ABSTRACT BOOK

ESVV2022

12th International Congress For Veterinary Virology

Sept. 20-23, 2022
Ghent, Belgium

The organisers of ESVV2022 thank the following sponsors for their crucial support:

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ESVV european society for veterinary virology

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WELCOME TO GHENT!

Dear colleagues and friends,

On behalf of the European Society for Veterinary Virology (ESVV), it is my great pleasure to welcome you in the historical city of Ghent, Belgium, for the 12th International Congress for Veterinary Virology! This triennial congress is held on September 20-23, 2022, in the International Convention Center of Ghent (Gent ICC).

The ESVV was founded in 1988 in Belgium to promote exchange of information in the field of veterinary virology. The first ESVV meeting was organized in 1989 and recent editions were held in Vienna (2018), Montpellier (2015) and Spain (2012).
Twenty-four keynote speakers were invited at ESVV2022 to present their latest findings. Six plenary sessions will cover pathogenesis, viral immunology, epidemiology, diagnostics, vaccines, and (reverse) zoonoses. Three round tables about viral infections of swine, horse, cattle, wildlife and vector-borne viral diseases will take place. Over 300 participants including veterinarians, virologists and academics, authorities, pharmaceutical industries as well as PhD students are registered.

The organisation of ESVV2022 would not have been possible without the hard work of the Local Organizing Committee, but also without the major contribution of the Scientific Committee who has allowed us to put together an exciting, broad and balanced scientific program. Thank you to all of you and also to all the speakers and poster presenters for their scientific contribution! Finally, the sponsors of ESVV2022 are also acknowledged for their essential financial support.

As you will experience, most of Ghent's medieval architecture remains intact and is remarkably well preserved or restored, which explains why the National Geographic Traveler Magazine listed Ghent as one of the most authentic cities of the world. Because of its geographic localization at the confluence of the Rivers Scheldt and Leie, the city has also a vibrant life and nightlife, with the Port located in the North and the University that also attracted many research oriented companies. Most of the hotels are situated in the historic car-free center and efficient tram transportation connects them to the Citadel park where the ICC convention center hosting ESVV2022 is located.

With all the science going on, don't forget to enjoy the many pleasures that Ghent has to offer: dozens of pubs with impressive selections of Belgian beers, mouth-watering Belgian chocolates, a delicious cuisine, excellent musea and astonishing historic buildings. Also, make sure to attend the congress dinner followed by the evening entertainment with Open Bar on Thursday evening!

Welcome to Ghent and enjoy!

Prof. Dr. Hans J. Nauwynck
Congress Chair

ESVV2022 COMMITTEES

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Ghent University, Belgium

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INVITED KEYNOTE SPEAKERS

Sandra Blome (Friedrich-Loeffler-Institut, Germany)
René Bødker (University of Copenhagen, Denmark)
Linda Dixon (The Pirbright Institute, United Kingdom)
Douglas Gladue (Plum Island Animal Disease Center, U.S. Department of Agriculture, U.S.A.)
Miguel Ángel Jiménez-Clavero (Animal Health Research Centre (INIA-CISA), CSIC, Spain)
Benedikt Kaufer (Freie Universität Berlin, Germany)
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Enric Mateu (Autonomous University of Barcelona, IRTA-CReSA, Spain)
Thomas Mettenleiter (Friedrich-Loeffler-Institut, Germany)
Gerald Misinzo (Sokoine University of Agriculture, Tanzania)
Mercedes Mouriño (Zoetis Manufacturing & Research, Spain)
Morten Nielsen (Technical University of Denmark, Denmark)
Norbert Nowotny (University of Veterinary Medicine, Vienna, Austria)
Polly Roy (London School of Hygiene & Tropical Medicine, United Kingdom)
José Manuel Sanchez-Vizcaino (Universidad Complutense de Madrid, Spain)
Niek Sanders (Ghent University, Belgium)
Artur Summerfield (University of Bern, Switzerland)
Sebastiaan Theuns (PathoSense, Belgium)
Etienne Thiry (Liège University, Belgium)
Wim van der Poel (Wageningen Bioveterinary Research, The Netherlands)
Kristien Van Reeth (Ghent University, Belgium)
Piet van Rijn (Wageningen Bioveterinary Research, The Netherlands)
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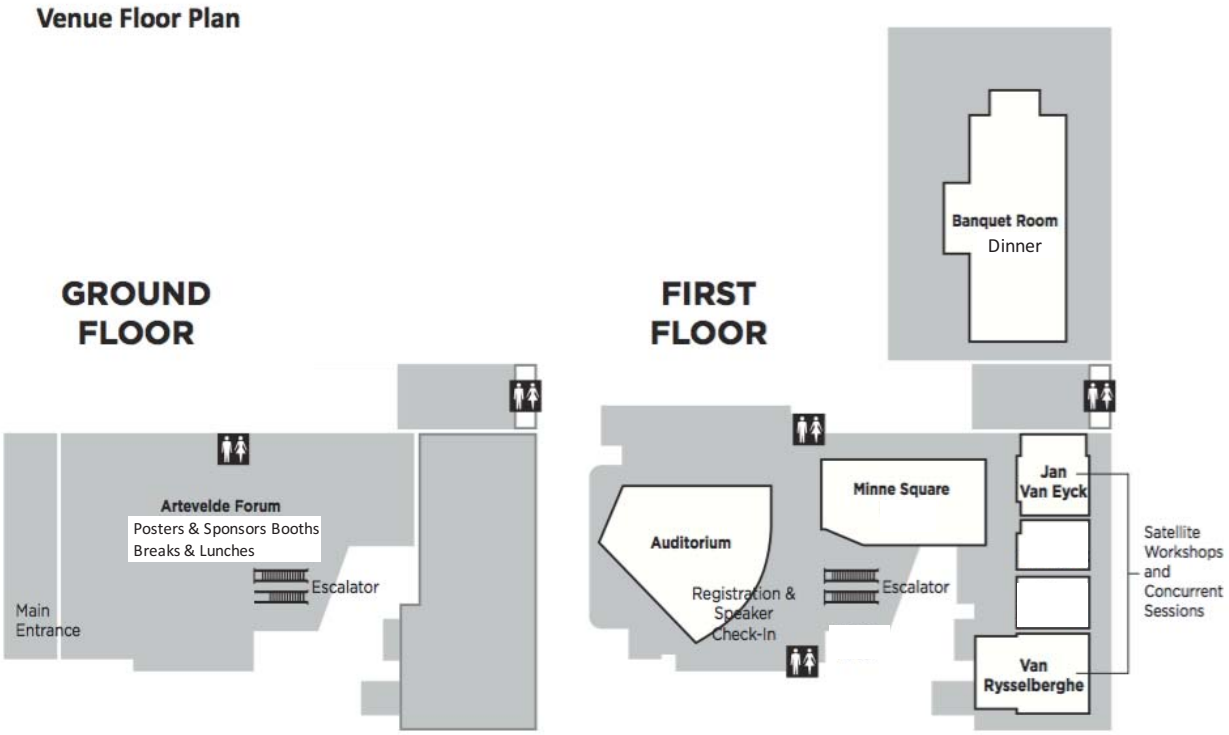
Tamaš Petrović (Scientific Veterinary Institute Novi Sad, Serbia)

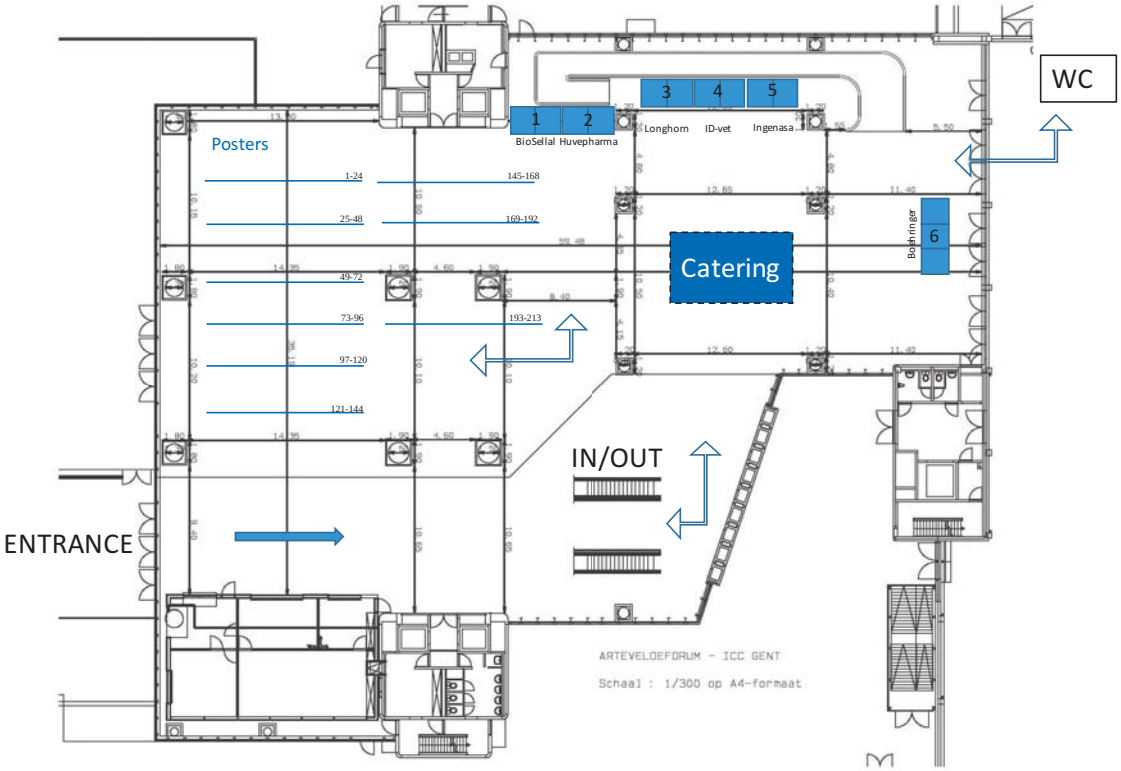
CONGRESS INFORMATION

CONGRESS VENUE

Ghent ICC - International Convention Center
Van Rysselberghedreef 2
9000 Ghent, Belgium
https://www.iccghent.com/
T: +32 (0)9 242 89 07
nina.vangerven@iccghent.com



POSTERS AND SPONSORS (ARTEVELDE FORUM)



ONLINE RESOURCES

- PDF of the Abstract Book

Available at: https://esvv2022.ugent.be/
Password: ESVVGhent2022
Free internet access in the ICC

ESVV2022 CONGRESS POLICIES AND INFORMATION

Name Badges

As a registered attendee, you will be issued an ESVV2022 name badge when you pick up your registration materials. You will be required to display your name badge for admission to all official functions. In the event of a lost badge, please visit the Registration Desk.

Liability

The Congress organizers cannot accept responsibility for personal injuries, or loss or, or damage to private property belonging to you or any accompanying people during or as a consequence of the Congress.

Programme Changes

ESVV 2022 reserves the right to make any necessary changes to the program at short notice. There will be no pro rata refund of registration fees for changes to the program.

Scientific Data
Information communicated by presenters should be considered "personal communication." Please seek permission from the presenter before quoting unpublished research results or using data as a basis for further investigations. Photographs or recording (audio or visual) of data, whether displayed on screens, in posters, or elsewhere is forbidden. Your cooperation is greatly appreciated.

Photography
By attending the Congress, you allow the congress photographer to take pictures of you. These pictures will only be made accessible to the participants of the Congress.

Other Considerations
As a courtesy to all Congress attendees, please make sure all mobile phones are turned off when sessions are in progress. Smoking: the Ghent ICC is completely smoke-free

Trams and Buses
Ghent has an extensive public transit system operated by the Flemish Transport Company De Lijn and consists of trams and buses. Plan your journey with De Lijn
https://www.delijn.be/en/routeplanner/
You can buy a ticket or pass on an electronic card from a ticket machine at the stop or in a Lijnwinkel store (outside Gent Sint-Pieters station or in de korenmarkt downtown). Always validate your ticket (yellow machine) before taking a seat on the bus or tram.

Tram directions
To the International Convention Center (ICC), from the centrum:
• Take the Tram Line 1: Direction 'Gent-Sint-Pieters/Flanders Expo
• Screens located on the ceiling will tell you which stop is coming next
• Exit at Van Nassaustraat (one block from Citadel Park)
• Walk half a block to Willem van Nassaustraat and turn left on this street
• Go to end of block and turn right on Koning Leopold II – laan
• Walk a short distance and you will see a park entrance on the left. Enter the park here and follow the signs to the ICC through the park.

From the ICC to centrum:
• Follow the signs from the ICC back through Citadel Park
• You will exit the park on Koning Leopold II – laan, turn right on this street
• Turn left on Willem van Nassaustraat Street and walk to the end of the street
• Turn right and you will see the tram station
• Take the Tram Line 1 from Van Nassaustraat in the direction 'Korenmarkt/Evergem'

Taxes
The Value-Added Tax, or VAT, is a general, broadly based consumption tax assessed on the value added to goods and services. It applies to most goods and services that are bought and sold for use or consumption in the European Union. The standard VAT rate in Belgium is 21%. Prices indicated are always including VAT.

Registration
Ghent ICC – International Convention Center, Van Rysselberghedreef 2, Ghent.
Minne Square, Level 1 for the registration desk.

Date/Registration Hours:
Tuesday, 20 September 14:00 - 20:00
Wednesday, 21 September 07:30 - 17:00

Workshop Registration Includes:
1. Name badge
2. Entry to all lectures, plenary sessions, round tables and poster sessions
3. Scheduled catering including seated dinner and open bar
4. Printed ESVV2022 Programme
5. Certificate of Attendance (available on page 18 of the Programme)
6. Congress bag filled with sponsor's flyers & items

Presenter Guidelines
• Arrive to your presentation room 15-minutes prior to the session start time to familiarize yourself with the microphone headset, laser pointer, slide advancer, speaker timer, and stage set-up.
• Please be seated at the front of the presentation room in the row reserved for speakers.
• The Session Chair will introduce you and monitor the length of the presentation.
• A speaker timer will be visible on the podium.
• A laser pointer and slide advancer will be available at the podium for your use.
• All mobile phones must be turned off while you are presenting. Mobile phones on silent will cause feedback with the microphones.

Posters
Ghent ICC – International Convention Center, Artevelde Forum, Ground Level

Poster Setup
Presenters may set up their posters on Tuesday 20 September between the hours of 15:00 – 18:30.

Presentation Requirements
All posters remain up for the duration of the congress. At least one of the authors must be present at the poster during the presentation session time as listed below.

Poster Session I, Wednesday 21 September 17:30-19:00, for all odd numbered abstracts in all 6 topics.
Poster Session II, Thursday 22 September 17:30-18:30 for all even numbered abstracts in all 6 topics.

Poster Dismantle
Posters can be removed beginning at 14:00 on Friday 23 September. Posters remaining after 17:30 on Friday 23 September will be discarded.

ESVV 2022 Agenda-at-a-Glance

Tuesday, 20 September	Wednesday, 21 September	Thursday, 22 September	Friday, 23 September
Registration & Speaker Check-in 14:00-17:00 Minne Square Setting Posters & Booths on display 15:00-17:00 Artevelde Forum	Registration & Speaker Check-in 07:30-17:00 Minne Square	Speaker Check-In 07:30-17:00 Minne Square	Speaker Check-In 07:30-16:00 Minne Square
	Plenary Session I: Pathogenesis and Viral Immunology **4 x 30' Keynote Lectures** 08:30 - 10:30 Auditorium	**Plenary Session III: Epidemiology, Diagnostics and Vaccines** **4 x 30' Keynote Lectures** 08:30 - 10:30 Auditorium	**Plenary Session V: (Reverse) Zoonoses** **3 x 30' Keynote Lectures** **2 x 15' Abstracts** 08:30 - 10:30 Auditorium
	Refreshment Break 10:30 - 11:00 Artevelde Forum	Refreshment Break 10:30 - 11:00 Artevelde Forum	Refreshment Break 10:30 - 11:00 Artevelde Forum
	Parallel Session A **Pathogenesis** 11:00 - 12:30 Auditorium **Parallel Session B** **Pathogenesis** 11:00 - 12:30 Jan Van Eyckzaal **Parallel Session C** **Viral Immunology** 11:00 - 12:30 Van Rysselbergh	**Parallel Session G** **Epidemiology** 11:00 - 12:30 Auditorium **Parallel Session H** **Diagnostics** 11:00 - 12:30 Jan Van Eyckzaal **Parallel Session I** **Vaccines** 11:00 - 12:30 Van Rysselbergh	**Parallel Session M** **Epidemiology** 11:00 - 12:30 Auditorium **Parallel Session N** **Vaccines** 11:00 - 12:30 Jan Van Eyckzaal **Parallel Session O** **Zoonoses** 11:00 - 12:30 Van Rysselbergh
	Lunch 12:30 - 13:30 Artevelde Forum	Lunch 12:30 - 13:30 Artevelde Forum	Lunch 12:30 - 13:30 Artevelde Forum
	Parallel Session D **Pathogenesis** 13:30 - 15:00 Auditorium **Parallel Session E** **Pathogenesis** 13:30 -15:00 Jan Van Eyck **Parallel Session F** **Viral Immunology** 13:30 - 15:00 Van Rysselbergh	**Parallel Session J** **Epidemiology** 13:30 - 15:00 Auditorium **Parallel Session K** **Diagnostics** 13:30 -15:00 Jan Van Eyck **Parallel Session L** **Vaccines** 13:30 - 15:00 Van Rysselbergh	**P.S. P Auditorium 13:30-14:00 Vaccines** **P.S. Q Jan Van Eyck Pathogenesis** **P.S. R Van Rysselbergh Pathogenesis** **ESVV General Assembly** 14:00-15:00 Auditorium
	Refreshment Break 15:00 - 15:30 Artevelde Forum	Refreshment Break 15:00 - 15:30 Artevelde Forum	Refreshment Break 15:00 - 15:30 Artevelde Forum
	Plenary Session II - Round Table I **Viral Infections of Swine** **ASFV** **1 x 30' and 4 x 15' talks + 30' discussion with audience** 15:30 - 17:30 Auditorium	**Plenary Session IV - Round Table II** **Viral Infections of Horses and Cattle** **Orbiviruses** **3 x 20' and 1 x 30' talks + 30' discussion with audience** 15:30 - 17:30 Auditorium	**Plenary Session VI - Round Table III** **Viruses in Wildlife & Vector-borne viral diseases** **3 x 15' talks + 30' discussion with audience** 15:30 - 16:45 Auditorium **Awards & Closing Ceremony** 16:45 - 17:15 Auditorium
Opening Lectures **Thomas Mettenleiter** **Evolution of virus research with pseudorabies virus as model** 17:00 - 18:30 Auditorium	**Poster Session I and reception with Belgian beers** 17:30-19:00 Artevelde Forum	**Poster Session II and reception with Belgian beers** 17:30-18:30 Artevelde Forum	
Welcome Reception 18:30 - 20:30 Artevelde Forum		**Seated diner** 18:30-21:00 **Evening Entertainment with Open Bar** 21:00-24:00 Banquet Room	

PROGRAMME

Tuesday, 20 September 2022

OPENING LECTURE **Auditorium**

17:00 - 17:30


Welcome address
Hans Nauwynck, Ann Martens
Ghent University, Belgium

Marisa Arias
President of the ESVV, INIA-CSIC, Spain

17:30 - 18:30

Evolution of virus research with pseudorabies virus as model
Thomas Mettenleiter
Friedrich-Loeffler-Institut, Germany

18:30 - 20:30

Welcome Reception **Artevelde Forum**

Wednesday, 21 September 2022

PLENARY SESSION I: **Pathogenesis and Viral Immunology** **Auditorium**
Chairs: Herman Favoreel (UGent, Belgium)
Benjamin Dewals (ULiège, Belgium)

08:30 - 09:00

The pathogenesis of fish alloherpesviruses
Alain Vanderplasschen
Liège University, Belgium

09:00 - 09:30

The pathogenesis and Evolution of Marek's Disease virus
Benedikt Kaufer
Freie Universität Berlin, Germany

09:30 - 10:00

The intriguing interplay of PRRSV with the host immunity
Enric Mateu
Autonomous University of Barcelona, IRTA-CReSA, Spain

10:00 - 10:30

Towards decoding the black box of immunity in veterinary species
Artur Summerfield
University of Bern, Switzerland

10:30 - 11:00

Refreshment break **Artevelde Forum**

11:00 - 12:30 **PARALLEL SESSION A - PATHOGENESIS** **Auditorium**
Chair: Bénédicte Machiels (ULiège, Belgium)

11:00 - 11:15 Cellular telomerase RNAs and viral RNAs possess common antiapoptotic functions that enhance herpesvirus-induced tumorigenesis
Ahmed Kheimar, Laetitia Trapp-Fragnet, and Benedikt B. Kaufer

11:15 - 11:30 AlHV-1 infection causes oligoclonal expansion and activation of $CD8^+$ T lymphocytes resulting in bovine malignant catarrhal fever via interaction with T cell signaling pathway
Meijiao Gong, Françoise Myster, Abdulkader Azouz, Guillem Sanchez Sanchez, Shifang Li, Justine Javaux, Sylvain Leemans, Olivier Nivelles, Willem van Campe, Stefan Roels, Laurent Mostin, Thierry van den Bergh, Pierre Kerkhofs, Laurent Gillet, Andrew J. Davison, David Vermijlen, Stanislas Goriely, Tim Connelley, Alain Vanderplasschen, Benjamin G. Dewals

11:30 - 11:45 Genomic Analysis and Replication Kinetics of the Closely Related EHV-1 Neuropathogenic 21P40 and Abortigenic 97P70 Strains
Eslam Elhanafy, Ines Zarak, Nick Vereecke, Sebastiaan Theuns, Kathlyn Laval, Hans Nauwynck

11:45 - 12:00 Virus-Induced Inhibition Of Superinfection As A Means For Accelerating Fitness-Based Selection Of Cyprinid Herpesvirus 3 Single Nucleotide Variants *In Vitro* And *In Vivo*
Yuan Gao, Noah Bernard, Bo He, Haiyan Zhang, Salomé Desmecht,, Catherine Vancsok, Maxime Boutier, Nicolás M. Suárez, Andrew J. Davison, Owen Donohoe, and Alain F.C. Vanderplasschen

12:00 - 12:15 Illumination of Cyprinid Herpesvirus 2 Infectious Cycle Using *In Vivo* Bioluminescent Imaging
Bo He, Owen Donohoe, and Alain F.C. Vanderplasschen

12:15 - 12:30 Impact of genetic diversity of the Rift Valley Fever virus, from the field isolates to a genetic determinant
Mehdi Chabert, Sandra Lacôte, Sreenu Vattipaly, Ana Filipe, Mohamed Bezeid Ould El Mamy, Moustapha Lo, Baba Doumbia, Philippe Marianneau, Catherine Cêtre-Sossah, Frédérick Arnaud, Maxime Ratinier

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11:00 - 12:30 PARALLEL SESSION B - PATHOGENESIS **Jan Van Eyck room**
Chair: Marisa Arias (CISA, INIA-CSIC, Spain)

11:00 - 11:15 Degree of PCV2 uptake by porcine monocytes is strain-dependent and is associated with amino acid characteristics on the outside of the capsid
Yueling Ouyang, Hans J. Nauwynck

11:15 - 11:30 ASFV-Host Protein Interaction Mapping Uncovers Novel Function of CP204L in Lysosome Fusion and Clustering
Katarzyna Dolata, Walter Fuchs, Grégory Caignard, Juliette Dupré, Katrin Pannhorst, Sandra Blome, Axel Karger

11:30 - 11:45 Artificial insemination as alternative transmission route for African swine fever virus: How infected boars could efficiently spread the disease
Friedrichs Virginia, Carrau Garreta Tessa, Deutschmann Paul, Christopher-Hennings Jane, Reicks Darwin, Beer Martin, Blome Sandra

11:45 - 12:00 Genome-wide CRISPR/Cas9 Knockout Screen in Porcine Cells to Identify Relevant Host Factors for African Swine Fever Virus Replication
Katrin Pannhorst, Jolene Carlson, Julia E. Hölper, Finn Grey, John Kenneth Baillie, Dirk Höper, Elisabeth Woehnke, Kati Franzke, Walter Fuchs, Thomas C. Mettenleiter

12:00 - 12:15 DNAJC14 independent replication of the atypical porcine pestivirus (APPV)
Carina M. Reuscher, Kerstin Seitz, Lukas Schwarz, Francesco Geranio, Olaf Isken, Martin Raigel, Theresa Huber, Sandra Barth, Christiane Riedel, Anette Netsch, Katharina Zimmer, Till Rümenapf, Norbert Tautz, Benjamin Lamp

12:15 - 12:30 Modeling porcine hemagglutinating encephalomyelitis virus infection in vivo and ex vivo
Juan Carlos Mora-Díaz, Pablo Piñeyro, Rolf Rauh, William Nelson, Zianab Sankoh, Edward Gregg, José Antonio Carrillo-Ávila, Huigang Shen, Rahul Nelli, Jeffrey Zimmerman, Luis Giménez-Lirola

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11:00 - 12:30 PARALLEL SESSION C - VIRAL IMMUNOLOGY **Van Rysselbergh room**
Chair: Simon Graham (The Pirbright Institute, United Kingdom)

11:00 - 11:15 *In Vitro* Recall Response to African Swine Fever Virus Reveals Immune Components Underlying Cross-protection

Jordi Argilaguet, Laia Bosch-Camós, Uxía Alonso, Anna Esteve-Codina, Beatriz Martín-Mur, María J. Navas, Marta Muñoz, Chia-Yu Chang, Sonia Pina-Pedrero, Francesc Accensi, Lihong Liu, Boris Gavrilov, Fernando Rodríguez

11:15 - 11:30 Dissecting differences and similarities in the host response to Rabies virus and other Lyssaviruses in the Syrian hamster model
Martina Castellan, Stefania Leopardi, Maira Zorzan, Petra Drzewniokova, Giampiero Zamperin, Isabella Monne, Ronald Mura, Lucas Brandao, Sergio Crovella, Paola De Benedictis

11:30 - 11:45 Inhibition of Arsenite-Induced Stress Granules by Cyprinid Herpesvirus 3
Yunlong Hu, Mamadou Diallo, Alain Vanderplasschen

11:45 - 12:00 Fast Isolation of Non-adherent, Lymphocyte-like Haemocytes in Shrimp for Immunological Studies during a WSSV Infection
Liping Zheng, Omkar Byadgi, Mostafa Rakhshaninejad Nejad, Hans Nauwynck

12:00 - 12:15 Virus-host interactome high-throughput mapping to identify new factors of pathogenicity and interspecies transmission for Bluetongue virus
Aurore Fablet, Cindy Kundlacz, Juliette Dupré, Edouard Hirchaud, Lydie Postic, Corinne Sailleau, Emmanuel Bréard, Stéphan Zientara, Damien Vitour, Grégory Caignard

12:15 - 12:30 Interplay between Foot-and-Mouth Disease Virus 3D polymerase and the type I interferon response: a contribution to viral persistence?
Morgan Sarry, Souheyla Benfrid, Cindy Bernelin-Cottet, Anthony Relmy, Aurore Romey, Anne-Laure Salomez, Gregory Caignard, Stephan Zientara, Damien Vitour, Labib Bakkali Kassimi, Sandra Blaise-Boisseau

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12:30 - 13:30 **Lunch** **Artevelde Forum**

13:30 - 15:00 **PARALLEL SESSION D -PATHOGENESIS** **Auditorium**
Chair: Gyula Balka (University of Veterinary Medicine Budapest, Hungary)

13:30 - 13:45 The Role of bovine ADAM17 in Pestivirus Infections and its Importance in the Pestivirus Resistance of CRIB-1 cells
Christiane Riedel, Marianne Zaruba, Hann-Wei Chen, Stefan Düsterhöft, Till Rümenapf

13:45 - 14:00 Infection kinetics of BTV-X ITL2021 (BTV-32) in small ruminants
Massimo Spedicato, Giovanni Di Teodoro, Liana Teodori, Alessandra Leone, Barbara Bonfini, Maura Pisciella, Ottavio Portanti, Emanuela Rossi, Tiziana Di Febo, Alessio Lorusso, Giovanni Savini

14:00 - 14:15 Subclinical PRRSV type 1 infection aggravates clinical course of *Streptococcus suis* infections in pigs
Norbert Stockhofe-Zurwieden, Manouk Vrieling, Jan Cornelissen, Helmi Feijten, Lisette Ruuls, Rob Zwart, Ditta Popma, Sandra Vreman

14:15 - 14:30 Pathogenesis of West Caucasian and Lleida bat viruses, two divergent lyssaviruses co-circulating in a widespread migratory bat species
Petra Drzewnioková, Barbara Zecchin, Dino Scaravelli, Francesca Festa, Martina Castellan, Maira Zorzan, Barbara Tramontan, Andrea Lombardo, Emmanuelle Robardet, Stefania Leopardi, Paola De Benedictis

14:30 - 14:45 Predicting infectious bursal disease virus pathotype: new models based on early changes in blood cell formula and bursa cells transcriptional activity
Molinet Annonciade, Céline Courtillon, Michel Amelot, Alassane Keita, Lucas Pierrick, Edouard Hirchaud, Yannick Blanchard, Béatrice Grasland, Nicolas Eterradossi, Sébastien Soubies

14:45 - 15:00 *In vivo* and *in vitro* characterization of neurotropism of highly pathogenic influenza virus H5N8 (clade 2.3.3.4b) in chicken and duck
Charlotte Foret-Lucas, Pierre Bessière, Amelia Coggon, Alexandre Houffschmitt, Maxence Delverdier, Thomas Figueroa and Romain Volmer

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13:30 - 15:00 **PARALLEL SESSION E - PATHOGENESIS** **Jan Van Eyck room**
Chair: Anette Gleitze Bøtner (University of Copenhagen, Denmark)

13:30 - 13:45 SARS-CoV-2 omicron infection induces decreased viral replication and inflammation in the upper and lower respiratory tract compared to the D614G and Delta variants in the Syrian hamster model.

Maxime Fusade-Boyer, Hélène Huet, Adèle Gambino, Audrey St Albin, Ophélie Grad, Nicolas Meunier and Sophie Le Poder

13:45 - 14:00 A deletion in the spike protein spanning the furin cleavage site alters SARS-CoV-2 virulence in K18-ACE2 mice
Giovanni Di Teodoro, Fabrizia Valleriani, Chiara Di Pancrazio, Flavio Sacchini, Maurilia Marcacci, Massimo Spedicato, Daniela Malatesta, Tetyana Petrova, Ottavio Portanti, Shadia Berjaoui, Emanuela Rossi, Francesco Bonfante, Alessio Lorusso.

14:00 - 14:15 Molecular determinants of ASFV hemadsorption and virulence
Daniel Pérez-Núñez, Raquel García-Belmonte, Elena Riera, Gonzalo Vigara-Astillero, Yolanda Revilla

14:15 - 14:30 Study of the potential of the *in vitro* replication capacity of porcine reproductive and respiratory syndrome virus PRRSV strains to predict their virulence *in vivo*
Jaime Castillo, Javier Martínez-Lobo, Javier Domínguez, Isabel Simarro, José María Castro, Concepción Revilla, Cinta Prieto

14:30 - 14:45 Neuraminidase and Trypsin Treatment of Highly Passaged Feline Enterocytes Enhances the Replication of FECV Type I Strains
Bixia Chen, Hans Nauwynck

14:45 - 15:00 The Histone-Like A104R Protein of African Swine Fever Virus is not Essential for Replication in Cell Culture
Walter Fuchs, Björn-Patrick Mohl, Tonny Kabuuka, Katrin Pannhorst, Günther M. Keil, Jan Hendrik Forth, Thomas C. Mettenleiter

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13:30 - 15:00 PARALLEL SESSION F - VIRAL IMMUNOLOGY **Van Rysselbergh room**
Chair: Falko Steinbach (APHA, United Kingdom)

13:30 - 13:45 PRRSV-induced CD8 T Cell Responses at the Maternal-Fetal Interface During Late Gestation
Melissa R Stas, Heinrich Kreutzmann, Kerstin H Mair, Michaela Koch, Christian Knecht, Maria Stadler, Simona Winkler, Katinka van Dongen, Masha A Razavi, Spencer N Sawyer, Clara Pernold, Sonia V Hernandez, Armin Saalmüller, Till Rümenapf, Wilhelm Gerner, and Andrea Ladinig

13:45 - 14:00 Frequent infection of cats with SARS-CoV-2 irrespective of pre-existing enzootic coronavirus immunity, Brazil 2020
Edmilson F. de Oliveira-Filho, Otávio V. de Carvalho, Ianei O. Carneiro, Fagner D'ambroso Fernandes, Sara N. Vaz, Célia Pedroso, Lilian Gonzalez-Auza, Victor C. Urbieta, Arne Kühne, Rafaela Mayoral, Wendy K. Jo, Andrés Moreira-Soto, Chantal B. E. M. Reusken, Christian Drosten, Carlos Brites, Klaus Osterrieder, Eduardo M. Netto, Luiz E. Ristow, Rita C. Maia, Fernanda S.F. Vogel, Nadia R. Almeida, Carlos R. Franke and Jan Felix Drexler

14:00 - 14:15 Cytotoxic $CD4^+$ and CD4/CD8 Double Negative T cells Correlate with Protection against PRRSV1 Transplacental Infection
Yanli Li, Gerard Martín-Valls, Ivan Díaz, Enric Mateu

14:15 - 14:30 Porcine nasal and lung macrophage subsets isolated by FACS and LCM show different transcriptomic profiles depending on tissue origin and location
Dayoung Oh, Nick Vereecke, Wim Trypsteen, Sieglinde Coppens, Ward De Spiegelaere, Heesoo Song, Bert Devriendt, Jo Vandesompele, Hans Nauwynck

14:30 - 14:45 Milk lactose protects against group A rotavirus infection
Xiaolei Ren, Waqar Saleem, Haes Robin, Jiexiong Xie, Sebastiaan Theuns, Hans J. Nauwynck

14:45 - 15:00 Alphaherpesvirus-Induced Inhibition of the m6A Writer Complex and Degradation of m6A Methylated Transcripts
Robert J. J. Jansens, Ruth Verhamme, Aashiq Mirza, Anthony Olarerin-George, Cliff Van Waesberghe, Samie R. Jaffrey, Herman W. Favoreel

15:00 - 15:30 **Refreshment break** **Artevelde Forum**

PLENARY SESSION II and ROUND TABLE I: Viral infections of swine - African Swine Fever
Moderator: José Manuel Sanchez-Vizcaino (Universidad Complutense de Madrid, Spain)
Auditorium

15:30 - 16:00

History of African Swine Fever, Europe & Asia
José Manuel Sanchez-Vizcaino
Universidad Complutense de Madrid, Spain

16:00 - 16:15

African Swine Fever in Africa: outsmarting the virus with fit-for-purpose technologies
Gerald Misinzo
Sokoine University of Agriculture, Tanzania

16:15 - 16:30

Pathogenesis of African Swine Fever
Sandra Blome
Friedrich-Loeffler-Institut, Germany

16:30 - 16:45

Molecular basis of African Swine Fever virulence/pathogenesis
Linda Dixon
The Pirbright Institute, United Kingdom

16:45 - 17:00

Vaccine development for the control of ASF; where are we and where to go?
Douglas Gladue
Plum Island Animal Disease Center,
U.S. Department of Agriculture, U.S.A.

17:00 - 17:30 **Discussion with audience**

POSTER SESSION I **Artevelde Forum**

17:30 - 19:00 **Posters viewing and reception with Belgian beers**

Thursday, 22 September 2022

PLENARY SESSION III: **Epidemiology, Diagnostics and Vaccines** **Auditorium**
Chairs: Tomasz Stadejek (Warsaw University of Life Sciences, Poland) & Laurent Gillet (ULiège, Belgium)

08:30 - 09:00

Epidemiology of emerging diseases
Norbert Nowotny
University of Veterinary Medicine, Vienna, Austria

09:00 - 09:30

Third generation sequencing, the new diagnostic power in the veterinary world
Sebastiaan Theuns
PathoSense, Belgium

09:30 - 10:00

Use of peptide analysis in the development of vaccines
Morten Nielsen
Technical University of Denmark, Denmark

10:00 - 10:30

Perspectives of mRNA vaccines in the veterinary world
Niek Sanders
Ghent University, Belgium

10:30 - 11:00 **Refreshment break** **Artevelde Forum**

11:00 - 12:30 **PARALLEL SESSION G -EPIDEMIOLOGY** **Auditorium**
Chair: Till Rümenapf (University of Veterinary Medicine, Austria)

11:00 - 11:15 Intra- and Inter-Cattery Epidemiology of Feline Coronavirus in Belgium between 2018 and 2021
Nick Vereecke, Veerle Stroobants, Marthe Pauwels, Aisha Van den Kerkhof, Sebastiaan Theuns, Hans Nauwynck

11:15 - 11:30 The Founder Variants Transmitted by Sows are the Main Source of PRRSV1 Genetic Diversity in an Unstable Infected Farm.
Hepzibar Clilverd, Gerard E Martín-Valls, Yanli Li, Marga Martín, Martí Cortey, Enric Mateu

11:30 - 11:45 Molecular epidemiology of West Nile virus lineage 1 (WNV-L1) in Spain. An update of the last decade.
Pilar Aguilera-Sepúlveda, Cristina Cano-Gómez, Amalia Villalba, Rubén Villalba, Montserrat Agüero, Miguel Ángel Jiménez-Clavero, Jovita Fernández-Pinero

11:45 - 12:00 Vaccination Strategy Framework Against African Swine Fever in Wild Boar
Marta Martínez, Jaime Bosch, Satoshi Ito, Ed van Klink, Edvins Olsevskis, Kevin Morelle, Jose Manuel Sánchez-Vizcaíno

12:00 - 12:15 Genetic and Antigenic Characterization of Respiratory Coronaviruses of Swine
Ruth Mumo, Anna Parys, Nick Vereecke, Bart Pardon, Sebastiaan Theuns, Kristien Van Reeth

12:15 - 12:30 Genetically Identical Strains of Four Different Honeybee Viruses have been Determined in Bumblebee and Honeybee Positive Samples
Ivan Toplak, Laura Šimenc, Metka Pislak Ocepek, Danilo Bevk

11:00 - 12:30 **PARALLEL SESSION H - DIAGNOSTICS** **Jan Van Eyck room**
Chair: Davide Lelli (IZSLER, Brescia, Italy)

11:00 - 11:15 Development and Validation of Four Duplex Real-Time PCR Assays for Sensitive Detection of Pathogens Associated with Equine Diarrhoea
Kees van Maanen, Rick Elbert, Sander Schuurman, Tara de Haan

11:15 - 11:30 Diagnosis and characterization of a novel strain of EHDV-8 in Tunisia in 2021.
Maurilia Marcacci, Soufien Sghaier, Corinne Sailleau, Stephan Zientara, Sarah Thabet, Emmanuel Breard, Liana Teodori, Massimo Spedicato, Damien Vitour, Lydie Postic, Ottavio Portanti, Salah Hammami, Valentina Curini, Francesca Di Giallonardo, Giovanni Savini, Alessio Lorusso

11:30 - 11:45 Assessing the health status of Belgian pig farms using an integrated, high-tech approach
Friso Griffioen, Nick Vereecke, Sieglinde Coppens, Gauthier Daneels, Jeroen Dewulf, Dominiek Maes, Sebastiaan Theuns, Hans Nauwynck

11:45 - 12:00 Characterization of the Subclinical Infection of Porcine Deltacoronavirus in Grower Pigs
Lu Yen, Juan-Carlos Mora-Díaz, Ronaldo Magtoto, Rolf Rauh, William Nelson, Jeffrey Zimmerman, Rahul Nelli, Luis Giménez-Lirola

12:00 - 12:15 Detection of Hepatitis E virus RNA in Belgian ready-to-eat pork meat and liver products
Tatjana Locus, Michael Peeters, Bavo Verhaegen, Koenraad Van Hoorde, Ellen Lambrecht, Thomas Vanwolleghem, Steven Van Gucht

12:15 - 12:30 Environmental sampling for the detection of Highly Pathogenic Avian Influenza H5 and Infectious Bronchitis Virus in poultry farms
Fabien Filaire, Daniel Akinsola, Laetitia Lebre, Perrine Pascal, Aurélie Secula, Charlotte Foret-Lucas, Timothée Vergne, Mathilde Paul, Guillaume Croville, Jean-Luc Guerin

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11:00 - 12:30 **PARALLEL SESSION I - VACCINES** **Van Rysselbergh room**
Chair: Emmanuel Albina (CIRAD, France)

11:00 - 11:15 The intradermal route induces full protection in pigs immunized with the attenuated African swine fever virus (ASFV) Lv17/WB/Rie1.
Carmina Gallardo, Alejandro Soler, Raquel Nieto, Nadia Casado, Marisa Arias

11:15 - 11:30 Comparative Evaluation of the Duration of Protective Immunity Induced by a Live Attenuated and an Inactivated Lumpy Skin Disease Virus Vaccine
Andy Haegeman, Ilse De Leeuw, Wannes Philips, Nick De Regge, Willem Van Campe, Laurent Mostin, Kris De Clercq

11:30 - 11:45 Pathogenesis Study in Goats with Recombinant Wildtype- or Vaccine-derived PPRV Expressing Enhanced GreenFluorescentProtein
Phaedra Eblé, Katie Schmitz, René van Gennip, Mieke Maris, Lucien van Keulen, Rory de Vries, Rik de Swart, Piet van Rijn

11:45 - 12:00 Developing a 'One Health' Nipah virus vaccine to protect animal and public health
Rebecca McLean, Miriam Pedrera, Nazia Thakur, Ahmed Mohamed, Sophia Hodgson, Sue Lowther, Tristan Reid, Shawn Todd, Brenton Rowe, Jemma Bergfeld, Lee Trinidad, Sarah Riddell, Sarah Edwards, Jean Payne, Jennifer Barr, Nick Rye, Matt Bruce, Tim Poole, Sheree Brown, Toni Dalziel, Gough Au, Megan Fisher, Rachel Layton, Teresa Lambe, Keith Chappell, Ariel Isaacs, Daniel Watterson, Mercedes Mourino, Ireen Sultana Shanta, Ayesha Siddika, Mst Noorjahan Begum, Sezanur Rahman, Abdulla Al Mamun Bhuyan, Muntasir Alam, Mohammed Ziaur Rahman , Mustafizur Rahman, Elma Tchilian, Sarah Gilbert, Paul Young, Dalan Bailey, Glenn Marsh, Simon Graham

12:00 - 12:15 Protection conferred by a DNA vaccine against highly pathogenic avian influenza in chickens : effect of vaccine schedule
Julie Valentin, Fabienne Rauw, Fiona Ingrao, Bénédicte Lambrecht

12:15 - 12:30 The telomeric repeats of Marek's disease virus vaccines are required for viral integration and genome maintenance
Yu You, Ahmed Kheimar, Luca D. Bertzbach, Benedikt B. Kaufer

12:30 - 13:30 **Lunch** **Artevelde Forum**

13:30 - 15:00 **PARALLEL SESSION J - EPIDEMIOLOGY** **Auditorium**
Chair: Roger Maes (Michigan State University, USA)

13:30 - 13:45 Analysis of canine parvovirus 2 isolates from Hungary reveals heterogenous phylogenetic origin
Dávid Géza Horváth, Ervin Albert, Gyula Balka, Ádám Dán

13:45 - 14:00 An Epidemiology Model of Influenza A Virus in Wild Birds Based on Surveillance of Black-headed Gulls (*Chroicocephalus ridibundus*)
Josanne H. Verhagen, Eline A.M. Vink, Marjolein J. Poen, Frank A. Majoor, Divya Kriti, Jayeeta Dutta, Harm van Bakel, Oanh Vuong, Rachel D. Scheuer, Mariëlle van Toor, Nicola S Lewis, Nichola N Hill, Justin Bahl, Lambodhar Damodaran, Jiani Chen, Thijs Kuiken, Ron A.M. Fouchier, Mart C.M. de Jong

14:00 - 14:15 Molecular Epidemiology of Porcine Parvovirus Type 1 (PPV1) and the Reactivity of Vaccine-induced Antisera against Historical and current PPV1 strains
Nick Vereecke, Elise Vandekerckhove, Lise Kirstine Kvisgaard, Guy Baele, Carine Boone, Marius Kunze, Lars Erik Larsen, Sebastiaan Theuns, Hans Nauwynck

14:15 - 14:30 Insights into the evolution and pathogenesis of A3B4 reassortants of infectious bursal disease virus (IBDV)
Anna Pikuła, Lester J. Perez

14:30 - 14:45 Occurrence and characterization of Rabbit Calicivirus (RCV) strains in Italy over 20 years
Patrizia Cavadini, Davide Mugetti, Alice Vismarra, Antonio Lavazza e Lorenzo Capucci

14:45 - 15:00 Large Scale Cross-Sectional Serosurvey of Hepatitis E Virus Infection in Belgian Pig Farms and Identification of Risk Factors for Herd Infection
Constance Wielick, Ravo Michèle Razafimahefa, Louisa Ludwig-Begall, Stefaan Ribbens, Claude Saegerman, Etienne Thiry

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13:30 - 15:00 **PARALLEL SESSION K - DIAGNOSTICS** **Jan Van Eyck room**
Chair: Kees van Maanen (Royal GD, Deventer, the Netherlands)

13:30 - 13:45 SARS-CoV-2 whole genome sequencing: evaluation of five different sequencing approaches

Valentina Curini, Massimo Ancora, Valeria Di Lollo, Silvia Scialabba, Barbara Secondini, Luigina Di Gialleonardo, Maurilia Marcacci, Marco Di Domenico, Iolanda Mangone, Alessandro De Luca, Adriano Di Pasquale, Alessio Lorusso, Cesare Cammà

13:45 - 14:00 Detection and Localization of Atypical Porcine Pestivirus in The Reproductive Tract of a Persistently Infected Boar
Lilla Dénes, Lukas Schwarz, René Brunthaler, Sandra Högler, Gyula Balka

14:00 - 14:15 Infectious bronchitis virus and its variants in Canada
Davor Ojkic, Emily Martin

14:15 - 14:30 Equine Coronaviruses – a common reason for equine colic?
Moritz Stummer, Vicky Frisch, Frauke Glitz, Jessika Cavalleri, Till Rümenapf, Angelika Auer

14:30 - 14:45 Detection of Alphamesonivirus 1 in two horses by a metatranscriptomics approach
Lucija Jurisic, Alessio Lorusso, Addolorato Ruberto, Valentina Curini, Giovanni Di Teodoro, Francesca Di Giallonardo, Maurilia Marcacci

14:45 - 15:00 Outbreak of Elephant endotheliotropic herpesvirus 6 (EEHV-6) associated disease in African elephants in a European zoo.
Angelika Auer, Christiane Riedel, Folko Balfanz, Annika Posautz, Azza, Abdelgawad, Jakob Trimpert, Tabitha Hoornweg, Thomas Voracek, Till Rümenapf

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13:30 - 15:00 **PARALLEL SESSION L - VACCINES** **Van Rysselbergh room**
Chair: Marylène Tignon (Sciensano, Belgium)

13:30 - 13:45 A Genotype II Live Attenuated Vaccine Candidate for African Swine Fever Based on Multiple Targeted Gene Deletions or Modifications
Anusyah Rathakrishnan, Ana Luisa Reis, Vlad Petrovan, Katy Moffat, Chris Chiu, Yuan Lui, Shinji Ikemizu, Simon Davis, Linda Dixon

13:45 - 14:00 Has the attenuation of CSFV C strain vaccine just resulted in a slow growing virus?
Falko Steinbach, Frederico Ferreira, Helder Nakaya, Helen Crooke

14:00 - 14:15 Characterization of Protective Immune Responses in Domestic Pigs following Intradermal Immunization with the Attenuated African Swine Fever Virus (ASFV) Lv17/WB/Rie1.
Miriam Pedrera, Alejandro Soler, Paloma Fernández-Pacheco, Nadia Casado, Alicia Simón, María Ana García-Casado, Marisa Arias, Carmina Gallardo

14:15 - 14:30 Study of the outcomes of infection with a new $H1_{av}N2$ genotype of swine influenza virus that emerged in France and evaluation of vaccine protection
Céline Deblanc, Stéphane Quéguiner, Stéphane Gorin, Séverine Hervé, Angélique Moro, Gérald Le Diguerher, Frédéric Paboeuf, Gaëlle Simon

14:30 - 14:45 Evaluation of PRRS MLV viremia and transmission for a better prevention of recombination between vaccine strains
Sophie Mahé, Patricia Renson, Mathieu Andraud, Mireille Le Dimna, Nicolas Rose, Frédéric Paboeuf, Olivier Bourry

