any farm. No positivity was found in the 83 swabs and 2 livers from wild birds collected in the Rescue Center.

Furthermore, four out of 61 human sputum collected from the farmers were positive for Chlamydia spp. Species typing is ongoing, but to date one *C. psittaci* infection was identified.

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P25 - Recent identification of Influenza D Virus in swine and cattle in Piedmont, Northwest Italy

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A new genus of the Orthomyxoviridae family, named Influenza D virus (IDV), was firstly identified in 2011 in Oklahoma in swine showing flu-like symptoms.

In the next few years, the IDV detection in cattle and pig farms in USA, France, China, Japan and Italy suggested a wide geographic distribution. Although IDV was initially isolated from pigs, cattle are suspected to be the main reservoir of the virus, likely implicated in the bovine respiratory disease complex.

The study investigates the circulation of IDV in pigs and cattle in Cuneo province (Piedmont region) by active and passive surveillance. In addition, a retrospective study was carried out on swine archived nasal swabs collected and extracted in 2014-2015 in the same province.

Thirty farms were sampled during the first year of the survey (2018-2019) and more than 300 samples from healthy and/or symptomatic subjects were collected. A total of 218 bovine nasal swabs, 24 bovine lungs and 58 swine oral fluids were processed for the identification of IDV's RNA. In the retrospective study, 857 swine nasal swabs and 22 samples of lungs from 43 farms were tested for IDV.

After RNA nucleic acid extraction, a Real-time PCR assay specific for Pb1 fragment of IDV was performed. Preliminary results show one cattle farm (for fattening) and one pig farm (for breeding) positive for IDV, from the active surveillance and the retrospective study, respectively. Furthermore, viral isolation on cell cultures is ongoing from starting material to identify the IDV strains circulating in the study area.

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P14 - Incidence of Taenia infections in Armenia from 2009 to 2018

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Human taeniasis is an infectious disease from ingestion of *Taenia saginata* (in beef) or *T. solium* (in pork) larvae. *T. solium* causes cysticercosis; neurocysticercosis (NCC) is a major cause of neurological morbidity worldwide. Although taeniasis have been reported in Armenia recently, *Taenia* infections are likely underdiagnosed, and the true prevalence of these diseases in this country is unknown. To this end, we conducted a retrospective study to determine the incidence in Armenia between 2009 and 2018.

Data sources were annual disease reports, urgent notification forms, epidemiological investigation forms. All cases were diagnosed coprologically by identifying *Taenia* eggs or proglottids in stool specimens.

From 2009 to 2018, 681 cases of human taeniasis were reported; 88% of these cases were caused by *T. saginata*, and the rest by *T. solium*. Incidence rates (annual per 100,000 population) ranged from 1.1 to 3.8, with the highest proportion of cases (12%) occurring in patients under 19 years of age. Annually, most cases (minimum 68% to maximum 82%) occurred among women as a result of tasting raw meat during cooking. The highest incidence rates were reported in the Tavush, Lori, and Aragatsotn regions, and annually between 20% and 37% of cases occurred in rural areas.

Given these results, there is an urgent need to establish active surveillance to detect *Taenia* infections in humans and animals. Intersectoral research and prevention programs should be implemented, public awareness should be improved, especially in areas where the incidence of taeniasis is the highest.

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P24 - Occurrence of a commercial N^{pro} and E^{rns} double mutant BVDV-1 live-vaccine strain in newborn calves

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Bovine viral diarrhoea (BVD) is one of the most important infectious diseases of cattle causing major economic losses and significant impact on animal welfare worldwide. The major source for disease spread are in-utero infected, immunotolerant, persistently infected animals since they shed enormous amounts of BVD-virus (BVDV) throughout their lives. During the sequence-based virus typing of diagnostic ear notch samples performed in the context of the obligatory German BVDV eradication program, the commercial N^{pro}/E^{rns} double mutant BVDV-1 live-vaccine strain KE-9 was detected in seven newborn calves; their mothers were immunized in the first trimester of gestation. Six calves either succumbed or were culled immediately, but the remaining animal was closely monitored for six months. Viral RNA was detected in the skin sample taken in its first and fifth week of life, but virus could not be isolated. Further skin biopsies that were taken at monthly intervals and every serum and urine sample, nasal, oral, and rectal swab taken weekly tested BVDV negative. However, neutralizing titers against BVDV-1 remained at a consistently high level. To further control for virus shedding, a BVDV antibody and antigen negative calf was co-housed which remained negative throughout the study. The missing viremia, a lack of excretion of infectious virus and negative follow-up skin samples combined with consistently high antibody titers speak against the induction of the classical persistent infection by vaccination with recombinant KE-9 during gestation. Hence, the epidemiological impact of the RNA/antigen positivity for an extended period in the skin is presumably very low.

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P18 - Rabbit Hemorrhagic Disease Virus-Like Particles, a versatile platform for multivalent foreign B-cell epitope display

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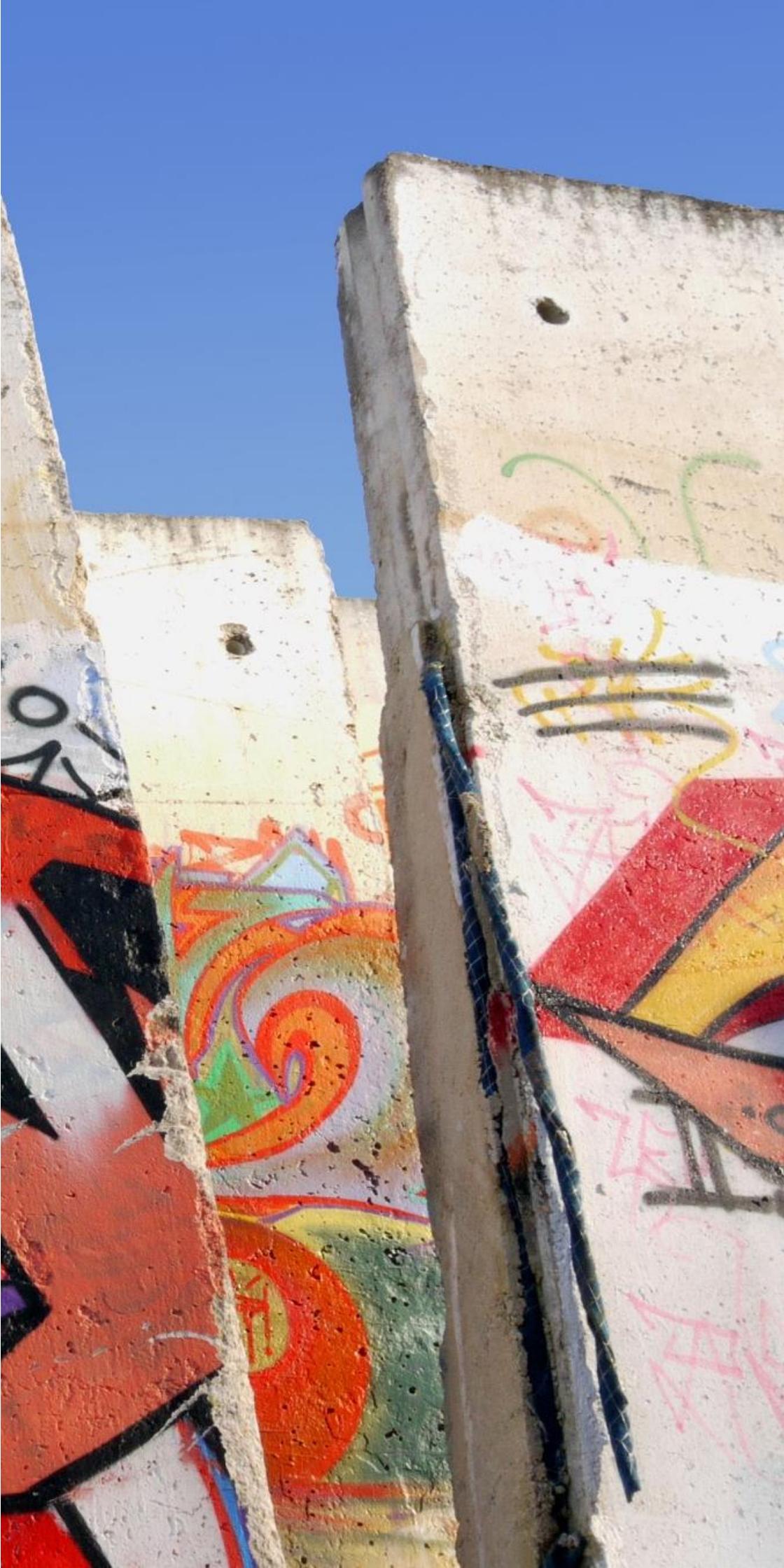
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Virus-like particles (VLPs) are viral structural protein capsids lacking genetic material. Replicative inability makes VLP-vaccines safer than classic ones and an attractive strategy for preventing disease outbreaks in disease-free status countries. In addition, VLPs can be engineered to display multiple foreign antigens, avoiding diagnostic proteins (DIVA vaccines). Our goal is to develop rabbit hemorrhagic disease virus (RHDV) VLPs as a delivery system for the multimeric presentation of immunogenic epitopes derived from animal-health relevant pathogens. Previously, we have identified four independent locations within the gene of the RHDV capsid protein (VP60), where we can insert foreign sequences without affecting the self-assembly of the resulting chimeric protein into VLPs and which can induce strong specific antibody responses against inserted foreign B-cell epitopes, including neutralizing antibodies.

In order to improve the potential of RHDV VLPs as platforms for foreign epitope presentation, we generated and characterized chimeric RHDV VLPs for the simultaneous display of two different model foreign B-cell antigens (2L21 from canine parvovirus and FCV22 from feline calicivirus). Resulting constructs were analyzed by SDS-PAGE, Western blot and electron microscopy. The immunogenic potential of the chimeric VLPs was analyzed in the mouse model. Sera from groups of immunized mice were assayed for antibodies specific for 2L21 and FCV22 epitopes by ELISA. Results have confirmed chimeric RHDV VLPs ability to induce specific humoral responses simultaneously against both model foreign B-cell epitopes. Thus, RHDV capsid might be a suitable vaccine platform for multivalent foreign B-cell epitope display.

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Posters

Threats at the European Borders

P7 - Mutations in conserved neuraminidase residues of avian influenza H5N1 virus naturally isolated from humans modulated sialidase activity and virulence in mice but not in chickens

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Highly pathogenic avian influenza virus H5N1 of clade 2.2.1 is endemic in Egypt since 2006 and distinct clades have evolved: clade 2.2.1.1 in commercial poultry and clades 2.2.1.2 and 2.2.1.2a in humans and poultry. Compared to the neuraminidase (NA) of the parental 2.2.1 viruses, avian viruses in clade 2.2.1.1 possessed one mutation (I168T) and human viruses in clades 2.2.1.2 and 2.2.1.2a had 4 mutations (A46D, L204M, S319F and S430G) and 16 mutations, respectively. Here, recombinant 2.2.1.2a viruses carrying different NA resembling those in clade 2.2.1, 2.2.1.1 or 2.2.1.2 or single mutations were generated. In vitro, no or minimal impact on replication in cell culture, plaque size, cleavability, receptor binding activity and oseltamivir resistance was observed. Viruses with human-like NA had significantly lower NA activity than viruses with avian-like NA. Reduced NA activity of 2.2.1.2a was due to L204M. Insertion of L204M in H1N1, H5N1 and H7N1 viruses also reduced the NA activity. All inoculated chickens died within 3 days post-infection. In mice, virus with L204M exhibited lower virulence and did not kill all animals, whereas S319F and S430G increased the virulence without remarkable difference in the cellular immune response. Together, H5N1 viruses in humans acquire NA mutations to maximize fitness in mammals without impact on replication in poultry.

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P1 - A risk assessment of human brucellosis infection in Shirak, Armenia from 2013 to 2018

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Brucellosis is a major public health concern in Armenia. Human and animal cases typically occur within well-documented epizootic risk areas; the incidence outside of these areas is less well-known. To compare the likelihood of contracting brucellosis within and outside of these risk areas, we conducted a serostudy among the residents of Shirak Marz, which contains known disease foci. Between 2013 and 2018, we collected blood samples from 1,358 randomly selected residents of 119 communities in Shirak Marz. We used Wright and Huddelson assays to test the samples. Participants with lab-confirmed brucellosis completed retrospective epidemiological questionnaires to determine associated risk factors; as a control, 2,210 healthy individuals also completed the questionnaire. We used ArcGIS 10.1 to develop risk maps.

During the study period, human brucellosis occurred in 36 of the 119 communities investigated. Of 1,358 subjects, 132 were confirmed to have brucellosis (including 28 chronic cases); of these, 104 reported direct contact with animals with laboratory-confirmed brucellosis, and 28 reported consumption of unprocessed dairy products. Among respondents exposed to sick animals, the infection risk odds ratio was 3.9 times higher than that of those who did not have contact with agricultural animals. We found that areas of integrated risk expanded over the course of the study: brucellosis was diagnosed in 24 communities in 2013, and 36 communities in 2018. These findings indicate that until effective brucellosis control measures are implemented in Shirak Marz, the risk of infection will remain high, both inside and outside of known disease foci.

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P9 - GENE SIGNATURES ASSOCIATED WITH FOOT-AND-MOUTH DISEASE VIRUS INFECTION AND PERSISTENCE PART I: PERSISTENT FMDV IN LONG-TERM MULTILAYER CULTURES OF SOFT PALATE CELLS

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Foot-and-mouth disease virus (FMDV) causes a highly contagious vesicular disease in livestock, with serious consequences for international trade. FMDV persistence in the oropharynx of cattle slows down the process to obtain an FMDV-free status after an outbreak. To study biological mechanisms, or to identify molecules that can be targeted to diagnose or interfere with persistence, we developed a model of persistent FMDV infection in bovine dorsal soft palate (DSP). Primary DSP cells were cultured in multilayers at the air-liquid interface (ALI). After 5 weeks of culture without passage, the cells were infected with FMDV strain O/FRA/1/2001. Infection was monitored until 28 DPI. Approximately 20% of cells still had a polygonal morphology and displayed tight junctions as in stratified squamous epithelia. Cells with similar morphology expressed cytokeratin. A limited cytopathic effect was induced, restricted to the upper cell layers. FMDV antigen, RNA and live virus were detected from day 1 to 28, with peaks at day 1 and 2. The proportion of infected cells was highest at 24 hours (3 % and 36 % of cells at an MOI of 0.01 and 1, respectively). At day 28 after infection, at a time when animals that still harbour FMDV are considered carriers, FMDV antigen was detected in 0.2% - 2.1% of cells, in all layers, and live virus was isolated from supernatants of 6/8 cultures. The ALI model of DSP brings new possibilities to investigate FMDV persistence in a controlled manner. Transcriptomic data are presented in a joint communication.

“Gene Signatures Associated with Foot-And-Mouth Disease Virus Infection and Persistence Part II: Proteogenomics Uncovers Critical Elements of Host Response in Bovine Soft Palate Cells” is presented as oral presentation (Topic: Threats at the European Borders).