14:45 - 15:00 Immunogenicity and efficacy of a novel universal influenza vaccine approach against H3N2 swine influenza infection in pigs
Constantinos S. Kyriakis, Vasilis Pliasas, Sarah Ives, Nick Bayless, Peter J. Neasham, J. Fletcher North, Maria C. Naskou, Virginia Aida, Rachel Neto, Dave Gangemi, Jacob Glanville

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15:00 - 15:30 **Refreshment break** **Artevelde Forum**

PLENARY SESSION IV and ROUND TABLE II: viral infections of horses & cattle-orbiviruses

Moderator: Piet van Rijn (Wageningen Bioveterinary Research, The Netherlands) **Auditorium**

15:30 - 15:50

Blue tongue virus in Europe and Africa
Alessio Lorusso
Istituto Zooprofilattico Sperimentale dell'Abruzzo, Italy

15:50 - 16:20 

A closer look at Bluetongue virus: what structures tell us for vaccine design against Bluetongue and African Horse Sickness disease
Polly Roy
London School of Hygiene & Tropical Medicine, United Kingdom

16:20 - 16:40 

Disabled Infectious Single Animal (DISA) vaccine platforms for Bluetongue and African Horse Sickness
Piet van Rijn
Wageningen Bioveterinary Research, The Netherlands

16:40 - 17:00 

Preparedness of the pharmaceutical industry for emerging orbiviruses - what is needed
Mercedes Mouriño
Zoetis Manufacturing & Research, Spain

17:00 - 17:30 **Discussion with audience**

POSTER SESSION II **Artevelde Forum**

17:30 - 18:30 **Posters viewing and reception with Belgian beers**

18:30 - 21:00 **Congress dinner** **Banquet room**

21:00 - 24:00 **Evening entertainment with Open Bar** **Banquet room**

Friday, 23 September 2022

PLENARY SESSION V: **(Reverse) Zoonosis** **Auditorium**
Chairs: Lars Erik Larsen (University of Copenhagen, Denmark)
Nick De Regge (Sciensano, Belgium)

08:30 - 09:00 

Reverse zoonotic infections of SARS-CoV2
Wim van der Poel
Wageningen Bioveterinary Research, The Netherlands

09:00 - 09:30 

Noroviruses at the interplay between veterinary and human public health
Etienne Thiry
Liège University, Belgium

09:30 - 10:00 

Influenza viruses in swine and humans: dangerous liaisons?
Kristien Van Reeth
Ghent University, Belgium

10:00 - 10:15 **Selected abstract 1 -** Vital roles of poultry vaccination in prevention of potential H7N9 avian influenza virus pandemic
Pengxiang Chang, Joshua E. Sealy, Jean-Remy Sadeyen, Sushant Bhat, Munir Iqbal

10:15 - 10:30 **Selected abstract 2 -** Emergence of SARS-CoV-2 variants in farmed mink during the epidemic in Denmark, June-November 2020
Thomas Bruun Rasmussen, Ann Sofie Olesen, Louise Lohse, Anette Bøtner, Graham J. Belsham, Anette Boklund

10:30 - 11:00 **Refreshment break** **Artevelde Forum**

11:00 - 12:30 **PARALLEL SESSION M - EPIDEMIOLOGY** **Auditorium**
Chair: Constantinos Kyriakis (Auburn university, USA)

11:00 - 11:15 Genetic and antigenic diversity of Rotavirus A in Danish pigs
Nicole Goecke, Kasper Pedersen, Pia Ryt-Hansen, Nicolai Weber, Lars Erik Larsen

11:15 - 11:30 An Equine Coronavirus Associated Epidemic of Infectious Pyrexia in Iceland
Vilhjálmur Svansson, Sigríður Björnsdóttir, Eggert Gunnarsson, Constance Smits, Kees van Maanen

11:30 - 11:45 Detection of porcine parainfluenza virus 1 (PPIV1): genetic diversity and co-infections with influenza A virus (IAV) and porcine reproductive and respiratory syndrome virus (PRRSV)
Aleksandra Woźniak, Piotr Cybulski, Tomasz Stadejek

11:45 - 12:00 Third generation nanopore sequencing of honeybee hemolymph as a new screening tool for honeybee viruses
Cato Van Herzele, Dirk de Graaf, Hans Nauwynck

12:00 - 12:15 Infection by a highly virulent PRRSV1 strain modifies the dynamics and shedding pattern of influenza A virus in an endemic pig farm.
Gerard Martín-Valls, Martí Cortey, Laia Aguirre, Enric Mateu

12:15 - 12:30 Circulation of Influenza A Virus in Wild Boars in the Emilia-Romagna Region (Northern Italy), between 2017-2022
Laura Soliani, Alice Prosperi, Elena Canelli, Laura Baioni, Valentina Gabbi, Camilla Torreggiani, Roberta Manfredi, Irene Calanchi, Giovanni Pupillo, Filippo Barsi, Patrizia Bassi, Laura Fiorentini, Matteo Frasnelli, Maria Cristina Fontana, Andrea Luppi, Chiara Chiapponi

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11:00 - 12:30 **PARALLEL SESSION N - VACCINES** **Jan Van Eyck room**
Chair: Yolanda Revilla (CBMSO-CSIC, Spain)

11:00 - 11:15 Detection of Genotype XIV.2 Newcastle Disease Viruses in Nigeria, Antigenic Characterization and Optimization of Vaccination Strategies
Ismaila Shittu, Eva Mazzetto, Chika Nwosuh, Alessandra Napolitan, Andrea Fortin, Maria Varotto, Alice Fusaro, Judith Bakam, Maryam Muhammad, Francesco Bonfante, Isabella Monne, Alessio Bortolami

11:15 - 11:30 Characterization of an attenuated strain of African swine fever virus providing sterilizing immunity after intramuscular or oronasal immunization
Olivier Bourry, Evelyne Hutet, Mireille Le Dimna, Pierrik Lucas, Yannick Blanchard, Amélie Chastagner, Frédéric Paboeuf, Marie-Frédérique Le Potier

11:30 - 11:45 Responses of PRRSv Vaccination in Piglets Born from PRRSv Vaccinated, ELISA Responding and Non-Responding Sows
Jorian Fiers, Dominiek Maes, Marylène Tignon, Ann-Brigitte Cay

11:45 - 12:00 High *in vitro* ASFV recombination rate in porcine alveolar macrophages
István Mészáros, Ferenc Olasz, Vivien Tamás, Tibor Magyar, Zoltán Zádori

12:00 - 12:15 A single oral immunization with a replication-competent adenovirus-vectored vaccine protects mice from influenza respiratory infection
Emeline Goffin, Xiang Du, Silvio Hemmi, Bénédicte Machiels, Laurent Gillet

12:15 - 12:30 Virulence properties of GI-23 infectious bronchitis virus isolated in Poland and efficacy of different vaccination strategies
Anna Lisowska, Anna Pikuła, Justyna Opolska, Katarzyna Domańska-Blicharz

11:00 - 12:30 **PARALLEL SESSION O - (REVERSE) ZOONOSES** **Van Rysselbergh room**
Chair: Tamaš Petrović (Scientific Veterinary Institute Novi Sad, Serbia)

11:00 - 11:15 Fitness and neurotropism of H5NX 2.3.4.4B in ferrets, human respiratory cells and human brain organoids
Francesco Bonfante, Alessio Bortolami, Eva Mazzetto, Matteo Pagliari, Cecilia Laterza, Marta Vascellari, Alessandra Napolitan, Valentina Panzarin, Andrea Fortin, Alice Fusaro, Jane Budai, Isabella Monne, Nicola Elvassore, Calogero Terregino

11:15 - 11:30 Isolation and Genome Characterization of Bat-borne Issyk-Kul Virus in Italy
Davide Lelli, Ana Moreno, Tiziana Trogu, Enrica Sozzi, Sabrina Canziani, Matteo Mauri, Luca Cavallari, Chiara Chiapponi, Antonio Lavazza

11:30 - 11:45 Determination of pathogenic potential of Spanish lineage 1 and 2 WNV strains in a mouse model
Raúl Fernández-Delgado, Rafael Gutiérrez-López, David Romero-Trancón, Pilar Aguilera-Sepúlveda, Desirée Dafouz-Bustos, Belén Gómez-Martín, Nuria Busquets, Miguel Ángel Jiménez-Clavero, Francisco Llorente

11:45 - 12:00 Vector competence of Belgian *Anopheles plumbeus* and *Culex pipiens* mosquitoes for Japanese encephalitis virus
Claudia Van den Eynde, Charlotte Sohier, Severine Matthijs, Nick De Regge

12:00 - 12:15 Study of the Effect of Bacterially Produced Secondary Metabolites on SARS-CoV-2 (COVID-19) *in Vitro*
Alexis C. R. Hoste, Aurélien Cugnet, Willy Smeralda, Magali Deleu, Mutien Garigliany and Philippe Jacques

12:15 - 12:30 Characterization of hepatitis E virus in Lithuanian human and animal populations
Juozas Grigas, Arnoldas Pautienius, Evelina Simkute, Indre Jasineviciute, Arunas Stankevicius

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12:30 - 13:30 **Lunch** **Artevelde Forum**

13:30 - 14:00 **PARALLEL SESSION P - VACCINES** **Auditorium**
Chair: Yolanda Revilla (CSIC-UAM, Spain)

13:30 - 13:45 Oral immunization with adenovirus-vectored vaccine induces a neutralizing antibody response in mice against Canine Distemper Virus infection
Xiang Du, Emeline Goffin, Lucie Gillard, Laurent Gillet

13:45 - 14:00 Bartha-K61 vaccine protects against novel Suid herpesvirus 1 strains
Konstantinos V. Papageorgiou, Margarita Michailidou, Ioannis Grivas, Evanthia Petridou, Efthymia Stamelou, Konstantinos Efraimidis, Lei Chen, Trevor W. Drew, Spyridon K. Kritas

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13:30 - 14:00 **PARALLEL SESSION Q - PATHOGENESIS** **Jan Van Eyck room**
Chair: Tamaš Petrović (Scientific Veterinary Institute Novi Sad, Serbia)

13:30 - 13:45 Evidence of Lumpy skin disease virus transmission from subclinically infected cattle by *Stomoxys calcitrans*
Haegeman A., Sohier C., Mostin L., De Leeuw I., Van campe W., De Regge N., De Clercq K.

13:45 - 14:00 Marek's disease virus virulence genes encode circular RNAs
Alexis S. Chasseur, Gabrielle Trozzi, Céline Istasse, Astrid Petit, Benoît Muylkens, Damien Coupeau

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13:30 - 14:00 **PARALLEL SESSION R - PATHOGENESIS** **Van Rysselbergh room**
Chair: Constantinos Kyriakis (Auburn university, USA)

13:30 - 13:45 Comparative pathogenesis of tick-borne encephalitis virus and louping ill virus in experimentally infected sheep
Nadjah Radia Adjadj, Mara Rocchi, Willem Van Campe, Nick De Regge

13:45 - 14:00 Are non-coding Simbuviruses RNA structures involved in transcription termination and translation?
Laura Bonil, Laetitia Wiggers, Hélène Dumont, Benoît Muylkens and Damien Coupeau

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14:00 - 15:00 General Assembly of the European Society for Veterinary Virology **Auditorium**

15:00 - 15:30 **Refreshment break** **Artevelde Forum**

PLENARY SESSION VI and ROUND TABLE III: Viruses in wildlife & vector-borne viral diseases

Moderator: Wim van der Poel (Wageningen Bioveterinary Research, The Netherlands) **Auditorium**

15:30 - 15:45

Are zoonotic tick borne encephalitis and West Nile virus in Northern Europa merely rare spillovers from wildlife reservoirs?
René Bødker
University of Copenhagen, Denmark

15:45 - 16:00

Recent events in the emergence of zoonotic viral diseases in Western Europe
Miguel Ángel Jiménez-Clavero
Animal Health Research Centre (INIA-CISA), CSIC, Spain.

16:00 - 16:15

Going global: the ongoing adaptation of the Goose/Guangdong lineage of highly pathogenic avian influenza virus to wild birds
Thijs Kuiken
Erasmus University Medical Center, Rotterdam, The Netherlands

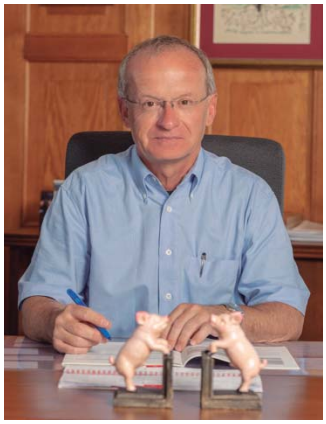
16:15 - 16:45 **Discussion with audience**

16:45 - 17:15 **Awards for 'Best Oral Presentations' and Closing Ceremony** **Auditorium**

KEYNOTE SPEAKERS

OPENING LECTURE
Tuesday 20 Septembre, 17:30 - 18:30, Auditorium

Evolution of virus research with pseudorabies virus as model
Thomas Mettenleiter, Friedrich-Loeffler-Institut, Germany



Presentation synopsis
It was exactly 120 years ago that Aladár Aujeszky first described the disease entity now associated with his name. The understanding of the disease and its causative agent, designated Aujeszky's Disease Virus, Pseudorabies Virus (PrV) or, taxonomically correct, Suid Alphaherpesvirus 1, evolved from demonstration of a filterable virus to the establishment of first ex-vivo culture techniques in the 1930s. Until then, the association between PrV and pigs as the natural reservoir had not yet been recognized. This changed in the 1950s with intensification of pig husbandry and in the 1960s and 1970s Aujeszky's Disease became a major factor in the pig industry prompting the use of attenuated live vaccines developed by classical means, e.g. passage in embryonated poultry eggs. Some of these early vaccines like the famous Bartha strain developed by Adorjan Bartha in Budapest, are still in use. In parallel, once the close relationship between PrV and human herpes simplex viruses has been recognized, research on PrV gained momentum as a model for herpesvirus biology, pathogenesis (in particular neuropathogenesis) and molecular biology. Pioneering molecular work led to the development of the first genetically engineered live attenuated vaccine viruses in use anywhere in the world. Concomitantly, studies on PrV have expanded our knowledge of herpesvirus neuroinvasion, as well as elucidated molecular steps relevant for lytic herpesvirus replication. The inception and use of the first ever 'marked' vaccines based on studies of PrV envelope glycoproteins allowed a novel DIVA (differentiating infected from vaccinated animals) approach which resulted in efficient eradication programs that finally led to disappearance of AD from several countries and regions including Europe and Northern America. Thus, PrV exemplifies not only the evolution of virus research over more than a century but also demonstrates successful 'translation' of research findings into practical use for the benefit of animals and humans alike.

Biography
Thomas C. Mettenleiter studied biology from 1977 to 1982 and earned his doctoral degree in genetics in 1985 at the Eberhard Karl University of Tübingen for his research work on pseudorabies virus conducted at the Federal Research Centre for Virus Diseases of Animals (BFAV) in Tübingen. With a research fellowship granted by the German Research Foundation (DFG) he went for a research stay with Tamar Ben-Porat and Albert Kaplan at Vanderbilt University, Nashville, TN, USA, from 1986 to 1987. After returning to BFAV, he obtained his post-doctoral habilitation in virology at the University of Tübingen in 1990. From 1994 to 2019 he chaired the Institute of Molecular Virology and Cell Biology at the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health (FLI), on the island of Riems. He has been leading the FLI since 1996, in 1997 he was appointed President of the FLI. His main field of research is virus infections of farm animals, in particular herpesviruses. In addition to studies on the basic processes of virus-host interactions, he is involved in the development of novel vaccines based on molecular biological techniques. He is a member of several international committees and working groups including the founding co-chair of the „One

Health High Level Expert Panel" jointly initiated by WHO, OIE, FAO, and UNEP. He is a member of the German National Academy of Sciences Leopoldina, the Academy of Sciences in Hamburg, the Polish Academy of Sciences, and the Royal Belgian Academy of Medicine. He has been awarded honorary doctorates from the University of Veterinary Medicine Hannover and from the Justus-Liebig-University Gießen, an associate professorship from the University of Greifswald and an honorary professorship from the University of Rostock. He is also a recipient of the Robert von Ostertag Medal of the German Federal Chamber of Veterinarians. He is senior editor of the scientific journal „Advances in Virus Research".

Wednesday 21 Septembre, 08:30 - 10:30, Auditorium

The pathogenesis of fish alloherpesviruses
Alain Vanderplasschen, Liège University, Belgium

Presentation synopsis
The order *Herpesvirales* is composed of the three families *Herpesviridae* (herpesviruses of amniotes: reptiles, birds and mammals), *Malacoherpesviridae* (herpesviruses of molluscs) and *Alloherpesviridae*. The family *Alloherpesviridae* consists of herpesviruses of amphibians and fish. Most alloherpesviruses described to date have been recognized because they cause disease outbreaks associated with mass mortalities that have serious impacts on the productivity and sustainability within the aquaculture sector. In this keynote lecture, Alain Vanderplasschen will discuss key aspects of the pathogenesis of fish alloherpesviruses (virulence, "host-pathogen-environment triad", virus transmission and immune evasion mechanisms) and compare them to what is generally accepted for the members of the family *Herpesviridae*.

Biography
Alain Vanderplasschen is Professor of Immunology at the Faculty of Veterinary Medicine of the University of Liège (ULiège). He is Doctor in Veterinary Medicine. After a PhD thesis (ULiège, 1995) under the supervision of Prof. P.-P. Pastoret on Bovine herpesvirus 4, he did a second thesis in 1998 ("*thèse d'agrégation*") at the University of Oxford (UK) in the laboratory of Prof. G.L. Smith on vaccinia virus (the virus used as vaccine against smallpox virus). In 1998, he became the first veterinarian scientist to obtain a permanent position in the history of the Belgian Fund for Scientific Research (FNRS). Prof. Vanderplasschen has a background and successful track record as a veterinarian virologist and immunologist. For nearly three decades, he has been studying host-virus interactions with three special interests: (i) understanding virus evolution, (ii) development of new veterinary vaccines and infectious disease mitigation approaches, and (iii) unraveling the mechanisms developed by viruses to evade the immune response of their host. Over the last ten years he has continuously demonstrated an ability to develop, in parallel, both fundamental and applied research in the field of fish virology and immunology. He is the laureate of several prestigious awards. Notably, in 2016, he became the first veterinarian scientist to obtain the prestigious GSK vaccines award (created in 1959) for his work: The infection of carp by Cyprinid Herpesvirus 3: a homologous host-virus model to study vaccinology and fundamental immunology. Other examples of the recent awards received in virology are the Francqui-Stichting Chair award in 2019 (University Catholique de Louvain) and the Collen-Francqui Chair award in 2021 (University of Ghent).

The Pathogenesis and Evolution of Marek's Disease virus

Benedikt Kaufer, Institut für Virologie, Freie Universität Berlin, Berlin, Germany

Presentation synopsis

Marek's disease virus (MDV) is a highly oncogenic alphaherpesvirus that causes polyneuritis, immunosuppression and deadly lymphomas in chicken. Tumors induced by MDV are considered to be one of the most frequent cancers in the animal kingdom. Over the years, a number of viral factors have been identified that are involved in pathogenesis and tumor formation. Intriguingly, MDV continuously evolved towards a greater virulence and has been able to overcome protection provided by different vaccines developed over the years; however, the evolutionary adaptations in the herpesvirus genome responsible for this increase in virulence have remained elusive. We recently demonstrate that few point mutations in the multifunctional meq gene acquired during evolution can significantly alter virulence. Defined mutations found in highly virulent strains also allowed the virus to overcome vaccinal protection. Concomitantly, the adaptations in meq enhanced virus shedding into the environment, likely providing a selective advantage for the virus. This presentation will highlight the recent advances in our understanding of MDV pathogenesis and its evolution towards a greater virulence.

Biography

Benedikt Kaufer is a professor and the director of the Institute for Virology at the Freie Universität Berlin. He received his Bachelor of Science at the Technical University of Munich (2005) and his PhD at the Department of Microbiology and Immunology at Cornell University (2010). In 2010 he established his own laboratory at the Institute for Virology of the Freie Universität Berlin and was appointed as an assistant professor for "molecular tumor virology" in 2011. He is currently an endowed Lichtenberg professor funded by the Volkswagen foundation. The Kaufer laboratory investigates various aspects of herpesvirus biology including pathogenesis, latency, virus integration and virus-induced tumorigenesis. The team uncovered various mechanisms involved in Marek's disease virus pathogenesis and lymphomagenesis, and discovered a mechanism that allows certain herpesviruses to integrate their genome into host telomeres during latency. For his work, he received a number of awards including the "Robert Koch Post-doctoral Award", the "Young Investigator Award" of the German Veterinary Society (DVG) and the "Loeffler-Frosch-Award" of the German Society for Virology (GfV). His research has been funded by various agencies including the German Research Foundation (DFG), European Research council (ERC) and the NIH.

The intriguing interplay of PRRSV with the host immunity

Enric Mateu, Autonomous University of Barcelona, IRTA-CReSA, Spain

Biography

I earned a Veterinary Medicine Degree in 1989 (Universitat Autònoma de Barcelona, UAB) and I got my Ph.D. in 1993 (UAB). I was a postdoc researcher at the University of Illinois (1994-95) where my research focused on the characterization of memory T cells in viral infections. In 1998 was tenured as a professor of Animal Health (UAB). Upon my return to Spain, I focused my research on viral infections of swine, especially in the aspects related to the immune response to these agents. This has been my main area of work since then. I participated in several national and international projects about *Porcine reproductive and respiratory virus (PRRSV), porcine circovirus 2, hepatitis E virus*, and *influenza A virus* in pigs among others. Our group published some of the first comprehensive descriptions of the immune response to PRRSV1 or PCV2 infections. Currently, my main interests are the investigation of T-cell correlates of protection in PRRSV infection, and the research on the factors that drive the maintenance of endemic Influenza A infections in pig herds with emphasis on the immunological aspects. Besides this, I have

contributed as a member of the organizing or scientific committees of several national and international congresses.

Towards decoding the black box of immunity in veterinary species

Artur Summerfield, University of Bern and Institute of Virology and Immunology, Mittelhäusern,, Switzerland

Presentation synopsis

The complexity of the immune system is based on many functional specialized cell types that interact with an enormous array of cell surface receptors and soluble cytokines as well as chemokines that regulate migration. Activation and de-activation processes are equally important for a well-targeted immune response that avoids causing unnecessary tissue damage. Migration of immune cells represents another key feature of such a response and includes migration from primary lymphoid tissue to the blood, from the blood to inflamed tissues, from inflamed to issue to draining lymph nodes and from activated lymph nodes back to the blood and inflamed tissues. Understanding this complexity in space and time requires a systems immunology approach that employs “omics” technologies, data science tools and bioinformatic pipelines that provide immunologically relevant and interpretable data. I provide examples for such an approach and demonstrate how such technologies can be applied to veterinary to measure and understand immune responses to vaccination and infection. This information is essential to rationally design new live attenuated vaccines, to optimize adjuvants or develop new immunotherapeutic drugs.

Biography

Artur Summerfield is a veterinary immunologist with particular interest on the immune response to infectious disease and vaccines in pigs and ruminants. His research focuses on antigen presenting cells, viral immunology as well as understanding and developing novel immunotherapeutics and vaccines using systems immunology approaches. After visiting schools in the US, France and Germany, he studied veterinary medicine in Berlin and obtained his PhD 1994 in Tübingen at the Federal Research Center for Viral Diseases (now Friedrich-Löffler-Institute) Germany. He then moved to the Institute of Virology and Immunology (IVI) in Mittelhäusern, Switzerland. In 2006, he became head of the Laboratory of Immunology and in 2010 head of the Research Department of the IVI. In 2013, he was nominated Professor for Veterinary Immunology at the Veterinary Faculty of the University of Bern.

Wednesday 21 Septembre, 15:30 - 17:30, Auditorium

History of African Swine Fever, Europe & Asia

José Manuel Sanchez-Vizcaino, VISAVET Centre. University Complutense of Madrid, Spain

Presentation synopsis

The global situation of ASF is worse than ever since 1921 when it was firstly described by Montogomery in Africa. The disease is already affecting the five continents: Africa, Europe (east and west), Asia (17 countries) and America (Dominican Republic and Haiti).

In this presentation special attention is dedicate to describe the ASF situation in East and West Europe and the Asia continent, as well as to update the situation of the EU H2020 –SFS-2019-862874 -VACDIVA project: “A safe DIVA vaccine for ASF control and eradication” to protect wild boar and domestic pigs”.

In Europe, two different epidemiological scenarios are described: East and West Europe, where currently 16 countries are affected and in Asia at this moment 17 countries are affected (data, 2022). The current main risk factors in Europe and Asia will be reviewed and the epidemiological implication evaluated.
In China both virulent and attenuated genotype II as well as attenuated genotype I ASFV strains are circulating related with the use of illegal vaccines. This situation created important epidemiological implication to all Asia and the rest of the world. The risk of infection due to potential pig meat and pig products contaminated with these attenuated circulating ASF isolates could create great difficulties for the early detection of ASF around the world.
Another Asian risk factor is the potential implication of wild boar and the transmission of the disease in the area. In general the high population of wild boar in the area is not correlated with the number of ASF cases declared, except in South Korea. In China only 9 cases of wild boar have been reported. This underreporting is an important factor because wild boar play an important role in the transmission of ASF.
Finally, the situation of the EU VACDIVA ASF VACCINE project will be presented. A description of the project consortium will be summarized as well as the objectives pursued and strategies in place and a current update to obtain a safe protective DIVA vaccine for domestic pigs (by injection) and wild boar (oral vaccine).

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Acknowledgements:

The research was funded by the EU's Horizon 2020 program, under the grant agreement No 862874 (VACDIVA).

Biography

José Manuel Sanchez-Vizcaino was born in Murcia, Spain. He obtained his DVM and Ph.D from the Complutense University of Madrid, completing his postgraduate studies in immunology and animal virology at Cornell University, New York. Upon his return to Spain, he joined the National Institute of Agricultural and Food Research (INIA) where he developed his scientific and research work as Head of the Department of Animal Virology (1985-1988), Head of the Department of Animal Health (1988-1993) and Founder Director of the BSL3+ at the Animal Health Research Center (CISA-INIA) (1993-2002). Since 2002, he is Full Professor of Animal Health at the Complutense University of Madrid (UCM) and Director of the Reference Laboratory of the World Organization for Animal Health (OIE) for African swine fever (ASF).
He has more than 240 scientific publications in high impact international journals. He is the author of 47 chapters in internationally renowned books. He has been the IP of more than 150 national and international competitive research projects and director of 34 doctoral theses. He has been awarded the Commendation of Number of the Order of Agrarian Merit (12/03/99) and of Food Merit (05/05/03), the Cross of Military Merit with white distinctive (03/01/03), all this is due to his contributions in animal health and in the control of infectious diseases, as well as internationally, with **the Medal of Merit from the World Organization for Animal Health (OIE) as international recognition for his outstanding services to world veterinary science (Paris, 5/24/2009)**. Likewise, as Doctor Honoris Causa by the University of Murcia (04/22/2010) and nominated "2013 George C. Poppensiek Visiting Professor in Global Animal Health" by Cornell University (09/2013), among other academic and scientific distinctions.
His current scientific interest is focused on the study of the epidemiology and preventive medicine of infectious diseases of animals, as well as the development of new diagnostic techniques, new generation vaccines and new strategies for their control. **He is currently the coordinator of the EU**

VACDIVA project for "a safe and DIVA, effective vaccine against ASF. More information: https://www.sanidadanimal.info/es/

African Swine Fever in Africa: outsmarting the virus with fit-for-purpose technologies

Gerald Misinzo, OR Tambo Africa Research Chair for Viral Epidemics, SACIDS Foundation for One Health, Sokoine University of Agriculture, Morogoro, Tanzania

Presentation synopsis

Most transboundary animal diseases (TADs) occur as pandemics characterised by their expanded geographical range since their first description and trans-continental spread. For instance, African swine fever (ASF) which was first described in Africa (Kenya) in 1921 has spread beyond Africa into Eastern and Central Europe, China, Southeast Asia, Oceania, and the Americas. Since Europe, Asia and Americas are globally the largest pork producers, the spread of ASF into these regions has inflicted serious economic losses, disrupted food systems due to animal mortality, reduced livestock productivity, and restricted international trade. Given the epidemiological and ecological complexity of ASF, especially in Africa where all the 24 known viral genotypes of ASF virus (ASFV) are known to exists, there is a pressing need to employ One Health, digital disease surveillance tools and genomics to timely detect, respond and contain disease outbreaks. The One Health approach is envisaged to pool resources and expertise across the human, animal (domestic and wildlife) and environmental health sectors. The use of digital disease surveillance tools such as AfyaData (http://afyadata.sacids.org) is envisaged to enhance early disease reporting and prompt containment. Furthermore, the use of fit-for-purpose genomic-based technologies such as battery-powered nucleotide amplification thermocyclers and heating blocks, and next-generation sequencing will enable confirmation of ASF outbreaks at community-level and mapping of the disease molecular epidemiology in domestic pigs, wild pigs and ticks. The combination of mobile phones digital technology for disease reporting and nanopore next-generation sequencing technology forms a combination of affordable technology to enhance the early detection and genotyping of ASFV in Africa.

Biography

Gerald Misinzo is a veterinary graduate from Sokoine University of Agriculture (SUA, Tanzania), MSc Molecular Biology from KU Leuven (Belgium) and holder of a PhD in Veterinary Virology from the University of Ghent, Belgium. A professor of virology, leading specialist in pathogen genomics of the SACIDS Foundation for One Health - an institute of SUA, and leader the World Bank-designated SACIDS Africa Centre of Excellence for Infectious Diseases of Humans and Animals in Eastern and Southern Africa.

He is the Oliver R Tambo Africa Research Chair for Viral Epidemics.

Professor Misinzo was member of the Special COVID-19 Committee appointed by Her Excellency Samia Suluhu Hassan, the President of the United Republic of Tanzania. A member of the Tanzania National Immunization Technical Advisory Group (NITAG) and the Vigilance Technical Advisory Committee (VAC) of the Tanzania Ministry of Health.

He is the Vice President of the Panel for the State of Laboratory Biosafety and Biosecurity in the Southern African Development Community (SADC) Region of the Academy of Science of South Africa (ASSAf) and a member of the Tanzania National Biosafety Committee. He is a member of PPR Global Research and Expertise Network (PPR-GREN) for the global eradication peste des petits ruminants.

His research interest is on viral infectious diseases of humans and animals with a focus, in humans: respiratory infections and acute febrile illnesses, and in animals on transboundary animal diseases

that affect livelihoods and food security. He employs genomics and metagenomics to study pathogen molecular epidemiology, host-pathogen interactions and diagnostics development.
To-date he has mentored two postdocs, 27 PhDs and over 59 MSc/MPhil.
On own merit and as coordinator of SACIDS Foundation for One Health, Professor Misinzo is a recipient of the Sokoine University of Agriculture Vice Chancellor's Award for best researcher at the University in attracting research funds, for two consecutive years, 2020 and 2021.

Pathogenesis of African Swine Fever

Sandra Blome, German National Reference Laboratory for ASF and CSF, Friedrich-Loeffler-Institut, Germany

Presentation synopsis

African swine fever (ASF) is one of the most complex viral diseases affecting livestock and has tremendous socio-economic impact. Over the last decade, the highly lethal, hemorrhagic disease has conquered several new areas and keeps spreading as true pig pandemic.
Although the speed at which our knowledge is increasing has not kept pace with the speed at which the virus is spreading, we have learned quite a bit about the pathogenetic relationships in recent years, at the level of the whole organism and also at the molecular level.
When it comes to our understanding of pathogenesis, it has been known for a long time now that ASF virus (ASFV) invades the body through the dorsal pharyngeal mucosa or the tonsils upon oro-nasal contact. After local replication, the virus spreads through viremia and hemadsorbing ASFV isolates are found associated with erythrocytes, but also with lymphocytes and neutrophils. Nowadays it is generally accepted that the massive destruction of macrophages plays a major role in the impaired hemostasis due to the release of active substances including cytokines, complement factors and arachidonic acid metabolites. Chronic disease may have an auto-immune component and lesions might result from the deposition of immune-complexes in tissues such as kidneys, lungs and skin with their subsequent binding to complement.
Among the lessons learned in recent times is that we need to incorporate vertical transmission pathways into our risk assessments. It has recently been shown that the virus can be transmitted to sows in the semen of infected boars that are not yet clinically apparent, and that these sows also pass the virus on to the fetuses. Furthermore, it was shown that the hygiene status of the host can significantly influence the course of disease, especially with moderately virulent viral strains. The direction of the cellular immune response is a key to further progression. Moreover, the impairment of interferon responses and other antiviral mechanisms has been further studied.
Integration of genomic and transcriptomic data into applied research is needed to help our understanding of beneficial and detrimental host reactions.

Biography

Sandra Blome studied veterinary medicine at the University of Leipzig, Germany, and has a doctorate degree in veterinary medicine. Since 2008 she is senior scientist at the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health (FLI), Germany, and responsible for the national reference laboratories for classical and African swine fever. Sandra Blome has long-term experience in working with transboundary viruses under high containment conditions up to BSL 3+ including animal experiments. She is deputy head of the Institute of Diagnostic Virology since 2015. Her research focuses on studies on pathogenesis of viral infectious diseases with particular emphasis on virus-host interaction and diagnostics/vaccine development.

Molecular basis of African Swine Fever virulence

Linda Dixon, The Pirbright Institute, Surrey UK

Presentation synopsis

African swine fever virus (ASFV) infection can result in an acute haemorrhagic fever resulting in death of almost all infected domestic pigs and wild boar. However, few clinical signs are observed in the natural hosts in E. Africa including warthogs and soft ticks. In domestic pigs and wild boar moderate and low virulence virus isolates have been described which cause reduced or no fatality. ASFV replicates in the cytoplasm of monocytes and macrophages, key cells involved in activating host innate and adaptive immune responses. Estimates are that more than a third of the 190 proteins coded for are not essential for replication in cells but have important roles in virus survival and transmission in the hosts. Comparisons of genome sequences identified large deletions, including members of five different multigene families, in attenuated compared to virulent isolates. These multigene families contain together more than 40 genes. Understanding their functions and interactions represents a key challenge for predicting the virulence of ASFV. Genes coding for a CD2 like protein and a C-type lectin domain containing protein, are also interrupted in some attenuated isolates.

Research on the functions of virus encoded proteins has identified many inhibitors of the host innate response. Many inhibitors of the main host antiviral pathway, the type I interferon response, have been identified. Targeted deletions of single or multiple genes from the ASFV genome have identified those which are not essential for replication and established the impact of the deletions on virus replication in macrophages and virulence in pigs. This research has led to an improved understanding of the molecular basis of virulence of different isolates and has identified candidate modified live vaccines. Current results and future areas of research will be summarised.

Biography

Dr Linda Dixon is Head of the African swine fever virus research group at the Pirbright Institute. Her research focuses on the role of ASFV proteins in evading host defences and application of this knowledge to understanding virulence and development of modified live vaccines. She has many publications in this field spanning more than 25 years. She is a WOAH disease expert on ASFV and an Investigator of the Jenner Institute. She studied Biochemistry at Sussex University and obtained a PhD in Molecular Biology at Edinburgh before completing postdoctoral research in Edinburgh and in Basel Switzerland.

Vaccine development for the control of ASF; where are we and where to go?

Douglas Gladue, Plum Island Animal Disease Center, U.S. Department of Agriculture, U.S.A.

Presentation synopsis

African swine fever virus (ASFV) is the etiological agent of a contagious and often lethal disease of domestic pigs that has significant economic consequences for the swine industry. The disease is devastating the swine industry in Central Europe and East Asia, with current outbreaks caused by circulating strains of ASFV derived from the 2007 Georgia isolate (ASFV-G), a genotype II ASFV. In the absence of any available vaccines, African swine fever (ASF) outbreak containment relies on the control and culling of infected animals. Limited cross-protection studies suggest that in order to ensure a vaccine is effective, it must be derived from the current outbreak strain or at the very least from an isolate with the same genotype. The discovery that the deletion of a previously uncharacterized gene, I177L, from the highly virulent ASFV-G produces complete virus attenuation in swine. ASFV-G-ΔI177L is one of the few experimental vaccine candidate virus strains reported to be able to induce protection against the ASFV Georgia isolate. The first generation of this vaccine candidate has been further tested in field trials and in additional safety studies including reversion to

virulence. The second-generation vaccine ASFV-G-ΔI177LΔLVR is able to be grown in a in house cell line PIPEC cells. Further developments in these two vaccine platforms will be presented

Biography
Dr. Douglas Gladue is an internationally recognized leader in the field of African swine fever (ASF). He is an inventor of the first commercially produced live-attenuated vaccine for ASF. He was elected as the Scientific Director for the global African Swine fever Alliance (GARA) and Treasurer for the world society of virology (WSV). He is the Director of the center of excellence for ASFV genomics, and has for 15 years conducted research for the United States Department of Agriculture (USDA) at Plum Island Animal Disease Center, a biosafety level 3 facility focusing on rationally designed vaccines for foreign swine diseases and was recently awarded the prestigious Arthur S. Flemming award for his accomplishments. Dr. Gladue's research has resulted in four effective live attenuated vaccine platforms against ASFV and one live attenuated vaccine platform for Classical swine fever (CSF) . His recent accomplishments include discovering cell lines for both ASFV diagnostics and for growth of ASFV vaccines. He has discovered over one hundred host-viral protein interactions and has used this information combined with a custom computational pipeline involving both bioinformatics and functional genomic data, to identify critical domains in viral proteins as genetic targets, that when mutated could change the pathogenesis of the virus. Dr. Gladue regularly serves on multiple scientific committees, grant review panels, and academic program review Committees. He has authored over 80 peer-reviewed scientific publications, as serves as editor for several scientific journals.

Thursday 22 Septembre, 08:30 - 10:30, Auditorium

Epidemiology of emerging diseases
Norbert Nowotny, University of Veterinary Medicine Vienna, Austria

Presentation synopsis
I will limit my talk to the epidemiology of selected viral diseases which emerged in Europe. I selected four, the mosquito-borne Usutu and West Nile virus infections, and the recently emerging SARS-CoV-2 and monkeypox, because they differ significantly in their epidemiological features.
Usutu virus (USUV) and West Nile virus (WNV) lineage 2 emerged in Europe 25 and 20 years ago, respectively. Both viruses circulate naturally between birds and bird-feeding mosquitoes. USUV infection leads to bird, mainly blackbird mortality. Severe human disease is rare. Meanwhile several independently introduced genetic lineages circulate, and the virus spread to large parts of Europe. In case of WNV, humans and horses are dead-end hosts, which may be subclinically infected, develop West Nile fever or rarely West Nile neuroinvasive disease (WNND), which may be fatal. Indicators for WNV-2 infections in a region are birds of prey, in particular goshawks, which frequently die due to WNND. WNV-2 dispersed to large parts of central, eastern and southern Europe and to a limited extent also to western Europe.
SARS-CoV-2 was first identified in the city of Wuhan, China, in November 2019. The World Health Organization declared the outbreak a pandemic on 11 March 2020. With almost 600 million diagnosed cases and 6.5 million associated deaths (as of 15.08.2022) COVID-19 is the most disastrous pandemic since the Spanish flu more than 100 years ago. During the more than 2.5 years since its emergence involved virologists learned a lot, not only about the virus itself, the emergence of new variants, waves of infection, variety of symptoms, development of novel types of vaccines and therapeutic agents, but also about human nature, how to deal with politicians and the media, and how to handle virologists-bashing in the social media.

Monkeypox is known as a human disease since more than 50 years with occasional outbreaks in the endemic regions of Central and Western Africa and a limited number of exported cases to non-endemic countries. Starting in May 2022 and ongoing, an unprecedented multi-country outbreak occurred in non-endemic countries with (as of 15.08.2022) 35,000 confirmed cases in 82 countries. On 23 July 2022, WHO declared the outbreak a public health emergency of international concern. Monkeypox spreads through close direct contact with rashes or body fluids from an infected person or through respiratory secretions. While anyone can get monkeypox, to date the vast majority of confirmed cases occurred in young or middle-aged men who have sex with men (MSM) and recent sexual contacts with new or multiple partners. Among other features, this novel epidemiological aspect will be discussed.

Biography

Norbert Nowotny is an Austrian-born virologist with broad teaching and research interests and experience in all aspects of viral infectious diseases. He was educated at the University of Vienna and conducted his Ph.D. studies at the University of Vienna Medical School. He graduated to PhD in January 1982 and completed his Habilitation (equivalent to DSc) in 1997. He joined the Institute of Virology of the University of Veterinary Medicine Vienna, Vienna, Austria, in 1981. 1996-1997 he was Guest Researcher at Stanford University School of Medicine, USA. From 2001-2005 he served as Professor of Virology at the College of Medicine, UAE University, from 2012-2016 at the College of Medicine, Sultan Qaboos University, Muscat, where he was also Head of the Department of Microbiology and Immunology, and from April 2016 to May 2019 he served as Professor of Virology at the College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai. Thereafter he continued to work at the Institute of Virology, University of Veterinary Medicine Vienna, Vienna, Austria.

Prof. Nowotny's research interests include virus infections at the environment / animal / human interface, emerging viral infections, viral zoonoses, and vector-borne virus infections. He has established international reputation in the areas of West Nile virus, Usutu virus, Borna disease virus, and coronavirus research.

Prof. Nowotny has authored a significant number of high-impact peer-reviewed research publications. In detail: 473 of his publications are currently listed in Google Scholar: https://scholar.google.ae/citations?user=oHMASRkAAAAJ&hl=en, 277 in Scopus: https://www.scopus.com/authid/detail.uri?authorId=7005482650, and 257 in PubMed: https://pubmed.ncbi.nlm.nih.gov/?term=Nowotny+N&sort=date.

Third generation sequencing, the new diagnostic power in the veterinary world

Sebastiaan Theuns, PathoSense, Belgium

Presentation synopsis

Identification of the causal infectious agents that are responsible for disease in animals has been a challenge for veterinarians since decades. Throughout history, virus diagnostics has gone through a revolution, from in vivo to in vitro inoculations, electron microscopy, antibody detection, and the development of (real-time) PCR to Sanger sequencing. In the past years, next-generation sequencing technologies have increased the ability to analyse genomic sequencings of viruses. DNA sequencing technologies include short (eg. Illumina) and long-read (eg. Oxford Nanopore Technologies) platforms, each having specific advantages and disadvantages that will be discussed. Such technologies have the potential to revolutionize the way we perform diagnostics of infectious diseases in veterinary medicine but have until recently only been available to R&D environments. Recently, a nanopore sequencing-based diagnostic platform became available to veterinary practitioners. With one analysis, and without prior selection a complete, semi-quantitative

overview of viruses and bacteria can be reported. This novel method is applicable to all animal species and its usability and interpretation will be positioned against other diagnostic tools for viruses.
Having the (complete) genomic sequences of viruses at hand, new opportunities for real-time epidemiological surveillance in animals become increasingly available. First of all veterinary medicine will have a better understanding of the strain diversity of enzootic viruses and their impact on management and vaccination strategies. Veterinary medicine will also be well prepared to trace and combat epizootic diseases. Some of these emerging viruses even have zoonotic potential. From a One Health perspective, real-time pathogenomic surveillance in enzootic and epizootic cases is thus extremely important and the possibilities for improvement of animal and human health will be discussed.

Biography
Dr. Sebastiaan Theuns is co-founder of PathoSense, a spin-off from Ghent University which offers complete diagnostics of infectious diseases in veterinary medicine. The technology makes use of third-generation sequencing (Oxford Nanopore Technologies) and allows veterinarians to make a diagnosis without having to make a prior selection of which pathogens to test for. The spin-off was generated from the Laboratory of Virology of Prof. Hans Nauwynck.
In 2010, Sebastiaan obtained his diploma as a veterinarian, after which he successfully defended his doctorate in 2015. He then developed expertise in veterinary vaccine development during a postdoctoral VLAIO Innovation Mandate. Sebastiaan has always shown interest in the diagnostics of infectious diseases in different animal species and saw a huge opportunity for innovation with the emergence of new DNA/RNA sequencing technologies. Since 2017, his research has led to the development of a complete 'sample-to-diagnostic-interpretation' platform for infectious diseases in veterinary medicine, which culminated in the spin-off PathoSense in October 2020. Since then, PathoSense has been used in more than 20 animal species by veterinarians in Belgium, the Netherlands and several other European countries. We believe that innovation is crucial and that is why scientific R&D will remain an important spearhead in our further development & scaling. The diagnostic discoveries have also led to new science opportunities and emphasize the importance of collaborations between companies and universities. Sebastiaan and PathoSense have already been selected for several prestigious start-up accelerator programs, including the Belgian MedTechAccelerator in 2018 and StartIt@KBC in 2020-2021. Recently he was also admitted to the Vlerick Business School Scale-Up Masterclass of 2022. Sebastiaan is a co-author of >30 scientific publications and a patent. He is also a co-promoter of several doctoral candidates.

Use of peptide analysis in the development of vaccines
Morten Nielsen, Technical University of Denmark, Denmark

Presentation synopsis
MHC peptide binding and presentation is the single most selective event defining the landscape of T cell epitopes. Consequently, understanding the diversity of MHC alleles and the set of ligands that can be bound and presented by each of these MHCs (the immunopeptidome) in a given population has an enormous impact on our capacity to predict and manipulate the potential of protein Ags to elicit functional T cell responses. Liquid chromatography mass spectrometry analysis of MHC-eluted ligand data has proven to be a powerful technique for identifying such peptidomes, and methods integrating such data for prediction of Ag presentation have reached a high level of accuracy for both MHC class I and class II.

In my talk, I will illustrate how these techniques when combined with refined immunoinformatics prediction methods can be applied to learn the rules of MHC antigen presentation also in the context of BoLA molecules for both class I and class II.

Further, using *Theileria parva* (the causative agent of East Coast Fever) as a showcase, I will illustrate how these techniques and tools can be used to guide the search of novel BoLA specific T cell antigens.

Biography
Morten Nielsen holds a position as Professor of Immunoinformatics and machine learning at the Department of Health Technology, Technical University of Denmark. He graduated with a master in physics from the University of Copenhagen. Obtained his PhD (also in physics) from the University of McGill, Canada. The core of Morten Nielsen's research deals with the development of novel and advanced data-driven prediction methods for pattern recognition in biological systems. Morten Nielsen is a pioneer in the field of immunoinformatics and a key inventor of several state-of-the-art methods for T and B cell epitope discovery including the NetMHC-suite currently used worldwide for rational epitope discovery. He has published more than 200 articles, books, and book chapters within the fields of immunology, immunoinformatics, computational biology, data mining and machine learning. Morten Nielsen is further CEO and co-founder of Eir Sciences, a company that offers contract research and epitope services within immunogenicity assessment, immunogenicity optimization, de-immunization and epitope prediction.

Perspectives of mRNA vaccines in the veterinary world
Niek Sanders, Laboratory of Gene Therapy, Faculty of Veterinary Medicine, Ghent University, Belgium

Presentation synopsis
Current veterinary vaccines are often based on old platforms, like inactivated and live-attenuated pathogens. These platforms have several drawbacks like a slow production, the need to culture pathogens and safety risks with especially live-attenuated vaccines. The availability of commercial veterinary DNA vaccines demonstrates that the veterinary sector is also open for medical innovation. The latter and the success of the human COVID-19 mRNA vaccines has triggered a lot of interest in mRNA vaccines in the animal health sector. In this presentation the different mRNA vaccine platforms and the different delivery systems will be discussed from a veterinary perspective. The stability and storage requirements of mRNA vaccines will also be tackled. Finally, the current experience with mRNA platforms in veterinary medicines will be reviewed.

Biography
Prof. Niek Sanders received his master degree in pharmaceutical sciences in 1997 and his PhD degree in 2001. During his PhD research he studied the barrier properties of cystic fibrosis (CF) lung mucus towards CF gene therapy. **From 2002 onwards**, he worked as a **postdoctoral fellow** of the *Research Foundation Flanders* (FWO) at Ghent University (Belgium), at the Ludwig-Maximilians-Universität (Munich, Germany), and at the University of Utrecht (the Netherlands). The **main focus of his postdoctoral research** was the development and in vitro evaluation of **delivery systems for DNA, siRNA and mRNA**. In **2008** he became research professor at Ghent University and started to **build-up the Laboratory of Gene Therapy** from scratch. The design and preclinical evaluation of **mRNA therapeutics, mRNA vaccines and cancer immunotherapies** are the cornerstones of his current research team. His research on cancer immunotherapies focuses on the design and preclinical evaluation of mRNA vaccines and mRNA encoding immune stimulating proteins. These cancer immunotherapies are evaluated in murine models and pets with spontaneous tumors. With regard to the **design of mRNA therapeutics** the team of Sanders is one of the leading teams in the world. During the past 8 years they made **several synthetic mRNA platforms** (unmodified, chemical modified and self-amplifying mRNAs) and engineered together with MIT **smart mRNAs that can be switched ON and OFF on-demand**. Moreover, since 2019 the team is also focusing on the development and in vivo evaluation of **novel delivery systems for mRNA** vaccines and therapeutics. This unique and broad knowhow and the possession over this wide range of synthetic mRNAs

enabled the group to initiate several ongoing projects. In more detail Sanders's team is **evaluating mRNA-based immunotherapies against cancer (e.g. breast cancer, melanoma), viral infections (e.g. Zika virus, SARS-CoV-2) and bacterial infections. Moreover, mRNA that encode therapeutic proteins are also made and applied in different fields.**
Prof. Niek Sanders is the author or co-author of more than 130 A1 paper in high impact journals and he was an invited speaker at several international meetings. He has also several patents or patent applications and his research has been awarded with several prizes. Prof. Niek Sanders is the scientific founder of Ziphius Vaccines, a Flemish start-up company that focuses on development of mRNA vaccines and mRNA therapeutics.

Thursday 22 Septembre, 15:30 - 17:30, Auditorium

Blue tongue virus in Europe and Africa
Alessio Lorusso, Istituto Zooprofilattico Sperimentale dell'Abruzzo, Italy

Presentation synopsis
Arboviruses are important causes of disease in humans and animals, and it is proposed that climate change will increase the distribution and severity of arboviral diseases. Orbiviruses are the cause of important and apparently emerging arboviral diseases of livestock, including bluetongue virus (BTV), African horse sickness virus (AHSV), equine encephalosis virus, and epizootic hemorrhagic disease virus (EHDV) that are all transmitted by haematophagous *Culicoides* insects. Since 1998, Southern Europe has experienced multiple incursions of different serotypes and topotypes (western (w) or eastern (e)) of BTV. Strains of BTV-1e, BTV-4w, BTV-9e and BTV-16e have all entered the eastern Mediterranean region. In addition, strains of BTV-1w, BTV-2w, BTV-3w and BTV-4w have entered Southern Europe because of wind-driven dissemination of infected midges from Northern African countries. Specifically, the virus had been likely introduced to Europe from Northern Africa via two major gateways: (i) from Morocco to Spain through the Straits of Gibraltar, (ii) from Tunisia or Algeria to Italy and Spain through Sardinia, Sicily, and Balearic Islands, respectively. In recent years (2012-2021), multiple BT outbreaks caused by BTV-4 strains have been notified in several countries facing the Mediterranean basin including Tunisia, Spain (mainland and Balearic Islands), France (mainland and Corsica), and Italy (mainland, Sardinia, and Sicily). BTV-4w strains collected in 2021 in Italy (mainland and Sardinia), France (Corsica) and Spain (Balearic island) were remarkably close (>99.56 % of nucleotide identity in all genome segments) to homologous strains collected in Tunisia in 2019, 2020 and 2021. These novel BTV-4w, along with a different genome constellation, were slightly divergent in Seg-2 (97.85% of nt identity) with respect to Balkanic BTV-4w strains isolated from 2014 onward in Europe including also recent French (Corsica, 2020) and Italian (Sicily, 2021) BTV-4w strains. The novel BTV-4w differed also from Spanish BTV-4w strains which have circulated in mainland Spain since 2010 as these latter were related to BTV-4w strains collected in Morocco and Tunisia in 2012 and 2013, respectively. The novel BTV-4w strains from 2021 had likely originated in southern Europe as a consequence of a novel wind-driven dissemination of infected midges from Northern African countries. This and novel evidences regarding BT in Europe and Africa will be discussed.

Biography
DVM-PhD, leading scientist at the Virology Unit of the IZS of Teramo-Italy which represents one of the WOAH-Reference Laboratory for BTV. Areas of interest: **1**-Virus discovery and characterization **2**-Genome manipulation via reverse genetics of RNA viruses and pathogenesis studies in animal models **3**-Zoonoses, emerging viral diseases, public health **4**-Diagnosis of infectious diseases via

innovative molecular methods. He has also been recently appointed as Deputy Director of the joint IZSVe-IZSAM FAO Reference Laboratory for zoonotic coronaviruses.

A closer look at Bluetongue virus: what structures tell us for vaccine design against Bluetongue and African Horse Sickness disease

Polly Roy, Department of Infection Biology, London School of Hygiene & Tropical Medicine, United Kingdom

Presentation synopsis

A detailed knowledge on virus structure and functions of viral proteins in replication process offers the best possibility for control. In our studies on Bluetongue virus, we have gained detailed knowledge of all aspects of the virus, its genetics, structure and replication. Specifically, we determined the complex nature of the virion through three-dimensional structure reconstructions; atomic structure of proteins and particles; the definition of the virus encoded proteins responsible for cell entry and enzymes required for RNA replication and the ordered assembly of the capsid shell. Based on the information gained, it has been possible to produce several types of subunit vaccine but more importantly to build recombinant virus like particles (VLP) that mimic the normal virus but have no ability to multiply. BTV VLPs, a first in the field (1990), had a characteristic icosahedral structure produced by co-expression of four structural proteins and were used for a series of vaccination trials. Collectively, the data form both sheep and cattle studies demonstrated that VLPs provided a higher level of protection against virulent virus challenge than subunit vaccines. Based on the molecular understanding of the genome packaging and RNA replication, we then developed the first BTV reverse genetics (RG) system, and used it to design 'entry competent but replication abortive' (ECRA) virus strains that offer a wholly new form of vaccine for BTV and, subsequently, for AHSV. Again, a series of vaccination trials in animals (in sheep, cattle and ponies) demonstrated protective efficacy and long-term protection induced by either single serotype or cocktail vaccines. Moreover, as both VLPs and ECRA vaccines use common "core" elements, new serotypes can be readily developed by exchange of only the serotype determinant to allow rapid completion of all regulatory hurdles and be ready for rapid roll-out should an outbreak occur.

Biography

Polly Roy has been Professor of Virology at the London School of Hygiene and Tropical Medicine since 2001. Her education began in Calcutta, India and was followed by a PhD fellowship at New York University in Molecular Biology. Following a 3-year postdoctoral research fellowship in RNA virology at Rutgers University, Roy joined the University of Alabama at Birmingham to establish her own research group, becoming a full Professor in 1985. In 1987, Roy took an International Fogarty fellowship to study at the University of Oxford where she established a UK-based research group, becoming Professor at the University in 1996. She leads a large research group working on the molecular biology of RNA viruses and including basic virology and applied vaccine research. The majority of the work features the segmented dsRNA Bluetongue virus both as a model and as the cause of significant agricultural losses. She is the leading exponent of the virus's structure, assembly and replication and is the originator of several innovative vaccine developments which offer improved options for control. The group's output includes pioneering work on co-expression of viral proteins to form immunogenic virus-like-particles, the development of reverse genetics for Orbiviruses, the *in vitro* assembly of infectious virus particles, the basis of genome segment packaging and novel single replication cycle vaccines.

Roy has trained more than 100 researchers, published over 365 manuscripts in refereed and high impact journals and contributed numerous chapters to virology books and encyclopedias. She is an editorial board member for a number of academic journals and is an organizer of several international scientific meetings. Polly Roy's contribution in Virology has been noted with the award of various honors and awards, including being elected as a Fellow of the Academy of Medical Sciences,

receiving the Indian Presidential **Gold Medal** for her contribution to science and technology and being made an **Officer of the Order of the British Empire (OBE),** for services to Virus Research in the **Queen's Birthday honors' list**.

Disabled Infectious Single Animal (DISA) DIVA vaccine platforms for Bluetongue and African Horse Sickness

Piet van Rijn, Wageningen Bioveterinary Research, The Netherlands & Centre for Human Metabolomics, North-West University, Potchefstroom, South Africa

Presentation synopsis

Bluetongue virus (BTV) and African horse sickness virus (AHSV) (genus *Orbivirus*, family of *Reoviridae*) cause economically and socio-emotionally important diseases in ruminants and equines, respectively. Orbiviruses are nonenveloped virus particles consist of three protein layers and contain ten genome segments of double stranded RNA (Seg-1 to 10). Many serotypes of BTV and AHSV are known and show poor cross-protection, which challenges the development of broad protective vaccines. Outer shell proteins VP2 and VP5 determine the serotype, which correlates with the genetic diversity of Seg-2[VP2]. BTV1-24 and AHSV serotypes are OIE-notifiable. Control of these vector borne diseases is hardly possible without vaccination. Transmission occurs by culicoides biting midges depending on viremia in the mammalian host for uptake and on virus secretion in midge saliva for infection.

Nonstructural protein NS3/NS3a is involved in virus release and suppression of immune responses. We discovered that NS3/NS3a is not essential for *in vitro* virus replication, whereas virulence and viremia in the hosts and virus propagation in midges require functional NS3/NS3a protein. These findings initiated the development of vaccine platforms for Bluetongue and African Horse Sickness and are applied for many serotypes by exchange of Seg-2[VP2] and Seg-6[VP5]. An in-frame deletion in Seg-10[NS3/NS3a] abolishes viremia and virus secretion ensuring safety regarding adverse effects and vaccine spread. These platforms are therefore named Disabled Infectious Single Animal (DISA) vaccine platforms. DISA vaccines for different serotypes share the NS3/NS3a deletion allowing safe formulations of multi-serotype cocktail vaccines. Further, the absence of viremia after DISA vaccination prevents rise of virulent, vaccine-related reassortants. Finally, deletion of dispensable immunogenic NS3/NS3a enables cost-competitive vaccine production and differential detection of infected animals in vaccinated populations (DIVA).

A prototype AHS DISA/DIVA vaccine partially protected ponies and will be further improved within the EU-grant SPIDVAC. The prototype BT DISA/DIVA vaccine meets all criteria for modern veterinary vaccines. Furthermore, pentavalent BT DISA/DIVA vaccine also induce sterile immunity in sheep and cattle against several studied serotypes. In conclusion, DISA/DIVA vaccine platforms pave the way to develop tailored-made as well as broad protective DIVA vaccines for Bluetongue and African Horse Sickness.

Biography

Prof. dr. Piet A. van Rijn is molecular virologist and since 1990 employed at Wageningen Bioveterinary Research (WBVR) in Lelystad, the Netherlands. He is also extraordinary professor at the North West University, Potchefstroom, South Africa. Since 2002, he is head of the dedicated National Reference Laboratory (NRL) for many exotic OIE-notifiable viral diseases of animals, such as BT, AHS, EHD, RP, PPR, LSD, GP, SP, EIA, WNF, RVF and Rabies. By lack of field samples of exotic diseases, he has developed an automated *in silico* validation tool using freely available software for in-house developed PCR diagnostics. Piet is also founder of the former European Network of Excellence EPIZONE, now continued as the European Research Group EPIZONE.

In addition, he has performed molecular virology research and development of (DIVA) diagnostics. His research at WBVR combines previous inventions like live-attenuation and DIVA with reverse genetics to improve vaccines. He started with the development of reverse genetics for CSFV and

performed a detailed epitope mapping on envelope glycoprotein E2. This research resulted in DIVA vaccine based on live-attenuated CSF vaccine. After the incursion of BTV serotype 8 in 2006 in NW Europe, Piet started research on Bluetongue and African Horse Sickness. Again, reverse genetics was implemented as key technology. His research group developed a new generation replicating DIVA vaccines for these midge-borne animal diseases, named Disabled Infectious Single Animal (DISA) DIVA vaccines, since these are not spread by midges. The DISA/DIVA vaccine platforms were applied for many serotypes of BTV or AHSV, respectively, and prototypes were successfully studied in target livestock species. More recently, his group implemented reverse genetics for PPRV and generated tagged PPRVs to study pathogenesis in goats. His research has been published in >100 high-ranking publications (ORCID ID: 0000-0002-2594-1232).

Preparedness of the pharmaceutical industry for emerging orbiviruses - what is needed

Mercedes Mouriño, Research and Emerging Diseases Senior Manager, Veterinary Medicine R&D Global Biologics at Zoetis Manufacturing & Research, Spain

Presentation synopsis

The most frequently diseases caused by orbivirus are BlueTongue (BT) and African Horse Sickness (AHS). There is no specific treatment for BT or AHS, prevention by vaccination remains the mainstay for control in endemic countries. Live-attenuated vaccines are commercially available, however, there are increasing concerns regarding its use because of their proven potential for reversion to virulence, capacity for transmission by vectors, and reassortment of their gene segments with other vaccine and field strains, leading to the creation of novel virus progeny and outbreaks.

For that reason, after BTV incursions into northern Europe, the industry focused on the development of inactivated vaccines. These played a major role in controlling transmission.

However, current commercially available vaccines lack the capability to differentiate between vaccine and field virus infection, leading to diagnostic complications in outbreaks in endemic areas where vaccination has occurred.

Recombinant vaccines (subunit, vectored, virus-like particle–based vaccines…) could avoid these complications and would likely be used after an incursion into previously unaffected regions. However, they are not commercially available, and more research and development is needed regarding their efficacy and duration of immunity.

The immediate availability and distribution of vaccines on the market and the possibility for rapid vaccination is critical for outbreak control and/or disease eradication. To address potential availability concerns in the face of an outbreak of new or re-emerging infectious diseases in Europe with the potential for severe impact on animal or public health, two key actions have been taken by the EU Medicines Agencies Network Strategy: the introduction into the legislation of the ‘exceptional use’ and of the ‘multi-strain’ dossier.

In preparedness for emerging orbivirus and to address emerging health threats, both the Authorization for emergency use and the multi-strain dossier guidelines provide some flexibility to the animal health industry. For BTV vaccines, the industries applied for multi-strain dossiers: these allow to adapt the formulations to the variable distribution of serotypes in the EU and the quicker registration of new vaccines against new serotypes. This approach can also be used now for vaccines against AHS.

Biography

Mercedes received a degree in Biology in 1992 and a PhD in Microbiology and Molecular Biology from the University of Barcelona (Spain) in 1997.

Mercedes joined Zoetis, a world leader animal health company, in 1998. She is part of the R&D team located in Spain. This team has a specific focus on veterinary vaccines and in particular, Mercedes

supervises the Research and Emerging Diseases group leading the activities related with the development in Europe of vaccines against Transboundary and Emerging Infectious Diseases.
With 24 years of experience, she and her team have brought several vaccines to the European markets: Suvaxyn® CSF Marker (a DIVA vaccine against the Classical Swine Fever virus), Zulvac® SBV (against the disease caused by the Schmallenberg virus in sheep and cattle) and several Zulvac® BTV vaccines (against the disease caused by several serotypes of the Bluetongue virus intended for sheep and cattle).
Through her professional career, she has been collaborating in several projects funded by the European Union. Currently, she is representing Zoetis in the DEFEND project. DEFEND is a Horizon 2020 project receiving funding from the European Union's Horizon 2020 research and innovation programme, that target two viruses of livestock which are emerging into Europe: African swine fever virus (ASFV) and lumpy skin disease virus (LSDV).

Friday 23 Septembre, 08:30 - 10:00, Auditorium

SARS-CoV-2 zoonosis and reverse zoonosis.

Wim HM van der Poel and the Netherlands Centre for One Health (NCOH) Consortium
Wageningen Bioveterinary Research, Wageningen University and Research, Lelystad, the Netherlands

Presentation synopsis

Coronavirus disease (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in December 2019 in Wuhan, China, and has since spread rapidly, evolving into a full-blown pandemic. Since viral nucleic acid sequences from Rhinolophus affinis bats, collected in Yunnan province showed a 96% resemblance to SARS-CoV-2, these bat species are considered the most likely natural reservoir of SARS-CoV-2. At first clinical infections in animals were rare but soon SARS-CoV2 was detected by RT-PCR in dogs and cats in several countries. Currently SARS-CoV2 infections have been detected in a range of animal species, besides cats and dogs, including hamsters, rabbits, ferrets, mink and other species. Especially mustelid species appear to be very susceptible to the virus. Zoonotic risks of such species therefore need to be studied and controlled.
SARS-CoV-2 caused outbreaks in mink farms in Europe and North America. These outbreaks were associated with respiratory disease and increased mortality and in the Netherlands more than 2 million mink have been culled because of these outbreaks. In the first farms, workers had shown coronavirus disease-associated symptoms before the outbreak. Viruses from humans exposed to mink clearly clustered with the animal viruses and not with the viruses detected in unrelated SARS-CoV-2 patients living in the vicinity of the farms. Sequences from infected mink farms fell into five distinct clusters, showing between mink farm transmission. The research showed evidence of ongoing SARS-CoV-2 transmission in mink farms and spill over events back to humans.
After the outbreaks in mink several more spill back events of SARS-CoV-2 have been reported. In Hong Kong transmission from a pet hamster to a human, led to onward human transmission. In the US it was demonstrated that free-ranging white-tailed deer (*Odocoileus virginianus*) as well as captive fallow deer (*Dama dama*) were exposed to multiple SARS-CoV-2 variants from humans. The circulation of SARS-CoV-2 among deer could result in spill over to other wildlife species or spill back to humans with unknown impact for the conservation and public health.
The possible risk of spillover of SARS-CoV-2 between humans and animals indicates there is an urgent need to establish comprehensive 'One Health' programmes to monitor wildlife and other hosting animal species globally. Considering the fact that SARS-CoV-2 mutations occurring in

animals may increase its pathogenesis or transmissibility in humans it is imperative to closely monitor mutations in the viral genome from infected animals and humans, particularly the genome regions affecting diagnostic tests, antiviral drugs, and vaccine development.

Biography

Prof. Wim H.M. van der Poel, DVM, PhD, is senior scientist at Wageningen Bioveterinary Research and special Professor of 'Emerging and Zoonotic viruses' at Wageningen University. He is a principal investigator within the Netherlands Centre of One Health (NCOH), member of the Project Management Board of the European Joint Program One Health (EJPOH) and coordinator of the EPIZONE European Research Group, the network on epizootic animal diseases research. The research work of Prof. Van der Poel involves three main areas: New and emerging viruses, Foodborne and Zoonotic viruses and 'Global One Health'. In these areas his research has primarily been focusing on the detection and characterization of viruses in the different hosting animals, as well as in food and environmental matrices. Controlling the risks of infectious diseases and chronic diseases are crucial to food security, public health, climate change and biodiversity. A Global one Health research approach is needed to achieve that.

Noroviruses at the interplay between veterinary and human public health

Etienne Thiry, Veterinary Virology and Animal Viral Diseases, FARAH, ULiège, Belgium

Presentation synopsis

As members of the *Caliciviridae* family of small, non-enveloped, positive sense, single-stranded RNA viruses, the genetically diverse noroviruses infect a broad range of mammalian hosts. Human noroviruses are recognised as the leading global cause of sporadic and epidemic viral gastroenteritis. They cause significant morbidity and mortality in low-income countries and engender enormous economic losses in high-income countries. Animal noroviruses have been linked to gastroenteritis outbreaks and diarrhoeic episodes in domestic cattle, pigs, cats, and dogs, and have been detected in wild animal and bird faeces; murine noroviruses, combining the advantages of easy *in vivo* infection of a native host and robust *in vitro* culture, are used as human norovirus surrogates. The lack of certitude regarding the source of newly emerging human noroviruses and the close genetic relatedness between certain animal and human strains have generated interest in the possible role of animals as a zoonotic reservoir for emerging noroviruses. Such a zoonotic potential has long been discussed, conceivable interfaces of shared species exposure being food (with a particular focus on the simultaneous presence of animal and human noroviruses in bivalve molluscs), water, or animal contact. Despite known noroviruses exhibiting distinct host specificity, the discussion about interspecies and/or zoonotic transmission is fuelled by the close relationship of certain animal and human strains, detection of human norovirus RNA in animal faeces, detection of antibodies against human noroviruses in swine and antibodies against bovine and canine noroviruses in humans, and the demonstration of experimental human norovirus infection in non-human primates, gnotobiotic pigs and calves. Transmission of noroviruses from animals to humans would have wide-ranging implications; however, conclusive data supporting such transmission is yet lacking (detection of viral RNA or antibodies involves inherent caveats). Equally, while the strong species barrier, determined to lie at a structural level between virus and host receptor for human and murine noroviruses, may theoretically be overcome by chance recombination events, this hypothesis remains to be validated. Meanwhile, the proven risk of food-borne human contamination via sources of animal origin poses a very real public health concern and is thus of relevance to the veterinary community.

Biography

Etienne Thiry was born on the 4th of April 1957 in Etterbeek (Brussels). He was graduated as doctor in veterinary medicine in 1980 and in veterinary sciences (PhD) in 1985. He is retired diplomate

member of the *European College for Veterinary Public Health*. From 1st October he is emeritus ordinary professor of veterinary virology and viral diseases, Liège University, Belgium. He is also emeritus professor of veterinary virology at the free university of Brussels. He won the *International Pfizer award* by the international committee of the World Buiatrics Society in 1996. He won the *Gaston Ramon* award by the French Academy of Veterinary Medicine in 2008. He was recognized by the European Society for Veterinary virology as honorary member in 2009. He received the award *Prix de la Francophonie* by the *Fédération des Associations francophones des vétérinaires d'Animaux de Compagnie* (FAFVAC) in 2011.

Etienne Thiry is associated member of the Royal Academy of Medicine of Belgium. He is also member of the National Committee for Microbiology of the Belgian Royal Academy; former chairman of the scientific committee at the Belgian Food Safety Agency (Afsca) (2014-2021); former chairman of the Risk Assessment Group Covid Animals (2020-2021); former chairman of the expert committee for animal health and welfare at the French Agency for Food, Environmental and Occupational Health and Safety (Anses) (2012-2018). He is vice-chairman of the board of directors of the federal scientific institute Sciensano and also chairman of the board of directors of the non-profit association Formavet active in the veterinary continuing education. He is consulting member, previously acting- and vice-chairman, of the *European Advisory Board on Cat Diseases*. He was also coordinator of the European Union "Better Training for Safer Food" project on "Animal Health Prevention and Control of Emerging Animal Diseases" (2012-2013).

His research interests cover several aspects of animal virology, especially the study of animal virus-host interactions and the evolution of viral populations in noroviruses and hepeviruses; in applied research, virucidic activity and the quality of personal protective equipments, especially in the context of the Covid-19 pandemic. These scientific activities generated more than 500 papers in specialised scientific journals (h-index 44).

Influenza viruses in swine and humans: dangerous liaisons?
Kristien Van Reeth, Ghent University, Belgium

Biography
Kristien Van Reeth was graduated as doctor in veterinary medicine in 1991 and in veterinary sciences (PhD) in 1998. She is Full Professor at the Laboratory of Virology, Department Translational Physiology, Infectiology and Public Health at the Faculty of Veterinary Medicine, Ghent University, Belgium. Her main research fields include the pathogenesis and immune response to swine influenza virus, public health aspects of swine influenza virus, pathobiology of the porcine respiratory disease complex, surveillance for influenza and coronaviruses of swine. She is a swine influenza expert for OFFLU (OIE/FAO network of expertise on animal influenza) and ex-chair of the OFFLU swine influenza virus group. She is a member and expert of the Chamber of Veterinary Medicines, Belgium, member of the Royal Academy for Medicine of Belgium and member of professional societies for virology, microbiology and swine health: European Society for Veterinary Virology (ESVV), American Society for Microbiology (ASM), American Association for the Advancement of Science (AAAS), Belgian Society for Virology (BELVIR).

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O2. Virus-Induced Inhibition Of Superinfection As A Means For Accelerating Fitness-Based Selection Of Cyprinid Herpesvirus 3 Single Nucleotide Variants In Vitro And In Vivo
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4. Evaluation of New Routes of Usutu Virus Infection in Birds
Aude Blanquer, Felipe Rivas, Ute Ziegler, Martin Groschup and Mutien-Marie Garigliany

O5. Are non-coding Simbuviruses RNA structures involved in transcription termination and translation?
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O6. Impact of genetic diversity of the Rift Valley Fever virus, from the field isolates to a genetic determinant
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O7. Marek's disease virus virulence genes encode circular RNAs
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O8. Neuraminidase and Trypsin Treatment of Highly Passaged Feline Enterocytes Enhances the Replication of FECV Type I Strains
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O11. ASFV-Host Protein Interaction Mapping Uncovers Novel Function of CP204L in Lysosome Fusion and Clustering
Katarzyna Dolata, Walter Fuchs, Grégory Caignard, Juliette Dupré, Katrin Pannhorst, Sandra Blome, Axel Karger

O12. Pathogenesis of West Caucasian and Lleida bat viruses, two divergent lyssaviruses co-circulating in a widespread migratory bat species
Petra Drzewnioková, Barbara Zecchin, Dino Scaravelli, Francesca Festa, Martina Castellan, Maira Zorzan, Barbara Tramontan, Andrea Lombardo, Emmanuelle Robardet , Stefania Leopardi, Paola De Benedictis

O13. Illumination of Mouse Adenovirus-1 lifecycle
Emeline Goffin, Xiang Du, Bénédicte Machiels, Laurent Gillet

O14. Genomic Analysis and Replication Kinetics of the Closely Related EHV-1 Neuropathogenic 21P40 and Abortigenic 97P70 Strains
Eslam Elhanafy, Ines Zarak, Nick Vereecke, Sebastiaan Theuns, Kathlyn Laval, Hans Nauwynck

O15. In vivo and in vitro characterization of neurotropism of highly pathogenic influenza virus H5N8 (clade 2.3.3.4b) in chicken and duck
Charlotte Foret-lucas, Pierre Bessière, Amelia Coggon, Alexandre Houffschmitt, Maxence Delverdier, Thomas Figueroa and Romain Volmer

O16. Artificial insemination as alternative transmission route for African swine fever virus: How infected boars could efficiently spread the disease
Friedrichs Virginia, Carrau Garreta Tessa, Deutschmann Paul, Christopher Hennings Jane, Reicks Darwin, Beer Martin, Blome Sandra

O17. The Histone-Like A104R Protein of African Swine Fever Virus is not Essential for Replication in Cell Culture
Walter Fuchs, Björn-Patrick Mohl, Tonny Kabuuka, Katrin Pannhorst, Günther M. Keil, Jan Hendrik Forth, Thomas C. Mettenleiter

O18. SARS-CoV-2 omicron infection induces decreased viral replication and inflammation in the upper and lower respiratory tract compared to the D614G and Delta variants in the Syrian hamster model.
Maxime Fusade-boyer, Hélène Huet, Adèle Gambino, Audrey St Albin, Ophélie Grad, Nicolas, Meunier and Sophie Le Poder

19. Peripheral Inoculation of Usutu Virus Generates a Neuroinvasive Disease in 129/Sv Pups
Mazarine Gerardy, Aude Blanquer, Ute Ziegler , Martin Groschup and Mutien-Marie Garigliany

O20. Modeling porcine hemagglutinating encephalomyelitis virus infection in vivo and ex vivo
Juan Carlos Mora-Díaz, Pablo Piñeyro, Rolf Rauh, William Nelson, Zianab Sankoh, Edward Gregg, José Antonio Carrillo-Ávila, Huigang Shen, Rahul Nelli, Jeffrey Zimmerman and Luis Giménez Lirola

O21. AlHV-1 infection causes oligoclonal expansion and activation of CD8+ T lymphocytes resulting in bovine malignant catarrhal fever via interaction with T cell signaling pathway
Meijiao Gong, Françoise Myster, Abdulkader Azouz, Guillem Sanchez Sanchez, Shifang Li, Justine, Javaux, Sylvain Leemans, Olivier Nivelles, Willem van Campe, Stefan Roels, Laurent Mostin, Thierry van den Bergh, Pierre Kerkhofs, Laurent Gillet, Andrew J. Davison, David Vermijlen, Stanislas Goriely, Tim Connelley, Alain Vanderplasschen, Benjamin G. Dewals

22. Illumination of Cyprinid Herpesvirus 2 Infectious Cycle Using In Vivo Bioluminescent Imaging
Bo He, Owen Donohoe, and Alain F.C. Vanderplasschen

23. In vitro evaluation of nine antiviral compounds for their potential effect against equid alphaherpesviruses EHV-4 and EHV-3
Camille Normand, Christine Fortier, Gabrielle Sutton, Côme Thieulent, Christel Marcillaud-Pitel, Loïc Legrand, Stéphane Pronost and Erika Hue

24. Spatiotemporal pathogen-host interactions during African swine fever virus infection
Joshua Hui, Rob Noad, Pippa Hawes, Raquel Portugal, Chris Netherton

O25. Cellular telomerase RNAs and viral RNAs possess common antiapoptotic functions that enhance herpesvirus-induced tumorigenesis
Ahmed Kheimar, Laetitia Trapp-Fragnet, and Benedikt B. Kaufer

26. Canine Adipose Derived Mesenchymal Stem Cells Support Canid Alphaherpesvirus 1 Infection In Vitro
Nina Krešić, Marina Prišlin, Ivana Ljolje, Šimun Naletilić, Petar Kostešić, Dunja Vlahović, Željko Mihaljević, Dragan Brnić, Nenad Turk, Boris Habrun

O27. DNAJC14 independent replication of the atypical porcine pestivirus (APPV)
Carina M. Reuscher, Kerstin Seitz, Lukas Schwarz, Francesco Geranio, Olaf Isken, Martin *Raigel, Theresa Huber, Sandra Barth, Christiane Riedel, Anette Netsch, Katharina Zimmer, Till Rümenapf, Norbert Tautz and Benjamin Lamp*

28. Analysis of Synchronous and Asynchronous In Vitro Infections with Homologous Murine Norovirus Strains Reveals Time-Dependent Viral Interference
Louisa Ludwig-Begall, Elisabetta Di Felice, Barbara Toffoli, Chiara Ceci, Barbara Di Martino, Fulvio Marsilio, Axel Mauroy, Etienne Thiry

O29. Study of the potential of the in vitro replication capacity of porcine reproductive and respiratory syndrome virus PRRSV strains to predict their virulence in vivo
Jaime Castillo, Javier Martínez-Lobo, Javier Domínguez, Isabel Simarro, José María Castro, Concepción Revilla, Cinta Prieto

O30. Predicting infectious bursal disease virus pathotype: new models based on early changes in blood cell formula and bursa cells transcriptional activity
Annonciade Molinet, Céline Courtillon, Michel Amelot, Alassane Keita, Lucas Pierrick, Edouard Hirchaud, Yannick Blanchard, Béatrice Grasland, Nicolas Eterradossi, Sébastien Soubies

31. Equine herpesviruses in the environment: what are the risks in terms of contamination?
Camille Normand, Pauline Defour, Aude Bonassies, Philippe Ciantar, Christine Fortier, Romain Paillot, Christel Marcillaud-Pitel, Pierre-Hugues Pitel, François Meurens, Stéphane Pronost, Erika Hue

O32. Degree of PCV2 uptake by porcine monocytes is strain-dependent and is associated with amino acid characteristics on the outside of the capsid
Yueling Ouyang, Hans J. Nauwynck

33. T-lymphoblasts from Landrace pigs are more susceptible to porcine circovirus type 2 (PCV2) than T-lymphoblasts from Piétrain pigs and the replication is strain-dependent
Yueling Ouyang, Hans J. Nauwynck

O34. Genome-wide CRISPR/Cas9 Knockout Screen in Porcine Cells to Identify Relevant Host Factors for African Swine Fever Virus Replication
Katrin Pannhorst, Jolene Carlson, Julia E. Hölper, Finn Grey, John Kenneth Baillie, Dirk Höper, Elisabeth Woehnke, Kati Franzke, Walter Fuchs, Thomas C. Mettenleiter

35. Age-depended Pseudorabies Virus Invasion in Porcine Central Nervous System
Konstantinos Papageorgiou, Ioannis Grivas, Maria Chiotelli, Alexandros Theodoridis, Emmanuel Panteris, Dimitris Papadopoulos, Evanthia Petridou, Nikolaos Papaioannou, Hans Nauwynck, Spyridon K. Kritas

O36. Molecular determinants of ASFV hemadsorption and virulence
Daniel Pérez-Núñez, Raquel García-Belmonte, Elena Riera, Gonzalo Vigara-Astillero, Yolanda Revilla

37. Photoconvertion and proximity biotinylation assays are powerful tools to track intracellular mobility and identify molecular interactants of a viral protein in living cells
Sébastien Pirotte, Mamadou Diallo, Alain Vanderplasschen

38. In vitro replication of the white spot syndrome virus in a cuticular explant model of shrimp (Litopenaeus Vannamei)
Mostafa Rakhshaninejad, Liping Zheng, Hans Nauwynck

O39. The Role of bovine ADAM17 in Pestivirus Infections and its Importance in the Pestivirus Resistance of CRIB-1 cells
Christiane Riedel, Marianne Zaruba, Hann-Wei Chen, Stefan Düsterhöft, Till Rumenapf

40. Age-dependent expression of the coronavirus receptors APN, DPP4, ACE2 and TMPRSS2 in different regions of porcine intestines
Waqar Saleem, Xiaolei Ren, Hans Nauwynck

O41. Evidence of Lumpy skin disease virus transmission from subclinically infected cattle by Stomoxys calcitrans
Haegeman A, Sohier C, Mostin L, De Leeuw I, Van campe W, De Regge N, De Clercq K

O42. Infection kinetics of BTV-X ITL2021 (BTV-32) in small ruminants
Massimo Spedicato, Giovanni Di Teodoro, Liana Teodori, Alessandra Leone, Barbara Bonfini, Maura Pisciella, Ottavio Portanti, Emanuela Rossi, Tiziana Di Febo, Alessio Lorusso, Giovanni Savini

43. Hepatitis E Virus Oral Infection of Pigs
Falko Steinbach, Charlotte L. Marsh, Sylvia S. Grierson, Bhudipa Choudhury

44. Feline herpesvirus type 1: Trails to enter the host cell
Aleksandra Synowiec, Krzysztof Pyrc

O45. Subclinical PRRSV type 1 infection aggravates clinical course of Streptococcus suis infections in pig
Norbert Stockhofe-Zurwieden, Manouk Vrieling, Jan Cornelissen, Helmi Feijten, Lisette Ruuls, Rob Zwart, Ditta Popma, Sandra Vreman

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O46. In Vitro Recall Response to African Swine Fever Virus Reveals
Immune Components Underlying Cross-protection
Jordi Argilaguet, Laia Bosch-Camós; Uxía Alonso; Anna Esteve-Codina; Beatriz Martín-Mur; María J. Navas, Marta Muñoz, Chia-Yu Chang; Sonia Pina-Pedrero; Francesc Accensi; Lihong Liu; Boris Gavrilov; Fernando Rodríguez

47. Influence of gammaherpesvirus infections on the antibody repertoire of their host.
Jérôme Baiwir, Xue Xiao, Céline Lété, Bénédicte Machiels, Laurent Gillet

48. The interference of West-Nile virus in vitro replication in mosquito cells due to Lammi virus an insect-specific flavivirus
Pontus Öhlund, Nicolas Delhomme, Juliette Hayer, Jenny C. Hesson, Anne-Lie Blomström

O49. Virus-host interactome high-throughput mapping to identify new factors of pathogenicity and interspecies transmission for Bluetongue virus
Aurore Fablet, Cindy Kundlacz,, Juliette Dupré, Edouard Hirchaud, Lydie Postic, Corinne Sailleau, Emmanuel Bréard, Stéphan Zientara, Damien Vitour, Grégory Caignard

O50. Dissecting differences and similarities in the host response to Rabies virus and other Lyssaviruses in the Syrian hamster model
Martina Castellan, Stefania Leopardi, Maira Zorzan, Petra Drzewniokova, Giampiero Zamperin, Isabella Monne, Ronald Mura, Lucas Brandao, Sergio Crovella, Paola De Benedictis

51. Molecular mapping of antigenic determinants of RHDV2
Vittoria Di Giovanni, Giulia Pezzoni, Roberto Benevenia, Antonio Lavazza, Lorenzo Capucci and Patrizia Cavadini

52. High-throughput mapping of virus-host interactions to identify new factors of virulence and pathogenicity for ASFV.
JulietteDupré, Aurore Fablet, Evelyne Hutet, Soumaya Messaoudi, Olivier Bourry, Yves Jacob, Stéphan Zientara, Damien Vitour, Marie-Frédérique Le Potier and Grégory Caignard

53. Study of the importance of the pORF63 tegument proteins in the biology of gammaherpesvirus infection.
Gillard L., Latif B., Rathakrishnan A., Myster F., Lemmens I., Tavernier J., Machiels B. and Gillet L

54. Replication characteristics of porcine reproductive and respiratory syndrome virus (PRRSV) in a new vein explant culture system
Shaojie Han, Dayoung Oh, Jiexiong Xie, Hans Nauwynck

55. Rotavirus Diagnostic - a View of the Mucosal and Systemic Immune Response.
Maxi Harzer, Antje Rückner, Martin Metzner, Thomas W. Vahlenkamp

O56. Inhibition of Arsenite-Induced Stress Granules by Cyprinid Herpesvirus 3
Yunlong Hu, Mamadou Diallo and Alain Vanderplasschen

57. Cross-Reactivity of Pre-and Post-Pandemic Human and Farm Animal Sera to SARS-CoV-2 and Other Coronaviruses.
Marcel Hulst, Arie Kant, Jean-Luc Murk and Wim H. M. van der Poel

58. Uncharacterised ASFV proteins expressed in mammalian cells serve as antigenic targets of the antibody-mediated immune response
Kirill Lotonin, Matthias Liniger, Nicolas Ruggli, Artur Summerfield, Charaf Benarafa

59. Influenza Pathogens: Improvement of Influenza A Detection Kit to Follow Virus Mutations.
S. Lesceu, K. Magré, C. Camus, H. Bourdeau, P. Pourquier

O60. Cytotoxic CD4+ and CD4/CD8 Double Negative T cells Correlate with Protection against PRRSV1 Transplacental Infection
Yanli Li, Gerard Martín-Valls, Ivan Díaz, Enric Mateu

61. Identification of MHC-I presented PRRSV peptides reveals epitopes within several non-structural proteins recognized by CD8+ T cells
Marlene Mötz, Melissa R. Stas, Sabine E. Hammer, Tereza Duckova, Frederic Fontaine, Alexandra Kiesler, Kerstin Seitz, Andrea Ladinig, André Müller, Christiane Riedel, Armin Saalmüller and Till Rümenapf

O62. Porcine nasal and lung macrophage subsets isolated by FACS and LCM show different transcriptomic profiles depending on tissue origin and location
Dayoung Oh, Nick Vereecke, Wim Trypsteen, Sieglinde Coppens, Ward De Spiegelaere, Heesoo Song, Bert Devriendt, Jo Vandesompele, Hans Nauwynck

O63. Frequent infection of cats with SARS-CoV-2 irrespective of pre-existing enzootic coronavirus immunity, Brazil 2020
Edmilson Oliveira-Filho, Otávio V. de Carvalho, Ianei O. Carneiro, Fagner D'ambroso Fernandes, Sara N. Vaz, Célia Pedroso, Lilian Gonzalez-Auza, Victor C. Urbieta, Arne Kühne, Rafaela Mayoral, Wendy K. Jo, Andrés Moreira-Soto, Chantal B. E. M. Reusken, Christian Drosten, Carlos Brites, Klaus Osterrieder, Eduardo M. Netto, Luiz E. Ristow, Rita C. Maia, Fernanda S.F. Vogel, Nadia R. Almeida, Carlos R. Franke and Jan Felix Drexler

64. Cross-protection among porcine enteric coronavirus
Hector Puente, Margarita Martín, Ana Carvajal, Ivan Díaz

O65. Milk lactose protects against group A rotavirus infection
Xiaolei Ren, Waqar Saleem, Haes Robin, Jiexiong Xie, Sebastiaan Theuns and Hans J. Nauwynck

66. Hemadsorption and type I IFN control are determinants for ASFV virulence
Daniel Pérez-Núñez, Raquel García-Belmonte, Elena Riera, Gonzalo Vigara-Astillero, Yolanda Revilla

67. A new avian in vitro model for the study of Flavivirus infection
José Rivas, Aude Blanquer, Axel Dubois, Ute Ziegler, Martin Groschup Luc Grobet, Mutien-Marie Garigliany

O68. Interplay between Foot-and-Mouth Disease Virus 3D polymerase and the type I interferon response: a contribution to viral persistence?
Morgan Sarry, Souheyla Benfrid, Cindy Bernelin-Cottet, Anthony Relmy, Aurore Romey, Anne-Laure Salomez, Gregory Caignard, Stephan Zientara, Damien Vitour, Labib Bakkali Kassimi et Sandra Blaise-Boisseau

O69. PRRSV-induced CD8 T Cell Responses at the Maternal-Fetal Interface During Late Gestation
Melissa R Stas, Heinrich Kreutzmann, Kerstin H Mair, Michaela Koch, Christian Knecht, Maria Stadler, Simona Winkler, Katinka van Dongen, Masha A Razavi, Spencer N Sawyer, Clara Pernold, Sonia V Hernandez, Armin Saalmüller, Till Rümenapf Wilhelm Gerner, and Andrea Ladinig

O70. Alphaherpesvirus-Induced Inhibition of the m6A Writer Complex and Degradation of m6A-Methylated Transcripts
Robert J. J. Jansens, Ruth Verhamme, Aashiq Mirza, Anthony Olarerin-George, Cliff Van Waesberghe, Samie R. Jaffrey, Herman W. Favoreel

O71. Fast Isolation of Non-adherent, Lymphocyte-like Haemocytes in Shrimp for Immunological Studies during a WSSV Infection
Liping Zheng, Omkar Byadgi, Mostafa Rakhshaninejad Nejad, Hans Nauwynck

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72. A longitudinal filed study of Swine Influenza A Virus in two Danish sow herds
Marianne Viuf Agerlin, Lars Erik Larsen Nicolai Rosager Weber, Pia Ryt-Hansen

73. Identification of IBV genotype I-23 in Brazilian chicken
Pedro Henrique Talassi, Ana Caroline Souza Barnabé, Ana Paula de Moraes, Michael Edward Miller, Soledad Palameta, Paulo Vitor Marques Simas, Clarice Weis Arns

74. Porcine paramyxovirus 1 (species Porcine Respirovirus 1) in Europe
Gyula Balka, Lilla Dénes, Aleksandra Woźniak, Tomasz Stadejek

O75. Analysis of canine parvovirus 2 isolates from Hungary reveals heterogenous phylogenetic origin
Dávid Géza Horváth, Ervin Albert, Gyula Balka, Ádám Dán

76. Preliminary data from a serological and molecular survey of Hepatitis E virus in stray cats and dogs in North-East Italy
Laura Bellinati, Letizia Ceglie, Elisa Mazzotta, Claudia Sandonà, Mery Campalto, Laura Lucchese, Alda Natale

77. Prevalence, Biosecurity and Risk Management of Bovine Coronavirus Infections on Dairy Farms in Europe
Anna Catharina Berge, Geert Vertenten

78. Risk Assessment of Low Pathogenic Avian Influenza Virus Dissemination in Duck Farms in France
Kateri Bertran, Luc Robertet, Alexandre Vove, Hugues Duret, Alize Mouchez, Guillaume Croville, Jean-Luc Guerin

79. SARS-CoV-2 Infection in Companion Animals: Serological Survey and Risk Factor Analysis in France
Pierre Bessière, Timothée Vergne, Matéo Battini, Jessie Brun, Julien Averso, Etienne Joly, Jean-Luc Guérin, and Marie-Christine Cadiergues

80. Preliminary Data on Potential Zoonotic Viruses in Cats in North East Italy: Mammalian Orthoreovirus and Rotavirus
Mery Campalto, Monica Mion , Elisa Mazzotta, Lara Cavicchio, Marilena Carrino, Letizia Ceglie, Maria Serena Beato, Alda Natale

O81. Occurrence and characterization of Rabbit Calicivirus (RCV) strains in Italy over 20 years
Patrizia Cavadini, Davide Mugetti, Alice Vismarra, Antonio Lavazza e Lorenzo Capucci

82. Sheep and goat breeders' opinions about testing for viral diseases at sale
Francois claine, Simon Boisdenghien, Jean-Yves Houtain

83. Limits to the participation of Small Ruminant Lentiviruses eradication program in Belgium
Francois claine, Mégane Létendart, Jean-Yves Houtain

O84. The Founder Variants Transmitted by Sows are the Main Source of PRRSV1 Genetic Diversity in an Unstable Infected Farm
Hepzibar Clilverd, Gerard E Martín-Valls, Yanli Li, Marga Martín, Martí Cortey, Enric Mateu

85. Time course analysis of the anti-ASFv effect of medium chain fatty acids
Maartje De Vos, Ha Thi Thanh Tran, Romain D'Inca, Kobe Lannoo, Hoang Vu Dang

86. A Serological Report on Bovine Herpes Virus Type 1 (BHV-1) in Mid Black Sea Region of Turkey: Traces from Cattle and Sheep local Herds
Ahmed Eisa Elhag Ibrahim, Zafer Yazici

87. Distribution of Rotavirus C Genotypes in German Pig Farms
Belinda Euring, Maxi Harzer, Antje Rückner, Thomas W. Vahlenkamp

88. Genetic investigation of the HPAI H5N1 viruses responsible of HPAI epidemic in Italy in 2021-2022
Bianca Zecchin, Alice Fusaro, Giacomo Barbierato, Edoardo Giussani, Diletta Fornasiero, Francesca Scolamacchia, Paolo Mulatti, Annalisa Salviato, Alessia Schivo, Elisa Palumbo, Maria Varotto, Federica Gobbo, Isabella Monne, Calogero Terregino

89. West Nile Fever antibody surveillance in horses in Finland
London L, Gadd T

90. Molecular characterization of emerging asfv genotype II strains in different Italian territories.
Monica Giammarioli, Alessandro Dondo, Cesare Cammà, Maria Teresa Scicluna, Loretta Masoero, Claudia Torresi, Maurilia Marcacci, Simona Zoppi, Valentina Curini, Marcello Giovanni Sala, Elisabetta Rossi, Cristina Casciari, Michela Pela, Claudia Pellegrini, Carmen Iscaro and Francesco Feliziani

O91. Genetic and antigenic diversity of Rotavirus A in Danish pigs
Nicole Goecke, Kasper Pedersen, Pia Ryt-Hansen, Nicolai Weber, Lars Erik Larsen

92. Detection and prevalence of novel porcine parvoviruses (PPV2-7) in Hungarian pig herd
Barbara Igriczi, Lilla Dénes, Gyula Balka

93. Swine influenza virus evolution and infection dynamics in intensive pig production systems
Paula Lagan, Anthony Hanrahan, Ken Lemon

94. Seroepidemiological Survey and Complete Genome Sequence of Equine Arteritis Virus in Horses in Serbia
Diana Lupulović, Tamaš Petrović, Gospava Lazić, Milena Samojlović, Vladimir Gajdov, Sava Lazić, Delphine Goudaire, Aymeric Hans

95. First Description of SARS-CoV-2 Infection in Two Feral American Mink (Neovison vison) Caught in the Wild.
Elisa Maiques, Miguel Padilla-Blanco, Consuelo Rubio-GuerriJordi Aguiló-Gisbert, Victor Lizana, Marta Muñoz-Baquero, Eva Chillida-Martínez, Jesús Cardells

O96. Vaccination Strategy Framework Against African Swine Fever in Wild Boar
Marta Martínez, Jaime Bosch, Satoshi Ito, Ed van Klink, Edvins Olsevskis, Kevin Morelle, Jose Manuel Sánchez-Vizcaíno

97. Pathotyping and Genotypic Characterization of Avian Orthoavulavirus 1 (Newcastle Disease Virus) Viruses from Wild Birds, Poultry and Captive Pigeons in Denmark
Karen Martiny, Lars E. Larsen, Charlotte K. Hjulsager

O98. Infection by a highly virulent PRRSV1 strain modifies the dynamics and shedding pattern of influenza A virus in an endemic pig farm.
Gerard Martín-Valls, Martí Cortey, Laia Aguirre, Enric Mateu

99. Interest of Non-lethal Sampling Methods in Detection and Genotyping of Carp Edema Virus in Koi Carp Trade
Laetitia Montacq, Guillaume Croville, Sokunthea Top, Stéphane Bertagnoli

O100. Genetic and Antigenic Characterization of Respiratory Coronaviruses of Swine
Ruth Mumo, Anna Parys, Nick Vereecke Bart Pardon, Sebastiaan Theuns and Kristien Van Reeth

101. Detection of SARS-CoV-2 in a Dog with Hemorrhagic Diarrhea
Miguel Padilla-Blanco, Consuelo Rubio-Guerri, Elisa Maiques, Santiago Vega, Luis Enjuanes, Alfonso Morey, Teresa Lorenzo, Clara Marin, Carmen Ivorra, Vicente Rubio

102. Genetic Diversity of PRRSV in Northern Italy from 2011 to 2021
Giovanni Parisio, Giovanni Alborali, Sonia Manenti, Debora Campagna, Maria Beatrice Boniotti, Ilaria Barbieri

103. Outbreak of Neuroinvasive Strain of Equid Alphaherpesvirus 1 in Serbia
Tamaš Petrović, Dejan Bugarski, Branislav Gagić, Zoran Petei, Biljana Đurđević, Gospava Lazić, Milena Samojlović, Diana Lupulović, Vladimir Gajdov, Vladimir Polaček, Aleksandra Nikolić, Sava Lazić

O104. Insights into the evolution and pathogenesis of A3B4 reassortants of infectious bursal disease virus (IBDV)
Anna Pikuła, Lester J. Perez

105. The Finding of SARS-CoV-2 in a Wild Eurasian River Otter (Lutra lutra) Highlights the Need for Viral Surveillance in Wild Mustelids
Consuelo Rubio-Guerri, Miguel Padilla-Blanco, Elisa Maiques Jordi Aguiló-Gisbert, Vicente Rubio, Víctor Lizana, Eva Chillida-Martínez, Jesús Cardells

106. Bovine Viral Diarrhea in dairy cows in Croatia – seroprevalence, virus detection and genotyping
Nevenka Rudan, Evica Marković, Zvonimir Hajek, Damir Rudan

107. Case of Bringing Aujeszky's Disease Virus via Feed to Pig Farms
Nataliia Rudova, Andrii Buzun, Oleksii Solodiankin, Boris Stegniy, Anton Gerilovych

O108. Molecular epidemiology of West Nile virus lineage 1 (WNV-L1) in Spain. An update of the last decade.
Pilar Aguilera-Sepúlveda, Cristina Cano-Gómez, Amalia Villalba, Rubén Villalba, Montserrat Agüero, Miguel Ángel Jiménez-Clavero, Jovita Fernández-Pinero.