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P10 - Vaccination of goats with a thermotolerant experimental vaccine confers a full protection against a PPR virulent challenge

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Peste des petits ruminants (PPR) is an OIE-listed disease of small ruminants, endemic in Africa and Asia, causing high economic impacts. Vaccination is an effective way to control PPR, but hot ambient temperatures associated to possible power cuts in developing countries may alter the vaccine stability during storage and transportation. Thus, making available an effective and thermotolerant vaccine is a priority.

Lyophilized form of an experimental vaccine (EXP-VAC), formulated to be thermotolerant, was exposed at 37°C for three days before use, mimicking a cold-chain rupture.

Ten PPRV-naïve goats received a dose of well-established PPR-VACTM[1], ten others received a dose of EXP-VAC and another ten remained unvaccinated. Twenty one days later, all goats were infected intranasally with a virulent PPRV (Morocco 2008). Clinical signs and viral ocular excretion (RT-qPCR) were monitored for 14 days.

Controls presented PPRV ocular shedding and severe clinical signs related to PPR such as nasal and ocular discharge, mucosa lesions, diarrhea, and weight loss.

EXP-VAC-vaccinated goats only presented few mildly swollen lymph nodes. EXP-VAC completely prevented viral ocular excretion. No difference in efficacy was observed between EXP-VAC and PPR-VACTM used according to label.

Thus, efficacy of EXP-VAC was demonstrated. This thermotolerant vaccine may be an aid to allow epidemiological control of PPR preventing economic losses in areas where the cold chain cannot be guaranteed.

[1] Enchéry, et al. Vaccine 37 (2019) 1667-1673.

PPR-VACTM: registered trademark of Botswana Vaccine Institute.

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P8 - Identifying the role of Complement Receptor 2 on Follicular Dendritic Cells in the persistence of Foot and Mouth Disease Virus

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Over 50% of cattle, regardless of vaccination status, become persistently infected after exposure to Foot and Mouth Disease Virus (FMDV). As well as allowing animals to become persistently infected carriers of FMDV, the current inactivated FMDV vaccines are also limited in that they only protect for 4-6 months. It is well known that in persistently infected animals, live FMDV is detected in germinal centers of lymphoid tissues from the head and neck; however, the mechanisms associated with establishment of persistent infections are poorly understood. FMDV immune complexes trapped by follicular dendritic cells (FDCs) may represent a possible source of infectious material upon natural infection, and this may be important for maintaining high titres of neutralising antibody.

In the present study, we tested the hypothesis that complement receptor 2 (CR2) on FDCs are involved in the trapping and long-term persistence of FMDV, and that the role of retained antigen is essential for inducing long term antibody responses after infection. A pilot study was carried out in an FMDV mouse model in which groups of BalbC mice were infected with FMDV after treatment with a monoclonal antibody 4B2 to block CR2. We assessed FMDV antigen retention on splenic FDCs using bioimaging and qPCR techniques, and measured the generation of the specific immune response by ELISA. We were able to demonstrate that 4B2 blocks CR2 in spleen cells *ex vivo* and *in vivo*; and that the blockade of CR2 by 4B2 *in vivo* impeded the trapping of FMDV in GC in the spleen.

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P11 - Studies on the evaluation of a molecular pen-side test for PPRV

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Peste des petits ruminant (PPR) is a highly contagious disease, especially in goats and sheep. The PPR virus belongs to the genus of Morbillivirus in the Paramyxoviridae family. The major distribution areas of this fatal disease extend to Africa and Asia. Due to the socio-economic impact of PPR in developing countries and similar to the successful eradication of rinderpest virus, FAO and OIE strives to eradicate the disease worldwide by 2030.

For a fast molecular detection of PPRV under field conditions, we started to shorten the nucleic acid extraction time of a magnetic bead based protocol from 18 to nearly 7 minutes. For this purpose, three different extraction kits were evaluated on a BioSprint 15 platform. Furthermore, specific real-time RT-PCR for the high-speed detection of PPRV in about 30 minutes was established. Initially, several primer-probe-combinations were tested in the high-speed RT-qPCR by using a commercially available lyophilized master mix. With a device test of various qPCR cyclers, differences in the required duration of an identical protocol, the sensitivity and the detection limit were evaluated. Comparative validations with regard to the robustness of various rapid detection methods for PPRV (Lateral Flow Devices, Antigen-ELISA, RT-qPCR) were carried out. In summary, the presented molecular pen-side test for PPRV, based on a rapid nucleic acid extraction and high-speed RT-qPCR, deliver highly sensitive and specific results in less than one hour. Based on this concept for a molecular pen-side test, the detection of further important pathogens with clinically similar symptoms can be easily realized.

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P2 - DISTRIBUTION OF BRUCELLOSIS IN BARDA-YEVLA KH REGION OF LOWLAND ECONOMIC ZONE OF AZERBAIJAN

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Brucellosis causes significant economic losses due to mass abortions, infertility, culling of productive animals in many countries, including Azerbaijan. The vaccination program available in the country covers only sheep. However, it's not enough to eradicate brucellosis in Azerbaijan. In the period of 2009-2015, most brucellosis cases in cattle and sheep occurred in Barda-Yevlakh region of lowland economic zone. The goal of this study is determining the prevalence of brucellosis in Barda-Yevlakh region.

The study was carried out in November-December 2015, covered 5 districts, including 25 villages and 15 winter pastures. Sample collection sites were selected by randomized method. Blood samples were collected from cattle, sheep and goats. Data were analyzed in Excel MS and Access MS.

The blood samples of 3,425 cattle and 5,565 sheep and goats were collected. The prevalence of brucellosis was 1.9% [n=65] among cattle, and 2.9% [n=162] among small ruminants. Seropositive animals were detected in 22 villages and 11 winter pastures. The infection rate was 100% for the districts, 88% for the villages and 73% for the winter pastures. The age of seropositive cattle was 7 years (median:5, mode:5) and small ruminants 3 years (median:3, mode:2) in winter pastures and villages.

The results show a relatively high prevalence among cattle, sheep and goats in the study area. It is important to emphasize that the prevalence of brucellosis among small ruminants was higher than prevalence among cattle, despite available in the country the small ruminants' vaccination program. We also recommend conducting a pilot project on vaccination program among cattle in lowland zone.

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P3 - Prevalence of *Cl. botulinum* and *Cl. perfringens* in animal food samples in Azerbaijan in the period of 2015-2019

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Botulism is a dangerous disease caused by *Clostridium botulinum* toxin and it has been described in most animal species. Two dead cattle were diagnosed with botulism cases in Gakh and Shaki regions of Azerbaijan in 2015. Therefore, starting from 2015, the Central Veterinary Laboratory (CVL) randomly collects and tests the animal feed for *Clostridium botulinum* and other species of *Clostridium* that are harmful for animals. The aim of this study was to evaluate the prevalence of *Cl. botulinum* and *Cl. perfringens* in animal food samples in Azerbaijan in the period of 2015-2019. The animal food samples were randomly collected by the regional veterinary inspectors, transported to the CVL for testing. All samples were cultured on Meat Peptone Agar. Grown cultures were stained using Romanovski Gimza and examined under the microscope. In total, 364 animal food samples were collected in the period of 2015-2019. 84 feed samples were positive for *Cl. Perfringens*. During this period, the CVL revealed the agent of enterotoxemia, *Cl. Perfringens* in 28 animal pathological material. No positive cases of animal botulism were detected until 2019. In the period of January-March 2019, CVL detected *Cl. botulinum* in five food samples collected from Agsu, Agdash, Baku and Siyazan. This samples were discarded from animals feed ration.

To analyze the situation with food borne botulism it is important to collect the samples from all regions regularly and to use the modern methods as ELISA for testing of the food samples. Also, recommended to follow up sanitary standards in feed storage.

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P4 - Using indirect hemagglutination to detect *F. tularensis* near Lake Sevan, Armenia

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Rodents and arthropods are known carriers of tularemia, a zoonosis. Humans are very susceptible to this disease through exposure to infected animals. To identify high-risk tularemia areas near Lake Sevan in Armenia, we used indirect hemagglutination to detect *Francisella tularensis* in rodents and mites.

In September 2018, we collected 40 *Microtus arvalis* (common vole) by trapping and excavation of burrows in two villages near the western edge of Lake Sevan: Chambarak, and Shorzha. In the laboratory, we collected 350 gamasid mites from these rodents and processed them by the photo eclectic method. We used indirect hemagglutination to test rodent serum for antibodies and tick suspension for antigen to *F. tularensis*. To exclude non-specific agglutination, formalized sheep erythrocytes were used.

Preliminary positive results (titers between 1:40 and 1:320) were observed in six rodent samples. Three of these samples were ultimately confirmed to be positive: one from Chambarak (1:40) and two from Shorzha (1:40 and 1:320). From the locations of the positive results, the field team collected additional material, from which a culture of *F. tularensis* was isolated.

The high-sensitivity, low-cost indirect hemagglutination method was effective in detecting *F. tularensis* in rodents. The Armenian Ministry of Health ultimately conducted a preventive vaccination program amongst residents of the areas where the positive samples were collected.

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P5 - Comparative investigation of zoonotic leishmaniasis in wild canids and domestic dogs in Armenia

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Visceral leishmaniasis (VL) is disease caused by the intracellular parasite *Leishmania* and transmitted by phlebotomine sand flies. Almost 1,000 cases of VL have been recorded in Armenia since it was first detected in the country in 1913. Canids are known as reservoirs. Studies indicate that seroprevalence of *Leishmania* is as high as 20% among domestic dogs in some areas of Armenia, but no data are available about incidence in wild canids. To address this gap, we conducted a serosurvey of jackals and domestic dogs in three regions of Armenia that are most often affected by VL.

Under veterinary supervision, we collected blood samples in Syunik, Tavush, and Lori regions from a total of 90 jackals and 90 domestic dogs. We tested all samples for the presence of the *Leishmania* recombinant K39 strip-test according to the manufacturer instructions.

15 of 90 jackals (17%) were seropositive for *Leishmania*, as well as 10 of 90 dogs (11%). In Syunik, 19% of jackals (7 of 36) and 9% of dogs (3 of 35) were seropositive. In Tavush, 11% of jackals (3 of 27) and 21% of dogs (6 of 28) were seropositive. In Lori, 19% of jackals (5 of 27) and 4% of dogs (1 of 27) were seropositive.

Given these seropositivity rates, it is possible that both wild and domestic canids are reservoirs for human leishmaniasis in Armenia. Further investigation is needed to determine seroprevalence among canids countrywide, and to clarify the role that these animals play in the transmission of *Leishmania*.

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P6 - Reassortment is the driver: Detection, incursion and spread of highly pathogenic avian H5 influenza viruses in Europe 2016-2018

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Highly pathogenic avian influenza viruses (HPAIV) of subtype H5 clade 2.3.4.4b were present in Europe starting in 2016, continuing in 2017 and ongoing until 2018, and showed high mortality in wild and domestic birds causing the most severe HPAIV epizootic ever reported in Europe. Analysing genome sequences of these viruses is the tool to gain insights into phylogenetic relationships, geographic spread and potential incursion routes and therefore can serve as a prerequisite for both effective outbreak forecast and control. For the segmented genomes of influenza viruses reassortments are a frequent source of new viruses that may complicate analyses and control of outbreaks. Here we present a fast workflow to detect and classify influenza reassortants via Supernetworks (IRIS) combined with geographic analysis. The algorithms were applied to sequences from H5 viruses from wild birds, poultry and other housed birds collected worldwide during the epizootic from Summer 2016 to Summer 2018. We analysed over 440 whole H5 genomes of different neuraminidase (segment 6) subtypes, mostly H5N8 viruses, but also H5N5, H5N6 and H5N2 viruses. These have a mix of different polymerase segments (segment 1-3) and nucleoproteins (segment 5) originating from a wide variety of LPAI viruses. The reassortant groups show a spatio-temporal distribution that points to independent incursions into Europe and spread between European regions. More than 15 distinct reassortants were identified with this method indicating multiple routes of globally occurring HPAIV H5NX viruses.

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P12 - Middle East respiratory syndrome coronavirus (MERS-CoV) early immune responses in an alpaca infection model

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Background: Middle East respiratory syndrome coronavirus (MERS-CoV) causes fatal infections to humans. Although dromedaries are considered the main animal reservoir, alpacas are also reported as potential hosts for MERS-CoV. This work aimed to investigate the early immune events triggered upon MERS-CoV infection in the airway epithelium of alpacas.

Materials and Methods: Twelve alpacas were inoculated with 10⁷ TCID₅₀ of MERS-CoV/Qatar 2015, and three extra animals served as negative controls. From 1 to 4 days post-inoculation (dpi), 3 alpacas were euthanized each day. Nasal swabs were collected for viral RNA detection and virus titration. Respiratory tissues were obtained to perform immunohistochemistry (IHC) and cytokine quantification by RT-qPCR. We also evaluated IFN- λ 3 responses on infected and non-infected nasal epithelium, as assessed by IHC, obtained by laser microdissection (LMD), from the same tissue section.

Results: All infected alpacas excreted infectious MERS-CoV, notably 2 dpi onwards. Maximum loads of virus were observed on epithelial cells from nasal turbinates at 2 dpi. Concomitant to virus resolution (3 dpi onwards), a moderate to high up-regulation of type I/III IFNs, interferon induced genes, RNA sensors and proinflammatory cytokines was observed. Similarly, IFN- λ 3 was up-regulated in infected epithelial cells obtained by LMD comparing to non-infected ones.

Conclusions: An early IFN and proinflammatory responses were triggered in epithelial cells upon infection with MERS-CoV. This innate immune response takes place shortly after viral infection at the nasal epithelium and might explain the subclinical infection occurring in camelid species, since it parallels with a decrease in viral loads in nose.

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P13 - An improved method for the genetic modification of Capripoxviruses

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Lumpy skin disease (LSD) is a disease of cattle caused by the LSD virus (LSDV), genus Capripoxvirus (CPPV) of the family Poxviridae.

Currently, LSD vaccines are live-attenuated, obtained via several in vitro passages of the virus. No other methods for rapid and efficient LSDV vaccine production have been developed so far. While homologous recombination (HR) is a standard technique for genetic manipulation of Orthopoxviruses, this is less efficient and more time-consuming for CPPV which have an in vitro slow growth rate and produce smaller and more indistinct plaques. This hampers the development of rationally designed live-attenuated vaccines. We aimed to establish improved methods for genetic manipulation of CPPV.

We combined HR with sorting of single infected cells in order to produce an eGFP-tagged LSDV. The eGFP sequence was fused to the LSDV ortholog of the VACV-A5L. BS-C-1 cells were infected with the Neethling strain of LSDV and then transfected with the recombinant construct. BS-C-1 carrying the recombinant virus were single sorted for green fluorescence twice. Fluorescent analysis of limiting dilutions were then used to isolate the recombinant virus and eliminate the wildtype virus. The generated eGFP-tagged LSDV was sequenced to confirm the correct eGFP insertion and absence of off-target mutations.