O109. Genetically Identical Strains of Four Different Honeybee Viruses have been Determined in Bumblebee and Honeybee Positive Samples
Ivan Toplak, Laura Šimenc, Metka Pislak Ocepek, Danilo Bevk

110. Determination and Analysis of Twenty-two Complete Genome Sequences of Viruses Found in Diseased and Healthy Honeybee Colonies in Slovenia
Laura Šimenc, Ivan Toplak

O111. Circulation of Influenza A Virus in Wild Boars in the Emilia-Romagna Region (Northern Italy), between 2017-2022
Laura Soliani, Alice Prosperi, Elena Canelli, Laura Baioni, Valentina Gabbi, Camilla Torreggiani, Roberta Manfredi, Irene Calanchi, Giovanni Pupillo, Filippo Barsi, Patrizia Bassi, Laura Fiorentini, Matteo Frasnelli, Maria Cristina Fontana, Andrea Luppi, Chiara Chiapponi

112. First Isolation of the Influenza A Virus H16N3 in Ukraine
Larysa Usova, Borys Stegniy, Alexander Rula, Semen Tkachenko, Olena Kolesnyk, Anton Stegniy, Mary J. Pantin-Jackwood, Martin Beer, Sasan Fereidouni, Denys Muzyka

O113. Third generation nanopore sequencing of honeybee hemolymph as a new screening tool for honeybee viruses
Cato Van Herzele, Dirk de Graaf, Hans Nauwynck

O114. An Equine Coronavirus Associated Epidemic of Infectious Pyrexia in Iceland
Vilhjálmur Svansson, Sigríður Björnsdóttir, Eggert Gunnarsson, Constance Smits and Kees van Maanen

O115. Intra- and Inter-Cattery Epidemiology of Feline Coronavirus in Belgium between 2018 and 2021
Nick Vereecke, Veerle Stroobants, Marthe Pauwels, Aisha Van den Kerkhof, Sebastiaan Theuns, and Hans Nauwynck

O116. Molecular Epidemiology of Porcine Parvovirus Type 1 (PPV1) and the Reactivity of Vaccine-induced Antisera against Historical and current PPV1 strains
Nick Vereecke, Elise Vandekerckhove, Lise Kirstine Kvisgaard, Guy Baele, Carine Boone, Marius Kunze, Lars Erik Larsen, Sebastiaan Theuns, and Hans Nauwynck

117. Success of Influenza A Virus Whole Genome Sequencing from Oral Fluids Depends on Sample Storage and Sequencing Protocol
Nick Vereecke, Aleksandra Woźniak, Marthe Pauwels, Sieglinde Coppens, Hans Nauwynck, Sebastiaan Theuns, and Tomasz Stadejek

O118. An Epidemiology Model of Influenza A Virus in Wild Birds Based on Surveillance of Black-headed Gulls (Chroicocephalus ridibundus)
Josanne H. Verhagen, Eline A.M. Vink, Marjolein J. Poen, Frank A. Majoor, Divya Kriti, Jayeeta Dutta, Harm van Bakel, Oanh Vuong, Rachel D. Scheuer, Mariëlle van Toor, Nicola S Lewis, Nichola N Hill, Justin Bahl, Lambodhar Damodaran; Jiani Chen; Thijs Kuiken, Ron A.M. Fouchier, Mart C.M. de Jong

O119. Large Scale Cross-Sectional Serosurvey of Hepatitis E Virus Infection in Belgian Pig Farms and Identification of Risk Factors for Herd Infection
Constance Wielick, Ravo Michèle Razafimahefa, Louisa Ludwig-Begall, Stefaan Ribbens, Claude Saegerman, Etienne Thiry

O120. Detection of porcine parainfluenza virus 1 (PPIV1): genetic diversity and co-infections with influenza A virus (IAV) and porcine reproductive and respiratory syndrome virus (PRRSV)
Aleksandra Woźniak, Piotr Cybulski, Tomasz Stadejek

121. Detection of influenza A virus (IAV) in nasal swabs and oral fluids cross-sectionally collected in 35 Polish swine herds
Aleksandra Woźniak, Piotr Cybulski, Tomasz Stadejek

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O122. Equine Coronaviruses – a common reason for equine colic?
Moritz Stummer, Vicky Frisch, Frauke Glitz, Jessika Cavalleri, Till Rümenapf, Angelika Auer

O123. Outbreak of Elephant endotheliotropic herpesvirus 6 (EEHV-6) associated disease in African elephants in a European zoo.
Angelika Auer, Christiane Riedel, Folko Balfanz, Annika Posautz, Azza, Abdelgawad, Jakob Trimpert, Tabitha Hoornweg, Thomas Voracek Till Rümenapf

124. Fast Identification of Viruses Using Nanopore Sequencing: Application to Avian Diseases
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ABSTRACTS

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O1.

Comparative pathogenesis of tick-borne encephalitis virus and louping ill virus in experimentally infected sheep

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Tick-Borne encephalitis virus (TBEV) and louping ill virus (LIV) are genetically and antigenically closely-related flaviviruses. Sheep play an important role in the epidemiology of both diseases but their susceptibility to both viruses is different. LIV causes a febrile illness in sheep that can progress to fatal encephalitis whereas TBEV infection is usually asymptomatic.
In this study, 8 month-old sheep were inoculated with a dose of $10^5 TCID_{50}$ of TBEV Neudoerfl strain or LIV LI/31 strain. Clinical signs were monitored and viral spread was followed over time in the Blood, visceral and lymphoid organs, brain tissues and skin biopsies collected at the site of inoculation. Although sheep seroconverted at 7 dpi with TBEV, no TBEV RNA was detected in serum and in the examined tissues except for the skin, the lymph nodes and the spleen. In contrast, LIV RNA was detected in serum from 2 dpi and peak viremia was reached at 5 dpi but dropped by 7 dpi, correlating with the first detection of neutralizing antibodies. The skin, lymphoid and visceral organs were LIV RNA-positive and LIV RNA was detected in all CNS regions starting from 5 dpi. The medulla oblongata and the pons harboured the highest viral loads of LIV RNA at 10 dpi compared to the others CNS regions.
These results confirm the difference in disease outcome after TBEV and LIV infection in sheep and suggest that TBEV replication and spread is counteracted early upon infection whereas LIV tends to replicate efficiently in sheep and disseminates to the brain.

72.

A longitudinal filed study of Swine Influenza A Virus in two Danish sow herds

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Swine Influenza A virus (swIAV) impacts the health and economy of the global swine industry. swIAV causes respiratory disease with symptoms such as lethargy, coughing, sneezing, nasal discharge and weight loss. SwIAV is one of several pathogens of the porcine respiratory disease complex. Studies have revealed that swIAV is endemic in many herds, due to the consistent supply of naïve piglets, which makes swIAV difficult to control.
The objectives of this study were to investigate the swIAV transmission dynamics and the impact on health and productivity in two Danish sow herds.
Two longitudinal field studies were carried out in commercial sow herds by following 228 pigs from birth until the end of the nursery. In each herd, two batches of ten sows were selected before farrowing. From each sow, three cross-fostered and three of the sows´ own piglets were included. Nasal swabs, blood samples, weight and clinical registration were collected from all piglets at seven different time point between 1 and 10 weeks of age. In addition, udder swabs were obtained from the sows during all samplings in the farrowing unit. Nasal swabs were tested by real time rtPCR using nanoscale PCR, targeting 13 different respiratory pathogens including swIAV. The udder swabs were tested for IAV by RT-rtPCR. Blood samples were analyzed for IAV antibodies using a commercial ELISA, targeting the NP gene.
In both herds, nasal swabs and udder swabs were positive for swIAV just before and/or just after weaning. Moreover, a significant decay of maternally derived antibodies was observed already at weaning. There was a reasonable correlation between the results of the udder swabs and nasal swabs indicating that udder swabs is a reliable alternative to nasal swabs, when testing piglets for swIAV in the farrowing unit. Viral shedding coincided with an increase in clinical signs of respiratory disease confirming the clinical importance of swIAV. At weaning, piglets are very susceptible for infection, so to mitigate the clinical impact of swIAV in piglets, efforts should be implemented to ensure an effective delivery of maternally derived antibodies or by applying active immunization of piglets.

O108.

Molecular epidemiology of West Nile virus lineage 1 (WNV-L1) in Spain. An update of the last decade.

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West Nile virus (WNV) is a zoonotic arbovirus worldwide spread. Since late 1990's its growing presence in the Mediterranean Basin is provoking significant outbreaks in birds, horses, and humans more and more frequently. In Spain, WNV lineage 1 (WNV-L1) circulates continuously in the South-East since at least 20 years. This study reports a complete molecular epidemiology analysis of the Spanish WNV-L1 circulating strains collected from animal samples during these years.

Organs, feathers and/or CSF were collected from wild birds and horses with RT-PCR confirming WNV infections, in different Spanish provinces, and subjected to virus isolation. Full genomes of thirteen WNV-L1 isolates (from 2012 to 2018) were sequenced (Sanger) and phylogenetic analyses were performed including a wide range of WNV sequences from Mediterranean representatives.

The analyses demonstrated that at least three introductions of WNV-L1 have occurred in Spain. More in detail, the results confirmed that Western Mediterranean clade (WMed) has been able to spread and evolve, giving rise to new variants that caused outbreaks in numerous species from diverse countries. Moreover, WMed clade can be divided in different clusters. In Spain, there have been two introductions of WNV related to WMed. The first one corresponds to the WMed-2 cluster, which comprises former Spanish isolates (2007-2008), and seems to have become extinct, as related isolates have not been found anymore. On the other hand, the second introduction corresponds to the WMed-1 cluster, which comprises the most recent animal isolates (2010-2018). Finally, the divergence represented by an isolate obtained from a goshawk in Málaga province indicates a third introduction of WNV-L1 from an unknown origin, which circulated at least in the 2017 season and is not related with any WMed isolates or from other geographical regions.

To conclude, the obtained results confirmed that WNV is able to reach Spain from other regions from time to time. There has been and still there is an active circulation of diverse variants of WNV-L1 in the south, central and western regions of the country, confirming them as hotspots for WNV circulation.

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O46.

In Vitro Recall Response to African Swine Fever Virus Reveals Immune Components Underlying Cross-protection

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African swine fever (ASF) currently represents the number one threat for the swine industry. No commercial vaccine is available against the ASF virus (ASFV), and their rational development is hampered by the poor knowledge of ASFV immunity. The experimental use of live attenuated viruses is a valuable tool to analyze ASFV-specific immune responses. We previously generated an ASFV deletion mutant lacking the CD2v gene, namely BA71ΔCD2 (genotype I). Notably, BA71ΔCD2 confers heterologous protection against the genotype II ASFV currently circulating worldwide. Here we used this vaccine prototype to investigate the cross-protective *in vitro* cellular recall response against ASFV. Pigs were intranasally vaccinated with BA71ΔCD2, and blood and submandibular lymph node (LN) samples were harvested three weeks later. PBMC and submandibular LN cells were stimulated with ASFV and their transcriptomic signatures were analyzed by RNA-sequencing and single-cell RNA-sequencing, respectively. Bulk RNA-seq analysis of PBMC revealed a robust Th1 response in cells from BA71ΔCD2-vaccinated pigs. Concomitant with this adaptive immune response, we also distinguished an innate immune response marked by the expression of macrophage-related inflammatory genes. Immunophenotyping by flow cytometry demonstrated the presence of polyfunctional CD4+CD8+ memory T cells, and an IFNG-dependent inflammatory response mediated by TNF-producing macrophages. scRNA-seq analysis of submandibular LN cells further validated and extended these results: (i) we observed a rapid inflammatory response characterized by the upregulation of interferon-stimulated genes and the Th1 chemokine CXCL10 in several cell subsets; and (ii) we found cytotoxic CD8+ T cells within the cell clusters that were only present or overrepresented in the vaccinated animal. Indeed, flow cytometry analysis of perforin-expressing PBMC revealed the presence of cytotoxic CD4+CD8+ and gamma-delta memory T cells as well as increased levels of nonspecific cytotoxic CD8+ T and NK cells in vaccinated animals after in vitro stimulation with ASFV. Altogether, this study allowed elucidating the complex cellular response associated with cross-protection against ASFV. Our findings represent a step forward in the understanding of ASF immunology and provide important clues on the functional immune mechanisms that should be considered to more rationally design future ASF vaccines.

O160.

The intradermal route induces full protection in pigs immunized with the attenuated African swine fever virus (ASFV) Lv17/WB/Rie1.

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African swine fever (ASF) is currently the major concern of the global swine industry, as a consequence of which a reconsideration of the containment and prevention measures taken to date is urgently required. A great interest in developing an effective and safe vaccine against ASF virus (ASFV) infection has, therefore, recently appeared. The ASFV Lv17/WB/Rie1 strain is a non-HAD attenuated genotype II ASFV isolated in Latvia that is able to induced protection to challenge with a virulent HAD in pigs immunized intramuscularly. Further experimental studies with this non-HAD virus may provide new insights on mechanisms of protective immunity to ASFV. Therefore, within this study we compare different combinations of doses and routes of immunization of pigs with the ASFV non HAD-Lv17/WB/Rie1, including the intranasal and intradermal route, the latter not previously tested. The intranasal route did not efficiently immunize the pigs regardless of dose. In contrast, the intradermal immunization with moderate dose (10^2 $TCID_{50}$) efficiently immunized. All pigs seroconverted from day 10 reaching similar antibody titers of that previously reported using the intramuscular route. Viremia was sporadically detected in blood, oral, or nasal excretions but infectious virus was not isolated. Immunized pigs were fully protected against challenge with virulent genotype II Arm07 isolate without clinical signs. These results showed that factors including delivery route and dose determine the outcome of immunization with the naturally attenuated isolate Lv/17/WB/Rie1.

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73.

Identification of IBV genotype I-23 in Brazilian chicken

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ABSTRACT

Infectious Bronchitis Virus (IBV), now Avian Coronavirus, is a species of virus of the genus Gammacoronavirus that infects several avian species, mainly broiler chickens. A pathogenic strain of IBV identified in an outbreak in Sao Paulo state from Brazil was sequenced and the phylogenetic tree show that this Brazilian sample was grouped with other samples from genotype I-23 (GI-23). This genotype emerged and widespread in the Middle East and is currently enzootic throughout Europe and Asia (Ukraine, Lithuania, Poland, Armenia, Russia, Belarus, Tajikistan, Kazakhstan, Germany, Afghanistan) and Africa (Nigeria and Egypt). The widespread and prevalence of GI-23 (EGY VAR II type strains) in the MENA region (Middle East and North Africa) and recent detections in Asia, Europe and Africa illustrate the ability of the GI-23 strain. Although many protective actions against the circulation of certain IBV variants have been implemented, the high prevalence of GI-23 IBV strains has suggested the adoption of vaccination schedules using GI-23-based vaccines. In this context, it is very important to carry out epidemiological surveillance and identification of genotypes to implement effective vaccine strategies to control outbreaks of infectious bronchitis in commercial chicken.

Keywords: IBV; avian coronavirus; genotype GI-23; Brazil

O122.

Equine Coronaviruses – a common reason for equine colic?

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Background
Equine coronavirus (ECoV) is related to bovine coronavirus and belongs to the genus Betacoronavirus within the family Coronaviridae. It has been reported to cause lethargy, anorexia, fever, changes in fecal consistency and colic in adult horses. Colic is one of the major equine diseases worldwide and an important reason for intensive veterinary medical care and surgery. The prevalence of colic signs in ECoV infected horses is around 20 %. Yet, it was unclear, whether ECoV is causing colic in horses showing no specific signs of infectious disease such as high fever. The aim of our study was to determine whether undetected ECoV infections are a relevant cause of colic in horses.

Methods
In our study we included 108 horses with signs of acute colic and 33 control horses without current signs of internal medical abnormalities. Fecal samples of all horses were screened for Betacoronaviruses by RT-qPCR. Positive results were confirmed by an ECoV-specific RT-qPCR and sequence analyses of N gene.

Results
Betacoronavirus-specific nucleic acids were detectable in six out of 108 colic horses (5.6 %) and in one horses from control group. Viral loads ranged between 1 x 10^6 and 4 x 10^8 genome copies per g feces. ECoV could be confirmed in three samples from the colic group (2.8 %). N gene sequences of ECoVs show higher identities to Austrian and Irish sequences than to Asian strains and NC99. Interestingly, from one patient Bovine Coronavirus (BCoV) could be identified.

Conclusion
We detected ECoV specific nucleic acids in fecal samples of 2.8% of horses with acute colic signs. Hence, ECoV is not a major player in equine colic disease but nevertheless should be regarded as infectious cause. The role of other Betacoronavirus species in horses should be investigated in further studies. Betacoronaviruses are capable of crossing the species barriers so we should closely monitor them in our companion animals. None of those horses was suspicious to suffer from an infectious disease and isolated to prevent transmission to other horses.

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O123.

Outbreak of Elephant endotheliotropic herpesvirus 6 (EEHV-6) associated disease in African elephants in a European zoo.

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Background
Elephant endotheliotropic herpesviruses (EEHVs) are known to cause acute hemorrhagic and often fatal diseases in young Asian and - sporadically -African elephants. Since their discovery in the 1990s, hundreds of cases have been reported in Asian elephants (Elephas maximus), whereas information about EEHVs and associated diseases in African elephants (Loxodonta africana) are limited. Only four clinical cases in African Elephants are known to date, with a case fatality rate of 50%.
In summer 2021, a two-years-old, captive African elephant calf died unexpectedly at the Tiergarten Schönbrunn, Zoo, Vienna, Austria. Hemorrhagic lesions typical for EEHV haemorraghic disease were observed during necropsy. Organ samples were sent to the Institute of Virology, Vetmeduni Vienna, Austria, todiagnose the possible causative agent(s). A Pan-Herpesvirus-nested PCR known to detect a broad panel of alpha-, beta- and gammaherpesviruses did not generate a specific band but a pan-EEHV semi-nested PCR gave positive results. Sequence analysis showed 99% identities to EEHV-6 sequences from NCBI GenBank.
A qPCR was implemented for absolute quantification and further weekly monitoring of the elephant herd. In addition, a highly sensitive EEHV-6-specific nested PCR was designed to confirm positive and questionable qPCR results.
A few weeks later blood samples of another elephant were EEHV-6 positive and the animal instantly developed clinical signs. Quick and intensive medical treatment - coupled with intense surveillance of viral loads - led to complete recovery.
Diagnostic tools such as determination of the immune status, frequent monitoring of blood and trunk wash samples as well as preparedness of the zoo veterinarians for immediate, intensive treatment can therefore be considered essential to successfully manage and control clinical EEHV infections in captive African elephants.

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47.

Influence of gammaherpesvirus infections on the antibody repertoire of their host.

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Gammaherpesviruses (GHVs) are ubiquitous viruses that have co-evolved with their hosts. Although these infections remain asymptomatic in most of the individuals, they can cause major lymphoproliferative disorders in both humans and animals. After primary infection, most of GHVs undergo latent expansion in germinal center (GC) B cells and persists in memory cells. In this project, using next generation sequencing and Murid Herpesvirus 4 (MuHV-4), a mouse GHV, we characterized the effect of a GHV infection on the B cell compartment of its host. Firstly, we showed that B cells of MuHV-4 infected mice display distinct VDJ recombination frequencies and isotypes switching routes compared to the ones of non-infected mice. Secondly, using a YFP expressing MuHV-4 strain, we compared the repertoire of MuHV-4 infected and non-infected B cells. Surprisingly, we observed that infected cells displayed a distinct repertoire than the one of their non-infected counterparts with a different speed of clonal expansion. These results suggest therefore that MuHV-4 infection is not random and establishes preferentially in some B cells. In the future, identifying the common determinants of these infectable B cell subsets could help us to better understand GHVs lifecycle and the lymphoproliferative disorders that they induce. More generally, it could help us to better understand how our environment and especially some infections agents shape our immune responses.

74.

Porcine paramyxovirus 1 (species *Porcine Respirovirus 1*) in Europe

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The family *Paramyxoviridae* consists of viruses that are known to affect a wide range of species including humans, pigs, cattle, poultry and companion animals. Porcine parainfluenza virus type 1 (PPIV-1, species *Porcine respirovirus 1*) was first detected in rectal and nasopharyngeal swabs obtained from pigs in a slaughterhouse in Hong Kong. PIV-1 has a negative sense, single-stranded RNA genome approximately 15 kilobases in length, consisting of six genes that encode for major proteins: nucleocapsid, phosphoprotein, matrix, fusion, haemagglutinin–neuraminidase and large proteins. The virus has been reported from the USA and Chile as well. Recent phylogenetic studies revealed that two distinct PPIV-1 lineages may have evolved independently in Europe and North America, and they might have spread to Asia, probably with infected animals
PPIV-1 in Europe was first described by our research group in nasal swab samples that were collected during a neuroinvasive astrovirus screening campaign. Real time PCR analysis of 15 nasal swab samples collected from suckling piglets of 22 herds revealed a single positive farm. A second round of sampling in the positive farm revealed a relative high positivity rates in the 4–6–8 week-old age groups: 65, 40, and 20%, respectively. We have conducted a second screening in Hungary based on oral fluid collection from 10 and 20 week-old animals and we found the virus in 50% of the 20 herds sampled. Additional nasal swab sampling of two of these farms revealed different patterns of circulation among herds.

On the other hand, a study conducted in Poland revealed that the virus is highly prevalent, as 76.7% of the farms tested were positive. These prevalence data are more similar to the ones observed in Germany and the Netherlands (42.3%) and in Chile (100%) and also in the USA where 43.3% of the samples submitted to ISU VDL during 2016–2017 were positive for PPIV-1.
Based on the results published so far, the virus seems to be highly prevalent in some European countries, infecting animals in the nurseries and fattening units mostly. Further research is needed for its role in respiratory diseases.

O75.

Analysis of canine parvovirus 2 isolates from Hungary reveals heterogenous phylogenetic origin

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Canine parvovirus 2 (CPV-2) is a highly important pathogen of carnivores worldwide. It is a ssDNA virus with genetic variability with a mutation rate similar to RNA viruses. It seems that the identification of intraspecies subtypes according to amino acid substitutions no longer serves the understanding of the evolutionary changes. According to wide-scale analyses, phylogeny is superior to phenotype and resolves clustering ambiguities. Also, strong correlation was found between phylogeny and virulence. Since no recent data on Hungarian isolates is available we aimed to re-evaluate the current molecular epidemiology by investigating the latest strains.

Ten CPV2 VP2 sequences were obtained from faecal swabs (n = 3) and necropsy samples (n = 7) submitted to the Department of Pathology, UVMB, Hungary for routine diagnostic investigations between 2019 and 2022. To assess genetic relatedness of the recent isolates, a phylogenetic tree was built based on the full sequence of the VP2 gene. Ten former Hungarian strains and representative sequences of a worldwide collection of VP2 genes were also involved as described by Cságola et al. (2012) and de Oliveira Santana et al. (2022). Isolates were assigned to the formerly described phenotypic groups 2a, 2b, and 2c according to certain amino acid residues.

The half of our isolates (n = 5) clustered into W1 along with other former Hungarian strains from 2012 and were identified uniformly as 2a subtype. Four further isolates clustered into the W2 group and typed 2c (n = 3) or type 2b (n = 1). Interestingly, in this cluster no former Hungarian isolates could be found but two 2c isolates were closely related to strains isolated in Africa and China. One strain was classified into the W group. This ancestral group comprised mainly 2a and 2b isolates from all parts of the world including two former Hungarian isolates. In contrast to previous findings, no recent Hungarian isolates clustered within W4, possibly indicating that other strains became more dominant recently.

Based on our results, the phylogenetic classification is indeed superior to the amino acid-based approach when investigating the population dynamics of Hungarian CPV-2 strains.

184.

What is the role of companion animals in the COVID-19 pandemic?

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The disease produced by the severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) is currently one of the primary concerns worldwide. Taking into account the potential zoonotic origin of the disease and that several animal species, including dogs and cats, are susceptible to viral infection, it is critical to assess the relevance of pets in this pandemic. In this work, we perform a large-scale study on SARS-CoV-2 serological and viral prevalence in cats and dogs in Spain, a country that has been seriously affected by the virus. In addition, we performed genomic surveillance in the positive specimens in order to identify the virus variant involved in the infection. Among the more than 2000 animals tested, we only detected 15 dogs, 16 cats, and 6 ferrets positive for RT-qPCR, which represents 1.3% of dogs, 1.1% of cats, and 6.66% of ferrets. In addition, 66 animals (28 cats and 38 dogs) showed neutralizing antibodies. Using whole-genome sequencing, we identified a dog infected by the Alpha variant, one cat infected by the Delta variant, and seven cats and one dog infected by the Omicron variant. While animals infected by the Alpha and Delta variant showed clinical signs and/or high viral loads, animals infected with Omicron were asymptomatic, and low viral loads were detected. Our results evidenced a low prevalence of SARS-CoV-2 infection in animals, suggesting that their epidemiological role in the pandemic is not relevant. However, genomic surveillance is still necessary in order to identify possible recombination events of the virus in these hosts that potentially can affect virus virulence and transmission.

76.

Preliminary data from a serological and molecular survey of Hepatitis E virus in stray cats and dogs in North-East Italy

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Hepatitis E virus (HEV) infection is the most common cause of acute hepatitis worldwide. Transmission of HEV in the Western world is mostly foodborne and waterborne, with pigs and wild boar as the most well-known reservoirs. However, the host range of zoonotic HEV has been expanded to involve other species, including companion animals. Several studies have identified contact with dogs or cats as a risk factor for HEV infection in humans, but there is scarce information on HEV in pets in several countries, including Italy. To evaluate the prevalence of HEV and to assess a possible zoonotic risk for those caring for stray animals, this study was designed to investigate the serological and molecular prevalence of HEV in stray dogs and cats in northeastern Italy.
From May 2021 to May 2022, public veterinary services collected serum samples and, when possible, fecal samples in seven different districts in the Veneto and Trentino-Alto Adige regions. Sampling was performed on stray dogs newly introduced to shelters, and on feral cats captured for the catch-neuter-release program. Sera were screened for antibodies with a commercial indirect multi-species ELISA test kit (IDvet), while fecal samples were tested for the presence of HEV RNA with two real-time RT-PCR protocols specific for HEV strains, targeting HEV-A and HEV-C.
A total of 153 and 118 fecal samples were collected from dogs and cats, respectively, in which HEV RNA was never detected. From dogs, 171 sera were collected, and no seropositivity was observed. Meanwhile, 195 sera were collected from cats and 2 were positive for anti-HEV antibody (1%; 95%CI: 0.1-3.7). For one cat sample, feces were available for molecular investigation, but HEV RNA was not detected; thus, the genotype remained undetermined.
The low seroprevalence in cats and the negativity reported in dogs are in contrast with higher seroprevalence values found in other European countries, as well as in Italy, even when stray animals were investigated. Further analysis will be needed to understand the reasons of the discrepancy observed. Whereas, the data obtained in real-time RT-PCR for HEV are in agreement with the available bibliography.

77.

Prevalence, Biosecurity and Risk Management of Bovine Coronavirus Infections on Dairy Farms in Europe

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This cross-sectional field study of over 100 European dairy farms aim to estimate the prevalence of Bovine Coronavirus (BCoV) and to characterize farm-level risk factors in management and biosecurity that are linked to BCoV infection in neonatal and weaned dairy calves. Nasal and faecal swabs were collected for BCoV detection using semi-quantitative Real Time PCR (RT-PCR), blood and bulk tank milk (BTM) were collected for BCoV antibody detection using ELISA (expressed in % inhibition). One BTM sample, 10-20 samples from neonatal calves, 10-20 samples recently weaned calves and 5-10 samples from fresh cows were collected. An extensive questionnaire was performed to determine husbandry and management factors. Biosecurity was scored using the Biocheck (https://biocheckgent.com). Multiple logistic regression models (binary and ordinal) with random effects of herds were used to evaluate relationship between husbandry, management, and biosecurity on dairies. The study is ongoing. Interim results from 45 dairy farms in Belgium, Czech Republic, Denmark, France, Italy, Netherlands, Portugal, and Sweden have been analysed. Antibody levels in BTM were on average 86 (expressed in % inhibition). Mean antibody levels were 56 in preweaned calves, 39 in weaned calves and 62 in fresh cows. There was poor correlation (r=0,41) between BTM and mean serum antibody levels. Presence of BCoV was confirmed in 17% of nasal and faecal swabs with no significant difference between nasal and faecal swabs. No virus was present in 25% of herds. Coronavirus was present in 23% of samples from neonatal calves, 20% of samples from weaned calves, and 8% of samples from fresh cows. There was poor correlation between serum antibody levels and virus shedding (r=0,48). The biosecurity on 41 dairies had an overall Biocheck score of 58% (external score 69%, internal score 41%). The virus shedding tended to be higher in herds with overall higher biosecurity scores, whereas antibody levels in cattle were non-significantly lower in herds with higher biosecurity score. The preliminary results indicate that BCoV is commonly present in both the respiratory and enteric pathway of European dairy cattle, with all herds being seropositive to the virus, and the virus present in 75% of herds.

O2.

Virus-Induced Inhibition Of Superinfection As A Means For Accelerating Fitness-Based Selection Of Cyprinid Herpesvirus 3 Single Nucleotide Variants *In Vitro* And *In Vivo*

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Cyprinid herpesvirus 3 (CyHV-3) is the archetype of alloherpesviruses and is advantageous to research because, unlike many herpesviruses, it can be studied in the laboratory by infection of the natural host (common and koi carp). Previous studies have reported a negative correlation among CyHV-3 strains between viral growth *in vitro* (in cell culture) and virulence *in vivo* (in fish). This suggests the existence of genovariants conferring enhanced fitness *in vitro* but reduced fitness *in vivo*, and *vice versa*. Here, we identified syncytial plaque formation *in vitro* as a common trait of CyHV-3 strains adapted to cell culture. Comparison of the sequences of virion transmembrane protein genes in CyHV-3 strains, and the use of various recombinant viruses, demonstrated that this trait is linked to a single nucleotide polymorphism (SNP) in the antisense ORF131 (C225791T mutation) that results in codon 183 encoding either an alanine (183A) or a threonine (183T) residue. In experiments involving infections with recombinant viruses differing only by this SNP, the 183A genovariant associated with syncytial plaque formation was the more fit *in vitro* but the less fit *in vivo*. In experiments involving co-infection with both viruses, in addition to the more fit genovariant contributing to the purifying selection of the less fit genovariant by outcompeting the latter, we observed that this process may be accelerated by strong viral stimulation of superinfection inhibition at a cellular level, and stimulation of resistance to superinfection at a host level. Collectively, this study illustrates how the fundamental biological properties of some viruses and their hosts may have a profound impact on the degree of diversity that arises within viral populations.

78.

Risk Assessment of Low Pathogenic Avian Influenza Virus Dissemination in Duck Farms in France

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Since 2015, highly pathogenic avian influenza (HPAI) outbreaks have been reported in wild and domestic birds in Europe, France being one of the most affected countries. Duck farms have been particularly susceptible to HPAI virus, with dramatic economic consequences. Surveillance and control rely on termination of HPAI virus-infected flocks and intensive screening of low pathogenic AI (LPAI) viruses before transportation to the slaughterhouse, with stricter biosecurity measures to reach slaughter. Besides, previous infection with LPAI viruses in ducks can influence susceptibility to HPAI viruses. Thus, understanding LPAI virus infection dynamics in ducks is key for AI control. Here we evaluated the infection dynamics of LPAI viruses in ducks at the individual and flock levels to estimate the risk of virus spread. Ten duck flocks ready to enter the force-feeding stage and positive for matrix (M) gene PCR were selected (N=400 ducks) and sampled at different times of their force feeding. High seroconversion rates at day 0 suggest that virtually all ducks from M gene PCR positive flocks had been exposed to AI viruses before entering the force-feeding stage. Most flocks had low virus shedding prevalence for a duration shorter than the force-feeding period (<11 days), while two out of 10 flocks and one feeding unit had higher virus shedding prevalence and titres, often during the whole force-feeding period (≥11 days). Viral RNA was detected in manure samples of almost all flocks regardless of virus shedding dynamics, but no viable virus was recovered. These results indicate that the risk of viable AI virus dissemination by manure may be low at the sample level, but the virus in manure can accumulate to high titres at the flock level, with a realistic potential for dissemination yet to be determined. These results indicate that LPAI virus infection dynamics in ducks are heterogeneous and complex, and that manure from M gene positive PCR flocks may represent a higher risk of virus dissemination than ducks themselves. Our findings may provide the necessary data to improve AI surveillance and control.

79.

SARS-CoV-2 Infection in Companion Animals: Serological Survey and Risk Factor Analysis in France

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), responsible for COVID-19 in people, has been detected in companion animals on rare occasions. A limited number of large-scale studies have investigated the exposure of companion animals to SARS-CoV-2. The objective of this study was to estimate seroprevalence in privately owned dogs and cats presented in veterinary clinics in different French regions and to test the hypothesis that the occurrence of an episode of COVID-19 in the household and close contact with the owner would increase the chances of the animals being seropositive. A secondary objective was to compare two serological diagnostic methods: an enzyme-linked immunosorbent assay (ELISA) and a hemagglutination-based assay. One hundred and sixty-five dogs and 143 cats were blood-sampled between March 2020 and December 2021. Neutralizing SARS-CoV-2 antibodies were detected in 8.4% of cats (12/143) and 5.4% of dogs (9/165). Seven animals (three dogs and four cats) were seropositive in the absence of an episode of COVID-19 in the house-hold. Despite not being statistically significant (chi-square test, p-value = 0.55), our data may suggest that the occurrence of an episode of COVID-19 in the household could increase the risk of animal seropositivity (odds ratio = 1.48; 95% confidence interval = 0.54–4.46). We found that ELISA was more suitable for anti-SARS-CoV-2 antibodies detection in pets: the hemagglutination-based assay was more sensitive, but a high proportion of sera (6.8%) contained xenoreactive antibodies, making the test inconclusive. This survey in-directly shows that SARS-CoV-2 circulates in canine and feline populations, but its circulation appears to be too low for pets to act as a significant viral reservoir.

3.

Structural and Functional Analysis of the Programmed -1 Frameshift Signal of Porcine Respiratory Coronavirus (PRCV)

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A wide range of RNA viruses, including coronaviruses, use programmed -1 ribosomal frameshifting (-1 PRF) to achieve the expression of some of their non-structural proteins. Two elements, the "slippery sequence" and a stimulatory RNA pseudoknot play a vital role in the process of frameshifting in coronaviruses. Mutations that modify this process, and small molecules that inhibit -1 PRF activity have deleterious effects on virus replication. Hence, this activity may be therapeutically targeted.
Various studies have been conducted previously to study the structural and functional analysis of -1 PRF activity in betacoronaviruses, such as, severe acute respiratory syndrome coronavirus (SARS-CoV) Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2. However, no studies have been reported for porcine respiratory coronavirus (PRCV), an alphacoronavirus. In this study, we have initiated the structural and functional analyses of -1 PRF activity in alphacoronavirus using PRCV as a model.
We compared the "slippery" and RNA pseudoknot sequences of different coronaviruses and identified the slippery sequence of PRCV (UUUAAAC). Furthermore, the PRCV RNA pseudoknot sequence and structure was predicted based on the models for other coronaviruses. We prepared DNA-launched RNA replicons including different lengths of sequence (100 – 314 nucleotides) including these PRCV sequences linked to Renila luciferase (RLuc) and chloramphenicol acetyltransferase (CAT) reporter proteins. The constructs were analysed by transfection into BHK21 cells. Reporter protein expression was analysed using a CAT ELISA and western blot assays in parallel with similar constructs containing the SARS-CoV-2 frameshift element. We will conduct mutational analysis of the slippery and RNA pseudoknot sequences of PRCV to understand the role of these elements for the efficiency of -1 PRF activity in PRCV.

4.

Evaluation of New Routes of Usutu Virus Infection in Birds

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Usutu virus (USUV) is a Flavivirus known to be transmitted to avian species through mosquito bites, and responsible for massive die-offs in wild and captive bird populations. Many cases of positive dead birds are however recorded during the winter, a vector-free period, suggesting that other routes of transmission are involved. To explain how USUV "overwinters", the main hypothesis is a bird-to-bird transmission, through direct or indirect contacts, as shown for the closely-related West Nile virus in wildlife but also under experimental conditions. To assess this question, we first experimentally challenged adult canaries with an intranasal inoculation of USUV at different doses. We observed that this route of inoculation led to a systemic dissemination of the virus comparable to that observed after parenteral inoculation, provided the inoculated dose was sufficient ($> 10^2$ $TCID_{50}$). Moreover, just as we had observed after intradermal inoculation, we highlighted the oronasal excretion of infectious viral particles in infected birds after intranasal inoculation. Based on these data, we co-housed infected birds (after intranasal or intradermal inoculation) with naive sentinels, to determine if a horizontal transmission could be reproduced experimentally. However, neither seroconversion nor viral RNA were noticed in the sentinel group, so no direct transmission seemed to have occurred between birds. Shed titres might be insufficient to infect sentinels in our experimental conditions and/or contacts between individuals might to limited in birds to allow transmission. Since intranasal inoculation leads to systemic dissemination and disease, we aimed at defining the cellular tropism of the virus in the upper airways. We thus evaluated the permissivity of respiratory epithelial cells *in vitro* using canary tracheal explant. Although we used a high infectious dose, no signs of viral replication or amplification were observed. These cells thus seem to not be permissive to USUV, contrary to what has been shown for the human respiratory epithelium. Further research on the cell tropism of USUV is needed to identify the cell type(s) involved in the initial replication and dissemination of the virus after intranasal inoculation and to understand how the bird-to-bird transmission might occur in wildlife.

48.

The interference of West-Nile virus *in vitro* replication in mosquito cells due to Lammi virus an insect-specific flavivirus

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Arboviruses i.e. viruses spread by arthropod vectors such as mosquitoes, ticks and biting midges are an increasing global threat to human and animal health due to the diseases that they cause. For many of these viruses there is a lack of vaccines and antivirals as well as effective control strategies to reduce the circulation of arboviruses in nature. One such novel approach has been suggested to be the ability to reduce the vector competence for different arboviruses. This could be achieved by for example using genetically modified mosquitoes or by using biological control agents such insect-specific viruses (ISVs). However, further knowledge is needed regarding the virus-vector interactions during infection. Therefore, in the present study, we used high-throughput sequencing to investigate the *in vitro* cellular response to both Lammi virus (LamV), an insect-specific flavivirus, as well as to West Nile virus (WNV), an arbovirus also belonging to flaviviruses. In addition we investigated if LamV could interfere with the mosquito vector competence for WNV. In short, we infected the Aedes albopictus cell line U4.4 with either WNV, LamV or through an infection scheme whereby cells were pre-infected with LamV 24 h prior to WNV challenge. The qPCR analysis showed that the dual-infected U4.4 cells had a reduced number of WNV RNA copies compared to WNV-only infected cells. The transcriptome profiles of the different infection groups showed a variety of genes with altered expression. WNV-infected cells had an up-regulation of a broad range of immune-related genes, while in LamV-infected cells, many genes related to stress. The transcriptome profile of the dual-infected cells was a mix of up- and down-regulated genes triggered by both viruses. Furthermore, we observed an up-regulation of signal peptidase complex (SPC) proteins in all infection groups. These SPC proteins have shown importance for flavivirus assembly and secretion and could be potential targets for gene modification in strategies for the interruption of flavivirus transmission by mosquitoes. Further *in vitro* and *in vivo* studies are needed to investigate the effects on the transmission of WNV, of either a prior LamV infection or of the gene modification targets brought up in this discussion.

O185.

Fitness and neurotropism of H5NX 2.3.4.4B in ferrets, human respiratory cells and human brain organoids

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Introduction - Highly Pathogenic Avian Influenza (HPAI) viruses of the H5 subtype clade 2.3.4.4b are now the dominant clade across Europe, Africa, the Middle-East and North America. The high circulation in wild birds has favoured the generation of multiple reassortant viruses (H5NX), with an extremely diverse gene constellation. Up to 2019, experimental as well as epidemiological evidence indicated a scarce adaptation to mammals. However, the 2020/2022 epidemics in Europe recorded infections in carnivores, the selection of adaptive mutations to mammals and sporadic human cases. To assess the zoonotic potential of these viruses we performed a characterization of these viruses in comparison with human and avian influenza strains of known phenotypes.
Methods - European HPAI isolates (H5N5, H5N1, H5N8) from the 2020/2021 epidemic were compared to a 2.3.4.4.b H5N8 isolate from 2019, two H5N1 viruses of 2.2 and 0 clade, a seasonal human H3N2 virus and an avian H3N6 virus. Virus replication and cell tropism were studied in respiratory/enteric swine and human cells, ferret respiratory explants and iPSC human brain organoids. In addition, pathogenesis studies were conducted in ferrets with two 2.3.4.4.b HPAI strains isolated in 2019 and 2021.
Results - The 2020/2021 2.3.4.4b viruses showed superior fitness in human and swine cells, as well as in ferret explants. This behaviour was particularly evident in vivo, as ferrets infected with a 2021 isolate showed moderate-to-severe signs of disease as opposed to a self-limiting asymptomatic infection with the 2019 strain. The 2021 virus partially transmitted to contact sentinels causing death, 4 days after their introduction. Neurological signs and the detection of viral RNA in the olfactory bulb and brain suggests a peculiar neurotropism of this isolate. In agreement with these results, 2021 viruses were the only ones to reach high infectious titers in human brain organoids, comparable to those of a known neurotropic HPAI virus.
Conclusions - Current clade 2.3.4.4b viruses show high fitness in mammalian cells and tissues of different mammalian species despite their diverse gene constellation.
Given their widespread diffusion in wild birds, increased surveillance activities and additional genetic and phenotypic characterization efforts should be prioritized to inform risk assessment.

O5.

Are non-coding Simbuviruses RNA structures involved in transcription termination and translation?

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Following the Schmallenberg virus (SBV) emergence in 2011, this ruminant arbovirus rapidly disseminated, reaching a pan-European distribution. Since then, it has shown an endemic pulsation behavior. It is a member of the *Peribunyaviridae* family, genus *Orthobunyavirus*, which belongs to the Simbu serogroup. SBV virion contains a negative single-stranded RNA genome, divided into three segments named after their length: large (L), medium (M), and short (S). These segments are flanked by non-translated regions (NTRs), with crucial roles in viral RNA replication and protein synthesis. It is worth mentioning that the mRNA differs from the other types of viral RNA transcripts because it possesses a cellular-derived 5'cap, and the 3' untranslated region (UTR) is truncated. To better understand how SBV sets functional requirements on the S segment 3' NTR, multiple mutations were designed targeting two highly conserved RNA motifs among Simbuviruses. The first motif is a stem-loop structure localized two nucleotides upstream of the mRNA cleavage site. The second is a GC signal found downstream on the complementary RNA. Rescue of recombinant viruses was accomplished using a three plasmids-based reverse genetic system (RGS). The biological properties of the recombinant viruses were evaluated in the relevant SBV infection contexts: mammalian and insect cells. Viral fitness was compared by assessing their transcription termination profiles, plaque size, and replication kinetics. Overall in mammalian cells, the stem-loop structure is essential for the production of replicative virions. When the secondary structure was preserved, one SBV mutant displayed a similar fitness to the wild-type virus, despite its sequence being modified, thus the RNA motif is structure-dependent. Yet, the stem-loop mutants exhibited different replication kinetics according to the complementarity degree between the stem arms. On the other hand, the GC signal is not essential for SBV, though two GC signal mutants displayed a strongly attenuated phenotype, due to a replicative disadvantage given by the inhibition of the mRNA cleavage. Therefore, the GC signal modulates the composition of viral RNA pools within the cells. In contrast, no replication difference was found in the insect cells, suggesting that both RNA motifs have any regulatory role on the SBV arthropod vector.

O161.

Detection of Genotype XIV.2 Newcastle Disease Viruses in Nigeria, Antigenic Characterization and Optimization of Vaccination Strategies

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Newcastle Disease Virus (NDV) is a highly contagious avian virus belonging to the genus *Orthoavulavirus* with worldwide distribution in poultry and wild bird species. Vaccination programs have mitigated the impact on poultry production of the disease in many countries. However, in various parts of the developing world including Africa, NDV still poses a serious threat to poultry, causing severe losses. In this study, we analysed the genetic and antigenic characteristics of velogenic strains detected in Nigeria during an active surveillance campaign performed in 2020-21 with the aim to identify the best vaccination strategy to reduce the impact of NDV in Nigeria.
Methods: Swabs collected from different poultry species were analysed by RRT-PCR targeting the L gene of any avian orthoavulavirus-1 (AOAV-1) genotype. Partial sequencing of the F gene enabled the identification of velogenic strains (v-NDV), which were then isolated in SPF embryonated chicken eggs. NDV-positive allantoic fluids were submitted for whole-genome sequencing by NGS and used for antigenic characterization. NDV isolates and corresponding antisera raised in SPF chickens were analysed by microneutralization assay and used to generate antigenic maps comprising velogenic strains and available NDV vaccines. Seven different NDV vaccination protocols, which included, among others, boosting with a homologous experimental vaccine, were tested in a vaccination-challenge study in SPF chickens.
Results: All detected velogenic strains belonged to genotype XIV.2. Antigenic maps generated using antigenic data from the Nigerian v-NDV strains and from NDV vaccine seed strains showed antigenic diversity between the different strains belonging to the same genotype, with one strain showing considerable antigenic distance from available vaccine strains. Considerable differences were identified both in terms of clinical protection offered and ability to reduce the spread to unvaccinated birds by the different vaccination protocols.
Discussion: Although NDVs are considered to belong to the same serotype, multiple reports have demonstrated antigenic diversity between strains, possibly explaining outbreaks in vaccinated flocks. Our work has piloted the development of a possible strategy for the optimization of vaccination protocols in countries experiencing recurrent ND outbreaks. Overall, our work highlights the critical importance of phenotypic characterization of field viruses for the optimization of vaccination strategies.

O162.