In conclusion, combining HR and fluorescence activated single cell sorting facilitated and sped up the process for LSDV genetic manipulation, avoiding the use of drug selection and multiple rounds of plaque purification. The LSDV strain generated using this new method will be a useful tool for studying the LSDV biology

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P14 - TaqMan based qPCR assay for specific detection of Sheeppox virus

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Sheeppox virus, together with goatpox virus and Lumpy skin disease virus, is a member of the genus Capripoxvirus, family Poxviridae. SPPV strains cause sheep pox, disease primarily in sheep but can also cause disease in goats. It is characterized by fever, generalized papules or nodules, vesicles, internal lesions and death. Mortality can be up to 100%. Sheep pox are endemic in Africa north of the Equator, the Middle East and Asia, but recently there were outbreaks in European countries Bulgaria and Greece and also in other countries such as Chinese Taipei, Israel, Kazakhstan, Kyrgyzstan, Mongolia, Morocco and Russia. Because of severity of disease and great economic importance, Sheep pox is listed to OIE notifiable diseases. Rapid detection of the virus before appearance of clinical signs would be very important in managing and for surveillance of the disease.

We describe development of rapid TaqMan based real-time PCR assay for specific detection of SPPV, both field and vaccine strains. The assay does not detect GTPV or LSDV strains. Specificity of the assay was tested on 20 different SPPV, GTPV and LSDV strains with no false positive or false negative results. Sensitivity of the assay was determined comparing with Capripox qPCR assay (Bowden) and it is determined that the SPPV assay is 4 times less sensitive.

Despite slightly lower sensitivity this assay can provide fast and reliable detection of the virus and differentiation from GTPV and LSDV strains without need for gel electrophoresis or nucleotide sequencing.

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Posters

Vector-borne Diseases

P4 - West Nile virus situation in Spain. A One Health approach

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The incidence and geographical distribution of West Nile virus (WNV) have increased in the last decades. In the Mediterranean basin, the number of human and equine cases and deaths dramatically augmented in 2018.

Southwest Spain has become a WNV endemic area, with annual outbreaks in birds and horses caused by lineage 1 strains, with few human cases reported. Recently, WNV has spread to Central Spain affecting wild birds and horses. In 2017 lineage 2 emerged in Northeastern Spain and in 2018 the first WNV outbreaks in horses appeared there. Besides, Usutu virus (USUV) has been identified in the country, highlighting the complex epidemiological scenario of these closely related vector-borne flaviviruses.

A multidisciplinary Spanish research group, composed of specialists in epidemiology, wildlife, entomology, animal and public health, reference laboratories and veterinary sectors, is working together to advance in the knowledge of the behaviour and eco-epidemiology of WNV and to provide better tools to control the disease. In this context, preliminary results of molecular and serological studies suggest the hypothesis of two WNV lineage 1 introductions into the territory and the circulation of USUV in new areas.

The final goal of this one-health approach is to promote an integrated human, animal and vector surveillance at country level aimed to detect WNV circulation and report animal cases in order to implement prophylactic measures in human settlements.

Finally, all gathered information will contribute to raising awareness among all stakeholders and citizens to reinforce measures directed to control WNV transmission and reduce infections.

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P6 - Monoclonal Antibodies as diagnostic tools for Lumpy Skin Disease

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Lumpy skin disease (LSD) is an infectious disease of cattle, caused by LSD virus (LSDV), genus Capripoxvirus, family Poxviridae, listed as notifiable by the OIE due to its potential for rapid spread and substantial economic impact. In 2015, LSD emerged in the EU causing outbreaks in Greece and then, throughout 2016, in several Balkan countries.

Laboratory confirmation of LSD relies mostly on molecular tests, however there is a lack of simpler virological tests such as ELISA for antigen detection or Lateral Flow Devices applicable in field conditions, or ELISA assays suitable for serological surveillance. Since such assays greatly benefit from the use of monoclonal antibodies (MAbs), we have produced a panel of 75 MAbs against LSDV Neethling strain and evaluated their potential for the development of new diagnostic tools. Of them, 12 MAbs cross-reacted to different levels with the Neethling attenuated vaccine strain in indirect ELISA, 20 reacted in Western Blotting the majority recognizing a viral protein of 35 kD, none showed virus-neutralizing capability.

A few selected MAbs, evaluated as coating and peroxidase-conjugated antibodies in sandwich ELISA, were able to detect LSDV in the supernatant of infected cultures.

These results are promising for the development of simple diagnostic tests useful to control the diffusion of LSD; moreover, the availability of a panel of characterized MAbs for the LSDV may provide important resources for improving knowledge on the virus antigenicity and structural features.

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P13 - Evaluation of an IgM-specific ELISA for early detection of bluetongue virus infections in domestic ruminants sera

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Competitive-ELISA (cELISA) is the most widely used serological test for the detection of Bluetongue virus (BTV) viral protein 7 (VP7) antibodies (Ab). However, these BTV cELISAs cannot distinguish between IgG and IgM. IgM Ab, generated shortly after the primary immune response, indicate a recent infection. Because the BTV genome or anti-VP7 Ab can be detected in ruminant blood months after infection, BTV diagnostic tools cannot discriminate between recent and old infections. In this study, an IgM-capture ELISA prototype to detect ruminant anti-BTV VP7 IgM was evaluated on 1,650 serum samples from cattle, sheep, or goats. Animals were BTV-naive, infected, or/and vaccinated with different BTV strains. 30 cattle sera infected with Epizootic haemorrhagic disease virus (EHDV - serotype 6) were also tested.

The specificity was 99.4 (IC95% 98.6-99.7). The exclusivity, measured with BTV positive samples was 98.1% (95,8-99,3). BTV samples from experimental infections were tested; IgM were detected from 1 to 5 weeks post-infection. Interestingly, the anti-VP7 IgM peak was concomitant with the presence of infectious viruses and BTV RNA in blood, allowing BTV RNA detection in IgM positive sera from serum bank stored at -20°C. Using this strategy, we were able, after amplifying and sequencing partial BTV RNA segment 2, to determine the causative BTV serotype/strain. Therefore, BTV IgM ELISA can detect BTV introduction in an area with BTV-seropositive domestic animals. This approach may be of particular interest for retrospective epidemiological studies.

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P19 - BLV infection in selected herds in Hatay province, Turkey: Is there any role of *Culicoides* spp. on the transmission of BLV?

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BLV is known as the etiological agent of Enzootic bovine leukosis, which is the most common neoplastic disease of cattle. The major route of virus transmission is believed to be iatrogenic. In addition, BLV proviral DNA has been identified in nasal secretions, milk, colostrum, and semen; however, natural transmission of BLV through these secretions has not been clearly demonstrated. The role of flies is not exactly known though some studies about the horses flies were reported in the past. In this presentation, we report to the determination of the BLV or antibodies to BLV in cattle (n=224) and also in *Culicoides* spp pools collected in Hatay province, Turkey. Sera were tested for antibodies by ELISA. All blood samples and *Culicoides* pools were subjected to PCR by using the primers targeting the env gene. The PCR products were sequenced. Our results showed that 2,67% (6/224) of the sampled cattle and a *C. schultzei* pool were positive for BLV besides the seroprevalance rate as 1,33% (3/224). According to the phylogenetic tree, the sequences of the BLVs detected both animals and a *Culicoides* pool were clustered with genotype 1 BLVs. Even if these results do not reveal exactly the role of *Clucoides* and also other some flies, in the transmission of BLV, the simultaneously presence of the same genotype virus in these species is noteworthy. Further studies on the Env gene and other gene regions of BLVs from these species are still continuing to understand the possible relation between cattle and flies.

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P12 - Vaccination of cattle with BTVPUR® containing BTV-2 provides full protection for one year

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Bluetongue virus (BTV) causes an infectious disease which is transmitted between ruminants through biting midges (Culicoides). BTV cycle can be interrupted by the vaccination of susceptible species.