Characterization of an attenuated strain of African swine fever virus providing sterilizing immunity after intramuscular or oronasal immunization

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African swine fever (ASF) is a contagious viral disease of suids, which induces high mortality in domestic pigs and wild boars. The disease originated from Africa was reintroduced in Europe in 2007 and has since spread widely across the European continent, Asia and the Caribbean. Given the current spread of ASF, the development of a vaccine is a priority.
The ANSES Ploufragan laboratory has produced, from the Georgia 2007/1 strain circulating worldwide, an attenuated strain called ASFV-989. Compared to the Georgia strain, the ASFV-989 strain genome has a deletion of 7458 nucleotides located in the MGF 505/360, which allowed developing two PCRs specifically detecting each of the strains in the biological samples. In vitro, in porcine alveolar macrophages, the replication kinetics of the ASFV-989 strain was identical to that of the Georgia strain.
In vivo, specific pathogen free pigs inoculated with the ASFV-989 strain, either intramuscularly or oro-nasally, exhibited transient hyperthermia and slightly decreased growth performances, unlike animals inoculated with the Georgia strain, which all presented a fatal evolution. Animals immunized with the ASFV-989 strain showed a viremia 100 to 1000 times lower than those inoculated with the Georgia strain and developed a specific cellular and humoral response. In pigs immunized with the ASFV-989 strain then challenged 2 to 4 weeks later with the Georgia strain, no symptoms were recorded and no viremia for the challenge strain was detected.
Recent work has made it possible to adapt the ASFV-989 strain to multiply on a cell line, an essential step for industrial production of this vaccine candidate. Studies are in progress to verify the safety and vaccine efficacy of this cell-line adapted strain.

O49.

Virus-host interactome high-throughput mapping to identify new factors of pathogenicity and interspecies transmission for Bluetongue virus

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Bluetongue virus (BTV) is responsible for a disease that affects wild and domestic ruminants. It transmitted by blood-feeding midges of the genus *Culicoides*. BTV is remarkably variable in its host range and clinical manifestations. This variability is due to several factors related both to the infected host and the viral serotypes and strains. Despite the fact that BTV has been used studied extensively as a model to study the orbiviruses, we still have little understanding of the molecular determinants of BTV virulence.
We took advantage of functional proteomic approaches such as high-throughput yeast two-hybrid (Y2H) to map interactions between BTV and cellular proteins. All viral proteins encoded by two serotypes of BTV (BTV8 and 27) were used as baits to screen two cDNA libraries originating from hosts naturally infected by BTV: cattle and *Culicoides*. Therefore, 56 Y2H screens were performed allowing us to identify a hundred of new cellular interactors. A preliminary global analysis has uncovered many signal transduction factors involved in the modulation of autophagy (BECN1, GABARAPL), apoptosis (AATF, PAWR, CASP8AP2) and the ubiquitin-proteasome system (UBA7, UBE2I, UBA1). The most interesting cellular interactors are currently re-tested by Y2H and N2H (Nanoluciferase two-hybrid) with the viral proteins encoded by BTV8 and 27 but also other serotypes (BTV1, 4, 25 and 30). Specific interactions of a particular virus or group of viruses would shed light on the molecular mechanisms responsible for its virulence/pathogenicity and transgression of cross-species barriers. We have also identified Wilms' tumor 1-associated protein (WTAP) as a new interactor of the BTV-NS4. In contrast to BTV8, 1, 4 and 25, NS4 proteins from BTV27 and BTV30 are unable to interact with WTAP. This interaction with WTAP is carried by a peptide of 34 amino acids ($NS4^{22-55}$) within its putative coil-coiled structure. Most importantly, we showed that binding to WTAP is restored with a chimeric protein where BTV27-NS4 is substituted by BTV8-NS4 in the region encompassing residue 22 to 55. We also demonstrated that WTAP silencing reduces viral titers and the expression of viral proteins, suggesting that BTV-NS4 targets a cellular function of WTAP to increase its viral replication.

80.

Preliminary Data on Potential Zoonotic Viruses in Cats in North East Italy: Mammalian Orthoreovirus and Rotavirus

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Mammalian Orthoreoviruses (MRVs) and Rotaviruses (RVs) are segmented dsRNA viruses of the *Reoviridae* family, which infect mammals including humans, causing asymptomatic and symptomatic infections. The segmented nature of MRV and RV genomes and their ability to infect animals and humans pose concerns for their zoonotic potential. MRVs are often been described as the only cause of enteritis, respiratory infections and encephalitis in humans, but limited reports exist on MRV in cats. RVs are classified in ten species (group A to J): in particular, group A (RVA) is worldwide distributed and frequently associated with zoonotic transmission. Considering the extensive contact between humans and pets, the MRV and RVA circulation in pets should be expanded. Between May 2021 and May 2022, we conducted a study to investigate the MRV and RVA circulation in 242 cats of North East Italy targeting animals with sporadic contacts with humans but in strictly contact with each other. In 2021, 180 cats were tested for MRV and 194 cats for RVA, and in 2022, 48 were tested only for RVA. MRV was detected by a published End Point RT-PCR on faecal (n=178) and oral (n=180) swabs and RVA was detected by an ad hoc developed Real Time RT-PCR on faecal swabs (n=242). Six out of 180 cats tested for MRV resulted positive of which two in oral and faecal swabs and four only in faecal ones. Ten out of 242 cats, sampled in 2021 and 2022, tested for RVA resulted positive. One cat tested positive for MRV and RVA in the faecal swab. Partial genome sequencing by Sanger, confirmed PCRs data and virus isolation attempts are currently ongoing on all positive samples. Our preliminary data highlight the detection of MRV in Italian cats for the first time and the RVA circulation in the cat population of North East Italy in 2021 and 2022. Due to the zoonotic potential of these viruses, more attentions and efforts should be directed towards a more in-depth understanding of the ecology of these viruses.

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O50.

Dissecting differences and similarities in the host response to Rabies virus and other Lyssaviruses in the Syrian hamster model

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Rabies virus (RABV) is the prototype of *Lyssavirus* genus, which accounts for more than 17 identified species divided in three different phylogroups. Lyssaviruses are transmitted following a biting event and are expected to trigger a 100% fatal neurological disease that can be spread from reservoir species to other mammals, including humans. Currently, host response against Lyssaviruses has only partially been unveiled and the available data regarding their behaviour are associated to RABV, assuming all lyssaviruses perform the same.
Therefore, the main purposes of this study are: (i) to investigate and compare the host response elicited by different Lyssavirus species (i.e. RABV vs non-RABV lyssaviruses) and (ii) by different viral doses.
Eight-week old Syrian hamster (*Mesocricetus auratus*) females were infected by intramuscular route with alternatively RABV, Duvenhage lyssavirus (DUVV), a bat associated lyssavirus circulating in Africa), and a phylogroup three Lyssavirus, the West Caucasian Bat Lyssavirus (WCBV).
We registered clinical score in the three weeks after infection. At the onset of symptoms, animals were sacrificed and brain immediately collected. Half of the brain was fixed for immunofluorescence examination, while the remaining was stored for RNA-seq analysis. The obtained RNA-seq data were analysed to identify differentially expressed genes and enriched biological processes between infected and control animals. Real-time PCR and immunofluorescence were performed to validate RNA-seq results.
Transcriptomic analysis revealed activation of the classical processes associated to RNA viruses infection, both in RABV and Lyssaviruses-infected animals, such as *cellular response to interferon alpha and beta*, *cytokine–mediated signalling pathway* and *activation of innate immune response*.
However, we also observed that Syrian hamsters infected with non-RABV Lyssaviruses activate specific biological processes related to viral response that are not triggered by the immune system of RABV-infected animals.
At the same time, we noticed that animal inoculation with a lower dose of RABV is also influencing host immune response.
In conclusion, the transcriptomic analysis of Lyssaviruses host response depicted several differences that could justify peculiar viral behaviours in terms of pathogenesis and ecological distribution.

O81.

Occurrence and characterization of Rabbit Calicivirus (RCV) strains in Italy over 20 years

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Lagoviruses are a group of riboviruses from the Caliciviridae family whose host spectrum is represented by lagomorphs. Focusing on viruses affecting rabbits, both pathogenic and non-pathogenic viruses are phylogenetically distinguished: Rabbit Hemorrhagic Disease Virus (RDHV/GI.1) and Rabbit Hemorrhagic Disease Virus-2 (RDHV-2/GI.2) belong to the first group, while in the second several genotypes of Rabbit Calicivirus (RCV/GI.3-GI.4) are present. Regarding this last group, the first RCV strain was described in Italy in 1996 from the intestine of rabbits in a farm showing many seropositive rabbits but never affected by RHDV. From the first case, several studies have been carried out aimed to characterizing RCV strains both in Europe and Australia. Indeed, it has been then showed that for RCVs recombination events with other lagoviruses are possible and relatively frequent. Following these studies, our work is aimed to characterized RCV strains diagnosed in Italy in samples of feces and intestines of rabbits.
Samples of wild and farmed rabbits were collected in various part of Italy from 2000 to 2020. To detect the viral RNA a first RT-PCR was performed using the universal primers for lagovirus Rab1/Rab2 and the amplification products were sequenced in both directions. All the positive samples were than amplified using a series of primers drawn to amplify the VP60 gene and sequenced using the Sanger method.
Out of 215 analysed samples, 52 resulted positive for lagovirus. Nine of these were confirmed to be RCV strains, by complete VP60 sequencing, and phylogenetic analysis showed that two belong to the European genotype (RCV_E1) and five to the Australian-like genotype (RCV_E2) whereas the remaining two strains, based on their VP60 gene sequences, could form a separate cluster from the other known RCV genotypes. These strains both originated from the same first positive farm detected in 1996 but they were identified after several years of interval. These strains have a nucleotide identity of 98.99% (BS_ 2000) and 90.49% (BS_2007) respectively compared to the first RCV "Italian" strain Subsequently, we are going to complete the analysis of the non-structural part of the genomes to highlight the presence of recombinant strains and understand the phylogenetic relationships through full-genome data.

O6.

Impact of genetic diversity of the Rift Valley Fever virus, from the field isolates to a genetic determinant

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Rift Valley fever (RVF) is a vector-borne disease mainly transmitted by mosquitoes. In ruminants, RVFV is responsible of a high neonatal mortality and numerous abortions, with huge economic consequences for livestock farmers of the affected countries. The circulation of RVFV is particularly active in countries of western Africa (Senegal/Mauritania) where the disease is endemic. An epidemy occurred in 2010 in the Northern desertic region of Mauritania causing for the first-time high mortality in camels, an animal previously known as a not susceptible species.
Initially, in order to assess the virulence of circulating RVFV strains, two field isolates were investigated: MRU25010-30 of camel origin (2010) and MRU2687-3 of goat origin from Southern Mauritania (2013). Their replicative capacities and virulence were determined *in vivo* in mice BALB/c and *in vitro* on several types of cells (i) human hepatocyte cell lines (HepaRG), (ii) induced pluripotent stem (IPS) cells differentiated in multicultural of primary neural cells (astrocytes, neurons and oligodendrocytes), (iii) A549 human lung adenocarcinoma cells. In mice model we tested two routes of inoculation, intranasally (IN) or subcutaneously (SC). Regardless of the route of inoculation, the strain MRU25010-30 induces higher mortality rates in 4 days compared to 10 days by MRU2687-3 strain. The strain MRU25010-30 disseminates to the brain faster (i.e. 4 days earlier) than the strain MRU2687-3. In cellular models, the strain MRU25010-30 produced from 10 to 100 times more infectious virus particles compared to the strain MRU2687-3. Deep sequencing showed that these two strains belong to two distinct genetic lineages along with intra-strains polymorphisms. Thanks to reverse genetics, a viral genetic polymorphism has been identified as a putative determinant of virulence for the strain MRU25010-30. We are currently exploring the impact of this polymorphism on the RVFV viral cycle.

O186.

Vital roles of poultry vaccination in prevention of potential H7N9 avian influenza virus pandemic

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H7N9 avian influenza virus (AIV) that can infect humans emerged in China in 2013. Since then, there have been 1,568 confirmed human infections with an estimated 40% case fatality rate. Given the risk of H7N9 AIV to humans and poultry, the Chinese government implemented a mass vaccination program against H7N9 AIV in poultry in 2017. Since then, the number of H7N9 AIV outbreaks along with human infections have dropped dramatically with only sporadic isolation in China. To predict the possible antigenic drift that might come up under vaccine-induced immunity and assess its risk to humans and poultry before their emergence in nature, we imitated natural selection process by propagating prototype H7N9 AIV in embryonated chicken eggs with the presence of homologous ferret antiserum. The serum escape mutant containing mutations A125T, A151T, and L217Q in the hemagglutinin glycoprotein was detected as early as passage five and that these mutations persisted until passage ten. Antigenic analysis showed that L217Q mutation in hemagglutinin is the key mediator of virus to escape from vaccine-induced immunity. Importantly, three newly identified serum escape mutations (A125T+A151T+L217Q) have also been found in field isolates infecting poultry and humans in 2019. To investigate the potential threat of the serum escape mutant to humans and poultry, the impact of these HA substitutions on receptor binding, pH of fusion, thermal stability and virus replication were investigated. Our results showed the serum escape mutant grew robustly *in vitro* and *in ovo*. It had a lower pH of fusion and increased thermal stability. Of note, the serum escape mutant completely lost the ability to bind to human-like receptor analogues. In conclusion: (1) Amino acid residue 217 in the HA glycoprotein is a key mediator of H7N9 AIV antigenicity; (2) The H7N9 AIV that contains A125T+A151T+L217Q mutations in HA protein might pose a reduced pandemic risk but remain a heightened threat for poultry; (3) The serum escape mutant selection method is a feasible and powerful tool to predict the evolution of avian influenza viruses.

O7.

Marek's disease virus virulence genes encode circular RNAs

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Marek's Disease Virus (MDV) is an oncogenic alphaherpesvirus responsible of Marek Disease in chicken. Like others herpesviruses, natural MDV infection can be divided in different phases, the latency, the reactivation and the lytic phase. This virus uses all the regulatory mechanisms of the cells and encodes for different non-coding RNA such as microRNAs, several long non-coding RNAs (e.g. the latency associated transcripts (LATs) or the viral telomerase RNA subunit (vTR)), and circular RNAs (circRNAs).
CircRNAs are a new class of non-coding RNA re-discovered this last decade. CircRNAs appeared as a new keystone in transcriptional and post-transcriptional regulation and are involved in numerous biological processes and cancer development. They are formed by a back-splicing mechanism depending on the splicing machinery. Recent studies reveal spliceosome-dependent biogenesis and the vast majority of circRNAs production occurs at major U2 spliceosome splice sites (GT/AG).
We established inverse PCRs to identify and characterize circRNA expression from 28 loci in the repeated regions of the MDV genome encoding the majority of virulence factors of the virus and presenting plenty of splicing isoforms. We identified a large variety of viral circRNAs during viral replication, latency and reactivation through precise mapping of full-length circular transcripts matching sequences of several viral genes. Hot spots of circRNA expression included the transcriptional unit of the major viral oncogene encoding the MEQ protein and the LATs. Moreover, we performed genome-wide bioinformatic analyses to extract back-splice junctions from lymphoma-derived samples. Using this strategy, we found that circRNAs are abundantly expressed *in vivo* from the same key virulence genes. Strikingly, the observed back-splice junctions do not follow a unique canonical pattern compatible with the U2-dependent splicing machinery and can originate from the interior region of exons or introns, leading to the generation of a high number of circRNAs isoforms. Numerous non-canonical junctions were observed in viral circRNA sequences characterized from *in vitro* and *in vivo* infections.
Given the importance of the genes involved in the transcription of these circRNAs, our study contributes to our understanding of the complexity of MDV-induced tumorigenesis.

O8.

Neuraminidase and Trypsin Treatment of Highly Passaged Feline Enterocytes Enhances the Replication of FECV Type I Strains

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Feline coronaviruses cause a clinical picture varying from mild enteric infections to fatal systemic infections in cats. The enteritis-inducing feline enteric virus (FECV) is one of the pathotypes. Based on the antigenic properties of the S protein, FECV is classified into two serotypes. Serotype I is most prevalent but isolation and cell culture adaptation is difficult, making it understudied. Thus, it is important to establish a cell line supporting the propagation of FECV type I. In our lab, a feline enteric cell line was established. Feline enterocytes at low passages are fully susceptible to FECV type I strains after pretreating cells with neuraminidase (NA), which removes the sialic acids from the cell surface and, as such, enhances the viral binding and infection. However, highly passaged cells (>30) led to a loss of their susceptibility to FECV type I strains after pretreating cells with NA. We assumed that during passaging, cells lose their ability to produce and/or secrete proteases and as a result, the virus fails to fuse with the cell membrane since its S protein cannot be cleaved by host cell proteases. We found that FECV type I strain UCD did not grow in highly passaged feline enterocytes in the presence or absence of trypsin. When we added trypsin after pretreating cells with NA, the infection of FECV type I strain UCD increased in a dose-dependent way with increasing concentrations of trypsin, with an infection rate from 0% to about 15% after 12 hours infection at a m.o.i. of 0.2. Based on the results, we may conclude that, to get an effective infection, the sialic acids should be first removed from the cellular surface to guarantee a better virus-receptor interaction, and that afterwards an exogenous trypsin is needed to compensate for the absence of the cellular protease. After NA and trypsin treatment, highly passaged feline enterocytes can easily support the propagation of FECV type I. In the future, the cellular protease that cleaves the S protein will be identified.

82.

Sheep and goat breeders' opinions about testing for viral diseases at sale

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Testing animals for certain diseases at sale is one of the pillars of external biosecurity. In Belgium, few data are available on the level of biosecurity in small ruminant farms, and particularly on the subject of breeders' requirements relating to the health status of sold animals.
The objective of this study is to assess the opinion of sheep and goat breeders about the possibility of detecting certain diseases at sale.
An online survey was sent to sheep and goat farmers known in the ARSIA database. A total of 185 breeders (157 sheep breeders and 28 goat breeders) responded to the survey. The median number of animals kept by respondent farmers was 40 (min. 3 ; max. 1600).
Among responding farmers, 98% (181/185) say that they are interested in testing for diseases at sale, regardless of their age, sex, herd size and species bred. In addition, 79% (146/185) of these specify that they would be ready to use testing kits. In 2021, more than 3 out of 4 breeders introduced at least one animal into their herd without having tested it for diseases. Lentiviruses were the most searched pathogens. Among these breeders, more than half has not carried out quarantine before introducing the purchased animal into the herd. The costs of lab analyzes and vets'interventions have been mentioned as the main obstacle to testing for diseases at sale.
The results of this survey demonstrate (1) the interest of sheep and goat breeders for a better knowledge of the health status of the animals purchased, regardless of their breeders'profile and (2) the possibilities for improving the level of biosecurity in sheep and goat farms.

83.

Limits to the participation of Small Ruminant Lentiviruses eradication program in Belgium

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Small Ruminant Lentiviruses (SRLVs) include Maedi-Visna Virus (MVV) and Caprine Arthritis and Encephalitis Virus (CAEV). They are contagious and incurable pathogens for goat and sheep. A study conducted in 2018 in Belgium showed that 17% of sheep and 13% of goats are presumed positive for SRLVs. However, less than 3% of sheep farmers and 1% of goat farmers participate in the national eradication program.

In order to understand this low participation rate, a survey was sent in 2022 to Walloon sheep and goat farmers, asking them if they knew the disease, if they have detected it, if they knew the national eradication scheme and if they participate in it as well as the reason(s) of this choice. A total of 106 sheep farmers and 25 goat farmers responded to the survey.

Most of the sheep farmers (54%) are unaware of the eradication program. It is mainly purebred breeders who participate to this in order to take part in sheep competitions. These breeders mention the following limits of the program: the costs of analyses and vets 'interventions as well as and the difficulty of maintaining certification. On the other hand, almost one in two goat breeders is aware of the eradication program. However, only 8% of the goat farmers participate in it. Goat farmers find there are not enough CAEV-free farms and consider that the terms and conditions for participation are too complex. Concerning the presence of infected animals in herds, only 2% of the sheep farmers reported to have detected SRLVs cases while 36% of the goat farmers mentioned it.

To conclude, huge differences could be observed between sheep and goat farmers considering the knowledge of the eradication program, the reasons of participation (or not) and the number of affected animals in herds.

O84.

The Founder Variants Transmitted by Sows are the Main Source of PRRSV1 Genetic Diversity in an Unstable Infected Farm.

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Porcine reproductive and respiratory syndrome virus (PRRSV) continuously expands its genetic diversity with the evolutionary driving forces being elusive. The objective of the present study was to examine how PRRSV1 genetic diversity rose in a sow farm after a PRRSV1 outbreak. For this purpose, a longitudinal study was performed on a farm two months after the onset of an outbreak. The farm was implementing blanket vaccination (3 times a year) with a PRRSV1 MLV. Ten litters (6 animals/litter) of piglets from three farrowing batches were followed by collecting umbilical cords at delivery and blood at 2, 4, 6, and 9 weeks of age. PRRSV1 was analysed by RT-qPCR. The virus from selected serum samples (n=35) was whole-genome sequenced (Illumina Miseq) after isolation in porcine alveolar macrophages. Viral quasi-species and the complete consensus genome sequences were obtained. Additionally, a set of positive samples with Ct values ≤ 32 were ORF5 Sanger sequenced (n=134). Anti-PRRSV1 ELISA antibodies and neutralizing antibodies (NAb) against the farm strain were also examined. In the first batch, which was close to the outbreak, several viral clusters were consistently identified and could be linked to particular sows. This suggests the existence of vertically transmissible founder variants. The infection chains for the founder variants could be traced back phylogenetically. Interestingly, in the third batch, which was sampled 11 months after the first one, one variant had become predominant with no traceable founder variants or infection chains. Phylogenetically, the predominant variant possibly arose from a born-infected piglet which survived with viremia but no NAb throughout the follow-up. The levels of maternally derived NAb specific for the farm virus were very low in all batches. Collectively, the founder variant transmitted by the sow is possibly crucial to driving PRRSV1 diversity in the field after an outbreak, while animals born viraemic might be a further boosting force. Also, the results highlight the fact that some PRRSV1 strains are very poor inducers of neutralizing antibodies.

9.

Porcine Intestinal Organoids for the Study of Host-Pathogen Porcine Enteric Viruses

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The study of host-virus interactions is essentially performed in cell culture and animal models. These approaches come up against a double problem i) methodological, linked most of the time to the use of immortalized cell lines sometimes poorly related to the target cells of the virus and ii) societal, linked to the use of animals for which the experimentation can induce significant symptoms, generate suffering and inevitably ends in death. The recent development of organoids, three-dimensionally self-organized mini-organs makes possible ex-vivo models with experimental conditions significantly closer to physiological conditions compared to current cell cultures. Further, these organoids, of which several thousands are produced from a single animals, may contribute for a significant reduction in the use of animals, for experimental purposes, in line with the rules of the 3Rs (Reduction, Refinement, Replacement).
Pig is amongst the most important livestock production and diarrhea in piglets is currently one of the major problems of the pig industry. Porcine coronaviruses, due to their worldwide prevalence and their genomic variability, strongly contribute to these pathologies. The isolation of enteric coronaviruses on cell culture remains a laborious but above all random process. We hope that enteric organoids will allow the isolation and production, even on a small scale, of non-cultivable coronavirus strains. Doing so, organoids will open original perspectives on the comprehension of the physiopathology of virus infections, especially deciphering the host-pathogen interactions without relying systematically on extensive animal experiments.
Here, we used two models of enteric porcine coronaviruses: i) the porcine transmissible gastroenteritis virus (TGEv), for which we have several viral strains, more or less virulent and also cultivable on immortalized cells, ii) the virus porcine epidemic diarrhea (PEDv) for which we have recent French strains, obtained directly from shredded material and which cannot be cultivated on cell lines. Initially, TGEv served as a model for establishing infection protocols that will beused for PEDv in order to propose a methodology for infection and viral production for the isolation of strains that cannot be cultivated in cell culture. In a second step, those enteric organoids will serve for host-pathogen interactions study of enteric coronaviruses.

124.

Fast Identification of Viruses Using Nanopore Sequencing: Application to Avian Diseases

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Poultry health is increasingly challenged by emerging infectious threats associated with animal reservoirs and global transport of animal. The emergence of highly pathogenic avian influenza viruses is an example of infectious threats challenging global livestock sustainability.
Classical molecular detection approaches are based on real-time PCR techniques targeting viral genes for a fast detection and quantification. Despite its robustness, PCR will hardly generate information on subtypes or presence of minority mutants. Propagation of viruses on cells or embryonated eggs before PCR or NGS is also a robust and efficient solution but is time-consuming and not compatible with a field emergence requiring a fast analysis.
We had already tested unbiased NGS directly performed on samples to identify pathogens without previous knowledge: we were able to identify and type avian poxviruses and adenoviruses. Here, we tested workflows combining multiplex-PCR enrichment and the MK1C Oxford Nanopore Technologies sequencing device. The MK1C combined with the Flongle flowcell is an interesting tool allowing a rapid diagnostic, considering its convenience, price and data throughput.
We applied a pipeline from the sampling to the bioinformatics analysis, allowing a complete dissection of the pathogen's population in less than 48 hours. This pipeline was applied to the detection and typing of influenza but also to infectious bronchitis viruses and coinfecting agents, directly sampled in the respiratory tract of infected birds. This approach allows a fast and sensitive NGS-based detection and typing of AIVs or IBV, virtually applicable to any viral disease. We report here the results of several studies, including the challenging bioinformatics analysis.

O125.

SARS-CoV-2 whole genome sequencing: evaluation of five different sequencing approaches

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Genomic surveillance of SARS-CoV-2 is critical to monitor the emergence and global spread of viral variants. Millions of SARS-CoV-2 genomes have been sequenced worldwide and shared on the GISAID database (https://www.gisaid.org/). Various NGS-based approaches have been developed to perform SARS-CoV-2 whole genome sequencing using different protocols. Recently, numerous commercial kits have become available on the market and are being deployed in SARS-CoV-2 genomic surveillance. In this work, we analyzed the performances of five different sequencing protocols.
Methods. Nucleic acids purified from nasopharyngeal swabs collected from inpatients and outpatients of the Abruzzi region, central Italy, were tested by a SARS-CoV-2-specific commercially available Real Time RT-PCR. A total of 33 positive samples with threshold cycle (C_T) ranging from 16 to 33 was used to compare five different sequencing protocols including:
- the Sequence-Independent Single-Primer Amplification (SISPA) technique;
- the Arbor SARS-CoV-2 panel, a targeted enrichment approach;
- the ARTIC protocol, a targeted method based on specific multiplex amplifications with specific primers;
- two amplicon-based commercial protocols namely the Swift Amplicon® SARS-CoV-2 Panel kit and the Illumina COVIDSeq Test.

SISPA, Arbor and ARTIC libraries were prepared using Illumina DNA Prep kit and deep sequencing of all samples was performed onto Illumina platforms, MiniSeq or NextSeq500. Bioinformatic analyses were performed as recently described by Di Pasquale 2021.
Results. The SISPA protocol combined with NGS produced complete sequences only from swabs with $C_T \leq 20$, instead, Arbor and ARTIC protocols produced complete genome sequences from all samples, reaching optimal levels of vertical (5000x) and horizontal (100%) coverages. The Arbor protocol was more expansive and laborious than ARTIC. The Swift protocol was more cost-effective and less time-consuming than ARTIC but less efficient in producing reliable data (two constant deletions of 7 and 34 nucleotides). Finally, the COVIDSeq Test showed the highest performances as complete consensus sequences (100% horizontal coverage) obtained also from low-input samples ($C_T > 30$) with the best processing time and costs.
Discussion. We conclude that the COVIDSeq Test method is a versatile and scalable method that is immediately applicable for SARS-CoV-2 genomic surveillance and easily adaptable to other pathogens.

126.

Virus discovery in dogs with viral encephalitis of unresolved etiology by high-throughput sequencing based metagenomics

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Viral infections are the major cause of disseminated non-suppurative encephalitis in dogs. However, the specific viral agent initially responsible for the infection may remain unknown by using routine diagnostics due to extensive or complete clearance of the virus initially responsible or possibly the presence of unexpected novel viral agents. By combining high-throughput sequencing (HTS) and big data analysis, this project takes on a metagenomics approach of resolving the viral etiology of non-suppurative encephalitis in dogs.

Documented cases of dogs (n=50) between the years 1979 and 2021 with non-suppurative encephalitis suggestive of a viral origin but without definitive diagnosis, stored in the archive of the Division of Neurological Sciences of the Vetsuisse Faculty of the University Bern, Switzerland, were selected for metagenomics analysis. RNA was extracted from selected brain regions of formalin-fixated paraffin embedded (FFPE) material and underwent cDNA library preparation for HTS. HTS data was then analyzed by an established in-house bioinformatics virus discovery pipeline, which consists of sequence comparisons with virus nucleotide and amino acid databases of GenBank.

Initial sequencing at low read depths followed by data analysis led to preliminary results with the identification of Tick-borne encephalitis virus in 13 cases and Canine distemper virus in three cases, respectively. Detected viral sequences were confirmed by conventional diagnostic techniques and followed-up by re-sequencing at higher read depths to obtain comprehensive genomic sequences from FFPE material, which by nature is highly fragmented.

Our results demonstrate the utility of HTS to detect viral agents known to cause non-suppurative encephalitis in a subset of unresolved cases. Furthermore, this method enables a non-biased approach that could lead to the detection of novel viral candidates. These results shed new light on the prevalence of Tick-borne encephalitis infections in dogs and the neuropathological presentation of Canine distemper virus encephalitis. Moreover, increased identification of causative viral agents will expand the knowledge of the pathogenesis of viral encephalitis in dogs and ultimately lead to better-tailored therapeutic interventions and prophylactic measures.

O163.

Comparative Evaluation of the Duration of Protective Immunity Induced by a Live Attenuated and an Inactivated Lumpy Skin Disease Virus Vaccine

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Vaccines have proven themselves as an efficient way to control and eradicate Lumpy Skin Disease (LSD) under standardized experimental settings and in the field. In addition to safety and efficacy aspects of vaccines, it is important to know the period during which they confer protective immunity against an infection (duration of immunity), as this impacts the design of an efficient control and eradication program. Here, we evaluated the duration of immunity induced by a homologous LSDV-based live attenuated vaccine (LSDV LAV) and an inactivated vaccine (LSDV INAC). For this purpose cattle were vaccinated following manufacturer's recommendations and challenged after 6, 12 and 18 months for LSDV LAV or after 6 and 12 months for the LSDV INAC. The animals were continuously followed clinically and sampled at regular intervals during the complete duration of the trial. Both vaccines were well supported by the animals with limited side effects after vaccination. The LSDV LAV provided a robust stimulation of the host immune response as witnessed by a strong antibody response and high interferon gamma responsiveness up to 18 months. This induced immune response protected against an LSDV infection up till 18 months after vaccination as no clinical signs and viremia could be observed after a viral challenge. The LSDV INAC also provided a good immune response albeit for a shorter duration than the LSDV LAV. All INAC vaccinated animals were protected till 6 months, but 2 out of the 6 animals developed clinical signs and viremia when challenged after 12 months. In conclusion, our data support that an annual vaccination campaign could maybe be replaced by vaccinations with a longer time interval when the LSDV LAV is used, while a bi-annual vaccination seems necessary when using the LSDV INAC.

85.

Time course analysis of the anti-ASFv effect of medium chain fatty acids

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Scientific research has proven that African swine fever virus (ASFv) is very stable in commonly imported feed ingredients. Moreover, it has been shown that ASFv can be transmitted orally via contaminated feed and cause infection. Treatment of feed can therefore mitigate the risk of ASFv introduction in a pig farm. In order to reduce the risk of ASFv entry via this route, we developed a synergistic mixture of medium chain fatty acids (MCFA) as antiviral agent.

Commercial swine feed was inoculated with either phosphate-buffered saline (negative control) or with an ASFv field-strain to a final virus titer of 10^5 HAD50/g feed (positive control). The treatment groups were treated with MCFA (C6:C8:C10) at a dose of 0.090, 0.125, 0.250 and 0.375% which was either mixed or sprayed into the feed and afterwards spiked with ASFv. All tests were performed in triplicates. All samples were incubated at room temperature for 3h, 6h, 12h, 24h and 72h. At the respective time-point, samples were collected for DNA extraction and evaluated by real-time PCR. The 2 highest dosages in test were further analyzed by haemadsorption (HAD) assay for infectious particles based on OIE protocol.

Results demonstrated that MCFA significantly increased Cq values when compared to the positive control at doses of 0.125, 0.250 and 0.375 from 12h after virus incubation onwards. The peak was reached at 72h. At that timepoint, HAD assay was performed with the highest dosages (0.250 and 0.375%) which caused a significant decrease of viral load with 0.375% MCFA.
No significant differences were observed between mix and spray methods at DNA nor virus titer level.

In a previously published *in vitro* experiment, we observed a strong anti-ASFv effect of MCFA on DNA level. However, we were not able to show a reduction in living material at 24h post-inoculation. In this study, we demonstrated that at 72h post-inoculation, 0.375% MCFA significantly reduced ASFv in feed at both DNA and living virus levels. In conclusion, MCFA are promising desinfectants, either sprayed or mixed into the feed, to diminish the risk of ASFv introduction in a pig farm.

O164.

Study of the outcomes of infection with a new $H1_{av}N2$ genotype of swine influenza virus that emerged in France and evaluation of vaccine protection

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In 2020, the surveillance of swine influenza A viruses (swIAV) in France revealed the emergence of a new $H1_{av}N2$ virus (HA clade 1C.2.4), called $H1_{av}N2$#E genotype. This virus rapidly spread within the pig population and was responsible for the majority of influenza infections investigated in France in 2020-2022, supplanting the $H1_{av}N1$ virus (HA clade 1C.2.1) which was previously predominant for more than 40 years. Sequencing analyzes indicated that the eight encoding genes originated from swIAV lineages enzootic in Denmark but genetic modifications have been fixed compared to parental viruses, especially in the receptor binding site and antigenic sites. Antigenic subtyping of $H1_{av}N2$#E strains also showed a distance to other $H1_{av}$ viruses isolated in France, as well as a limited cross-reaction with antisera obtained from vaccinated animals.
To further characterize this new enzootic genotype, we compared the outcomes of $H1_{av}N2$#E and $H1_{av}N1$ infections in specified pathogen free pigs inoculated intra-tracheally, and assessed the protection conferred by the Respiporc Flu3® vaccine. Three groups of six unvaccinated or vaccinated pigs (two injections three weeks apart) were inoculated with $H1_{av}N2$#E or $H1_{av}N1$ or culture medium, respectively. Three weeks later, all swIAV-inoculated animals were re-inoculated with the $H1_{av}N2$#E virus in order to evaluate the impact of pre-existing immunity towards a second infection with the emerging genotype.
In unvaccinated pigs, the $H1_{av}N2$#E-infection induced a greater decrease in daily weight gain during the four days post-inoculation and virus excretion was detected earlier than in case of $H1_{av}N1$-infection. While the vaccination strongly reduced clinical signs and completely prevented virus shedding in $H1_{av}N1$-inoculated animals, such a protection was not observed following $H1_{av}N2$#E inoculation. However, all vaccinated and unvaccinated animals primo-inoculated either with $H1_{av}N1$ or with $H1_{av}N2$#E did not developed any infection after the subsequent inoculation with $H1_{av}N2$#E. The innate, humoral and cellular immune responses developed in the different batches are being investigated, but the initial results already suggest that the actual vaccine is less effective against $H1_{av}N2$#E than $H1_{av}N1$ in providing clinical protection and reducing virus shedding, which may have contributed to its rapid dissemination in pig farms.

O127.

Detection and Localization of Atypical Porcine Pestivirus in The Reproductive Tract of a Persistently Infected Boar

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Atypical porcine pestivirus (APPV) belongs to the genus *Pestivirus* within the family of the *Flaviviridae*. Recently, APPV has been identified as the causative agent of congenital tremor (CT) type AII. The disease is a neurological disorder that affects newborn piglets and is characterized by generalized trembling of the animals. However, little is known about the epidemiology of the infection, transmission and spread of the virus between herds. It has already been confirmed, that similarly to other Flaviviruses, APPV can be shed by semen. Previously, we have identified the interstitial Leydig cells, the peritubular myoid cells and the smooth muscle cells of medium-sized arteries as the target cells of APPV in the testicles of newborn, CT-affected, congenitally infected piglets.

In our present study, we examined FFPE tissue samples obtained from a persistently infected 6-months-old, sexually mature boar, born with CT, that still was shedding 2.1×10^9 GE/mL APPV in its semen. We detected viral genome by RNAscope *in situ* hybridization method in the T-cell zones of the reproductive lymph node and the MALT of the ileum, periarteriolar lymphoid sheaths of the spleen, and in undifferentiated cells of the crypts in the ileal glands. We also identified positive cells among the neurons of the cerebral cortex, in the molecular and granular cell layer of the cerebellum, including the Purkinje-cells and less intense signal in the neurons of the spinal cord. We found weak signal in the colon, in the cells of the zona arcuata of the adrenal cortex and none in the lungs and the liver.

In reproductive organs, virus was detected in the Leydig cells and peritubular myoid cells of the testicles, and also in the cells beyond the Sertoli cell barrier (Sertoli cells and germ cells) and in the epididymis. We found positive cells in the prostate and the bulbourethral gland. Our results show that APPV can cause persistent, congenital infection in pigs and boars can actively shed the virus upon infecting cells beyond the Sertoli cell barrier and accessory sexual glands. Further research is needed to highlight the underlying mechanisms of the persistent infection.

51.

Molecular mapping of antigenic determinants of RHDV2

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Rabbit Haemorrhagic Disease Virus (RHDV) is a very virulent virus of the genus *Lagovirus* causing a severe and fatal hepatitis in the European rabbit (*Oryctolagus cuniculus*), with 100% morbidity and 80-95% mortality. After its emergence in China in the 80s and the occurrence in Europe on 1986, a new RHDV-related virus, called RHDV2, emerged in Europe on 2010 and rapidly spread worldwide. Thanks to its specific antigenic profile, allowing to largely escape the heard immunity previously generated by RHDV, it became prevalent causing extended epidemics in wild and domestic rabbits, but affecting also some other lagomorphs species.
Indeed, since the first identification, RHDV2 virulence increased and it frequently underwent to recombination events.
Since lagoviruses do not replicate "in vitro", it is impossible to create escape mutants for the mapping of monoclonal antibodies' (MAbs) epitopes. To locate the major antigenic determinants on the viral surface, in a previous study, we used a typing ELISA test based on a panel of 22 RHDV2-specific MAbs to characterize more than 300 Italian isolates collected from 2010 to date. Thereafter, by comparing the reactivity of single MAbs with the VP60 aminoacidic sequence of the isolates, we preliminary mapped 3 putative epitopes on the capsid protein, located on the external VP60 loops that constitute the immunogenic surface of the virion. Aim of this work was to confirm that the different amino acid mutations identified in the analysis of RHDV2 isolates with the MAbs panel, actually constitute part of the epitope recognized by the corresponding MAb. In order to achieve this goal, we reproduced single mutations of the VP60, and we expressed the viral capsid via baculovirus system infecting *Spodoptera frugiperda* (Sf9) cells. We obtained Virus Like Particles (VLPs), for each mutant that we analyzed with a specific MAb. With this strategy, we confirmed that the change of a single amino acid at position, Ile347→Thr is responsible for the loss or gain binding specificity with some monoclonal antibodies. This amino acid is located on the loop L2 of the P2 subdomain of the VP60 and exhibits high variability within RHDV2 strains. The baculovirus expression system will be used to verify other potential epitopes.

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O10.

A deletion in the spike protein spanning the furin cleavage site alters SARS-CoV-2 virulence in K18-ACE2 mice

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SARS-CoV-2 uses the S glycoprotein to bind to its receptor and to induce host membrane cell fusion and virus entry. In this study, we compared the virulence properties of a SARS-CoV-2 FCS 10 amino acid-deletion mutant (Δ680SPRAARSVAS689; Δ680-689, ΔFCS) in K18-ACE2 mice to those of the parental viral isolate and of two major Delta variant of concern (VOC) isolates.
Methods: Mice were intranasally inoculated with 10^5 $TCID_{50}$/25 μl of four SARS-CoV-2 isolates: two early B.1 lineage isolates including wild type (wt)-B.1 and B.1-ΔFCS, and two Delta isolates. Mice were monitored for 14 days for weight change, lethality, and clinical score. Oral swabs were collected daily and tested for SARS-CoV-2-RNA. From succumbed mice and from mice sacrificed on day 3 and 7 post-inoculation (dpi) organs were collected for molecular and histopathological investigations.
Results: Both Delta VOC isolates showed high rates of lethality, clinical score, and severe body weight loss. As opposite, B.1-ΔFCS exhibited significant lower virulence properties than its parental wt-B.1strain. Oral swabs tested positive from 1 to 9 dpi, with B.1-ΔFCS demonstrating the lowest RNA loads. Delta VOC isolates showed the highest RNA copy numbers in all tested organs. In this regard, significant differences were also recorded between the two B.1 isolates, mainly in brain and lungs. Severe interstitial pneumonia was observed in all Delta VOC infected mice, while both B.1 strains caused mild pulmonary histological lesions.
Discussion: The B.1-ΔFCS isolate was obtained by natural deletion through serial passages on VERO E6 monolayers. In our experimental setting, the B.1-ΔFCS showed reduced shedding and lower virulence in the K18-hACE2 mice compared to wt-B.1 and Delta isolates. Similar results were also previously observed in mice and hamster infected with a recombinant SARS-CoV-2 without the four amino acid motifs (PRRA) in the S protein. Overall, our work highlights the critical nature of the FCS in understanding SARS-CoV-2 infection and pathogenesis.

128.

Phylogenetic analysis of BoHV-1 strains from Dutch cattle collected in 1995-2020 reveals high genetic heterogeneity

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Bovine herpesvirus-1 (BoHV-1) may cause infectious bovine rhinotracheitis (IBR), milk drop, vaginitis, balanoposthitis, abortion, and infertility in cattle, and subclinical infections also occur. In several European countries, monitoring and vaccination programmes are in place, and in several countries BoHV-1 already has been eradicated. In the period 1995 - 2020, BoHV1 positive samples (nasal swabs and/or virus isolates) obtained from Dutch cattle, were stored at -70 °C. Nasal samples had been tested using a real-time PCR targeting the gene encoding the glycoprotein B (gB) and targeting the gene encoding the glycoprotein E (gE). From this set, 200 samples were selected, distributed over the years. Subsequently, whole genome sequencing on an Illumina platform was performed on these samples to investigate genetic diversity. For comparison 14 BoHV-1 samples originating from Germany and obtained in 2013-2018 were included. All PCR positive samples were positive for both gB and gE by real-time PCR, indicating these were BoHV-1 field strains and not the BoHV-1 gE-deletion vaccine strain. Phylogenetic analysis based on nucleotide polymorphisms (SNPs) revealed that all Dutch isolates belonged to type BoHV-1.1. Three isolates obtained from Germany were of the BoHV-1.2 type. A high degree of genetic heterogeneity was demonstrated between Dutch strains and more than 10 different clusters could be distinguished. For 30 samples both the primary sample (nasal swab) and the isolated virus were available, and sequence comparisons demonstrated that these paired samples always showed an identical sequence.
Isolation, molecular characterization, and phylogenetic analysis of BoHV-1 strains provide valuable information to better understand the epidemiology of BoHV-1. In the end phase of a control programme and in cases of re-introduction in BoHV-1 free regions, such data can be very useful for molecular epidemiologic analysis of outbreaks.

O11.

ASFV-Host Protein Interaction Mapping Uncovers Novel Function of CP204L in Lysosome Fusion and Clustering

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African swine fever pandemic continues to cause significant economic losses. The lack of in-depth knowledge concerning function and cellular targets of African swine fever virus (ASFV) proteins hampers efforts to develop vaccines and treatments. To address this, we used affinity tag-purification mass spectrometry (AP-MS) to generate an ASFV-host protein-protein interaction map. We uncovered high-confidence host protein interactors for 24 most abundant ASFV proteins. Among these, the ASFV protein CP204L, also known as P30 or P32, was identified to interact with the VPS39, a component of the homotypic fusion and vacuole protein sorting (HOPS) complex. Domain mapping revealed a 270-residue region within VPS39 that binds to CP204L. Moreover, expression of CP204L promotes fusion and accumulation of lysosomes blocking their interaction with VPS39. In early phase of infection, CP204L and VPS39 colocalize at the site of virus replication and the knockout of endogenous VPS39 decreases the levels of early synthesized CP204L protein. These results indicate the potential mechanism by which ASFV escapes degradation and establishes its virus factories. In summary, our study gains new insights into ASFV-host protein interaction and virus biology. Furthermore, additional characterization of other protein-protein interactions revealed by this study is expected to identify more potential targets for novel therapeutic strategies.

O12.

Pathogenesis of West Caucasian and Lleida bat viruses, two divergent lyssaviruses co-circulating in a widespread migratory bat species

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In June 2020, a cat died with clinical rabies in Italy after infection with West Caucasian Bat virus (WCBV). WCBV was first described in 2003 in a miniopterus bat (*Miniopterus schreibersii*) from Russia, and never found again. Miniopterus is a migratory species whose geographical range spans across Southern Europe from Portugal to Russia. Other than WCBV, this bat is the natural host for Lleida bat virus (LLEBV). Both WCBV and LLEBV are divergent from rabies virus (RABV) and expected not to be fully covered by current vaccines.
After identifying the natural source of the spillover in an urban colony of miniopterus, we studied the circulation of WCBV and LLEBV in this host. We evaluated the pathogenesis of these viruses in the Syrian hamster after intramuscular (IM) inoculation, in comparison with RABV, using comparable titers determined using the mouse intracranial lethal dose 50.
We founded neutralizing antibodies against both WCBV and LLEBV, supporting the co-circulation of these viruses in the Italian urban population. Salivary swabs and carcasses occasionally found were all negative, confirming low prevalence and shedding. Experimental IM infection showed that IM lethality of WCBV was similar to RABV. WCBV caused a furious clinical manifestation with severe weight loss and spread from the CNS to the kidneys and salivary glands, with few individuals showing intermittent shedding through saliva. On the other hand, LLEBV was not able to kill a single hamster in 40 days, despite its comparable lethality after IC inoculation in mice.
In conclusion, we found that WCBV circulates in its bat host across a wide geographical range and is able to spill over terrestrial mammals. Experimental infection suggest that the pathogenesis of this virus is similar to RABV, with neurological manifestations, multi-organ involvement and salivary shedding. In addition, we showed that LLEBV circulates in the same host in Italy. However, our data suggest a peculiar pathogenesis compared to other lyssaviruses investigated so far, with a constraint to spread from the periphery to the central nervous system.

O165.

Oral immunization with adenovirus-vectored vaccine induces a neutralizing antibody response in mice against Canine Distemper Virus infection

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Canine Distemper Virus (CDV) is a fatal and highly contagious pathogen of multiple carnivores. While injectable vaccines are very effective in protecting domestic carnivores, their use in the wild is unrealistic. Alternative vaccines are therefore needed. Adenovirus (AdV) vectors are popular vaccine vectors due to their capacity to elicit potent humoral and cellular immune responses against the antigens they carry. In parallel, vaccines based on live human AdV-4 and -7 have been used in U.S. army for several decades as replicative oral vaccines against respiratory infection with the same viruses. Based on these observation, the usage of oral administration of replication competent AdV vectored vaccines has emerged as a promising tool especially for wildlife vaccination. Developing this type of vaccine is not easy, however, given the high host specificity of AdVs and their very low replication in non-target species. To overcome this problem, we tested the feasibility of this approach using murid adenovirus 1 (MAV-1) in mice as vaccine vector. We first constructed different vaccine vectors expressing the H or F proteins of CDV either in their native form, or in a shortened form presenting a shortened signal peptide for F, or only the globular part of H expressed or not as a structural protein of the vaccine. These different strains were then used as oral vaccines in BALB/c mice and the immune response to CDV was evaluated. Only the strain expressing the full length CDV H protein generated a detectable and neutralising immune response to CDV. Using this strain, we were able to show that although this type of vaccine is sensitive to pre-existing immunity to the vector, a second oral administration of the same vaccine boosted the immune response to CDV. Overall, this study was able to demonstrate the feasibility of using replicating AdVs as oral vaccine vectors to immunise against CDV in wildlife carnivores.

O13.

Illumination of Mouse Adenovirus-1 lifecycle

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Adenovirus pathogeny is highly variable depending of the host and the route of infection. Taking advantage of this last property, live human AdV-4 and -7 are used as oral vaccines to protect US soldiers against the severe respiratory disease caused by these same viruses when they enter through the airway. The mechanisms underlying the host and route dependence of AdV infections outcomes are still elusive. Murine AdV type 1 (MAV-1), a naturel mouse pathogen, allows to address such questions in an accessible and relevant form. In this study, a MAV-1 recombinant expressing the firefly luciferase (MAV-1 Luc) was generated in order to decipher the virus in vivo tropism following different routes of administration. BALB/c and SJL/J or C57BL6 mice, respectively described as resistant and susceptible, were infected with MAV-1-Luc by intraperitoneal (IP), intramuscular (IM), intranasal (IN), or oral administration, then daily assessed by whole-body imaging. Bioluminescence was detected from MAV1-Luc IM, IN and IP infected mice. IM and IN infections showed respectively local intramuscular and lung signals. IP administration gave rise to a generalized infection with a massive replication in the brain and nasal horns of SJL and C57BL6 mice, while these tissues remained uninfected in BALB/c mice. In all mouse strains, no signal was detected following oral administration. This model provides precious tool to study AdV infection in its host and better understand the variable AdV infection outcome depending on the route of entry into the host.