BTVPUR® is a range of inactivated vaccines registered in a “multi-strain” EU dossier, allowing formulation of monovalent or bivalent vaccines with different combinations of the registered serotypes BTV-1/2/4/8. In sheep and cattle, efficacy of BTVPUR® is substantiated through an official claim with one-year duration of immunity (DOI). Hereafter we present the experimental data supporting the BTV-2 DOI in cattle.

Thirteen BTV-naïve calves were vaccinated twice, three weeks apart, with a BTV2 vaccine; in parallel, seven control calves stayed unvaccinated. Twelve months later, seven vaccinates and seven controls were challenged with a virulent BTV-2, while the six remaining vaccinates were boosted with a single injection. Challenged animals were monitored for clinical signs, serology (VNT) and viraemia (real-time RT-PCR) for 28 days after challenge.

After challenge, all controls were viraemic, but not the vaccinates (prevention of viraemia). In addition, the increase of rectal temperatures in the control group was significantly higher than in the vaccinated groups.

Three weeks after the 12-month revaccination, BTV-2 neutralizing-antibody titres were significantly higher than those observed after the initial vaccination.

One-year DOI of BTVPUR® containing BTV-2 was demonstrated in cattle. With previous results, this shows that the BTVPUR® vaccine range is an effective and flexible tool to clinically and epidemiologically control BTV outbreaks in EU in ruminants.

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Merial is part of Boehringer Ingelheim.

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P7 - Optimised serological assays to quantify the humoral immune response to Lumpy skin disease virus (LSDV)

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Lumpy skin disease (LSD) is an important transboundary disease of cattle. Over the last decade LSD has spread further into the Middle East and more recently, eastern Europe causing significant production losses. LSD is caused by lumpy skin disease virus (LSDV), a member of the Capripoxvirus genus within the Poxviridae family. Following infection with LSDV, clinical disease can range from inapparent (subclinical) to moderate or severe (clinical). A complex humoral immune response is raised to LSDV with the production of neutralising (nAb) and non-neutralising antibodies (nnAb). The serum neutralisation test (SNT) is the most commonly-used method for measuring the nAb response to LSDV. Few methods exist for measuring nnAb. In order to improve the detection and quantification of nAbs and nnAbs to LSDV, an optimised immunofluorescent virus neutralisation test (IFVN) and immunofluorescent monolayer assay (IFMA) were developed. The IFVN calculates the nAb by measuring the reduction in the number of LSDV foci present after incubation with test serum. The IFMA determines antibodies to a wide spectrum of LSDV antigens by detecting LSDV-infected cells using the test serum. A comparative study was performed to characterise serum collected from cattle challenged with LSDV. Titres derived from the IFMA were higher for each animal compared to the IFVN in both clinical and subclinical animals indicating a high proportion of nnAbs. The detection and quantification of nAb and nnAb to LSDV provide vital information on the immune status of animals following infection or vaccination and contributes to the determination of correlates of protection.

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P15 - Assessment of the impact of vaccination interval on the serological response of sero-negative cattle vaccinated with BTPUR®

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Most inactivated vaccines require 2 injections to achieve their claimed level of protection in naïve animals. Typically, the Summary of Product Characteristics (SPC) of such vaccine will indicate, based on scientific demonstration that the interval between these 2 vaccinations should be of XX weeks.

The BTPUR® vaccine range makes no exception to this and, when 2 injections are required, the recommended vaccination interval is “3 to 4 weeks”.

However, for practical and logistic reasons, this interval is not always respected and it may happen occasionally that this interval is extended to 6 or even 8 weeks.

To assess the impact of this deviation on the immunogenicity of the vaccination program, one group of 10 seronegative cattle was vaccinated according to the recommend regimen (4 weeks interval). Another group of 10 seronegative cattle was vaccinated using a delayed revaccination interval (8 weeks interval). All animals were regularly blood sampled and their sera were assayed in virus neutralization tests against BTV-8.

The results of this field trial show that, with the tested vaccine:

- Both vaccination regimens (4 weeks or 8 weeks interval) induced a clear sero-response against BTV-8.
- The 8 week vaccination interval allowed a booster effect at least equivalent to the one observed with the recommended vaccination interval of 4 weeks.

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P2 - Flaviviruses in Germany: vector competence of northern and southern European *Culex pipiens* biotype *molestus* for Usutu and West Nile virus

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West Nile virus (WNV) and Usutu virus (USUV) are neurovirulent mosquito-borne flaviviruses posing a global threat to human and animal health. While research has predominantly focused on WNV, as the geographically most widespread arbovirus with a high zoonotic potential, USUV is considered a neglected pathogen. Both viruses originate from Africa and currently circulate throughout Europe, with sporadic outbreaks among resident wild and captive bird species as well as other susceptible mammals. The first isolation of USUV in Germany was in 2011, with recurrent outbreaks in the following years. In 2018, WNV antigen was detected for the first time in Germany in several bird species and two horses. In the same year, Serbia reported a significant number of human WNV cases, after both viruses had been introduced within the last decade.

To better comprehend and predict the spread of WNV and USUV strains throughout Germany, vector competence studies were performed with a native *Culex pipiens* biotype *molestus* colony from Lower Saxony. For comparison purposes, a Serbian *Culex pipiens* biotype *molestus* colony from Novi Sad was also tested. Mosquitoes were offered a blood meal spiked either with a German WNV lineage 2 strain isolated in 2018 or an USUV Africa 2 strain from 2015. After two to three weeks, individual samples of mosquito saliva, bodies and extremities were tested for the viruses by cultivation on mammalian cells or qRT-PCR. Both the German and the Serbian mosquito colonies were capable of transmitting WNV in their saliva 14 and 21 days post infection.

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P3 - Experimental West Nile and Usutu virus infections in a migratory bird, the European turtle dove (*Streptopelia turtur*)

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In this study, we investigated the effect of WNV and USUV infections in the migratory European turtle dove, to determine its potential role in viral dissemination.

Two groups (n=10) of doves were inoculated subcutaneously in the neck (10000 pfu/individual) with either Italy/08 WNV strain or Haut-Rhin7315 USUV strain. As control group, 9 animals were sham-inoculated with an equivalent volume of diluent. Birds were observed daily for clinical signs and a follow-up of viral load in blood, feathers, oral swabs and neutralising antibody titres was made up to 15 days post-inoculation. The birds were euthanized at 15 dpi and tissue samples were collected for viral load determination.

No significant mortality or morbidity were observed. In the USUV-infected group, only 60% of the animals showed a low viral load in blood, but no virus was detected in feathers or oral swabs. In the WNV-inoculated group a low viral load was detected only in the blood and feathers of 50% and 30% of the doves, respectively. All inoculated animals developed neutralising antibodies and viral RNA was detected in spleen at 15 dpi in 80% of USUV and in all WNV-infected birds.

These data indicate that (1) the infection caused by the assayed strains is asymptomatic in turtle doves and (2) they are not competent hosts. Consequently, a relevant role of the European turtle dove in the dissemination of these flaviviruses is not expected.