O166.

A single oral immunization with a replication-competent adenovirus-vectored vaccine protects mice from influenza respiratory infection

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The development of effective and flexible vaccine platforms is a major public health challenge as recently highlighted by the COVID-19 pandemic. Adenoviruses (AdVs) are easy to produce and have a good safety and efficacy profile when administered orally as demonstrated by the long-term use of oral AdV 4 and 7 vaccines in the US military. These viruses therefore appear to be the ideal backbone for the development of oral replicative vector vaccines. However, research on these vaccines is limited by the ineffective replication of human AdVs in laboratory animals. The use of mouse AdV type 1 (MAV-1) in its natural host allows infection to be studied under replicative conditions. Here, we orally vaccinated mice with MAV-1 vectors expressing the full length or the "headless" hemagglutinin (HA) of influenza to assess the protection conferred against an intranasal challenge of influenza. We showed that while the headless HA vector did not generate a significant humoral or cellular immune response to influenza, a single oral immunisation with the full-length HA vaccine generated influenza-specific and neutralizing antibodies and completely protected the mice against clinical signs and viral replication.

52.

High-throughput mapping of virus-host interactions to identify new factors of virulence and pathogenicity for ASFV.

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African swine fever (ASF) is a highly pathogenic disease causing hemorrhagic fever in domestic pigs and wild boar. It is responsible for numerous epizootics, particularly in Europe and Asia, causing major economic losses to the swine industry. The African Swine Fever virus (ASFV) is the etiological agent responsible for this disease. It is a large double-stranded DNA virus encoding for more than 150 proteins. Different works have shown that there is a close relationship between the ability of some viral proteins to modulate the host antiviral response and the attenuation and virulence processes of ASFV. However, only few protein-protein interactions have been described so far to explain how ASFV escape the host immunity, notably by inhibiting the type I interferon (IFN-I) response.

First, we used an unbiased screen to search for cellular partners of 100 viral proteins. We performed yeast two-hybrid screens using these viral proteins of the Georgia 2007/1 strain as baits and identified more than 50 new virus-host interactions. The global analysis of these interactions clearly shows an enrichment for cellular factors involved in the cytoskeleton (KIF15, FNLB, CENPF) and the innate immunity (COPA, TNIP2, TRIM7, CALCOCO2, BANF1).

In parallel, we were interested in the ability of ASFV proteins to individually inhibit the IFN-I pathway. For this purpose, we have screened 100 ASFV proteins using an IFN-luciferase reporter gene system. We showed that at least seven viral proteins (I267L, MGF360-11L, DP96R, MGF505-3R, R298L, DP71L, C962R) contribute to the inhibition of the IFN-I induction pathway. In order to characterize their antagonist effect, a split-nanoluciferase approach was used to screen these viral proteins with a library of 16 major proteins of the IFN-I response. This approach led us to identify IRF3, IRF7, NEMO as new putative targets of ASFV proteins.

By combining different screening approaches, we have already highlighted new mechanisms by which ASFV hijacks cellular pathway for replication and escapes the vigilance of the immune system. Later on, by comparing virus-host interactions that have been (and will be) obtained with attenuated strains of ASFV, we should identify specific targets that could explain the attenuation process at the molecular level.

O167.

Pathogenesis Study in Goats with Recombinant Wildtype- or Vaccine-derived PPRV Expressing Enhanced GreenFluorescentProtein

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Peste des Petits Ruminants Virus (PPRV) is a highly contagious morbillivirus that causes severe disease in small ruminants, predominantly goats and sheep. PPRV is closely related to rinderpest virus, which was eradicated in 2011. Since then the prevalence of PPRV has globally expanded, leading to a huge economic burden in endemic countries. We aimed to study the pathogenesis of two recombinant (r)PPRV strains, a wildtype pathogenic virus and a vaccine strain expressing enhanced Green Fluorescent Protein (eGFP), by experimental inoculation of goats. An improved understanding of the pathogenesis of PPRV will support future studies of the changing epidemiology of this impactful disease.

Pathogenic (Tbilisi/2016) and vaccine (Nigeria75/1) rPPRV eGFP-expressing viruses were generated by reverse genetics. Two groups of five goats were intratracheally inoculated with either virus. Goats were monitored over time for clinical signs and macroscopic fluorescence, and nose, throat, eye and rectal swabs and blood samples were collected. PPRV replication was assessed in blood and swabs, and virus-specific antibodies in serum were measured. Necropsies were performed at regular intervals for histological and immunohistochemical analysis. Clinical signs were exclusively observed in goats inoculated with the pathogenic PPRV, concurrent with the appearance of macroscopic fluorescence from 5 days post inoculation (dpi) onwards. PPRV-genomes were detected in swabs and blood samples from goats inoculated with both viruses. GFP-positive peripheral blood mononuclear cells were detected from 3 dpi. All goats of both groups developed high antibody titres. Post-mortem, macroscopically relatively few lesions were observed, although at 14 days post inoculation PPR-typical zebra stripes were detected in the rPPRV Tbilisi group. In contrast to the macroscopic lesions, evaluation of fluorescence and immunohistochemistry (IHC) at the peak of infection showed prominent PPRV infection of all lymphoid organs and the epithelia of the trachea, bronchi, gastro-intestinal tract, skin and mucosae of the mouth, nose and pharynx, while vaccine strain-inoculated goats remained almost completely fluorescence and IHC negative.
rPPRV-eGFP viruses are an excellent tool to study pathogenesis especially *in vivo* and in case of negative macroscopical findings at necropsy. They will be used in further pathogenesis studies in natural and alternative hosts such as swine and cattle.

86.

A Serological Report on Bovine Herpes Virus Type 1 (BHV-1) in Mid Black Sea Region of Turkey: Traces from Cattle and Sheep local Herds

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Bovine Herpes Virus Type 1 (BHV-1), one of the main causative pathogens of bovine respiratory disease complex (BRDC), a severe multifactorial syndrome, affects animals' productivity leading to significant economic losses in the livestock industry. Recently we detected fatal BRDC cases in many calves of our local herds attributed to a single etiology, and in most lung specimens, (BHV-1) was the dominant virus amid isolation and molecular characterization. Thus we aimed to examine how far the spreads of this obtained local isolate in our household ruminants herds from past to present; for this purpose, a total number of 1750 archived serum samples, cattle (n=1000), sheep (n=750), randomly selected from five provinces of the black sea region in Turkey between 2018-2021 to determine any antibodies circulation against our indigenous isolate (BHV-1 TR4/19) by using the virus neutralization test. Results indicated that the overall prevalence rate for (BHV-1) local isolate was 10.40 (182/1750)%. For cattle and sheep, the Seropositivity rates were 12.40 (124/1000)% and 7.73 (58/750)%, respectively. Following the serum neutralization test 50 (SN50) for the positive samples, neutralizing antibodies fluctuated between titers 1/4 to 1/512 for cattle and 1/4 to 1/128 for sheep, noticing that 1/8 was the most detected titer. The importance of this study arises from being the first research to be carried out with a domestic isolate in our country; given the fact that all previous serological research was achieved with international reference strains, accordingly, the obtained data provided important information about the retrospective situation of this virus local isolates in different states of our region. In addition, although BHV-1 causes a cattle-specific disease, there are many studies on its serological status in small ruminants; in this regard, the detected sheep seroprevalence updated previous results and confirmed the role of small ruminants as a reservoir in transmitting the virus to large ruminants. In this context, we recommend further comprehensive studies to determine the current national epidemiological situation of BHV-1 for the purpose of developing effective control and eradication programs for local livestock.

Keywords: BRD, IBR, BoHV-1, Cattle, Sheep, Seroprevalence, Nothern Turkey

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O14.

Genomic Analysis and Replication Kinetics of the Closely Related EHV-1 Neuropathogenic 21P40 and Abortigenic 97P70 Strains

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Equine herpesvirus type-1 (EHV-1) is a common horse infection resulting in abortion, neonatal foal death, respiratory illness and neurological problems. The number of outbreaks of neurological disorders, known as equine herpes myeloencephalopathy (EHM), has increased considerably since the turn of the century. The genomes of five Belgian and French isolates from the deadly 2021 neurological outbreak in Valencia, Spain, were reported. Surprisingly, none of the isolates showed the A2254G (N752D) mutation in open reading frame 30 (ORF30), which was thought to be a marker for neuropathogenic strains. Genomic analysis was performed on these strains, 6 other historical clade 10 strains (including the Belgian 97P70 abortion strain), the well-characterized neuropathogenic 03P37 (Belgium; clade 11) and Ab4 (UK; clade 1) strains and the abortigenic reference strain V592 (UK, clade 9). BLASTN and MAFFT were used for pairwise identity determination and multiple sequence alignment in the genomic investigation. With only mutations in ORFs 13, 24, 30, 32, 40, 65 and 71, EHV-1 21P40 (a Belgian Valencia isolate) and 97P70 (a Belgian abortigenic strain) revealed a high nucleotide identity (99.96%). Respiratory epithelial cells and monocytic cells ($CD172a^+$) are main target cells for EHV-1. Therefore, we used the equine respiratory mucosal explant model and the equine blood monocytic cells ($CD172a^+$) from isolated PBMCs, as *in vitro* models for EHV-1 infection to compare the replication kinetics of these two genetically closely related strains. At 24 hours post inoculation (hpi), tracheal mucosal explants infected with 21P40 showed fewer clusters of infected epithelial cells as compared to those infected with 97P70. However, a higher number of single infected leukocytes were detected under the basement membrane at 24 and 48 hpi upon infection with 21P40. The percentage of $CD172a^+$ cells infected with 21P40 (4%) higher than with 97P70 (1%) at 24 hpi. Based on the available data and literature, we may conclude that even though very few genomic differences between EHV-1 21P40 and 97P70 strains were shown, Valencia strain EHV-1 21P40 behaved differently form 97P70 but in a similar way as the reference neuropathogenic EHV-1 strain 03P37.

87.

Distribution of Rotavirus C Genotypes in German Pig Farms

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Rotaviruses are major pathogens causing gastroenteritis both in humans and in a large number of mammalian and avian species. The infection can lead to severe episodes of diarrhoea, especially in newborns, and thus to depression, weakness, anorexia and vomiting. In pig farms, these pathogens can cause great economic damage due to reduced growth rates and fatal infections. Suckling piglets up to eigth weeks of age are particularly affected, as maternal antibodies often do not provide sufficient protection. Rotaviruses are divided into different groups (A-D and F-J), of which groups A, B, C and H are circulating in pigs. Diagnostics are often limited to group A rotaviruses (RVA), as these are considered the most important group in pigs. However, research results show that group C rotaviruses (RVC) as leading agent or in combination with other viral and bacterial pathogens are quite capable of causing massive diarrhoea. A large amount of sequence data exists for RVA, on the basis of which a classification system has been established. In practice, the dual system based on viral proteins 4 (VP4; P-type) and 7 (VP7, G-type) is used to classify RVA into different genotypes and serotypes. For RVC, the available sequence data are still very limited, which makes genotyping much more difficult.
The aim of this study was to shed light on the RVC strains circulating in Germany. For that purpose RVC positive samples from different pig farms were collected and analysed by two RT-PCRs each covering the entire open reading frame of VP4 and VP7. The PCR products were sequenced and classified into different G and P types using phylogenetic analyses. So far, 28 VP7 and 15 VP4 sequences have been identified. It has been shown that of the samples analysed, the genotypes G6 and P21 occur most frequently with 75% and 86,6%, followed by G4 and P5 with 14,3% and 6,7%. In addition, a new genotype was found for VP7. For the establishment of an entire RVC classification system, the investigations are to be extended to other European countries.

129.

A novel Betaretrovirus discovered in cattle with neurological disease and encephalitis

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Newly emerging diseases increasingly have their origin in animals and many of them have the ability to infect the central nervous system (CNS). A major part of encephalitis cases in cattle is etiologically unresolved, posing not only a threat to animal but also human health due to the close contact between them. Therefore, the extension of our knowledge of the repertoire of neuroinfectious viruses in livestock is important for the early detection of emerging diseases. Using High-Throughput-Sequencing (HTS) and bioinformatics, we previously discovered a new retrovirus, Bovine retrovirus CH15 (BoRV-CH15), in the CNS of a single cow with non-suppurative encephalitis. Retrospective and prospective examinations by HTS, PCR, and RT-PCR showed the identification of BoRV-CH15 in six additional animals with clinical neurological disease. The cases differed in geographical location and were identified over a period of more than 20 years. Phylogenetic comparison of all BoRV-CH15 genomes with representative exogenous retroviruses showed an affiliation to the genus *Betaretrovirus*, but neither to other known neuroinvasive viruses belonging to the family *Retroviridae* nor to endogenous bovine retrovirus sequences. For *in situ* detection of BoRV-CH15 RNA, fluorescent and chromogenic in situ hybridization (ISH) was performed on formalin-fixed, paraffin-embedded (FFPE) brain tissue slides showing typical signs of non-suppurative encephalitis. We found a clear cytoplasmic distribution of BoRV CH15 RNA mostly in cells morphologically compatible with neurons. This finding supports a causative relation between BoRV-CH15 infection and non-suppurative encephalitis. The identification of six full-length or nearly full-length BoRV-CH15 genomes and the possible association with neurological disease provide a solid basis for further *in vitro* investigations. Continued screening of brain samples is indispensable to determine the significance of these findings and the causative association between BoRV-CH15 and neurological disease and/or non-suppurative encephalitis in cattle.

187.

Susceptibility of Pets to SARS-CoV-2 Infection: Lessons from a Seroepidemiologic Survey of Cats and Dogs in Portugal

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Betacoronavirus (β-CoV) are positive single-stranded RNA viruses known to infect mammals. In 2019, a novel zoonotic β-CoV emerged, the severe acute respiratory syndrome (SARS)-CoV-2. Although the most frequent SARS-CoV-2 transmission route is within humans, reverse zoonosis to domestic and wild animals has been reported. To understand the potential role of domestic animals in SARS-CoV-2 global transmission, as well their susceptibility to infection, a seroepidemiologic survey of cats and dogs in Portugal was conducted. Antibodies against SARS-CoV-2 were detected in 15/69 (21.74%) cats and 7/148 (4.73%) dogs. Of the SARS-CoV-2 seropositive animals, 11/22 (50.00%) were possibly infected by human-to-animal transmission, and 5/15 (33.33%) cats were probably infected by cat-to-cat transmission. Moreover, one dog tested positive for SARS-CoV-2 RNA. Data suggest that cats and dogs are susceptible to SARS-CoV-2 infection in natural conditions. Hence, a one-health approach is crucial in the SARS-CoV-2 pandemic to understand the risk factors beyond infection in a human–animal environment interface.

O168.

Responses of PRRSv Vaccination in Piglets Born from PRRSv Vaccinated, ELISA Responding and Non-Responding Sows

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Introduction
Porcine Reproductive and Respiratory Syndrome virus (PRRSv) vaccination is widely used to control production losses. A previous field study identified the presence of routinely PRRSv-vaccinated, ELISA seronegative sows. Based on this, a follow-up study was conducted, aimed at assessing maternally-derived antibodies (MDAs) and PRRSv-vaccine responses in piglets born from ELISA responding (E+) and non-responding (E-: ELISA seronegative/low seropositive) sows.
Methods
Three E+ and five E- sows were selected in a PRRSv-vaccinating sow herd without wild PRRSv circulation. Eighty-three piglets were followed-up: 29 born from E+ sows and 54 from E- sows. Two third of the piglets were vaccinated (V) at three weeks of age with homologous PRRSv-1 vaccine (20/29 and 36/54). The last third remained unvaccinated (C) and was housed separately. Piglets were blood sampled at three, six and eleven weeks of age; serum was analyzed using ELISA (IDEXX) and PCR.
Results
Before vaccination, 0/54 E- and 29/29 E+ piglets were seropositive in ELISA. The unvaccinated, E-C piglets remained seronegative (18/18) throughout the study, while the number of seropositive E+C piglets was reduced to 8/9 and 0/9 at six and eleven weeks of age, respectively.
Three weeks post-vaccination (wpv), 26/36 (72.2%) E- piglets seroconverted in ELISA, increasing to 28/36 (77.8%) at eight wpv. E+ piglets had a delayed vaccine response: 5/20 (25%) had increased ELISA S/p values at three wpv compared to before vaccination, increasing to 11/20 (55%) at eight wpv.
Early and persistent vaccine viremia was observed in E-V piglets: 27/36 (75%) and 17/36 (47.2%) were PCR positive at three and eight wpv. Three wpv, 6/20 (30%) E+V piglets tested PCR positive, of which five became negative eight wpv. Interestingly, four additional E+V piglets became PCR positive at eight wpv. All C piglets remained PCR negative during the study.
Conclusion
In E- piglets, MDAs were absent and vaccination resulted in early vaccine viremia and seroconversion in approximately ¾ of the piglets. In contrast, the presence of MDAs in E+ piglets inhibited vaccine viremia and early vaccine response. E+ piglets showed a delayed vaccine response, possibly facilitated by secondary vaccine exposure through contact with PCR positive E-V piglets.

O130.

Environmental sampling for the detection of Highly Pathogenic Avian Influenza H5 and Infectious Bronchitis Virus in poultry farms

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Despite implementation of biosecurity, the poultry industry is still facing major threats due to enzootic or emerging viruses. Since 2016, France faced multiple highly pathogenic avian influenza virus (HPAIV) epizootics (2016/2017, 2020/2021 and 2021/2022). Infectious bronchitis virus (IBV) is endemic in poultry, worldwide. Both viruses show a very high potential of dissemination in poultry populations and major consequences on animal health, HPAIV threating public health due to its zoonotic potential. In order to better understand and control the spread of these viruses, we investigated the role of the environment -mostly dust- as virus' vehicle and the relevance of environmental sampling matrix for early detection.
During the 2020/2021 HP H5N8 and the 2021/2022 HP H5N1 epizootics in France, more than 105 animal houses HPAI-infected or suspected were sampled for dry dust, using dry wipes. In addition to dust sampling, aerosols were collected, using 2 collector devices in more than 30 of these houses. Systematically, 20 tracheal swabs were realized for official surveillance analyses. All samples were analyzed by RT-qPCR using officially-approved commercial kit, to compare sampling detection sensitivity. In addition, viral egg isolation assays were realized to confirm the presence of infectious particles in the environment.
In 2022, 25 chicken flocks at high risk for IBV, based on serological surveillance, were monitored using dry wipes in complement to 20 cloacal swabs. Swabs and wipes were analyzed by RT-qPCR using officially-approved commercial kit to compare sampling detection sensitivity. Additionally, vaccination protocols and performance data were collected in order to investigate the relation between IBV infection, vaccination and performances. Finally, viral egg isolation assays and molecular characterization were realized to differentiate IBV vaccinal and wild-type strains.
In both cases, environmental samples, realized on HPAIV and IBV farms, showed highly consistent results with swabs, clearly indicating the high potential of dust sampling for early detection and surveillance. Dust sampling is a fast, simple, cheap and non-invasive strategy with, at least, similar sensitivity performances than swabs. Finally, our data suggest that dust and aerosols could play a major role in the within-farm or farm-to-farm virus dispersion.

O15.

In vivo and *in vitro* characterization of neurotropism of highly pathogenic influenza virus H5N8 (clade 2.3.3.4b) in chicken and duck

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Due to their polybasic cleavage site recognized by ubiquitous proteases, highly pathogenic avian influenza A viruses (HPAIV) replicate systemically, causing significant tissue damage which may involve severe Central Nervous System (CNS) injury. H5Nx HPAIV of clade 2.3.4.4b, in particular subtype H5N8, caused devastating epizootics in recent years, particularly in Europe. During the 2016 epizootic, H5N8 HPAIV infection was associated with severe neurological disorders in the majority of ducks, whereas these were almost non-existent in chickens. To explore the basis for the difference in neuropathology observed in the field between the two species, we analyzed viral replication and host response in the CNS following experimental infections with a 2016 H5N8 HPAIV *in vivo* and in primary cortical neurons culture *in vitro*.

Experimental infection with H5N8 HPAIV showed predominant neurological involvement in ducks whereas chickens were mainly affected by respiratory distress, confirming field observations. RT-qPCR and immunohistochemistry revealed that viral replication was early and intense in duck brain while brain colonization occurred at later stages of infection in chickens. Analyses of the host response in the brain showed more intense and prolonged innate immune and inflammatory responses in ducks compared to chickens. Accordingly, histological lesions were also more severe in the duck brain.

In order to explore the origin of the difference in neurological damage, we developed primary culture of avian neurons. H5N8 replication kinetics were equivalent in duck and chicken primary cortical neurons. In addition, expression of innate immunity and inflammatory genes was similar in chicken and duck neurons. These results suggest that the predominant neurological symptoms observed in ducks are not due to an increased sensitivity of duck neurons, but rather result from an earlier colonization of the brain in ducks compared to chickens, eventually leading to more intense inflammation.

O16.

Artificial insemination as alternative transmission route for African swine fever virus: How infected boars could efficiently spread the disease

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The rapid spread of African swine fever virus (ASFV), an enveloped, double-stranded DNA virus causing severe disease with often high fatality rates in Eurasian suids, remains a threat for pig populations and economies world-wide. Even though research advances steadily, major alternative transmission routes for ASFV have yet to be assessed. In this study, we present evidence that ASFV is detected in boar semen early upon infection and that it can efficiently be transferred from infected boars to naïve sows via the act of artificial insemination (AI). The pork industry heavily relies on semen derived from few boar studs to inseminate many sows at once, which bears the risk of ASFV being distributed nationwide in contaminated semen. We collected blood and semen from four boars daily after inoculation with the ASFV strain "Estonia 2014" and report detection of ASFV genome in the semen at 3 dpi (blood: 2 dpi). A total of 14 sows was inseminated with ASFV-positive semen on two days, using semen of day 4 and 5 pi, respectively. Upon semen transfer, seven sows got infected, all other sows have contracted the virus from their infected penmates on later days. Thirteen sows were pregnant after AI, but 12 aborted pregnancy upon developing high fever. We monitored ASFV detection in blood of all animals and semen of the boars and compared various kits for nucleic acid extraction as well as qPCR to determine the optimal pipeline for early ASFV detection in boar semen. Furthermore, as morphology of fetuses and amniotic fluid differed among the offspring of one sow, we analyzed the organ sacs of all fetuses individually to assess whether ASFV can migrate through the placenta and infect embryos/fetuses of an ASFV-positive sow. In conclusion, we show that AI is an efficient and probably underestimated route for ASFV transmission. Furthermore, utilization of the NucleoMag kit (Macherey-Nagel) for DNA extraction with the VetMAX kit (Thermo Fisher) for qPCR rendered to be most effective in detecting even low amounts of ASFV genome in different, and especially inhibitor-rich, matrices.

Keywords: African swine fever virus; early detection, matrix comparison, validation, alternative transmission ro

O17.

The Histone-Like A104R Protein of African Swine Fever Virus is not Essential for Replication in Cell Culture

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African swine fever virus (ASFV) causes a fatal disease with up to 100% mortality in domestic pigs and wild boar (species *Sus scrofa*), leading to significant economic losses for swine production. Unlike other eukaryotic viruses, ASFV encodes a histone-like nucleic acid-binding protein, pA104R. The A104R gene is highly conserved and present in all ASFV isolates of different genotypes. Moreover, A104R-like sequences have been identified in the genomes of soft ticks, which can replicate and transmit ASFV. Using a virulent genotype IX field isolate from Kenya, we analyzed the importance of A104R for viral replication in a permissive wild boar cell line (WSL), and in porcine peripheral blood mononuclear cells (PBMC) as natural host cells of ASFV. Although previous reports indicated an essential function, the A104R open reading frame (ORF) could be successfully deleted by homologous recombination between a reporter gene-containing plasmid and wild-type ASFV in transfected and subsequently infected WSL cells. Loss of A104R did not detectably affect viral DNA replication or RNA transcription, but led to a moderate reduction of virus titers (approx. 10fold) and plaque sizes (approx. 40%) in WSL cells. However, in PBMC the replication defect of A104R deletion mutants was more pronounced, leading to more than 100fold reduction of virus titers. Whereas reintroduction of the authentic A104R ORF fully restored wild-type virus growth properties, substitution by the similar A104R ORF (95% identity) encoded by an ASFV-like element derived from the genome of an *Ornithodoros moubata* soft tick was not capable of rescuing the deletion mutant phenotype. In line with this, initial experiments indicated a reduced DNA-binding activity of the tick-derived protein. Thus, our studies demonstrated that the A104R gene is not essential for DNA replication or formation of infectious ASF virus in cell culture. However, the observed *in vitro* attenuation of A104R-deleted ASFV indicates that removal or mutation of this gene could contribute to the development of safe live virus vaccines, which are urgently needed in view of the current spread of African swine fever in many parts of the world.

O18.

SARS-CoV-2 omicron infection induces decreased viral replication and inflammation in the upper and lower respiratory tract compared to the D614G and Delta variants in the Syrian hamster model.

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After the first detection of SARS-CoV-2 in 2019 in China, the genetic evolution of the virus led to the emergence of several variants of concern (VOCs) leading to increase in transmission, and/or change in virulence. Omicron, the latest VOC detected in November 2021, is the main variant currently circulating in the world. Using the Syrian hamster model, we investigated the pathology and innate immune response of different SARS-CoV-2 variants (D614G, Delta and Omicron) in the upper and lower respiratory tract at different days post-infection (dpi 1, 2 and 4). Animals were infected with 5.10^3 TCID50 with the different VOCs. Viral replication was assessed in the upper and lower respiratory tract by quantification of viral subgenomic RNA. Nasal swabs were collected up to 4 days after infection and titrated by $TCID_{50}$ assay. Histological analyses were also performed on the olfactory epithelium, (OE) and lungs. To study the innate immune response, the expression of IFNs, cytokines and ISGs was measured by RT-qPCR on days 1, 2 and 4 post-infection in different tissues (OE, lungs, steno glands and olfactory bulb). Omicron-infected hamsters did not lose weight in contrast to animals infected with the D614G and the Delta variants. In the OE, the viral load of Omicron variant is lower compared to Delta and D614G infection but is sustained over days post-infection, whereas from day 2 p.i, the amount of virus decreased about one log in the OE for the other variants infection. From day 1 post-infection, ISGs expression like ISG15 and MX2 is also significantly decreased in Omicron infected animals compared to other VOCs. Our results confirm the decrease of viral replication, induction of ISGs and pathogenicity of omicron. We show than in contrast to other variant, omicron viral load is sustained at the same level in the nasal cavity during several days, which could participate to its higher contagiousness. The low level of ISGs expression could explain the maintenance of omicron replication. Kinetic studies of VOCs infection, in particular investigation at day1 post-infection, are necessary for further comprehension of the physiopathology of SARS-CoV-2 VOCs, and the crucial role of ISGs.

88.

Genetic investigation of the HPAI H5N1 viruses responsible of HPAI epidemic in Italy in 2021-2022

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The last two epidemic waves caused by the H5Nx Highly Pathogenic Avian Influenza (HPAI) viruses of clade 2.3.4.4b occurring in Europe in 2020-2021 and 2021-2022 had devastating economic impacts on the poultry industry of several countries. Between October 2021 and April 2022, Italy reported 317 HPAI H5N1 outbreaks in poultry, which caused the death of 1670 domestic birds. Most of the outbreaks occurred in the highly density poultry populated areas of northern Italy. To shed light on the number of introductions and diffusion dynamics of the H5N1 in Italy, evolutionary analyses (BEAST v1.10.4, Network10, IQtree v1.6.6) were performed on the complete genomes of 342 viruses identified in poultry (N=320) and wild birds (N=22), generated using an Illumina MiSeq platform.
Our analysis revealed at least twelve distinct introductions (seven in domestic birds) which had been caused by six distinct genotypes originating from different reassortment events. We observed a clear clustering by province, which suggested the occurrence of several secondary outbreaks. The Bayesian phylogeographic analysis suggested that the provinces of Verona and Mantua (north eastern Italy) had been the main source of the virus for the other provinces. Furthermore, the Bayesian analyses showing the species contribution to the viral spread revealed that, among the infected species, turkeys acted as the most likely source of the virus for the other domestic birds. Some mutations associated with an increased binding to human-type receptors were detected in the HA protein of all (S137A, H3 numbering) or few (S159N/T160A, H3 numbering) viruses. Besides, two mutations associated to an increased polymerase activity in mammalian cells (NP-N319K and PB2-K482R) were detected in a H5N1 virus from a wild bird.
Our genetic investigation of the HPAI H5N1 viruses responsible of the 2021-2022 Italian epidemic revealed the incursions of multiple viral genotypes. Nevertheless, a single genotype accounted for most of the outbreaks in poultry. Mutations associated with mammalian adaptation were only sporadically identified in the analysed viruses from avian hosts. However, the persistent circulation and continuous emergence of new reassortant viruses in Italy and Europe raise concern for animal and public health.

89.

West Nile Fever antibody surveillance in horses in Finland

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Background

West Nile Fever (WNF) infection has never been detected in animals in Finland (1). Nearest WNF cases has been detected in Germany, where first cases in horses were reported in 2018 (2).

A survey of imported horses was done in Finland in 2012 - 2013. A total of 193 horses from intra EU trade and 8 horses imported from outside EU were tested negative for IgM WNV antibodies (acute infection). IgG antibodies were found in 29 horses from intra EU trade, and 6 horses imported from US. The vaccination status for WNV was known only in one horse in intra EU trade (3).

The purpose of this surveillance was to find out if WNF has spread to Finland in recent years.

Methods

Serum samples from Finnhorse stallions were used for surveillance. Samples were taken before breeding seasons 2021, 2020 or 2019 (N=70, 98 and 15 respectively) from stallions intended for artificial insemination. Finnhorse stallions were selected for WNF IgG and IgM analyze because it is a native breed in Finland. The breed of stallions was checked from breeding association's database.

176 serum samples were analyzed for IgM and 183 for IgG antibodies with commercial ELISA kits according to manufacturer's instructions. For IgM we used ID Screen® West Nile IgM Capture (ID VET Innovative diagnostics, France). Samples were tested for IgG antibodies with IgG ID Screen® West Nile Competition Multi-Species (ID VET Innovative diagnostics, France).

Results

All serum samples were negative for both IgM and IgG antibodies.

Conclusions

The IgM test is highly specific, but the low specificity of IgG ELISA kits meant that it could detect animals infected with other flaviviruses (4). As IgG test is not specific to WNFV-antibodies, this result indicates that those horses are also negative for gross-reactive viruses. Because horses are useful sentinel animals of WNF infection, this surveillance indicates that WNF has not yet spread to Finland.

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19.

Peripheral Inoculation of Usutu Virus Generates a Neuroinvasive Disease in 129/Sv Pups

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Usutu virus (USUV) is an emergent arthropod-borne flavivirus, mostly transmitted through the bite of *Culex spp*. mosquitoes. Originating from Africa, USUV extended its geographic distribution over many European countries in the last few decades. It is now becoming endemic and co-circulates with its close parent the West Nile Virus (WNV), causing seasonal episodes of mass mortalities in various bird species as well as occasional neurological diseases in both healthy and immunocompromised mammals, including humans. The threat that these Flaviviruses represent for public health has no longer to be demonstrated. Yet, the factors responsible for the development of these sporadnionic events are still poorly understood. The increasing number of cases diagnosed in European Union in the past few years thus generates growing interest in the establishment of a relevant and reproducible mammalian model of neuroinvasion following USUV inoculation. Adult immunocompetent mice proved to be quite resistant to USUV infection, with occasional individuals showing a sporadic neuroinvasive disease. Although young mice (< 1 week-old) display an apparent higher susceptibly, no study has specifically addressed this question yet. We thus assessed the age-dependent evolution of the susceptibility of wild type 129/Sv mice to USUV by comparing the outcomes of 6 age groups (5, 7, 9, 11, 13 or 15 days-old) of eight female pups. Every animal received a subcutaneous inoculation of one million TCID50 of Usutu virus (strain USU-BE-Seraing/2017, lineage Europe 3, *GenBank*: MK230892). While all pups infected at 5 days-old died between 6 and 10dpi, lethality dropped to 0% for the 15 days-old group, and the relationship between the age at infection and the mortality rate appeared linear in the intermediate groups. The clinical manifestations shared by all the symptomatic individuals suggested a neurotropic infection, which was confirmed by molecular data, highlighting evidence of neuroinvasion in every case. Interestingly, survivors were exempt from any clinical signs and presented a marked decrease in viral titers and dissemination, suggesting that the outcome of the infection is determined at a very early stage after inoculation. Future studies are needed to characterize the host-pathogen interactions involved in the development of susceptible or resistant phenotypes.

90.

Molecular characterization of emerging asfv genotype ii strains in different italian territories.

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In January 2022, ASF genotype II was first introduced in the nord-est Italy (Piedmont region) and recently, in May 2022, it was identified in central Italy (Lazio region), again in wild board (*Suis scrofa*). This study evaluates the use of Nanopore technology coupled with Illumina technology, for the identification and characterization of ASFV genotype II strains collected from different Italian territories.
Bone marrows and spleens of wild boars from the infected areas were tested by National Reference Laboratory for Swine Fevers (CEREP) in the Biosafety Level 3 laboratory for diagnostic confirmation. The samples were sequenced by Next Generation Sequencing in collaboration with National Reference Centre for Whole Genome Sequencing of Microbial Pathogens: database and bioinformatics analysis (GENPAT). Genome annotation was performed using the GATU software. The annotations were manually verified and curated using the Ugene software package. The maximum likelihood phylogenetic tree was constructed with the Tamura–Nei parameter model, as suggested by MEGA v.7.
The preliminary results confirm the remarkable genetic stability of ASFV genotype II, at least until now. Generally, in countries where multiple mechanisms of ASF transmission (mixed sylvatic and domestic cycles) play crucial roles in the epidemiology of the disease, higher levels of virus variation is observed among isolates. It was hardly unexpected that the samples analysed in this study have extremely high homology with the ones of genotype II strains circulating over a wide geographic area during the last fifteen years, where the involvement of ticks can be excluded.
The *full-length* sequences available in NCBI are not completely representative of the most recent EU and non-EU outbreaks; the same also involved the countries bordering the north-west of Italy, representing a disadvantageous lack for an exhaustive phylogenetic and epidemiological analysis aimed at tracing the Italian outbreaks occurring in January and May 2022. However, the complete sequences publically available analyzed together with the data obtained in this study remark the high sequence similarity of the ASFV strains and a low viral evolutionary rate, which lead, for the moment, to the improbability of tracing the exact origin of the ASFV genotype II outbreaks, which concerned different Italian territories.

53.

Study of the importance of the pORF63 tegument proteins in the biology of gammaherpesvirus infection.

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Gammaherpesviruses (gammaHVs) are widespread viruses that cause lifelong infections in many mammalian species and represent a significant cause of diseases. Thus, in humans, Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with several cancers and is therefore a major subject of research. Among the different constituents of the gammaHVs virions, tegument proteins play major roles in virus entry, morphogenesis and egress but also in early evasion of innate immune recognition. In particular, a potential role in immune evasion has been proposed for the tegument protein encoded by KSHV open reading frame 63 (ORF63). However, KSHV study is limited by the absence of established animal model and by the lack of permissive cell lines. In this work, we used Murid herpesvirus 4, a virus phylogenetically related to KSHV, to decipher the roles of ORF63. Surprisingly, our study revealed the existence of two distinct pORF63 isoforms. While we previously showed that the long pORF63 isoform was essential for the migration of viral capsids toward the nucleus during entry, a role for the short pORF63 isoform had never been addressed so far. In order to investigate the specific function of the short ORF63 protein during infection, we generated distinct viral strains: a full knock-out mutant (multistop mutant), a mutant only expressing the long isoform and a single stop mutant in which only the short form is expressed. A comparative analysis of these mutants revealed that the absence of both proteins was associated with a dramatic growth deficit *in vitro* mainly related to deficient capsid transport during entry and to absence of cell-cell spread. Deciphering the precise functions of pORF63 isoforms during infection could improve our understanding of the gammaHV biology.

O20.

Modeling porcine hemagglutinating encephalomyelitis virus infection *in vivo* and *ex vivo*

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Porcine hemagglutinating encephalomyelitis virus (PHEV) causes vomiting and wasting disease and/or encephalomyelitis in suckling pigs. This study characterized PHEV infection, pathogenesis, and immune response in CDCD 6-day-old pigs. Piglets (n=18) were randomly distributed into PHEV (n=12) or control (n=6) groups. Before inoculation and immediately prior to euthanasia, blood was collected to evaluate viremia and humoral response by RT-qPCR and ELISA, respectively. Viral shedding was evaluated daily using nasal and rectal swabs throughout the study. Piglets in each group were euthanized at 5, 10, or 15 days post-inoculation (dpi). A variety of tissue sections were collected for viral RNA detection, histopathological and immunohistochemical evaluation, respectively. Infected animals developed mild respiratory, enteric, and neurological clinical signs between 2 to 13 dpi. PHEV did not produce viremia, but virus shedding was detected in nasal secretions (1-10 dpi) and feces (2-7 dpi) by RT-qPCR. Of all the tested tissue samples, only liver tissue had no viral RNA detected. The detection rate and RT-qPCR Ct values decreased over time. The highest concentration of the virus was detected in turbinate and trachea, followed by tonsils, lungs, tracheobronchial lymph nodes, and stomach from inoculated piglets necropsied at 5 dpi. The most representative microscopic lesions were gastritis lymphoplasmacytic, moderate, multifocal, with perivasculitis, and neuritis with ganglia degeneration. A moderate inflammatory response, characterized by increased levels of IFN-alpha in plasma (5 dpi) and infiltration of T lymphocytes and macrophages, was also observed in stomach. Increased plasma levels of IL-8 were detected at 10 and 15 dpi, coinciding with the progressive resolution of the infection. Moreover, a robust antibody response was detected by 10 dpi. An *ex vivo* air-liquid porcine respiratory cells culture system showed virus replication and cytopathic changes and disruption of ciliated columnar epithelia, thereby confirming the upper respiratory tract as a primary site of infection for PHEV. This study provides a platform for further multidisciplinary studies of coronavirus infections.

O131.

Characterization of the Subclinical Infection of Porcine Deltacoronavirus in Grower Pigs

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Porcine deltacoronavirus (PDCoV) is a transboundary and emerging pathogen that replicates in small-intestinal enterocytes, causing enteric disease in suckling pigs. Although less severe, the gross and histological changes in the gut of pigs infected with PDCoV are similar to those observed in the other two major enteropathogenic swine coronaviruses, transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhea virus (PEDV). There are no previous reports of PDCoV outbreaks in grower or adult pigs. Thus, the objective of this study was to characterize the susceptibility and dynamics of PDCoV infection in grower pigs under experimental conditions using a combination of syndromic and laboratory assessments. Seven-week-old conventional pigs (n=24) that were pre-screened negative for different swine coronaviruses were randomly distributed into PDCoV (USA/IL/2014 strain, orally) (n=12) and control (culture medium, orally) (n=12) groups. Blood was collected from individual pigs at day post-inoculation (dpi) -7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42 to evaluate viremia and humoral response by RT-qPCR (Tetracore Inc.) and indirect ELISA (based on S1 recombinant protein), respectively. Viral shedding (RT-qPCR) was evaluated every other day between dpi 0 to 42 using pen feces and oral fluids. In addition, the potential infectivity of the temporal fecal and oral fluid samples was assessed in monolayers of swine testicular (ST) cells via observation of potential virus-specific cytopathic effect and expression of nucleoprotein (N) via immunofluorescence assay. No clinical signs were observed throughout the study. No viable virus was detected in pen feces and oral fluids. PDCoV viremia was also not detected, but virus shedding was detected in feces (6-22 dpi) and oral fluids (2-30 dpi), with a peak of detection (% positive pigs and Ct values) at dpi 10. A moderate IgG response was first detected at dpi 10, being significant after dpi 14 compared to the control group, and coinciding with the progressive resolution of the infection. This study demonstrated that exposure of grower pigs to PDCoV results in subclinical infection, making active surveillance based on systematic sampling and laboratory testing critical for accurate detection of PDCoV infection and circulation in grower and adult animals.

132.

Efficiency standardized PRRSV serum RT-qPCR results

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Consistency of PCRs has been improved by the use of commercial extraction and amplification kits, but further improvement may be possible by expressing results relative to a reference standard (RS) -- as is done in basic research when PCR results are expressed as "efficiency standardized Cq" (ECq):

$ECq = E^{-\Delta Cq}$ where E = amplification efficiency and ΔCq = (sample Cq - RS Cq).

That is, ECq represents the fold change in a sample relative to the plate RS. The objective of this study was to explore the ECq methodology for routine diagnostics.

Serum samples (n = 44) were collected from 4 pigs vaccinated with a PRRSV MLV (Ingelvac® PRRSV MLV) on days post-vaccination (DPV) 0, 5, 8, 11, 14, 17, 21 and 28.

Target-specific (PRRSV RNA) and matrix-specific (serum) reference standards (RS) were created by rehydrating and then diluting (1×10^4) PRRSV vaccine (Ingelvac® PRRS MLV) with PRRSV-free serum. Four reference standards were run on each plate.

Testing was performed in 48-well plates using commercial reagents (IDEXX Laboratories, Inc.) and the MIC PCR™ Cycler (Bio Molecular Systems, Australia).

Results, i.e., Cqs and amplification efficiencies (E), were provided by commercial software (MIC PCR™ , v2.10.4). The "E" for each plate was calculated as the mean E of the 4 RSs and the ΔCq for each sample was calculated as (sample Cq – mean RS Cq).

Across 8 plates, the mean plate RS Cq and E responses were 30.6 and 90%, respectively. All serum samples were negative on DPV 0 (ECq 0, Cq ≥ 40) and all were positive on DPVs 5 - 28 (mean ECq = 8.4, mean Cq = 31.1). In this case, an ECq of 8.4 indicated that the concentration of PRRSV RNA was 8.4 times the concentration in the RS.

Expressing Cqs in the context of an agree-upon reference standard could improve test consistency across laboratories. Moreover, all results have a numeric value, i.e., "indeterminate" Cqs become zeros. Therefore, it becomes possible to calculate cutoffs and statistically evaluate diagnostic performance.

134.

Heating or diluting swine oral fluid samples does not improve qPCR detection

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Reports in the literature describe improved detection of nucleic acids in human oral fluids (OF) by heating and/or diluting the sample. In this study, we tested the effect of heating or diluting swine OF samples on the detection of PRRSV, influenza A virus (IAV) and *Mycoplasma hyopneumoniae* (*MHP*).
In Experiment 1, OF samples containing PRRSV (n = 8), IAV (n = 8), or *MHP* (n = 8) were 2-fold serially diluted (neat, 1:2, 1:4, 1:8) using PRRSV, IAV and MHP-free OF as diluent (n = 32 aliquots per pathogen). Each aliquot was split into 4 and randomized to one of 4 procedures: (P1) heat (95°C × 30 m) and direct qPCR; (P2) heat, cool (25°C × 20 m) and direct qPCR; (P3) heat, cool, nucleic acid extraction, and direct qPCR; (P4, i.e., control) extraction and qPCR.
In Experiment 2, OF samples containing PRRSV (n = 9), IAV (n = 10), or *MHP* (n = 10) were split into three aliquots: (D1) undiluted; (D2) diluted 1:2 with OF free of PRRSV, IAV and *MHP*; (D3) diluted 1:2 with TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA). Samples were randomly ordered and then tested.
Nucleic acid extraction and qPCR were done using commercial products (IDEXX Laboratories, Inc.).
In Experiment 1, P4 (control) produced 32/32 positives for PRRSV and IAV and 31/32 for *MHP*. Cumulatively, P1, P2, and P3 produced 1/96 positive for PRRSV, 5/96 for IAV, and 47/96 for *MHP*. Among all positives, P4 produced the lowest Cqs.
The results (means) from Experiment 2 showed no gain with D2 or D3:
PRRSV - undiluted Cq = 32.3; D2 Cq = 34.2; D3 Cq = 36.8.
IAV - undiluted Cq = 29.0; D2 Cq = 30.0; D3 Cq = 29.9.
MHP - undiluted Cq = 33.0; D2 Cq = 33.6; D3 Cq = 33.9.
Contrary to some reports, heat and dilution treatments were detrimental to the qPCR detection of PRRSV, IAV, and *MHP* in oral fluid samples and best results were obtained using standard methods. Examination of the literature showed that studies often did not include comparisons with standard methods.

135.

Use of a endogenous reference gene (internal sample control) in a PRRSV RT-qPCR

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Endogenous reference genes, i.e., housekeeping genes inherent to a specimen, have been used extensively as internal sample controls (ISC) in PCR-based gene expression research and with increasing frequency in veterinary diagnostics. This study evaluated the detection of a porcine endogenous reference control in a commercial PRRSV RT-qPCR (RealPCR*NA PRRS Types1-2 RNA Mix, IDEXX Laboratories, Inc., USA) using samples of known PRRSV status collected under ideal (research) conditions.

Serum (n = 132) and individual oral fluids (n = 130) samples were collected from 12 14-week-old pigs from -7 to 42 days post vaccination (DPV; Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica, Inc., USA). Samples were tested using a commercial RT-qPCR that simultaneously detects both PRRSV RNA and the ISC. ISC Cq results were characterized in terms of distribution and frequency. Thereafter, the 95th, 97.5th, and 99th percentiles of the ISC Cqs were calculated for each specimen using R 4.1.0 (R core team, 2020).

All oral fluid and serum samples were negative for PRRSV RNA at -7 and 0 DPV, with the first positive at 3 DPV for both sample types. The ISC was detected in all samples (n = 262), with mean Cq values of 26.6 in oral fluid, and 27.1 in serum. The upper 95th, 97.5th, and 99th percentiles for ISC Cqs were 29.5, 30.1, and 30.7 in oral fluids and 29.1, 29.3, and 29.5 in serum.

Although not commonly described in veterinary diagnostics, the use of ISCs could be a useful addition to quality management in routine PCR testing. In this study, the ISC response in samples collected under the “best-case scenario” (i.e., under experimental conditions and immediately stored) was uniform over the time and unaffected by PRRSV replication. Thus, preliminary data suggest that failure to detect the ISC implies an irregularity with the sample integrity. Work in progress will evaluate the effect of sample "mishandling", e.g., adverse storage conditions on ISC detection.

133.

Effect of freeze-thaw on the detection of PRRSV RNA by RT-qPCR

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Re-testing samples is common in diagnostic and research settings, but the effect of freeze-thaw cycles on nucleic acid detection by PCR is largely unexplored. The objective of this study was to quantify the effect of multiple freeze-thaw cycles on the detection of PRRSV RNA and a porcine endogenous reference gene (internal sample control, ISC) in serum and oral fluids.

The serum samples (n = 10) used in the study were from pigs experimentally inoculated with wild-type PRRSV (n = 5) and corresponding negative control pigs (n = 5). Oral fluid samples (n = 10; 6 PRRSV positive and 4 negative) were from individually housed pigs vaccinated with a PRRSV modified-live vaccine (Ingelvac® PRRS MLV).

Samples were aliquoted (1 ml) into 2 ml tubes to create 4 complete sample sets. Sample sets were subjected to 2, 5, 10, or 15 freeze-thaw cycles. A freeze-thaw cycle consisted of freezing at -80°C for ~ 8 hours and then thawing overnight at 4°C. After all treatments were completed, samples were tested using a commercial RT-qPCR that detects both PRRSV and the ISC RNA simultaneously (RealPCR*NA PRRS Types1-2 RNA Mix, IDEXX Laboratories, Inc., USA). The effect of freeze-thaw on detection was quantified by linear regression on Cqs using SAS 9.4 (SAS Institute, Cary NC).

In serum, freeze-thaw cycles had little impact on either PRRSV or ISC Cqs, i.e., the slope of the regression was -0.135 (95% CI: -0.37, 0.34) for PRRSV, and 0.018 (95% CI: -0.06, 0.09) for the ISC. In oral fluids, the freeze-thaw effect was more discernable, i.e., the slope of the regression was 0.208 (95% CI: -0.02, 0.43) for PRRSV, and 0.193 (95% CI: 0.06, 0.32) for the ISC.