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P8 - Entomological and genetical studies of Lumpy Skin Diseases in the Samara region

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Lumpy skin disease (LSD) is a viral infection affecting cattle. LSD reached Russia in 2015. Currently, it is present mainly in the regions along the border with Kazakhstan and occurs as local epizooties. The long-distant spread of LSD virus is linked to illegal movements of animals while it's local spread is driven by mechanical transmitters - blood-sucking insects fed on infected animals. Therefore, the presence, abundance and ability of vectors to maintain the virus play a crucial role in the epidemiology of the disease. In this study, we identified the presence of potential vectors in the affected area as well as the vectors directly involved in the epizooty of LSD in the Samara region in 2018 and obtained a molecular characterization of the virus caused it. The most abundant potential vector species in the area is *Musca domestica*, followed by *Stomoxys calcitrans*, *Culicoides pulicaris*, *Culex pipiens*, and *Musca autumnalis*. PCR analyses revealed the presence of the viral DNA in the pulled samples of *S. calcitrans* и *M. domestica* sampled at the outbreak site. LSDV genome remained detectable within 21 days after the registration of the disease. We isolated this virus on cell culture. Its titer at 5th passage was of 5.5-6.0 lg TCID₅₀/ml. Phylogenetic analysis indicated that it belongs to the cluster of LSDV circulating in Europe since 2015. Kinelsky strain genome shares 99.9% homology with RNOA-15 isolate. The results provide valuable information for understanding the epidemiology of the disease and improving its prevention.

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P18 - In-vivo characterization of tick-borne encephalitis virus in the natural reservoir host

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Tick-borne encephalitis virus (TBEV) is an important tick-borne arbovirus, causing severe neurological symptoms in humans. To successfully circulate in nature, the virus supposedly needs an amplifying host, to be spread among the tick-population. One of these suspected hosts is the bank vole, a small mammalian that is found in woodland areas all across Europe. To explore their role in the complex transmission cycle of TBEV, outbred bank voles were inoculated with various TBEV isolates, originating from ticks as well as bank voles. Housed in groups of four, three bank voles each were infected with the remaining animal acting as an in-contact control to monitor for virus shedding. None of the animals showed any neurological symptoms during the experiment. 28 days post infection dissection was performed. The majority of infected animals tested positive for TBEV-RNA by RT-PCR in whole blood samples, while no viral genome was detected in the corresponding serum samples. In addition, several brain samples tested positive, primarily from animals inoculated with an isolate originating from a bank vole brain. Virus was successfully re-isolated in cell culture from selected organ samples. All inoculated animals seroconverted, showing remarkable high titers in a serum neutralization assay. In contrast, in-contact animals showed neither positive RT-PCR results nor seroconversion. These findings suggest that even though infected bank voles do not shed infectious virus, they seem to play an important role in the transmission of TBEV, since a prolonged viremia could possibly be a source for infection of feeding naïve ticks.

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P5 - Suitability of individual and bulk milk samples to investigate the humoral immune responses to LSD vaccination

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The commercially available ID Screen® Capripox double antigen ELISA from IDvet® validated and authorized for serum and plasma, recently improved the options for assessing the humoral immune response against lumpy skin disease (LSD). In this study, the suitability of milk (individual and bulk) and colostrum as samples for this ELISA was investigated.

Samples (serum, milk and colostrum) were collected three times (before, one and five months post re-vaccination) in LSDV-field strain free regions in Serbia. Test specificity was confirmed by analyzing of 352 individual milk samples from Germany.

From 154 collected serum samples from Serbia, 75 were detected as positive by the Capripox ELISA. Milk samples were tested using protocol for serum and with a modified protocol (increased incubation time). Obtained results of milk and colostrum samples were analyzed with reduced cut-off value. Out of 154 milk samples, 38 samples were detected as positive using the protocol for serum, whereas with modified protocol the number of positive sample increased up to 48. Only two milk samples from Germany had border line results with the modified protocol. Sensitivity and specificity of the ELISA for milk samples reached values in-between 88% to 91%, with significant statistical difference ($p < 0.05$) between both protocols. In addition, antibody detection was possible in bulk milk and colostrum samples.

In principle, the commercially available Capripox ELISA, developed for antibody detection in serum and plasma, shows to be suitable for detection of LSD-specific antibodies in milk samples and can be a helpful tool in LSDV monitoring programs.

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P16 - Investigating the relationship dynamics between serological responses and bluetongue virus replication in cattle and sheep

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Bluetongue is a non-contagious, haemorrhagic disease of ruminants caused by the Orbivirus, Bluetongue virus (BTV). Whilst cattle are often sub-clinically infected, sheep typically develop moderate to severe clinical disease. Throughout previous BTV infection studies, cattle appear to develop BTV-specific antibodies much later in infection than sheep; either against the group antigen, VP7, or neutralising antibodies against VP2. However, experimental variations (including BTV strain, tissue culture adaptation, inoculation route) make comparing these species-specific serological responses very difficult. Even within host species, systematic correlation of the dynamic relationships between these immunological responses and viral replication or strain virulence has been underexplored and may prove valuable in establishing correlates of protection or disease.

Here, we have used archived time-course serum obtained from sheep and cattle across several BTV infection studies where respective BTV strains were all transmitted to individual animals via blood-feeding of infected *Culicoides sonorensis*, thereby simulating natural infection most closely. The detection of anti-VP7 and neutralising antibodies within sera of these BTV-infected animals was correlated to respective viral blood RNA levels (qRT-PCR) and clinical manifestation. Indirect ELISAs were developed using a BTV (VP7) milk ELISA platform (ID-Vet, Grabels, France) to determine respective immunoglobulin isotype subclasses (such as IgM, IgG) and class-switching dynamics throughout infection. Seroconversion dynamics of individual animals most closely related to respective onset and progression of detectable RNA levels in the blood, with animals of earlier and higher RNAemia seroconverting earlier. Those sheep most clinically affected tended to seroconvert earlier, reflecting the dynamics of respective BTV blood RNA levels.

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P9 - ONE-RUN REAL TIME PCR ASSAYS FOR THE DETECTION OF CAPRIPOXVIRUSES, FIELD ISOLATES AND VACCINE STRAINS OF LUMPY SKIN DISEASE VIRUS

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The cattle and sheep industry is economically important for sustainable economic growth. Lumpy skin disease (LSD) has recently expanded its range into the Northern Hemisphere. Considering the current situation, it's important to have reliable diagnostic tools for successful control and eradication.

In this work, we present for the first time, a set of PCR assays that can simultaneously identify the genome of Capripoxviruses (PCR-CAPR), field isolates of LSD viruses (PCR-LSDV) and the Neethling vaccine strain (PCR-NEE). The PCR-CAPR assay targets the conserved P32 gene, the PCR-LSDV assay the LSDV126 (EEV gene) and the PCR-NEE assay the LSDV008 gene. The amplification efficiency for all assays was more than 90%, with detection limit of 0.3 lg TCD₅₀ / cm³.

An evaluation of the assay repeatability revealed that the intra-run variation was between 0.16 to 0.54 and the inter-run variation ranged from 0.39 to 0.52 over five repetitions across three runs. These assays were successfully validated on a large number of samples collected during LSDV outbreaks experimental studies, supported by sequencing and virus isolation results.

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P10 - Identificaton of Neethling-like vaccine strains in Russia in 2017

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Lumpy skin disease (LSD) is a dangerous transboundary infectious disease of cattle, accompanies with significant morbidity but low mortality [Tageldin et al., 2014; Sevik et al., 2016].

LSD has affected many regions throughout Russia since its first occurrence in 2015. The largest number of outbreaks for Russia was 2016 when the virus resurged following a modified stamping out campaign, causing 313 outbreaks in 16 regions. To avoid unwanted adverse reactions following the use of live attenuated vaccines against LSD virus (LSDV), sheep pox-based vaccines were administered during vaccination campaigns. As a result, LSD was successfully contained throughout all Russian regions in 2017. In the same year, however, LSD emerged anew in a few regions of the Privolzhsky Federal District of Russia along the northern border of Kazakhstan, which then necessitated vaccinating cattle with a live attenuated LSDV vaccine. Although live attenuated LSDV vaccines are prohibited in Russia, several vaccine-like LSDV strains had been identified in the 2017 outbreaks, including commercial farms and backyard animals exhibiting clinical signs consistent with those of field LSDV strains. Three vaccine-like LSDV strains clearly aligned with corresponding RPO30 and GPCR gene sequences of commercial attenuated viruses. How vaccine-like strains spread into Russian cattle remains to be clarified.