Samples in diagnostic laboratories commonly undergo freeze-thaw cycles in the course of testing and retesting, but the effect of this process on the results is largely unquantified. This study demonstrated that PRRSV and ISC RNA was relatively resistant to multiple freeze-thaw cycles, albeit more resistant in serum than oral fluids. Further studies are needed to address the freeze-thaw effect on other pathogens and other specimen matrices.

O91.

Genetic and antigenic diversity of Rotavirus A in Danish pigs

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Group A rotavirus (RVA) can cause gastroenteritis in piglets. The virus is genotyped according to variations within each of the eleven double-stranded RNA gene segments. RVA genotypes are often only described by the VP7 (G-genotype) and VP4 (P-genotype) genes, encoding the two outer capsid proteins. A total of 12 G-genotypes and 14 P-genotypes have been detected in pigs [1-3]. The only porcine RVA vaccine (Rokovac Neo, Bioveta) available in Denmark is based on the G5P[7] genotype. The level of heterogenicity between the field strain and the vaccine stain could potentially play a role in the efficacy of RVA vaccines and therefore, it is important to determine the diversity of circulating RVA strains [4].

The objective of this study was to describe the genetic diversity of RVA in Danish pigs including genotyping and comparison of amino acids differences in antigenic epitopes between the field viruses and the vaccine strain. One RVA positive real-time RT-PCR fecal (sock or rectal swab) sample from 63 Danish herds were tested by newly designed conventional PCR assays detecting the VP7 and VP4 genes and subsequently sequenced by Sanger sequencing and/or NGS (Illumina MiSeq).

The sequencing analysis showed that the VP7 genotypes G3-G5, G9-G11 and G26 were detected with G5, G9 and G11 being most prevalent. P[13] and P[23] were the VP4 genotypes most often detected, but P[6], P[7], P[26] and P[32] were also present, and a possible new VP4 genotype P[X] was discovered in one of the herds. Furthermore, several of the samples (30%) contained more than one VP7 and/or VP4 genotype. The G9, P[6], P[13] and P[23] genotypes were only 42%, 25%, 40% and 48% identical to the vaccine strain in the antigenic epitopes, respectively.

This study indicates that there is a diverse distribution of RVA genotypes in the Danish pigs, and mixed genotype infections were prominent. Considering the amount of genetic variance in the circulating genotypes and the major differences in antigenic sites compared to the vaccine strain it is conceivable that vaccine derived antibodies may have an incomplete binding to some parts of the antigenic domains of VP7 and VP4.

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O21.

AlHV-1 infection causes oligoclonal expansion and activation of CD8$^+$ T lymphocytes resulting in bovine malignant catarrhal fever via interaction with T cell signaling pathway

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Alcelaphine herpesvirus 1 (AlHV-1) is a member of the *Gammaherpesvirinae* subfamily and establishes asymptomatic latent infection in its natural host species, the wildebeest. Cross-species transmission to various ruminant species including cattle can occur, resulting in the induction of malignant catarrhal fever (MCF), a deadly peripheral T cell lymphoproliferative disease. Here, we first confirmed in the bovine species that AlHV-1 latency-associated gene expression is essential for persistent infection of CD8$^+$ T cells and MCF development. Next, we performed an in-depth characterization of peripheral CD8$^+$ T cells during bovine MCF. T cell receptor sequencing of both CDR3α and β revealed oligoclonal expansion of CD8$^+$ T cells, and we observed severe transcriptomic and epigenetic changes in CD8$^+$ T cells using RNA-seq and ATAC-seq analyses. MCF was associated with significant enrichment of gene expression involved in proinflammatory cytokine signaling, cell cycle, TCR signaling, chromatin remodeling but reduced expression of genes involved in adhesion. We observed upregulation of *MKI67*, as well as effector molecules like *GZMA*, *GZMK* and *GNLY*. Whereas *TCF7*, *CCR7* and *CD226* were downregulated, exhaustion genes like *PDCD1*, *EOMES* and *TOX2* were upregulated in MCF, confirmed by analysis of open chromatin. Such unique MCF-related transcriptomic program was confirmed in clusters containing infected CD8$^+$ T cells by single-cell RNA-seq analysis. Analysis of the viral genome transcription identified viral genomic regions being expressed in infected bovine CD8$^+$ T cells, such as the region predicted to encode the gene A10. A10 encodes a transmembrane protein containing an immunoreceptor tyrosine-based activation motif (ITAM) and a SRC homology 3 domain (SH3), suggesting interaction with intracellular T cell signaling. We demonstrated that A10 is phosphorylated in T cells *in vitro* and affects T cell signaling. Impaired expression of A10 did not affect AlHV-1 replication *in vitro* but rendered AlHV-1 unable to induce MCF in the rabbit model. Furthermore, AlHV-1 expressing mutated forms of A10 devoid of ITAM and/or SH3 domains induced MCF with a significant delay compared to a wildtype virus. Overall, we provide a thorough description of CD8$^+$ T cell responses during MCF to uncover a novel mechanism explaining how AlHV-1 dysregulates T cell signaling leading to MCF.

136.

Potential Target Antigens for Development of a DIVA Assay Accompanying a Prototype Marker Vaccine for ASFV.

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African swine fever (ASF) is an infectious disease that causes high mortality rates in domestic pigs and wild boar and in consequence, important economic losses in the swine industry. ASF virus (ASFV) is a very complex virus with around 50 structural proteins, some of which are used as target antigens in the available commercial diagnostic tests. At the moment, there are no specific treatment or vaccine available against ASFV.
Under the VACDIVA European project, several promising prototype marker vaccines based on Lv17/WB/Rie1[1], a modified live attenuated genotype II ASFV field isolate, have been produced. The candidate vaccines have gene deletions replaced by a cassette containing a reporter gene to improve their DIVA (differentiation of infected from vaccinated animals) characteristics.
In the present study, we have evaluated the immunogenicity of the proteins codified by the deleted and the reporter genes and their potential as DIVA targets for the further development of a serological DIVA assay. Moreover, the highly immunogenic viral antigen p72, was used as control for the detection of infection and monitoring immunity in vaccinated animals. In this context, the target and the reporter antigens have been recombinantly produced in mammalian and insect cells, respectively, and their immunogenicity were evaluated by indirect ELISA.
A total of 62 serum samples from 7 domestic pigs (DP), experimentally inoculated with the parental virus and collected between 0- and 54-days post-infection (dpi) were analysed: 100 % of the pigs seroconverted against the target antigen after 24 ± 5 dpi and against protein p72, after 15 ± 2 dpi. All pigs resulted negative against the reporter antigen.
In the case of vaccinated pigs, a panel of 193 serum samples from 14 DP vaccinated with VACDIVA candidate vaccines and collected between 0- and 64- dpi were analysed. All animals resulted negative against the target antigen and positive against protein p72 since 15 ± 6 days post-vaccination (dpv). Moreover, concerning immunogenicity of the reporter antigen, the 14 animals tested seroconverted at different times after 16 dpv. Currently, serum samples from wild boar have been tested and the data is under analysis.
These preliminary results of the evaluation of the selected proteins are promising and they seem to be good candidates for the development of a companion serological DIVA assay for the prototype vaccines.

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137.

Improving African Swine Fever Surveillance using Fluorescent Rapid Tests.

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African Swine Fever (ASF) is a highly infectious disease of swine, caused by the ASF virus (ASFV). Infection with ASFV correlates with a wide range of clinical syndromes from almost unapparent disease to haemorrhagic fever with high fatality rates. To date, there are no commercially available vaccines worldwide and therefore, ASF control is based on early diagnosis and the enforcement of strict sanitary measures. Lateral flow assays are user-friendly, low cost, provide rapid results, and have long-term stability, what makes them one of the most widely used techniques for point-of-care testing, accelerating the final diagnosis.

In this work, we described the optimization of two lateral flow assays based on the use of europium-labelled nanoparticles. Using specific anti-p72 monoclonal antibodies, a fluorescent lateral flow assay was developed for the direct detection of ASFV antigen (**Ag-assay**). For the development of the rapid test for the detection of specific ASFV antibodies, p72 ASFV antigen was also bounded to europium-labelled nanoparticles (**Ab-assay**). Analytical sensitivity was determined using a negative blood sample spiked with p72 and with a highly positive serum, respectively.
Finally, a group of samples was analysed. For Ag-assay, a total of 15 experimental positive blood samples were evaluated, and 34 field negative blood samples. For antibodies detection, a total of 48 positive serum samples, and 84 experimental and field negative samples (34 bloods and 50 sera) were evaluated. In the following months this panel will be increased.
For results reading, an UV-lamp or rapid test reader (Pacific Image Electronics) were used indistinctly.

The optimized assays exhibited a significant sensitivity increase compared to colorimetric assays, (up to 16-fold increase in analytical sensitivity). For Ag-assay, sensitivity improved especially for samples with Ct values above 20, reaching an 80 % compared to OIE PCR. Ab-assay showed the same sensitivity as IPT, increasing ELISA and colorimetric LFA's sensitivities.

In conclusion, the assays described in this work exhibited a huge increase in their sensitivity conserving the advantages of rapid tests. Therefore, they are a useful tool for improving ASFV surveillance through a fast and highly sensitive identification of infected animals, and an active surveillance of serological status.

O169.

Developing a 'One Health' Nipah virus vaccine to protect animal and public health

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Nipah virus (NiV) causes a severe and often fatal neurological disease in humans. Whilst Old World fruit bats are the natural reservoir, NiV also infects pigs and may cause a mild/unapparent disease. Pig-to-human transmission was responsible for the first and most severe NiV outbreak in Malaysia and Singapore in 1998-99. This outbreak caused severe and lasting economic costs to the local pig industry. Despite the threat NiV poses to some of the most pig dense regions of the world, no vaccines are currently available. To address this, we are developing a vaccine for use in pigs that would reduce the risk that NiV poses to the pig industry, livestock keepers and public health. We evaluated the vaccine potential of three NiV vaccine candidates in pigs: (1) adjuvanted soluble NiV G protein (NiV sG); (2) adjuvanted pre-fusion conformation stabilised NiV F protein (NiV mcsF); and (3) replication-deficient adenoviral vectored NiV G (ChAdOx1 NiV G). NiV sG induced the greatest NiV-neutralising antibody titres, NiV mcsF induced an antibody response best able to neutralise NiV glycoprotein mediated cell-cell fusion, and ChAdOx1 NiV G induced significant $CD8^+$ T-cell responses. Despite these differences in immunogenicity, prime-boost immunisation with NiV sG protein, NiV mcsF protein, or ChAdOx1 NiV G all conferred a comparable high degree of protection against challenge infection, with significant reduction in NiV RNA and no isolation of live virus from vaccinated pigs. However, neither vaccine was capable of providing effective protection after a single immunisation. A follow-up study of immune responses comparing a single shot to prime-boost immunisation showed a clear boosting effect on T cell and antibody responses for all three vaccines, and both immunisation regimens induced durable responses. Finally, the three vaccine candidates were evaluated, using a prime-boost regimen, under field conditions in Bangladesh where NiV is known to circulate. Indigenous backyard pigs mounted antibody responses comparable to European pig breeds under controlled conditions. In addition to providing a platform for the development of a NiV vaccine for pigs, we hope these studies will also benefit ongoing human vaccine development efforts.

O138.

Assessing the health status of Belgian pig farms using an integrated, high-tech approach

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Infections with pathogens in combination with poor management, and biosecurity can result in significant production losses on pig farms. At present no objective parameters exist to define the health status of a farm. Conversely, research has shown reduced pathogen circulation on farms with improved biosecurity and management practices.

The aim of this study was to investigate pathogen circulation and assess biosecurity (BioCheck.UGent, Biorisk) on five healthy pig farms in Belgium, using an integrated approach with screening tools, and longitudinal sampling. The herds did not suffer from major clinical problems in any age group, nor from poor performance. Within each farm, three sows of the same farrowing room were selected. Two days after farrowing, three viable piglets of each sow were selected and ear tagged. One (n=3) was sampled weekly (nasal-, rectal-, and tracheobronchial swabs) until 12 weeks of age. Samples were transported at 4°C, pooled, and analyzed by nanopore metagenomics sequencing at PathoSense BV. Metagenomics provide genetic information for viruses and bacteria present in the samples. Additionally clinical scoring was performed weekly. Each pen was scored twice for coughing and sneezing during five minutes. Furthermore fecal consistency was scored through visual observation.

As compared to the Belgian overall biosecurity average (74/100), the selected farms scored slightly better (77.8/100). Preliminary data showed that clinical scores fluctuated over time, with mean scores of 14.6/100, 4.4/100, 18.9/100 for sneezing, coughing, and fecal consistency, respectively, indicating good overall health. However, short episodes of elevated clinical scores (>30) were observed on every farm, with a major contribution of increased sneezing scores in the nursery unit (10/54 of total scorings performed compared to 2/54 and 5/54 for coughing and fecal consistency, respectively). In cases with elevated sneezing scores porcine cytomegalovirus (7/10) and porcine hemagglutinating encephalomyelitis virus (4/10) were most often observed. Aforementioned viruses were regularly found together with bacteria of the *Glaesserella* and *Mycoplasma* genus.

These findings shed light on potential roles of rather neglected pathogens in the development of disease in swine. Also, our results reaffirm the importance of co-infections and emphasize the potential of metagenomics monitoring and diagnostics.

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O188.

Characterization of hepatitis E virus in Lithuanian human and animal populations

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Hepatitis E virus (HEV) is a causative agent of hepatitis E infection, prevalent in both human and animal populations of Europe. Wild and domestic animals are often considered to be primary reservoirs for human HEV infections in Europe, mainly associated with transmission via food chain. Molecular characterization and comparison of strains isolated from animal and human samples provide information about cross-species transmission dynamics, while isolation using *in vitro* cell models help explain underlying mechanisms of HEV pathobiology.
As a continuation of previous animal studies in Lithuania, data from pig (n = 384) and wild animal (n = 620) populations was compared to novel seroprevalence and HEV RNA data from humans (n = 366). Following ELISA and reverse-transcription PCR testing, HEV RNA-positive human samples were subsequently selected for analysis by partial ORF2 sequencing and compared to previously acquired strains prevalent in local animal population. Genetically similar strains originating from human serum and wild boar liver samples were subsequently used for isolation in primate (MARC-145 and Vero), pig (PK-15) and murine (Neuro-2a) cells of renal and nervous tissue.
Lower HEV seroprevalence rate in humans compared to wild boars and pigs, but not deer species has been observed. Inflammatory bowel disease (OR=4.541), organ transplantation (OR=4.042) and age (OR=1.032) has been associated with elevated risk of anti-HEV IgGs in humans. Acquired partial ORF2 sequence from the human sample shared high genetic similarity with local animal strains. However, differences in capacity for infection and replication have been observed in cell cultures, potentially associated with quasi-enveloped and nonenveloped forms of HEV.
In addition to capacity of wild boar-derived strain to successfully infect cells of different animal species, including primate cells, genetic similarities between human- and animal-derived HEV strains in Lithuania suggest a capacity for cross-species transmission of local strains. Moreover, infectivity rates of similar strains may be greatly influenced by the tissue of origin used for virus isolation rather than genetic differences.

139.

Outbreaks of African Swine Fever in Ukraine during 2019-2021

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African swine fever causes significant economic damage to pig farming and destroys wild and domestic pig populations. The causative agent is a DNA-containing virus of the *Asfarviridae* family, *Asfivirus* genus. Since 2007 ASF virus has spread from Africa through Georgia to European and Asian countries. In Ukraine, the first outbreak of ASF was registered in 2012. Current outbreaks of ASF are registering in Ukraine and neighboring countries. Given this difficult epizootic situation a State Monitoring Program for ASF has been implemented.

The study aimed to analyze results of ASF testing conducted by authorized State Laboratories of the State Service of Ukraine on Food Safety and Consumer Protection in frame of the State Monitoring Program in 2019-2021.

Methods. DNA were extracted from pathological and biological materials (spleen, lymph nodes, bone marrow, blood) of wild and domestic pigs using following commercial kits: Art RNA MiniSpin (LLC "ArtBioTech"), QIAamp cador Pathogen Mini Kit (QIAGEN), Patho Gene-spin DNA/RNA Extraction kit (iNtRON). Detection of P72 gene of ASFV was performed by qPCR using commercial kits: VetMAX™ African Swine Fever Virus Detection Kit (Applied Biosystems), ASF PCR-RT (LLC "Ukrvetprompostach"), Biocore ASFV (LLC Biocor Technology LTD).

Results. From 2019 to 2021, 129250 pigs were tested for ASF (122744 domestic pigs and 6506 wild pigs). In total, 197 animals tested positive (157 domestic and 40 wild pigs) from 97 outbreaks. In 2019 87/46699 (0.19%) of domestic pig and 22/1949 (1.13%) of wild pig samples tested positive. In 2020 43/38780 (0.11%) of domestic pig and 14/1700 (0.82%) of wild pig samples tested positive. In 2021 27/37265 (0.06%) of domestic pig and 4/2857 (0.14%) of wild pig samples tested positive.

Conclusions. From 2019-2021, 97 ASF outbreaks were registered: 78 in domestic pigs and 19 in wild pigs. The prevalence of the ASF outbreaks among domestic pigs may be attributable to improper measures taken to avoid the entrance and spread of the pathogen. In comparison, lower number of ASF outbreaks among wild pigs may be in consequence lower animal density. More studies are needed to fully understand ASF dynamics in Ukraine.

54.

Replication characteristics of porcine reproductive and respiratory syndrome virus (PRRSV) in a new vein explant culture system

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically important pathogens in swine industry. Macrophages are the sole host cell that can be infected. Sialoadhesin (Sn) and scavenger receptor CD163 mediate virus attachment, internalization and disassembly. Compared to the low virulent Lelystad strain (LV), the highly virulent Lena strain has a broader macrophage tropism in the host as both $CD163^{+}Sn^{+}$ macrophages and $CD163^{+}Sn^{-}$ macrophages can be infected in the respiratory tract. Vessel pathology may be observed with PRRSV but data concerning the primary replication in the environment of blood vessels are absent. In the present study, *ex vivo* models with swine ear and hind leg vein explants were established to study the interaction of PRRSV(LV/Lena) with macrophages in the vicinity of the veins. The replication characteristics of the low virulent PRRSV LV strain and the highly virulent subtype 3 PRRSV Lena strain were analyzed in the established model. At 0, 12, 24 and 48 hours post inoculation (hpi), the viability of the explants was analyzed, and the infected cells were quantified and identified by immunofluorescence stainings.

No significant changes were found in the occurrence of apoptosis in the cells of the vein explants over time. Lena replicated much more efficiently than LV in the macrophages surrounding the veins. More than 90% of the infected cells were $CD163^{+}Sn^{+}$ positive. In addition, Lena strain infected also $CD163^{+}Sn^{-}$ macrophages suggesting that an alternative entry receptor may contribute to the wider cell tropism of the highly pathogenic PRRSV strain.

In this study, an *ex vivo* vein model was successfully established, and PRRSV replication was found in macrophages in the surroundings of the vein, with PRRSV Lena being more virulent than PRRSV LV. This model can now be used with other viruses with a tropism for macrophages and causing vessel pathology such as African swine fever virus.

140.

Development of Serological IgG and IgM Antibody Profiling Assays for Bovine Parainfluenza-3 Virus

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Bovine respiratory disease (BRD) is endemic in young calves leading to substantial economic loses to the sector through impacts on animal health and performance, with a range of viral pathogens including bovine parainfluenza-3 (BPI-3) involved in the early stages of BRD development. Serological-based screening is an important strategy to monitor and maintain herd health. Assays that can profile IgG and IgM levels to such infections can assist with diagnosis and inform therapeutic treatment. This work utilised recombinant forms of viral subunit proteins to develop assays capable of measuring BPI-3 associated IgG and IgM levels in sera and profile changes in BPI-3 specific antibody profiles in pre- and post-vaccination calves.

Methods: Development of indirect ELISA-based assays utilising purified recombinant BPI-3 Hemagglutinin Neuraminidase (HN) subunit protein as antibody capture antigen and HRP-conjugated anti-bovine IgG and IgM as secondary reporter antibodies, with assay specificity demonstrated using viral specific control sera. IgG and IgM antibody levels were time-profiled using developed assays in early (19-32 days old) and late (6 months old) vaccinated calves with levels in BPI-3 viral challenged animals determined. Anti-bovine antibodies were fluorescently (Alexa 647) conjugated to adapt assays for alternative platforms assessing levels based on fluorescent intensity.

Results: HRP- and fluorescent-antibody conjugated ELISA assays using recombinant BPI-3 HN protein as capture antigen demonstrated significant viral specificity when compared with viral specific positive sera. BPI-3 HN antibody profiling showed steeper decline in maternal derived IgM levels relative to IgG levels post-birth. BPI-3 HN IgG assay was shown to profile long-term antibody immune-reactivity with significant levels detectable 14-20 days post vaccination/challenge. The BPI-3 HN IgM assay was found capable of detecting recent infections with significant levels detectable within 8 days of vaccination/challenge, even in the face of maternal antibodies. The adapted fluorescent BPI-3 HN assay showed potential for integration into other fluorescent platforms available for veterinary diagnostics.

Conclusion: HRP- and fluorescent-based serological assays were successfully developed to profile BPI-3 HN specific IgG and IgM antibodies in sera. Antibody profiling showed BPI-3 HN IgM levels were less affected by maternal antibodies with significant changes post-vaccination/challenge detectable in reduced time relative to corresponding IgG levels.

55.

Rotavirus Diagnostic - a View of the Mucosal and Systemic Immune Response.

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Pigs play a major role as meat suppliers for the world population. For economical production farmers need to use the genetic potential of daily weight gain. This requires good health management, hygiene and housing conditions. Despite many efforts' diarrhoea is still often seen in the first days of life which significantly influence the average daily weight gain of the affected animals. This results in a constant economic loss and a reduced health and welfare status of the pigs.
Besides nutritional factors, various pathogens (viruses, bacteria, parasites) play an important role in causing diarrheal diseases. Among them, we have identified Rotaviruses as a partially underestimated trigger. To prevent clinical disease in piglets, it is considered common practice to use maternal vaccines. Despite effective reports this approach does not always lead to the desired success. The lack of cross-immunity between different Rotavirus serotypes and the completely different stimulation of the immune system by parenterally administered vaccines, as opposed to natural infections, may be a reason for lower protection against the clinical manifestation of RVA infection lead.
Two independent studies were conducted to examine this issue in more detail. Firstly, saliva, serum and intestinal mucus samples were obtained from natural infected pigs to investigate the correlations between the antibody concentrations in the different secretions. Secondly, the introduction of a stable-based rotavirus vaccine was monitored diagnostically and the time course of the humoral immune response was followed over 5 months. Immunofluorescence and neutralization test were carried out to determine IgG and IgA specific and serotype-specific neutralizing antibody titers, respectively.
Neutralizing rotavirus-specific titers in saliva provide a better correlate to intestinal ratios than serum titers. Within three months after basic immunization salivary and serum neutralizing titers decreased. In addition, there are different titer courses in saliva and serum.
We assumed that the combination of serum and saliva-based diagnostics can be used to obtain an impression of the serotype-specific stimulation of the systemic and mucosal immune system. In the end, this allows a differentiated conclusion to be drawn about the immune status of the sow and possibly the quality of the colostrum and milk.

22.

Illumination of Cyprinid Herpesvirus 2 Infectious Cycle Using *In Vivo* Bioluminescent Imaging

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During the past two decades, as a consequence of international trading, Cyprinid herpesvirus 2 (CyHV-2) reached a worldwide distribution. This virus causes outbreaks associated with high mortality rate in golfish (*Carassius auratus*) and related fish species (*Carassius gibelio and Carassius carassius)*. Its high economic impact on the aquaculture sector has subsequently stimulated an increasing number of fundamental and applied researches. In the present study, we aimed to use bioluminescent *in vivo* imaging to gain an understanding of some important fundamental aspects of CyHV-2 pathogenesis, such as, for example the portal of viral entry into the host. With that goal in mind, we produced a recombinant strain expressing luciferase and copepod GFP as reporter proteins. Several approaches were developed to compare the recombinant strain produced to its parental strain in term of genome structure, expression of genes flanking the insertion site, replication in cell culture and virulence. All together, these results suggest that the LucGFP recombinant produced exhibits adequate properties for the study of the pathogenesis of CyHV-2. Consequently, this recombinant was used to study CyHV-2 pathogenesis in goldfish using IVIS following different inoculation modes mimicking different epidemiological conditions. These experiments suggested that the skin and the gills are the main portals of entry of the virus into its host and that the digestive track does not act as a portal of entry but rather a portal of egress from the infected host.

O189.

Study of the Effect of Bacterially Produced Secondary Metabolites on SARS-CoV-2 (COVID-19) *in Vitro*

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In the past 20 years, three coronaviruses have emerged with the potential to cause pandemics, as showcased by the newly emerged SARS-CoV-2, the causative agent of COVID-19. Vaccines have rapidly been developed, however, to further reduce viral transmission, and mortality, effective antiviral treatments are still needed.
Lipopeptides are secondary metabolites produced by *Bacillus* spp., which are promising microbial biosurfactants and were shown to have antiviral properties against a wide range of enveloped DNA and RNA viruses, including coronaviruses. In the present work, lipopeptides from the surfactin family (surfactin, lichenysin and pumilacidin) were produced to test their antiviral activity against SARS-CoV-2. Different surfactin-like isoforms, varying in their fatty-acid chain length and their peptide chain composition, were purified. The *in vitro* cytotoxicity of each isoform was determined, and each was tested for its antiviral activity against SARS-CoV-2 on Vero E6 cells.
Surfactin isoforms exhibited the lowest cytotoxicity of the surfactin family, followed by lichenysin and pumilacidin, respectively. Based on these cytotoxicity results, variants were selected and tested for their antiviral activity. Some variants significantly reduced the viral RNA concentration in infected cells by 3-log to 6-log compared to control infected cells. Further experiments on SARS-CoV-2 were carried out to determine the mechanisms by which these lipopeptides inhibit SARS-CoV-2. Some isoforms significantly reduced the binding of SARS-CoV-2 to the cells, with some of them inhibiting the binding of the virus to levels comparable to neutralizing antibodies. Finally, experiments to study the impact of these lipopeptides on the fusion and on the budding steps are being carried out to fully understand the inhibitory mechanism of these lipopeptides.
The present work provides insights to better understand the link between structure and antiviral activity of surfactin-like lipopeptides and could lead to the design of new lipopeptides exhibiting a low cytotoxicity and a high antiviral activity, and, potentially, an effective treatment.

O56.

Inhibition of Arsenite-Induced Stress Granules by Cyprinid Herpesvirus 3

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Eukaryotic cells can express specific mechanisms that quickly block protein synthesis when facing various types of stress, including viral infections. Stress granules (SGs) are discrete cytoplasmic inclusions into which stalled translation initiation complexes are dynamically recruited in cells subjected to stresses. Incubation of cells with arsenite is a well-known process to induce the formation of SGs. Recently, we observed that Cyprinid herpesvirus 3 (CyHV-3) prevents the formation of SGs induced by arsenite treatment of infected cells. This phenomenon was proved to be time-dependent. Resistance to arsenite treatment was observed after at least 12 hours of infection. We also observed that transfection of CCB cells with the entire genome of CyHV-3 cloned as a bacterial artificial chromosome (BAC) conferred protection to arsenite treatment applied 48 hours after transfection. With the goal in mind to identify CyHV-3 ORF(s) causing this phenotype, we produced a series of recombinant CyHV-3 BAC plasmids, each plasmid being deleted for approximately 10 consecutive ORFs. We found that all plasmids were able to inhibit SGs induced by arsenite with exception of the plasmid deleted for ORF100 to ORF112. These results suggested that one or several proteins encoded by this genome region is/are responsible for the resistance observed. Next, we tested the ability of the proteins encoded by ORF100 to ORF112 expressed ectopically in CCB cells to prevent the formation of arsenite induced SGs. We observed that both ORF101 and ORF104 inhibited SGs formation. Finally, BAC plasmids deleted for ORF101 and/or ORF104 were produced and tested for their ability to inhibit arsenite-induced SGs. The single deleted plasmids and the double deleted plasmid conferred partial protection and no protection, respectively. These data demonstrated that ORF101 and ORF104 are the proteins responsible for the resistance to arsenite induced SGs conferred by CyHV-3 infection. Finally, recombinant BAC plasmids were produced to test whether ORF101 and ORF104 are essential or not for viral replication in cell culture. These experiments demonstrated that ORF101 and ORF104 are non-essential and essential proteins, respectively. This study is the first to identify proteins of the *Alloherpesviridae* family able to block the innate immune response of the host cell.

23.

***In vitro* evaluation of nine antiviral compounds for their potential effect against equid alphaherpesviruses EHV-4 and EHV-3.**

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Introduction: Equine herpesvirus 4 (EHV-4) is a common respiratory pathogen of the horse, sporadically causing abortions or neonatal deaths. Despite preventive measures such as vaccination, the resurgence of EHV-4 infection remains a significant threat to the equine industry. Equine coital exanthema is an acute viral infection of the horse caused by EHV-3. Despite the limitations or absence of vaccines, very few studies have been conducted on the search for antiviral molecules against these viruses.
Objectives: to evaluate the efficacy of nine antiviral compounds previously described with antiviral properties against EHV-1 against EHV-4 and EHV-3.
Study design: Determination of the effect of 8 concentrations of nine selected compounds (previously identified for their effect on EHV-1) by Real-Time Cell Analysis (RTCA) on 2 *in vitro* models**:** CCL26 infected with EHV-4 and E.derm cells with EHV-3. Cytopathic effects were monitored by RTCA (xCELLigence® and Incucyte®) and the viral load (EHV-4 and EHV-3) was quantified by qPCR. EC_{50} values for both xCELLigence® and qPCR methods were determined.
Results: The EC_{50} values show that seven molecules have antiviral potential to prevent EHV-4 infection of CCL26 *in vitro*. The nucleoside analogues aciclovir and vidarabine are not effective in preventing EHV-4 infection of CCL26. Aphidicolin is the most effective compound with an EC_{50} value of 1.63±0.76 μM determined by xCELLigence and 0.25±0.12 μM when determined by qPCR. All 9 compounds are effective against EHV-3. Pritelivir and cidofovir are the least effective molecules against EHV-3 (EC_{50} >50 μM). Aphidicolin is also the most effective compound against EHV-3 with an EC_{50} value of 2.43±0.02 μM (xCELLigence) and 1.63±0.48 μM (qPCR).
Conclusions: Seven antiviral compounds (ganciclovir, valganciclovir, decitabine, idoxuridine, pritelivir, cidofovir and aphidicolin) prevent the cytopathic effect of EHV-4 on CCL26 cells *in vitro*. Aciclovir, the most widely used antiviral against alpha-herpesviruses *in vivo*, does not appear to be effective against EHV-4 *in vitro*. The 9 compounds are effective against EHV-3. Aphidicolin is also the most effective compound against EHV-4 and EHV-3.
Declarations: The authors declare no conflicts of interest.

24.

Spatiotemporal pathogen-host interactions during African swine fever virus infection

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African swine fever (ASF) is a disease causing high mortality in domestic pigs and wild boar, and is of global concern due to its large-scale economic, animal health impact, and the fact there is currently no commercial vaccine against the disease. ASF is caused by African swine fever virus (ASFV) a large double-stranded DNA virus, which is the only member of the *Asfarviridae* family, and codes for between 150-167 proteins depending on the genotype. Targeted gene deletion and exogenous expression have been used to characterise the function of many of the viral genes that have been studied, and so there is little information on virus-host pathogen interactions in infected cells and their roles in pathogenesis. This project focuses on several genes, including ASFV genes E183L and A179L. E183L is a model structural gene linked to viral entry into host cells, and may have additional roles in inducing apoptosis and the recruitment of viral membrane precursors. A179L is a model immune evasion gene associated with apoptosis inhibition. Sequences were designed containing E183L and A179L genes fused to the self-labelling tags HaloTag, SNAPf and Strep tag. Transfer plasmids containing the fusion gene, a LacZ reporter gene and viral flanking sequences were generated to recover recombinant viruses expressing the fusion genes in place of the wild type gene. We successfully generated two recombinant ASFV viruses (A179L fused with the SNAPf tag and E183L fused with a twin Strep tag) without wildtype contamination through multiple rounds of selection using a LacZ reporter gene. We were able to demonstrate expression of these tagged genes from modified virus, which displayed growth kinetics comparable to the wildtype virus. These recombinant viruses will be used to identify interacting partners of our proteins of interest using affinity purification mass spectrometry at different times during the ASFV replication cycle. This will further allow us to uncover the host pathways that these ASFV proteins utilise and exploit in order to cause pathogenesis in host cells.

57.

Cross-Reactivity of Pre-and Post-Pandemic Human and Farm Animal Sera to SARS-CoV-2 and Other Coronaviruses.

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Pre- and post-pandemic sera of farm animals and humans, including SARS-CoV-2 convalescent human sera collected from PCR-positive individuals with known SARS-CoV-2 antibody VNT titer, were tested for cross-reactivity with the beta-CoVs Bovine Coronavirus (BCV), Porcine hemagglutinating encephalomyelitis virus (PHEV) and SARS-CoV-2, and with the alpha-CoV Transmissible gastroenteritis virus (TGEV). Neutralization tests were validated using sera from naive animals (cesarian derived/colostrum-deprived and SPF animals), sera from experimentally infected animals with BCV, TGEV, PHEV and Porcine epidemic diarrhea virus (PEDV) and sera from SARS-CoV-2 infected minks as negative and positive controls. A high percentage of human pre- (37%) and post-pandemic (78%) sera and sera from cows, pigs, sheep and rabbits (e.g. 60% for rabbits) contained antibodies able to (cross)-neutralize beta-CoV BCV infection *in vitro*. Except for pig sera, these BCV-neutralizing sera failed to neutralize infection with the alpha-CoV TGEV. Sera with cross-neutralizing activity towards BCV were further tested for their ability to stain PHEV infected cells, bind to immobilized recombinant spike (S1-S2) and NP proteins of SARS-CoV-2 in ELISA's, and to neutralize SARS-CoV-2 *in vitro*. A high percentage of these human and animal sera stained PHEV infected cells and some of them also reacted positive with recombinant NP and S1-S2 proteins from SARS-CoV-2 in the ELISA. However, none of the pre-pandemic human sera and sera collected from farm animals were able to effectively neutralize SARS-CoV-2 infection *in vitro*. This highlights the unique surface structure to which SARS-CoV-2 evolved from its beta-CoV ancestors, especially regarding the immunodominant region on top of the spike protein containing the receptor bindings domain. The higher percentage and increase in strength of BCV neutralization and reactivity for PHEV we observed for post-pandemic convalescent human sera compared to pre-pandemic human sera suggested that SARS-CoV-2 infection activated a "memory" antibody response toward common β-CoV's epitopes. Characterization of these epitopes may contribute to the development of vaccines for humans and animals that confer a broad protection against future emerging "threatful" beta-CoV's and SARS-CoV-2 VOC's.

92.

Detection and prevalence of novel porcine parvoviruses (PPV2-7) in Hungarian pig herds

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Porcine parvoviruses belong to the *Parvovirinae* subfamily within the *Parvoviridae* family. Porcine parvovirus (PPV1) is considered one of the most important cause of reproductive disorders in swine. Infection with PPV1 leads to stillbirth, mummification, embryonic death and infertility (SMEDI). In the last two decades 6 novel parvoviruses were identified in pigs and designated as porcine parvovirus 2–7. The presence of these emerging viruses has been reported in several countries around the world, although their pathogenic role and clinical and economical relevance is largely unknown. So far, PPV2, PPV3, PPV4 and PPV6 have been identified in Hungarian herds. Our aim was to monitor the prevalence of the novel PPVs in Hungary, to investigate the within-herd infection dynamics of the virus.

For the estimation of the prevalence altogether 1855 serum samples from different age groups, 178 oral fluid and 97 processing fluid samples were collected in a systematic, cross-sectional method from 20 large scale swine herds, and tested by real-time qPCR.

PPVs were present in at least one type of diagnostic material in 18 out of the 20 (90%) pig farms. Comparing the viruses PPV1 and PPV4 was detected in 10, PPV2 in 18, PPV3 in 15, PPV5 in 12, PPV6 in 13 and PPV7 in 16 herds, but the within-herd prevalence rates were quite different. The highest detection rates were observed in oral fluid samples, as the proportion of the positive samples ranged from 15% (PPV4) to 54% (PPV7). Most PPVs were also found in the serum samples of all ages, but 10-, 14- and 18-week-old pigs were the age groups with the most frequent detection rates. PPV2, PPV3 and PPV6 were the most prevalent viruses (25%, 18% and 17% positivity, respectively), detected with particularly low Ct values in serum samples.

Our results indicate that all novel PPVs are present and some of them are widely spread in Hungarian pig population. The virus seems to circulate subclinically on the examined farms, as no overt clinical disease was reported in the herds during the period of the samplings.

O141.

Detection of Alphamesonivirus 1 in two horses by a metatranscriptomics approach

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In October 2021, an 18-month-old foal and a 7-years-old mare (Haflinger breed) died after acute respiratory distress syndrome in Miranda (Molise region, southern Italy). Post-mortem examination, histopathology, molecular tests, and metatranscriptomics analysis were performed to elucidate the cause of death.
Methods. For histology, lung and bronchial lymph nodes were collected from the two individuals, fixed in 10% neutral buffered formalin, and processed for H&E.
Specific molecular tests were carried out with nucleic acids purified from retropharyngeal, submandibular, bronchial lymph nodes, lungs, and spleen for detection of *Equine Herpesvirus 1 and 4*, *West Nile Virus*, *Usutu virus*, *Equine Arteritis virus*, *Alphainfluenzavirus,* and *African horse sickness virus*. Metatranscriptomics by high-throughput Illumina sequencing was performed only from the lungs of both individuals.
Results. Post-mortem examinations revealed severe gelatinous subcutaneous oedema in the neck region, hemorrhagic pleural effusion, pulmonary oedema and thickness of interlobular septa, splenomegaly, enlargement of bronchial, submandibular and retropharyngeal lymph nodes and petechial hemorrhages of the mucosa of the large intestine.
Histopathology showed interstitial pneumonia, multiple foci of bronchopneumonia, inflammatory pulmonary perivascular cuffs, and necrotic lymphadenitis. Both individuals showed the same gross and microscopic lesions. More in details, histopathology suggested the occurrence of a viral infection. All virus-specific molecular assays turned out negative.
Illumina sequencing data were analyzed by CZ IDseq (v4.0). After quality filter and host depletion, taxonomically classification was performed and 14,683 (foal) and 746 (mare) reads, respectively, were assigned to *Alphamesonivirus 1 species*, a mosquito-specific virus with extensive geographic distribution and host range. Mapping vs best reference (MF281710) produced nearly complete consensus sequence for both samples (foal HCov 99%, VCov 37.22; mare HCov 87%, VCov 5). Pan-mesonivirus real time RT-PCR confirmed the presence of *Alphamesonivirus 1* RNA in both samples. Phylogenetic analysis using 45 complete genome sequences publicly available placed *Alphamesonivirus 1* sequence of this study with an extant *Alphamesonivirus 1* sequence obtained from an Italian Culex pool in 2018.
Discussion. As far as we know, this is the first identification of *Alphamesonivirus 1* RNA in a vertebrate host. Although also the histology suggested the presence of a viral infection, more studies are reasonably warranted.

O25.

Cellular telomerase RNAs and viral RNAs possess common antiapoptotic functions that enhance herpesvirus-induced tumorigenesis

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Marek's disease virus (MDV) is a highly oncogenic herpesvirus that causes deadly lymphoma in chickens. MDV encodes a telomerase RNA subunit (vTR) that is highly expressed in MDV-induced tumor cells and plays a crucial role in lymphomagenesis. However, the transformation mechanism remains poorly understood. vTR has a high homology to the chicken telomerase RNA (chTR) of 88%, suggesting that it has been pirated from the host genome. In addition, vTR also shares the four main functional domains with all other telomerase RNAs (TR) including human TR (hTR). In this study, we investigated if cellular TRs can complement the loss of vTR in MDV-induced tumorigenesis. Therefore, we replaced vTR with either chTR or hTR in the genome of the very virulent RB-1B strain. Resulting recombinant viruses replicated comparable to wild type virus and the respective TRs were highly expressed in MDV-infected cells. Intriguingly, overexpression of chTR restored disease and tumor formation close to wild type levels. Strikingly, both vTR and chTR inhibited apoptosis in chicken T cells, a property that likely contributes to tumorigenesis. In contrast, overexpression of hTR did not complement the loss of vTR in MDV-induced tumorigenesis. Consistently, hTR only inhibited apoptosis in human cells but not in chicken cells, indicating that the underlying mechanism is not conserved between men and chickens. Our study revealed that overexpression of a cellular TR (cTR) can drive tumor formation and that TRs possess antiapoptotic functions that appear to be host-specific.

142.

Safe and rapid method for sampling African swine fever virus genome and its inactivation in animals´ skin and environmental samples

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African swine fever (ASF) is currently the most dangerous disease for the pig industry, causing huge economic losses. The disease is spreading globally owing to the fact that an effective vaccine or treatment is still unavailable. Only the early detection of ASF virus (ASFV) and proper biosecurity measures are effective as regards reducing expansion in new and endemic areas. Viral introduction into a disease-free territory is primarily facilitated by infected animals (wild and domestic) and their products. In addition, one of the most widely recognized risks, as regards the introduction ASF, is contaminated livestock vehicles.
To improve ASF surveillance at different risk points, we have assessed the capacity for the detection and inactivation of ASFV by using Dry Sponges (3M) pre-hydrated with a new surfactant liquid. In order to take effective action for the rapid identification and further control of ASF, we have sampled different types of surfaces in ASFV-contaminated facilities, including animal skin. The results obtained in two independent studies confirmed that this sampling method allows the viral genome detection from different surfaces with a sensitivity similar to other commonly used methods. The surfactant liquid successfully inactivated the virus, and ASFV DNA was well-conserved at different storage temperatures during more than 30 days, thus allowing the subsequent detection using a quantitative PCR assay.
This is, therefore, an effective method with which to systematically recover loads of ASFV from different surfaces, which has a key applied relevance as regards evaluating the effectiveness of disinfection in vehicles transporting live animals or products at risk of being contaminated. This method provides an important basis for the validation testing of ASFV that can be assessed without the biosafety requirements of a BSL-3 laboratory, which may substantially speed up the early detection of the pathogen and the number of laboratories needed to perform the test. This simple, rapid and economic sampling method will reduce the true risk of ASFV transmission between farms, improve animal welfare and avoid significant economic losses.

26.

Canine Adipose Derived Mesenchymal Stem Cells Support Canid Alphaherpesvirus 1 Infection *In Vitro*

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Since the 1960s Canid alphaherpesvirus 1, a *Varicellovirus* has been recognized to cause fatal hemorrhagic disease in puppies, ocular, respiratory and reproductive disorders in adult canines.
Massive infection of fetal tissues, cell-associated viremia and replication in vascular endothelium are highlights of CHV-1 infection *in vivo*.
Mesenchymal stem cells are located in perivascular area, tether, circulate and home to injured tissues and it is unknown if they could present CHV-1 targets. We sought their permissiveness and ability to support CHV-1 infection *in vitro* in comparison to MDCK, a CHV-1 primary choice for infection *in vitro*. Furthemore, infection-induced changes in gene expression using array technology were analysed.
To deliver the aims, canine mesenchymal stem cell cultures from abdominal adipose tissue (cASCs) of ten females were established in line with ISCT requirements. Initiated cultures were exposed in two passages to CHV-1 field strain, isolated from 6-day-old golden retriever organs.
Spindle-shaped morphology, a lifetime up to six passages in culture, preserved trilineage differentiation and immunophenotype characterized cASCs cultivation.
The first signs of CHV-1 CPE in cASCs ocurring at 30h p.i. in the form of cell rounding, further progressing to clustering already rounded cells and developing characteristic CPE to the full extent in 72-96h were hallmarks of CHV-1 infection *in vitro*. CHV-1 achieved stable titers in MDCK at MOI 0,1, 0,5 and 1 while CHV-1 titers in cAD-MSCS were variable between donors at different MOI and on average lower than in MDCK.
CHV-1 significantly affected expression in 20,23% of genes with overexpression of genes involved in cell growth, chondrocyte differentiation, encoding signaling proteins, maintenance of cell shape and cytoplasm integrity. Downregulated genes regulate cell survival, proliferation, are a component of class I major histocompatibility complex involved in the presentation of peptide antigens to the immune system and membrane receptors involved in cell adhesion.
The effects of CHV-1 seen on cASCs monolayers are confirmed also at the mRNA level proving that CHV-1 directed cell shape, integrity and survival. Variations in infection intensity between biological replicates reflect naturally variable permissiveness to CHV-1. Canine ASCs hold promise in modeling CHV-1 infection and its response to new environment.

O170.

Immunogenicity and efficacy of a novel universal influenza vaccine approach against H3N2 swine influenza infection in pigs.

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Influenza A viruses (IAVs) pose a significant threat to both public and animal health. Seasonal influenza outbreaks, caused by constantly drifting viruses, and the potential of the emergence of novel zoonotic IAVs necessitate the development of a broadly protective influenza vaccines. Current human and swine commercial influenza vaccines albeit protective against homologous viruses, often fail to elicit robust immune responses and protection against even slightly antigenically distinct viruses. The goal of this study was to assess a recently developed influenza vaccine strategy in the swine model as a candidate universal influenza vaccine for both humans and pigs. This novel vaccine platform is based upon sequential administration of low doses of multiple recombinant hemagglutinin (H1 and H3) proteins derived from pre-2009 swine flu pandemic viruses. Additionally, we evaluated the performance of licensed or experimental adjuvants in combination with our antigenic formulation. A total of 72 influenza-seronegative piglets were used in the study and were divided into 9 groups of 8 pigs per group. Animals were vaccinated two to four times, with different combinations of antigens and adjuvants. Eight weeks after the final immunization, pigs were intranasally challenged with A/sw/MO/A01476459/2012, a North American H3N2 swine IAV field isolate, and euthanized 5 days later. Immunogenicity and vaccine-induced protection were evaluated based on five parameters, (i) hemagglutination inhibition (HAI) and (ii) virus neutralization (VN) antibody titers, (iii) virus titers in nasal swabs and tissue homogenate samples, (iv) bronchoalveolar lavage fluid (BALF) cytology at euthanasia (day 5), and (v) respiratory tract histopathology. Our results demonstrated that the addition of squalene (an adjuvant approved for use in humans), and veterinary licensed oil-in-water (OW) and water-in-oil-in-water (WOW) adjuvants significantly improved the breadth of immune responses, and conferred strong virological protection against heterologous challenge.

O27.

DNAJC14 independent replication of the atypical porcine pestivirus (APPV)

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Atypical porcine pestiviruses (APPV, *Pestivirus K*) are a recently discovered, very divergent species of the genus *Pestivirus* within the family *Flaviviridae*. The presence of APPV in piglet producing farms is associated with the occurrence of so-called "shaking piglets" suffering from mild to severe congenital tremor type A-II. Previous studies showed that the cellular protein DNAJC14 is an essential cofactor of the NS2 autoprotease of all classical pestiviruses. Consequently, genetically engineered DNAJC14 knockout cell lines were resistant to all tested non-cytopathogenic (non-cp) pestiviruses. Surprisingly, we found that the non-cp APPV can replicate in these cells in the absence of DNAJC14 suggesting a divergent mechanism of polyprotein processing. A complete laboratory system for the study of APPV was established to learn more about the replication of this unusual virus. The inactivation of the APPV NS2 autoprotease using reverse genetics resulted in non-replicative genomes. To further investigate whether a regulation of the NS2-3 cleavage is also existing in APPV, we constructed synthetic viral genomes with deletions and duplications leading to the NS2 independent release of mature NS3. As observed with other pestiviruses, the increase of mature NS3 resulted in elevated viral RNA replication levels and increased protein expression. The replication-optimized synthetic APPV genomes might be suitable live vaccine candidates, whose establishment and testing warrants further research. Taken together, our data suggest that APPV exhibit a divergent mechanism for the regulation of the NS2 autoprotease activity most likely utilizing a different cellular protein for the adjustment of replication levels.

143.