The present work has been the first to report on the presence of widespread vaccine-like LSDV strains in Russian cattle despite prohibitions in the use of live LSDV vaccines.

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P11 - Determination of lumpy skin disease virus in bovine meat and offal products following experimental infection

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Lumpy skin disease has recently expanded its range northwards to include The Balkans, South Europe, Turkey, and Russia. Given the absence of conclusive evidence on the transmission mechanism and natural ability of LSDV to infect subclinically, viraemic animals and their products represent a risk as an indirect transmission pathway.

In this work, we used virulent lumpy skin disease virus (LSDV) strain Russia/Dagestan/2015 to evaluate the safety of meat and offal products in terms of PCR positivity and infectivity in clinical and subclinical infection.

At day 21 post-inoculation, seven animals developed the generalized disease, and four animals became latently infected without clinical signs. Upon necropsy, animals with the generalized disease had skin lesions, enlarged lymph nodes, lesions in lungs, trachea, and testicles. Subclinically ill animals had enlarged lymph nodes.

The skeletal meat in both disease presentations was negative by PCR and virus isolation, whereas in cattle with skin lesions, meat parts with gross pathology physically connected underneath the site of a skin lesion carried the live virus. In subclinical infection, only enlarged lymph nodes carried the infectious virus; the other internal organs were negative.

Overall, viral genomes and infectious virus were recovered in organs with gross lesions. Infectious virus was found in testicles regardless of the presence of pathology or the type of disease presentation. Our findings demonstrate that non-treated skeletal meat from LSDV infected animals considered a safe commodity, whereas internal organs carry the live virus and should be appropriately handled.

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P14 - The Bluetongue virus strain related to TOV in healthy goats in south Germany

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In the last years, several 'atypical' Bluetongue virus (BTV) serotypes were discovered and serologically defined like TOV in Switzerland, BTV-26 in Kuwait and BTV-27 on Corsica. In addition, putative novel serotypes based on the genetic data from China and Italy were found. All the 'atypical' BTV were only identified in small ruminants, mostly without any clinical sign.

In July 2018, several goats from a holding in Baden-Wuerttemberg were screened for the presence of BTV and two goats were tested positive in a BTV-25 serotype specific PCR. The following experimental diagnostic inoculation of goats using the BTV-25 positive field blood samples improved the quality and the propagation of the BTV-25 related virus in cell culture was successfully for the first time since the initial detection of TOV in Switzerland in 2008. By applying the sequence- independent single-primer amplification (SISPA)-NGS method, full genome of this BTV-25like isolate of Germany was obtained. Furthermore, we could surveille over a longer time period a BTV-25 occurrence in a goat flock in the southwest of Bavaria with a size of approximately 120 goats. The EDTA blood was screened with RT-qPCR by using a new developed BTV-25 assay targeting segment-2 and for serological surveillance, the serum was screened with a commercial cELISA. In beginning of 2019, a goat imported from Italy to a farm in Baden-Wuerttemberg was tested positive for BTV-25 and for this time, the virus propagation was for the first time successfully from a field blood sample.

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P20 - Functional characterization of Simbu virus nonstructural proteins in the insect vector cycle

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Shuni virus (SHUV) and Schmallerberg virus (SBV), members of the Simbu serogroup within the family Peribunyaviridae, genus Orthobunyavirus, are transmitted by *Culicoides* biting midges. Infection of dams during a critical phase of gestation may lead to severe congenital malformations in ruminants. For SHUV, also neurological symptoms were described and a general zoonotic potential cannot be ruled out. While SBV is endemic in Europe, SHUV is distributed in Africa and the Middle East and a further northern spread seems likely.

The tri-segmented RNA genome of SHUV and SBV codes for four structural and two nonstructural proteins, namely NSs and NSm. NSs is a virulence factor in vertebrate hosts, counteracting the interferon induction, while NSm plays a role in virus assembly and morphogenesis and a function in the insect host is supposed. For SBV, a reverse genetics system including NSs and NSm deletion mutants is already available. To investigate the function of both SBV nonstructural proteins in the insect vector, multistep growth studies with NSs and NSm deletion mutants were performed in *Culicoides sonorensis* cells. For the establishment of a SHUV reverse genetics system, cDNA copies of the three RNA segments of a recent Israeli isolate were cloned into a plasmid vector and deletion mutants lacking NSs and/or NSm will be generated and in vitro characterized.

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P1 - Detection and molecular characterization of West Nile Virus from wild bird in Serbia 2018

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West Nile fever is zoonotic arthropod-borne disease affects birds, humans and horses. Causative agent is virus, which belongs to family Flaviviridae, genus Flavivirus. Up to today nine genetic lineages were described. It's been detected in Africa, Europe, Australia, the Middle East, North, South and Central America and West Asia. Virus circulates between mosquitoes and birds, whereas humans and horses are dead end hosts. The presence of clinical symptoms in birds varies from absence to fatal neurological symptoms. Horses and humans can be affected, with signs of encephalitis and encephalomyelitis. Migratory birds are responsible for the widespread distribution of virus.

The aim of this study is identification and molecular characterization of West Nile virus detected in dead hooded crow (*Corvus cornix*) in Serbia during the monitoring of the disease in mosquitoes and wild birds, in July 2018. In order to determine genotype of the virus, sequencing of the envelope gene was performed. Homology of obtained nucleotide sequence with known isolates identified in Serbia and neighbour countries in previous years was assessed. The phylogenetic analysis showed that this isolate belongs to lineage 2 WNV, and showed 99,92% homology with sequences of West Nile virus isolates 341/2010-Greece, Serbia/Novi Sad 24/2013, Vojvodina_2013_03 and Nea Santa-Greece-2010.

Results obtained during the monitoring of WNV and consequential phylogenetic analysis are important for the understanding of WNV molecular epizootiology and could be used for the determining the origins and geographical distribution of the virus.

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P17 - Experimental infection of calves with seven serotypes of Epizootic Hemorrhagic Disease virus: production, characterization of reference sera and evaluation of a new competitive ELISA

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Epizootic Hemorrhagic Disease is an infectious non-contagious disease of ruminants caused by Epizootic hemorrhagic disease virus (EHDV). Seven serotypes are officially recognized and at least 2 putative new serotypes have been reported. These reports highlight the need for efficient diagnostic tools to identify the causal agent.

The aim of this study was to produce reference sera against serotype EHDV-1/3, 2, 4, 5, 6, 7, and 8. Seven Prim'Holstein calves were inoculated at day 0 (D0) with the seven reference strains of EHDV (1 serotype per calf). Blood samples (EDTA and whole blood) were periodically taken from D0 until the end of the experiment (D31). Sera were tested both with a commercially available competitive ELISA (c-ELISA) and a new c-ELISA kit. Viral genome was detected from EDTA blood samples and typed using in-house real-time RT-PCRs. Sera taken on D31 were tested by seroneutralisation (SNT) and virus neutralisation test (VNT) (for calibration of reference sera).

Viral RNA was first detected at D2 in five of seven calves then in all at D7. Seroconversion was observed between D10 and D23 according to the EHDV serotype. SNT and VNT have allowed to determine the neutralizing antibody titers of each serum and the potential cross-reactions between serotypes. The two c-ELISA used in this study showed similar results. The specificity, sensitivity and exclusivity of the new c-ELISA have also been evaluated. These calibrated sera are now available for the serological identification of an EHDV isolated on culture cells or for positive control in SNT assay.

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