Development of a Bead based fully automated Multiplex tool to simultaneously diagnose FIV, FelV and FIP/FCoV

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Introduction: Feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and feline coronavirus (FCoV) are serious infectious diseases affecting cats worldwide. Transmission of these viruses occurs primarily through close contact with infected cats (via saliva, nasal secretions, faeces, etc.).
FeLV, FIV and FCoV infections can occur in combination and are expressed in similar clinical symptoms. Diagnosis can therefore be challenging:
- Symptoms are variable and often non-specific.
- Sick cats show very similar clinical symptoms: apathy, anorexia, fever, immunodeficiency syndrome, anemia, etc.
Sample volume for small companion animals for diagnostic purposes can be challenging to collect. In addition, multiplex diagnosis of diseases can contribute to an easier, cheaper and faster workflow in the lab as well as to better differential diagnosis of diseases

Methods: M2 Multiplier from Dynex techonogies (USA) has been used as fully automated platform for Multiplex diagnostics. Plastic beads have been coated with antigens for FIV and FIP/FCoV as well as antibodies for FeLV. Beads are then inserted at fixed positions in a 96well microtiter plate. Feline serum or plasma samples are diluted and processed by the automated system and incubated with the beads. After automated washing a mixture of HRP conjugated antibodies for feline IgGs and FeLV antigen is added, followed by another washing step. Read out of results is performed via chemiluminescence after the addition of Luminol.

Results: Simultaneous detection of antibodies for different diseases as well as antigen detection is possible with a fully automated system. A 100% concordance with singleplex methods like ELISA or IFA can be observed. The system shows a high precission (intraassay and interassay) as well as a high stability (accelerated stability testing) and robustness (no interference with disturbing substances like hemoglobin and triglycerides)

Conclusion: The new tool can be used for multiplex diagnostics of the most important feline infectious diseases. Only a very small sample volume is required. Fully automation results in a very convenient and fast method for diagnosing animal diseases.

O190.

Isolation and Genome Characterization of Bat-borne Issyk-Kul Virus in Italy

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Issyk-Kul virus (ISKV), *Nairoviridae* family, was firstly isolated in 1970 from a *Nyctalus noctula* bat trapped near Lake Issyk-Kul, Kyrgyzstan. ISKV was subsequently detected in nearby countries in Central Asia in several bat species and bat ticks, and it has been described to cause sporadic outbreaks of febrile illness in humans. It has been assumed that bats and ticks are both reservoirs of ISKV with transmission to humans being associated with tick bites and exposure to bat feces and urine. Following the detection of portions of ISKV genome in *Eptesicus nilssonii* in Germany it has been recently suggested that its geographical range expanded to Europe.
In this study an adult female of *Hypsugo savii* spontaneously dead in a wildlife rehabilitation center was sampled and fully necropsied within a surveillance program for emerging viruses associated with bats implemented in Italy. Anamnesis reported sensory depression, inappetence and weight loss. The death occurred eleven days after the admission to the rehabilitation center and no traumatic or pathological lesions were observed at necropsy. After exclusion the presence of lyssavirus from brain, tissue samples from internal organs (lung, hearth, intestine) were subjected to viral isolation on cell culture. CPE was observed on MARC-145 cells and negative staining electron microscopy performed on cells supernatants revealed the presence of spherical enveloped virions of 80-100nm morphologically referable to Nairovirus. Virus identification was confirmed by NGS-sequencing.
Molecular and phylogenetic analyses were also performed. The complete genome sequence revealed the 3 typical Nairovirus segments L(11,978nt), M(4,907nt) and S(1,457nt) encoding protein corresponding to the polymerase protein, polyglycoprotein and nucleoprotein respectively. Blast analysis showed the highest nucleotide identity with strain ISKV LEZ 86-787 for L gene (95.34%), strain LEIV-315K for M gene (81.34%) and strain ISKV PbGER for S gene (97.51%). Phylogenetic trees based on full-length of L, M and S genome sequences confirmed that the new isolate belongs to the ISKV clade within the Keterrah group, genus Orthonairovirus.
ISKV isolation from a synanthropic bat species such as *Hypsugo savii* suggests possible implications for public health and the need for further investigation aimed to better define the viral ecology and diffusion in bats population and its zoonotic potential.

93.

Swine influenza virus evolution and infection dynamics in intensive pig production systems

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Swine influenza virus (SIV) is associated with outbreaks of acute respiratory disease and contributes to chronic conditions such as porcine respiratory disease complex. Recent intensification of pig production systems poses a challenge for the control of SIV due to large population sizes and the continuous introduction of naïve animals. However, little is known about SIV infection dynamics within intensive systems nor how SIV evolves under these conditions. Therefore, a longitudinal SIV surveillance study was performed on three intensive farms comprised of breeding units and their associated weaning and finishing facilities. Farms were visited on 12 occasions over an 18 month period and pigs non-invasively sampled (nasal wipes, udder wipes and oral fluids) from each production stage (gilts, suckling pigs, weaned pigs and finishing pigs). Samples were tested for the presence of SIV by RT-qPCR and virus isolation attempted in eggs for all positive samples. Complete viral genomic or partial (HA and NA segments only) sequences were determined for viral isolates or directly from original clinical material where possible. Levels of SIV specific antibodies present in oral fluid samples were measured by ELISA.
RT-qPCR data revealed that SIV was mostly absent from breeding and finishing sites but was highly prevalent on two weaning units. Although SIV was in continuous circulation on these units throughout the study period, two epidemic peaks separated by a period of low prevalence were observed. SIV specific antibodies in weaner oral fluid samples negatively correlated with viral prevalence at the population level. Sequence analysis revealed that initially, a single strain of SIV circulated on each site, with pH1N1 present on one unit and human-like H1N2 on the other. Comparison of viral sequences from different time-points showed that during the first epidemic wave, viral HA and NA sequences were relatively stable. However, numerous mutations emerged during the second epidemic wave that may be associated with antigenic drift and immune escape. These results suggest a central role for weaned piglets in the evolution of SIV and will contribute to an improved understanding of infection dynamics in intensive pig rearing systems.

144.

Detection of a Low Pathogenicity Avian Influenza Virus H6N1 in Poultry in N. Ireland & parallel infections in the Republic of Ireland & Great Britain

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In 2020 the poultry population in Northern Ireland (NI) exceeded 24 million birds, 63% of which was made up of broilers. The output value in the poultry sector including egg production exceeded €½ Billion; an industry of distinct significant value to NI economy, remaining the single biggest employer in the country.
In January 2020 a small broiler breeder operation Co. Fermanagh in South West NI reported a large number of poultry deaths. Reduced appetence and an increase in egg deformation in addition increased mortality suggested the possible presence of a notifiable avian disease (NAD). NAD presents a major threat to this key industry; particularly in the form of Highly Pathogenic Avian Influenza (HPAI).
HPAI was not suspected as although mortality had increased, it remained relatively low; unusually pronounced diarrhoea was present throughout. Post mortem recorded pale, friable livers; marked congested carcasses but no evidence of petechiation or ecchymosis and only slight congestion in tracheas. Although lungs were congested, there was no consolidation apparent.
Further analysis would reveal NI's first detection of a low pathogenicity H6N1 avian influenza. What followed was the identification of 15 separate infected premises (IPs) in NI resulting in testing of over 3,000 birds and the culling of in excess of 680,000 in efforts to control the spread between early January and late March.
One hundred metres from the first identified case in NI the 1st IP in RoI was detected, resulting in the identification in 14 separate IPs, many clustering near to the NI / RoI border. While these outbreaks progressed a parallel outbreak also of H6N1 started in early February centred mainly in the Scottish Borders; although extending to fewer IPs (7 total), large scale commercial layer operations were impacted. In excess of 1.4 million birds were affected. Overall this rapidly spreading outbreak would require the culling of over 2 million birds across the United Kingdom and the Republic of Ireland before it was brought under control. Here we describe the background and response to that outbreak.

O191.

Determination of pathogenic potential of Spanish lineage 1 and 2 WNV strains in a mouse model

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West Nile virus (WNV) is the most widespread emerging arbovirus in the world. Its transmission cycle is complex and is driven by factors associated to the virus, the vectors, the hosts and the environment. The incidence and spread of WNV have increased in Europe in the last three decades with two genetic lineages (L1 and L2) currently circulating. L1 was first identified and isolated in Spain in 2007. Since then it is spreading in south-western Spain, where cases in horses and humans have been reported since 2010, including an unprecedented outbreak of WNV meningoencephalitis with 77 human cases and 8 deaths in 2020. Meanwhile in Catalonia (north-eastern Spain), L2 has been detected repeatedly in goshawks and mosquitoes since 2017, with no human cases reported yet. In this study, the pathogenic potential in mammals of recent Spanish isolates, (year 2020) from L1(Andalusia) and L2 (Catalonia), was evaluated in a mouse model and compared with previous Spanish isolates.

Groups of twelve four-week-old female Swiss outbred mice were intraperitoneally inoculated with 1000 pfu of each viral isolate and monitored daily for symptoms and death, up to 3 weeks after inoculation.

First clinical signs appeared at 6-7 days-post-infection (dpi) and mortality started between 7 and 9 dpi. Overall, Catalonian L2 isolates showed lower virulence profiles as compared to Andalusian L1 isolates in this animal model. Statistically significant differences in survival curves, and differences in mortality rates and median survival times were found between these two groups of isolates. Pathogenicity profiles of L2 isolates from Catalonia were similar to the observed for B956 strain considered of moderate virulence. Pathogenicity of southern lineage 1 isolates of 2020 were similar to that of isolates obtained previously from the same area, and could be considered as of high virulence.

The results obtained using this mouse model point out that the recent WNV L2 strains circulating in Catalonia seem to pose a lower risk for humans and horses to cause severe illness in case of infection as compared to recent L1 isolates from the area and year where severe human outbreaks occurred in 2020.

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O145.

Detection of Hepatitis E virus RNA in Belgian ready-to-eat pork meat and liver products

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Introduction
Hepatitis E virus (HEV) is a major cause of viral hepatitis worldwide. Most infections in Western countries are caused by genotype 3 (gt3). Foodborne transmission is considered to be a significant route of infection. Pigs and wild boars are identified as the main source of human HEV gt3 infection. However, information about the risk of HEV contamination of specific food products is limited.
Theoretical risk analyses (i.e. product contamination risk and overall infection risk by Risk Ranger analyses) indicated that ready-to-eat products pose the highest risk for HEV contamination.

Materials and methods
High risk pork meat products were purchased from various supermarkets. In total 54 products were collected: 23 liver pâtés, 18 raw dried sausages and 13 raw dried hams. Food matrixes were disrupted by using the TRIzol method with some modifications (i.e. an extra chloroform step for removing fats), followed by RNA extraction (NucliSENS) and HEV RNA detection by RT-PCR. HEV-positive samples were re-analyzed by nested RT-PCR, a fragment of 531 nucleotides from ORF2 was Sanger sequenced and aligned against HEV reference genomes.

Results and conclusion
No HEV RNA was detected in raw dried sausages, but 65% of liver pâtés and 15% of raw dried hams tested positive for HEV RNA. All sequenced viruses belonged to gt3c.
These data indicate a high prevalence of HEV RNA in pork liver pâté products, and suggests ongoing zoonotic infections in Belgium.
Additional experiments (i.e. capsid integrity and cell culture assays) are ongoing to determine infectivity of food borne HEV and ways to circumvent this.

58.

Uncharacterised ASFV proteins expressed in mammalian cells serve as antigenic targets of the antibody-mediated immune response

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African swine fever (ASF) is a devastating disease of wild boars and domestic pigs, which causes massive economic loss in affected countries. Eradication of the rapidly spreading disease caused by the genotype II African swine fever virus (ASFV) is currently not feasible due to a lack of effective treatment. Therefore, vaccine development and improvement of diagnostic methods are of priority importance at the moment. Since previously developed vaccine candidates have been shown to confer only partial protection, the search for novel viral antigens is necessary. The ASFV genome is characterised by complex organization and a highly ordered expression pattern. The functions of the encoded proteins include viral particle assembly, maintenance of genome integrity, viral transcription and host defence evasion. For a range of these proteins, as well as for unstudied ones, antigenic properties remain unknown. For our study, we selected fifteen ASFV proteins, for the most part uncharacterised, to be expressed in three mammalian cell lines (HEK293T, WSL and BHK-21). Protein expression was compared in HEK293T and WSL cells by fluorescence microscopy and further validated by western blotting and ELISA. The expression of several structural proteins has also been optimized in BHK-21 cells. Furthermore, we established an efficient assay using nickel-coated ELISA plates to test the binding of IgG antibodies from ASFV-positive serum to the viral proteins. We used serum samples collected from specific pathogen-free (SPF) pigs infected either with the low-virulence “Estonia 2014” strain or with the highly virulent “Armenia 2008” strain. As a result, six novel antigens (CP123L, I177L, B117L, B169L, EP84R and B125R) have been identified by ELISA, indicating their potential role in the development of the humoral immune response to ASF.

28.

Analysis of Synchronous and Asynchronous *In Vitro* Infections with Homologous Murine Norovirus Strains Reveals Time-Dependent Viral Interference

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Human noroviruses are recognised as a leading global cause of sporadic and epidemic viral gastroenteritis. Viral recombination is a key mechanism shaping the evolution and diversity of noroviruses. Single-cell coinfection by multiple viruses is the ultimate prerequisite to viral recombination. Under natural conditions, various environmental, host, and virus factors may influence the probability of synchronous coinfections and may determine the delay or even the absolute achievability of asynchronous cellular superinfections. Here, we determine the effect of a temporal separation of *in vitro* infections with the two homologous murine norovirus (human norovirus surrogate) strains MNV-1 WU20 and CW1 on the composition of nascent viral populations. WU20 and CW1 were either synchronously inoculated onto murine macrophage cell monolayers (coinfection) or asynchronously applied (superinfection with varying titres of CW1 at half-hour to 24-hour delays). Both quantification of genomic copy numbers and discriminative screening of plaque picked infectious progeny viruses 24 hours after initial co-or superinfection, demonstrated a time-dependent predominance of primary infecting WU20 in the majority of viral progenies. Our results indicate that a time interval from one to two hours onwards between two consecutive norovirus infections allows for the establishment of a barrier that reduces or prevents superinfection; this first demonstration of time-dependent viral interference for noroviruses has clear implications for norovirus epidemiology, risk assessment, and potentially treatment. Further work will focus on the mechanism of the observed interference (pre-or post-entry mode of action analysis using fluorescently tagged viruses) and aims to thus pinpoint the barrier to superinfection.

94.

Seroepidemiological Survey and Complete Genome Sequence of Equine Arteritis Virus in Horses In Serbia

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Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a contagious viral disease that affects equids, mostly horses. The majority of cases are subclinical, although some horses develop clinical signs of acute infection with fever, oedema, conjunctivitis and nasal discharge. In pregnant mares EAV can cause abortions while in young foals fulminating pneumonia and death. Infected stallions after recovery may remain persistently infected, shedding the virus via semen.
The aim of our study was to investigate the EAV circulation in horses in Serbia, as well as to conduct full- genome sequencing of an EAV RS1 strain, isolated from the sperm of a Lipizzaner stallion in the Vojvodina Province.
Blood samples from 340 horses, including 168 mares and 172 stallions, were collected during 2013 and 2014 from 37 locations in Vojvodina , and from cities of Belgrade and Požarevac in Central Serbia. In parallel, 17 semen samples were taken from three Lipizzaner stallions from one stud farm in Vojvodina. Serum samples were tested by virus-neutralization test, while semen samples were analyzed by real-time RT-PCR and subsequently, full-genome sequencing was conducted on one isolated EAV strain by MiSeq mashine and BLASTN program to search nucleotide databases.
The results of our research revealed that the overall percentage of seropositive horses was 15.88% (54/340). The antibody titres varied from 1:4 to 1:512. The seropositivity rate was higher in mares comparing to stallions (18.45 vs.13.37%). Seropositivity increased with age, being 3.03% in young animals up to 34.21% in older horses. Phylogenetic analysis of the complete genome of EAV strain showed that this virus is classified into the European subgroup 2 and is forming a new cluster together with the GB_Glos_12 isolate from United Kingdom from 2015, sharing 86.2% nucleotide identity.
This is the first seroepidemiological study that encompasses a large number of horses and the first report of full-genome sequence of EAV strain isolated from a stallion semen in Serbia. We concluded that EAV is circulating in our region and is necessary to implement an effective surveillance program.

Key words: Equine arteritis virus, horses, serology, whole-genome sequence, Serbia.

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59.

Influenza Pathogens: Improvement of Influenza A Detection Kit to Follow Virus Mutations.

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KEY WORDS: Influenza A, innovative tests, virus mutation,

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 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 (subtypes H1 to 18) are low pathogenic, such as H9. Co-infection between avian respiratory diseases and low pathogenic viruses can lead to significant losses in poultry flocks. In addition, some subtypes containing H5 and H7 are associated with highly pathogenic forms of the disease, with a high mortality rate.
To control influenza A outbreaks, PCR kits have been developed. These kits, designed to target a portion of the gene M, can detect any subtype of influenza A virus.

RNA viruses have high mutation rates and influenza A viruses are known to be genetically labile viruses. During the last influenza season of winter 2021/2022, in Europe, different influenza A strains were circulating and some of them carried mutations on the gene M. These mutations led to a decrease in the performance of the existing kits on the market causing by domino effect, an important health risk for European poultry.

To solve this problem, Innovative Diagnostic has worked on a new formula of the IDFLUA in order to have a kit able to detect with a high sensitivity all types of Influenza A virus including the latest mutations.

95.

First Description of SARS-CoV-2 Infection in Two Feral American Mink (*Neovison vison*) Caught in the Wild.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, has infected several animal species, including both domestic and wild animals. The most susceptible animals to be infected by this virus have been the mustelids and, particularly, the American mink (*Neovison vison*). In fact, numerous fur farms where these animals are caged have reported multiple infections, resulting in their sacrifice to avoid mink to human transmission that has been previously demonstrated. The first reported free-ranging wild American mink which resulted infected was captured in Utah (USA) in August 2020, but this animal was captured in the surrounding of a fur farm where several infected mink cases were previously detected. Therefore, we report here the first SARS-CoV-2 infected feral mink whose infection was not directly related to humans or fur farms. We detect the virus in 2 of 13 brown American mink captured in the Valencian Community (Eastern Spain) during an invasive species trapping campaign. SARS-CoV-2 RNA was detected in the mesenteric lymph nodes of both animals by two-step RT-PCR and was confirmed by sequencing a 397-nucleotide amplified region of the *S* gene, yielding identical sequences in both animals. Furthermore, molecular phylogenetic analysis showed that this sequence was identical to the consensus SARS-CoV-2 sequence from Wuhan. Given that the closest fur farm is about 20 km away from the trapping site, both animals were captured in a pandemic period (January 2021) where the incidence of SARS-CoV-2 in the human population of the neighboring villages was extremely high (about 1,000 per 100,000 habitats), and the high dependence of these animals on water, we suggest wastewater as the source of infection. Thus, our findings appear to represent the first example of SARS-CoV-2 infection acquired in the wild by feral American mink in self-sustained populations and highlight the need for active surveillance in wild mustelids.

O146.

Diagnosis and characterization of a novel strain of EHDV-8 in Tunisia in 2021.

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Epizootic haemorrhagic disease (EHD) is an infectious disease of wild and domestic ruminants sustained by Epizootic haemorrhagic disease virus (EHDV), an *Orbivirus* existing in multiple serotypes transmitted by *Culicoides* midges. In late September 2021, EHDV was reported in cattle farms in central/western Tunisia. It rapidly spread all over the country with more than 200 confirmed outbreaks. Clinical signs included fever, conjunctivitis, lacrimation, drooling, erythema of nasal and oral mucosa, teat erosions. In this work, we describe the identification and characterization through two different Next Generation Sequencing (NGS) approaches of a novel EHDV-8 strain directly from positive blood samples.
Methods. A total of 174 whole blood samples were collected from symptomatic cattle and tested for EHDV RNA by real time RT-PCR. Positive samples were used for virus isolation and NGS analysis by Illumina and MinION technologies. A total of 415 serum samples of cattle were tested by c-ELISA and serum neutralization (SN). Isolates were tested by virus neutralization (VN) for serotype identification.
Results. Complete consensus sequences of the ten genome segments were obtained from six blood samples. Thus, we were able to identify a novel EHDV-8 strain otherwise not typeable with serotype specific molecular tests. VP-2 of EHDV-8 TUN2021 clustered (84% aa identity) with the only available VP-2 of EHDV-8 this latter being the Australian reference EHDV-8 isolated in 1982. Analysis of the other segments placed EHDV-8 TUN2021 more closely to EHDV-6 from South Africa (1996) and Tunisia (2006). Thus, the Tunisian strain is most likely a reassortant between serotypes 6 and 8. Shotgun Metagenomic by MinION produced reads classified as EHDV within twenty minutes from the beginning of the run but was unable to identify the serotype due to limitation of the reference database. VN suggested that these isolated strains belong to serotype 8 whereas SN did not allow the identification with certainty of the serotype as for well-known cross-reactivity between EHDV-6 and 8.
Discussion. This is the first evidence of EHDV-8 in the field. Certainly, more surveillance is needed to assess the origin of this strain.

O96.

Vaccination Strategy Framework Against African Swine Fever in Wild Boar

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African swine fever (ASF) continues to expand threatening wild and domestic pig populations worldwide. The insidious nature of ASF virus allows the infection to perpetuate in the environment and silently spread (e.g. through ingestion of infected waste food), particularly in wild boar populations which have maintained an own epidemiological cycle in most of the European Union (EU). The difficulty of implementing control measures such as stamping out or scanning surveillance in wild boar have boosted research in ASF vaccines, together with the fast worldwide expansion of ASF in the last years. Wild boar vaccination has been applied successfully in the EU in the past against classical swine fever (CSF) or tuberculosis, and vaccination strategies have been modelled for some other wildlife species and diseases (i.e. rabies, foot-and-mouth-disease). CSF and ASF share the same population at risk when there is no vector involvement and routes of transmission are also similar. Here, we propose a vaccination strategy framework to contain ASF in the wild boar population. First, we identified three different epidemiological scenarios in the EU, according to prevalence and incidence trend, risk of re-introduction, spatio-temporal analysis of notifications and population at risk. Second, we identified the eco-epidemiological models that could help elucidate who, when, where and how to vaccinate. Finally, we identified and fed the population, habitat and transmission model parameters based on a literature review, an epidemiological survey, field data of wild boar movement and interactions, and data analysis from experimental studies with acute and attenuated ASF strains and vaccine candidates. This work in progress is developed under the EU funded project VACDIVA and will continue with simulating the epidemic in the different scenarios with and without a vaccine candidate that is under research, together with a sensitivity analysis to account for data uncertainty.

O29.

Study of the potential of the *in vitro* replication capacity of porcine reproductive and respiratory syndrome virus PRRSV strains to predict their virulence *in vivo*

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The etiological agent of Porcine Reproductive and Respiratory Syndrome (PRRS) is the PRRS virus (PRRSV), a member of the *Arteriviridae* family. One of the main characteristics of PRRSV is its high variability, which leads to the appearance of important differences in virulence among isolates. Thus, several studies have showed the existence highly virulent isolates that induce a more severe disease, while data from the field suggest the existence of isolates of lower virulence. However, up to date pathogenicity can only be accurately determined by *in vivo* studies. Therefore, the development of methods that allow predicting the virulence of a particular isolate *in vitro* would represent an important advance in the investigation of this disease and help to fulfill the 3R principle. Consequently, the objective of this study was to determine whether the *in vitro* replication dynamics of PRRSV-1 strains in different naturally existing PRRSV-permissive pig cell types can be used as a virulence predictive factor.
To achieve this objective, a total of 7 PRRSV-1 isolates previously characterized *in vivo* and classified as isolates of high (n=3) or low (n=4) virulence were used to infect primary cultures of porcine alveolar macrophages (PAM), splenic CD163+CD169- macrophages, splenic CD163-CD169+ macrophages, unfractionated bone marrow cells (BM) and porcine blood monocytes. The cultures were collected at different time-points post-infection (p.i.) to determine growth curves and the *in vitro* replication capability of each virus.
The results of this study indicate that highly virulent isolates replicate to a higher titers than those of lower virulence. In addition, the results confirm that the dynamic of infection differs between cell types. Thus, viral titers reached their peak at 24-48 hours p.i. in PAM cultures while in BM cultures viral titers increased progressively until 72-96 hours p.i. Nonetheless, it is remarkable that cells from different pig donors differ in their susceptibility to infection and in their capability to reveal differences in virulence between PRRSV isolates. Nonetheless, and altogether, the results show the relevance of the study of the *in vitro* replication capability of PRRSV isolates to initially screen their potential virulence.

97.

Pathotyping and Genotypic Characterization of Avian Orthoavulavirus 1 (Newcastle Disease Virus) Viruses from Wild Birds, Poultry and Captive Pigeons in Denmark

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Newcastle disease is a notifiable poultry disease caused by a virulent form of Avian Orthoavulavirus 1 (AOaV-1), also known as Newcastle disease virus (NDV), that belong to the paramyxoviridae family. AOaV-1 viruses can infect a variety of wild birds, pigeons and poultry. The clinical signs in affected birds can vary and virulent strains may cause up to 100% mortality, thus posing a serious threat to animal welfare and poultry production. Even avirulent strains can mutate and become virulent when infecting poultry. To control Newcastle disease, many countries, including Denmark, use mandatory vaccination. However, there is growing concern that commonly used vaccines may be ineffective against AOaV-1 strains emerging in Europe. Surveillance and knowledge of currently circulating strains is therefore critical for predicting and preventing new outbreaks.

In this study, we pathotyped and genotyped AOaV-1 viruses detected in wild and domestic birds in Denmark during the last decade. The viruses were detected in sick or apparently healthy wild birds and in other captive birds. RNA was extracted from the viruses, and the full-length fusion (F) gene was amplified by RT-PCR and sequenced on an Illumina MiSeq platform. The viruses were genotyped by phylogenetic analysis and pathotyped based on the sequence of the F-gene cleavage site. Preliminary data show that an AOaV-1 virus from captive pigeons was virulent and belongs to Class II genotype VI.2.1.1.2.2, while the viruses found in Danish wild birds and poultry were avirulent strains belonging to Class I genotype 1.2 and Class II genotype I.2.

O60.

Cytotoxic $CD4^+$ and CD4/CD8 Double Negative T cells Correlate with Protection against PRRSV1 Transplacental Infection

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Vertical transmission is one of the key events in *porcine reproductive and respiratory virus* (PRRSV) pathogenesis. Vaccination provides partial protection against transplacental transmission, but vaccinated sows can be infected by heterologous strains. The present study investigated the role of cytotoxic lymphocytes, particularly cytotoxic T lymphocytes (CTL), in the protection against vertical transmission under field conditions in an PRRSV1 infected farm. With this aim, a cohort of primiparous sows (n=18) never exposed to PRRSV except for two doses of vaccination (Porcilis PRRS) was bled on the 10^{th} week of gestation, that is before the critical period for transplacental infection could happen. Sows were followed until the end of gestation to determine which sows delivered infected (n=8) or healthy piglets (n=10). Next, we compared the proportions and functionality of CTL, NKT, and NK cells through a range of assays, including phenotyping, intracellular staining of IFN-γ and TNF-α, and proliferation assays. No differences in the proportions of NK, NKT or T-cells were observed. However, upon *in vitro* re-stimulation with the field-circulating virus, sows that delivered PRRSV1-free piglets displayed a higher frequency of virus-specific $CD107a^+$ IFN-γ-producing T cells, which accumulated in the $CD4^+$ compartment, including the CD4 single positive (CD4 SP) and CD4/CD8 double positive (CD4/CD8 DP) subsets. Also, the same group of sows harboured a higher proportion of $CD107a^+$ TNF-α-producing T cells that mainly accumulated in the CD4/CD8 double negative (CD4/CD8 DN) subset. Consistently, the proliferative response of CD4 SP and CD4/CD8 DN T cells to the virus was significantly higher in sows that delivered PRRSV1-free piglets. As regards NKT or NK cells, no difference was observed between two groups of sows. Our data strongly suggest that CTL responses are correlated with the protection against PRRSV1 transplacental infection, being executed by CD4 T cells or CD4/CD8 DN T cells.

O98.

Infection by a highly virulent PRRSV1 strain modifies the dynamics and shedding pattern of influenza A virus in an endemic pig farm.

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Influenza A virus (IAV) is endemic in pig farms of Europe. Circulation of the virus may start in the farrowing units and then spreads to nurseries. Pigs with prolonged nasal shedding (up to 3 weeks) are commonly found. Moreover, some animals infected at early ages got reinfected later. It has been proposed that the presence of maternally-derived antibodies may result in impaired development of the immune response to the virus after the infection. Here we describe how the infection by a highly virulent *Porcine reproductive and respiratory syndrome virus* (PRRSV) strain changed the dynamics of IAV and increased the proportion of prolonged shedders and reinfected animals. The affected farm was a 1,400-sow farrow-to-wean operation. H1avN2 IAV has been detected in weaners and growers. Nurseries had recurrent episodes of cough. IAV was monitored in a longitudinal follow-up (October-December 2021). Nasal swabs (NS) and blood were collected at 1, 3, 5, and 8 WOA from 40 animals and analyzed for IAV (NS) and PRRSV (NS and blood). A second follow-up was performed in February-April 2022. Forty animals were followed at 1, 3, 4, 5-8, and 12 weeks of age as above. In the first study, IAV infections concentrated (50% of positive NS) at 3 weeks of age (WOA) with only 1 additional positive later. No prolonged shedders or reinfected animals were observed. Mortality (birth to 8 weeks) was 17.5% (71% of it coincidently with the circulation of IAV). In the second follow-up, the farm had been infected by a highly virulent PRRSV1 strain, derived from the Italian strain PR40. IAV was found in NS of animals from 3 until 8 WOA. Prolonged shedders (2-4 weeks of shedding, 42.5%) were always found after 3 WOA, and reinfections (30%) were observed after 6 WOA. Mortality reached 50%. PRRSV was found in 80% of the animals at 3 weeks of age. Of note, nasal shedding of PRRSV persisted 4-5 weeks in >25% of pigs (average Ct: 31.1±4.1). This case illustrates the impact that virulent PRRSV1 had on the health of the farm in general and in the IAV infection pattern.

171.

Outbreak investigation and genotyping of lumpy skin disease virus strains circulating in ethiopia: *rdna vaccine construct*

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Lumpy skin disease (LSD) is an OIE notifiable viral disease in cattle and buffaloes, under the genus *Capripoxvirus*, family *Poxviridae*. Its global distribution covers most areas of Africa, the Middle East and Southeast Europe. LSD is significantly an important cattle disease in Ethiopia which has an estimated economic losses of USD 1176 per affected herd, (USD 489) in the subsistence and (USD 2735) in the commercial farm type annually. LSD control highly depends on vaccination with a "live attenuated vaccines'' in Ethiopia. However, this vaccine has poor efficacy and can result in clinical complications for the animals. We have done a report based outbreak investigation in the central and south parts of Ethiopia from September 2021 to April 2022. From a total of 8 districts, 26 tissue (skin nodular) samples were aseptically collected from clinically positive animals. Virus isolation and identification were done on primary lamb kidney (LK) and MDBK cell lines at the National Vaccine Institute (NVI), Ethiopia and 4 isolates on LK and 11 MDBK cells had shown good cytopathic effect (CPE). LSDV DNA has been extracted from cell culture and transported to the laboratory of host-pathogen interaction (HPI), KU Leuven. Full-length LSDV RNA polymerase subunit (RPO30) and G-protein-coupled chemokine receptor (GPCR) genes were amplified and confirmed for their identity 560bp and 1158bp respectively and sent for Sanger sequencing. Genotyping and phylogenetic analysis were done for wild isolates with vaccine strains currently used in Ethiopia. The resulting phylogenetic tree revealed the new isolates were more related to LSD vaccine strain/KS-1/ and previous Ethiopian LSDV isolates (accessed from Gene bank). Alignments of amino acid (AA) sequences were also shown that conserved AA sequence within LSDV new and previous isolates. In the meantime, a highly immunogenic LSDV gene corresponding to vaccinia virus proteins A27L, B5R, and L1 expression is ongoing for the rDNA vaccine trial in animals. The information gained from this study can produce a valuable direction in the improvement of LSD control strategy, especially on the vaccine.

Keywords: *Cattle*, *Ethiopia, Lumpy skin disease, Genotyping, vaccine, virus*

147.

The first detection of PRRSV type 2 in Serbia

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most common and economically devastating infectious diseases in the pig industry worldwide. PRRSV is divided into two genotypes: Type 1 (European type) and Type 2 (North American type) PRRSV. PRRSV type 2 in Europe was first reported in Denmark followed by reports from many other countries. However, wild type of PRRSV type 2 was only confirmed in Germany and Hungary, while in other countries the PRRSV type 2 is described as a modified live vaccine (MLV)-related strain. The first evidence of PRRSV in Serbia dates from 2006. Since then only PRRSV type 1 has been detected, with the majority of strains belonging to lineage 3, clade 3C. To control PRRS, many farms in Serbia use active immunization against type 1. After the vaccination was implemented on a farm, the clinical signs kept occurring in weaners. In March 2022, by testing 80 serum samples from different categories, circulation of wild PRRSV type 1 was confirmed in weaners starting from the 3rd week after weaning. Genotype 2 specific real-time RT-PCR was not carried out. However, since PRRS was not brought under control using vaccination, and specific clinical signs had been manifesting in the intervals, PRRSV diagnostics was repeated by testing new 90 samples after 2 months after the first sampling. PRRSV type 1 specific real-time RT-PCR was negative, but type 2 specific PCR confirmed the genome of PRRS type 2 in weaners, again starting from the 3rd week after weaning. Retrospective real-time RT-PCR testing of earlier 80 samples revealed that the weaners were infected also with PRRSV type 2 at that time. Further to this finding, complete ORF5 and ORF7 were sequenced and confirmed that the detected virus belonged to genotype 2. This is the first evidence of PRRSV type 2, and the first report of PRRS type 1 and type 2 cocirculation on a farm in Serbia. Further investigation that will be carried out should reveal the way of the virus introduction and its relationship to other PRRSV type 2 circulating strains in Europe.

O30.

Predicting infectious bursal disease virus pathotype: new models based on early changes in blood cell formula and bursa cells transcriptional activity

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Infectious bursal disease virus (IBDV), the etiological agent of Gumboro disease, is a worldwide spread immunosuppressive virus. It infects young chicks (*Gallus gallus*) and targets B cells produced in the bursa of Fabricius, inducing immunosuppression in survivor animals. IBDV are classified into 4 pathotypes depending on the mortality and immunosuppression they induce: vaccinal (non pathogenic), strictly immunosuppressive, classical virulent and very virulent. No pathotype-specific predictive viral marker has been identified and the current approach to assess pathotype relies on animal experiments. This study first purpose is to use machine learning models to investigate to which extent early changes in host blood cell formula, uricemia and bursal viral load may be pathotype-specific and secondly to determine pathotype-specific transcriptional markers. Thus, specific-pathogen-free (SPF) chicken were infected with similar doses of very virulent, classical, strictly immunosuppressive or vaccinal IBDV strains. At 2 and 4 days post infection (dpi), the bursal viral load, uricemia and total blood cell counts (B cells, T cells, monocytes, granulocytes, thrombocytes and erythrocytes) were determined and bursas were sampled for transcriptomic analysis. The most performant machine learning model, random forest, determined that the 4 dpi variation of the blood cells formula was sufficient for pathotype determination.
Secondly, transcriptional activities of bursal cells at 2 dpi were investigated using 3'RNAseq. 8313, 9044 and 8096 genes were differentially expressed in strictly immunosupressive, classical and very virulent groups, compared to mock-infected group.
Several biological processes were found to be differentially affected depending on pathotype : B cell proliferation and differentiation, immune response and adherence junction. Those observations are consistent with IBDV lymphotropism and its associated histological lesions. Thirteen marker genes were identified to allow pathotype discrimination and are under investigation for validation.

To conclude, we propose a new *in vivo* protocol for pathotyping IBDV, quicker than the classical one, relying on early changes of the blood cell formula. We also identified transcriptional markers which are candidates for the development of an *ex vivo* pathotyping model using primary B cells culture. Both those models respond to the 3 R principle by reducing and refining animal uses in experimentation.

99.

Interest of Non-lethal Sampling Methods in Detection and Genotyping of Carp Edema Virus in Koi Carp Trade

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Koi Sleepy Disease (KSD) and Carp Edema are emerging diseases both caused by Carp Edema Virus (CEV), which belongs to the *Poxviridae* family. They may be associated with severe gill damages and high mortalities in common and koi carps. CEV has been detected in many countries, sometimes associated with trading context. As koi fish are often expensive and sometimes considered as pets, traditional CEV surveillance, which requires lethal sampling methods, is usually not performed in non-symptomatic batches. The aim of this study was the development of new non-lethal sampling methods for CEV early detection.
All samples were collected from a French wholesaler facility between 2019 and 2022. Since CEV has been detected in this facility following the first koi fish imports from Japan in 2017, sampling was limited to koi batches imported from Japan.
For all samples, CEV DNA detection and quantification were performed by qPCR. We collected different shipping environmental samples (water and bag swabs). Some symptomatic and asymptomatic batches were monitored few weeks after their arrival by gill swabbing and analysis of naturally dead fish gills. Partial P4a gene sequences were also analyzed to genotype positive samples.
CEV DNA was detectable in most shipping water and/or shipping bag swabs of batches coming from different Japanese breeders. When gill swabs were performed and/or dead body analyzed, results were not always consistent with shipping environment results. In some cases, monitoring shipping water showed higher sensitivity than analyzing few gill swabs per batch.
As expected, all genotyped samples clustered in genogroup II. Indeed, genogroup II is usually associated with koi carp and genogroup I with common carp. Despite all batches has originated from Japan, sequences were very similar to strains reported in various countries. We also noted that samples were genetically closer when Japanese breeders are located in the same prefecture.
We will keep monitoring CEV strains imported in this facility, looking for partial P4a sequence variation over time. We also aim to study other genes to confirm these analyses.

148.

Comparison of serological tests for SARS-CoV2 antibody detection in animals

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Several animal species are reported to be susceptible to SARS-CoV-2 infection, in particular mustelids, felines, dogs and some rodents, with potential zoonotic implications. The suitability of commercial and in-house kits for the serological diagnosis of SARS-CoV-2 in animals was investigated through the detection of different types of antibodies (Abs) such as IgG, total or neutralising antibodies against different SARS-CoV-2 antigens (nucleocapsid (N) protein or Spike Receptor-Binding Domain (RBD)).
Anti-N Abs were detected using a commercial indirect ELISA and two different double-antigen-ELISAs (DAS-EL), one commercial and one in-house, for the detection of total Abs in animal sera. For the detection of Abs against RBD, the virus neutralisation test (VNT) was used as the "gold standard" techniche. Other tests included two different ELISAs for total neutralising Abs (surrogate virus-neutralisation test (sVNT)) and one DAS-EL for total Abs anti-RBD. The comparison of the different serological methods was performed considering the method and type of Abs detected and was calculated using the inter-rater agreement statistic (K) and diagnostic accuracy (table 2x2) for each test. A panel of 300 samples with known status was used (120 minks, 61 humans, 51 dogs, 18 pigs, 16 lions, 9 guinea pigs, 9 rabbits and 5 hamsters) divided into 140 positive and 160 negative sera based on available information, such as anamnestic and clinical data, date of collection, experimental infections, PCR.
A better antibody response to RBD than to N was observed, as demonstrated by the higher sensitivity of the diagnostic tests for anti-RBD Abs compared to anti-N. Regarding ELISAs for anti-N Abs, a better diagnostic performance was observed when the two DAS-ELs N developed and validated for animal sera were used, showing a perfect agreement between them (K: 0.83, 95%CI:0.074). On the other hand, all the methods used to detect anti-RBD Abs produced better results, except for one sVNT test that showed poor diagnostic performance. In contrast, an excellent agreement was found between both VNT and the other sVNT (K: 0.97, 95%CI:0.027) and between VNT and DAS-EL S (K: 0.97, 95%CI:0.041).

61.

Identification of MHC-I presented PRRSV peptides reveals epitopes within several non-structural proteins recognized by CD8$^+$ T cells

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most relevant porcine pathogens worldwide. Active control of the disease relies on modified life virus vaccines (MLVs), as most inactivated vaccines provide limited protection. Neutralizing antibodies occur late in infection, hence CD8$^+$ T cells are considered important correlates of protection in PRRSV infected pigs. Our aim was to identify viral peptides naturally processed by the immunoproteasome and bound by the class I major histocompatibility complex (MHC-I), and confirm their ability to stimulate CD8$^+$ T cells. For this purpose, we immunoprecipitated the MHC-I/peptide complex of PRRSV infected porcine alveolar macrophages to isolate the viral epitopes and analyze them with liquid chromatography coupled to mass spectrometry. With the identified peptides, we stimulated peripheral blood mononuclear cells (PBMCs) and measured the PRRSV-specific CD8$^+$ T cell response with an intracellular cytokine staining. Our data reveals that PRRSV non-structural proteins encoded in open reading frame 1a and 1b present the major source of MHC-I presented peptides. The biological relevance is shown as some of these epitopes are able to trigger IFNgamma responses in PBMCs *in vitro*. Overall, we provide an elegant method for the identification of immunogenic MHC-I bound viral epitopes that could be generally applied to other porcine pathogens.

O100.

Genetic and Antigenic Characterization of Respiratory Coronaviruses of Swine

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Porcine respiratory coronavirus (PRCV) and porcine haemagglutinating encephalomyelitis virus (PHEV) are coronaviruses that cause respiratory illness in pigs. PRCV is associated with the porcine respiratory disease complex where coinfection with bacteria and other viruses exacerbates signs resulting in economic losses. PHEV also causes vomiting and wasting disease and encephalomyelitis particularly in piglets less than 3 weeks old, where infection can result in mortality. Globally, PRCV and PHEV are infrequently monitored. Thus there is limited data on the evolution of these viruses and their potential risk to public health despite all 3 major coronavirus epidemics being associated with zoonotic transmission.
In this study, we analyse the genetic divergence of 10 PRCV and 7 PHEV isolates from Belgium, Germany and the Netherlands collected from 2020 to 2022 from pigs exhibiting signs of respiratory illness. They were diagnosed by metagenomic analysis, isolated in cell culture and sequenced. The sequences of the spike protein were compared to previously circulating isolates and to human coronaviruses, HCoV-229E and HCoV-NL63 for PRCV and HCoV-OC43 and HCoV-HKU1 for PHEV. Antigenic relationships were determined by virus neutralization (VN) assays with swine hyper-immune sera raised against 1 historical and 1 contemporary isolate of PRCV and PHEV.
Hyper-immune sera raised against a PRCV isolate from 1991 still reacted with a PRCV isolate from 2020 in the VN assay. Similarly, the PHEV isolates from 1972 and 2020 showed cross-reactivity in the VN assay. Both for PRCV and PHEV, VN antibody titres against the homologous and heterologous strains showed ≤4-fold differences. These preliminary findings indicate that there have been minimal antigenic changes. Further analysis is still ongoing.

192.

Development Of An Immortalized Swine Respiratory Cell Line For Influenza A Virus Research

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The most frequent bidirectional transmission of H1N1, H1N2 and H3N2 influenza A virus (IAV) subtypes occurs at the human/swine interface. Swine serve as an important intermediate host species for generating novel IAVs with pandemic potential because of the host's susceptibility to IAVs of swine, human and avian origin. Primary respiratory cell lines are used in IAV research to model the host's upper respiratory tract *in vitro*. However, primary cell lines are limited by their passaging capacity and are relatively time-consuming for use in industry and research pipelines. We were interested in developing and characterizing a biologically relevant immortalized swine respiratory cell line that could be used for efficient propagation and characterization of swine IAV isolates. Lung tissue for the generation of primary swine respiratory cells were isolated from the bronchi of an 8-week-old Yorkshire/Hampshire pig, which were immortalized by transduction of the SV40 T antigen using a lenti-virus vector (abm). The transduction of the SV40 T antigen was confirmed by rt-qPCR in cells passaged greater than twenty times. Immortalized swine respiratory cells were shown to primarily express alpha 2-3 sialic acid receptors and were susceptible to swine IAVs of H1N1, H1N2 and H3N2 subtypes. In addition, we observed an increase in IL-6 and TNF-a fold-gene expression following individual inoculation from a swine H1N1 isolate (A/Sw/IA/EZ15/2015) compared to inoculation from either a seasonal human H1N1 (A/NewCal/1999) or pandemic (A/CA/07/2009) strain of the same subtype. We assessed the use of the immortalized swine respiratory cells for propagating a H3N2 swine field isolate (A/swine/NC/KH1552516/2016) from various lung tissue of swine used in experimental infections and found comparable virus titers to the gold-standard Madin-Darby Canine Kidney (MDCK) cells. In the future, we intend to investigate the ability of the immortalized swine respiratory cell line to propagate a H3N2 human field isolate (A/TX/50/12/2012) experimentally infected in swine. In addition, to utilize next-generation sequencing technology to compare the genome integrity of the human H3N2 experimental isolates propagated in either the immortalized swine respiratory cell line or MDCK cells.

31.

Equine herpesviruses in the environment: what are the risks in terms of contamination?

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Introduction: Nine equid herpesviruses have been described in horses (equid herpesvirus 1 (EHV-1) to EHV-9). EHV-1 and EHV-4 are highly contagious viruses with respiratory tropism that cause rhinopneumonitis. The primary form of the disease is an upper respiratory tract infection. However, EHV-1 can cause more severe secondary forms such as abortions and myeloencephalitis. Transmission of these viruses is mainly by direct contact with infectious secretions or by inhalation of aerosols. However, several studies highlight the involvement of inert surfaces as a source of contamination. The COVID-19 pandemic has highlighted the importance of taking into account the survival of the virus in the environment and has also underlined the scarcity of studies in this field.
Objectives: 1/ To study the survival of EHV-1 and EHV-4 in rainwater at three different temperatures. 2/ To investigate the presence of the virus during an episodic EHV-4 in a stud farm on different surfaces (leather, metal grid and trough).
Material and methods: The survival of EHV-1 and EHV-4 in rainwater was studied at +4°C, +20°C and +34°C for up to 21 days and 28 days, respectively. The infectivity of viruses was assessed by cell culture (conventional method and real-time cell analysis) and by qPCR. During an EHV-4 outbreak, samples were collected with wipes from different surfaces up to 45 days (D+7, D+11, D+17, D+24, D+45) after the start of the outbreak. The samples were analysed by qPCR.
Results: EHV-1 remained infectious for 21 days in rainwater at +4°C and +20°C. Infectivity was lost on day +7 in water at +34°C. EHV-4 remained infectious for 28 days at +4°C while infectivity decreased after 7 days at +34°C. The presence of the EHV-4 genome, during monitoring at the stud, was found on the different surfaces studied at all times.
Discussion and conclusion: These results demonstrate that equine herpesviruses can survive in water, and maintaining their infectivity, on different surfaces. The survival of the virus in water and on different surfaces should be a warning in case of an epidemic to take measures to avoid contamination by contact.

193.

Comparison of Replication Kinetics and Disease of Human and Swine Influenza A Viruses in Pigs

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Influenza A Viruses (IAVs) pose a significant threat to both animal and human health. Key species in the ecology of IAVs include, among others, wild and domesticated birds, swine, and humans. IAVs are characterized by (a) rapid genetic and antigenic changes (genetic drift), which reduce the protection acquired by previous exposures and reassortment (genetic shift), in which coinfecting viruses exchenge gene segments during replication. This process can lead to novel and potentially pandemic progeny viruses. Most threatening among these is reassortment between viruses from different species, leading to novel reassortant strains against which no previous immunity exists. Experimental data has shown that swine are susceptible to human IAVs (huIAV), and huIAV gene segments are found when sequencing circulating swine IAVs (swIAV). This indicates frequent reverse zoonotic events and subsequent increased risk of reassortment with circulating swine viruses. However, wholly human viruses are rarely isolated during surveillance efforts. This brings into question how susceptible swine really are to infection with huIAVs, and the frequency at which these reverse zoonotic events occur. To help answer these questions, we infected naïve piglets with either human (huIAV), swine (swIAV), or the pandemic 2009 virus (pdmH1N1). In contrast to infection with either the pdmH1N1 or swIAVs, infection with a huIAV resulted in extremely limited clinical disease. In agreement with this, huIAVs replicated poorly in the lower respiratory tract and resulted in less infiltration of inflammatory cell types in bronchoalveolar lavage fluid (BALF). Analysis of BALF also revealed a notable difference in the expression of pro-inflammatory cytokines, supporting a poor replication phenotype in the lower respiratory tract. huIAVs were also shed for a shorter duration, and at a lower titer as determined from nasal swabs. These results help explain the lack of detection of huIAVs in swine populations, and how these infections go unnoticed, allowing opportunities for reassortment.

O62.

Porcine nasal and lung macrophage subsets isolated by FACS and LCM show different transcriptomic profiles depending on tissue origin and location

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Macrophages have a pivotal role during viral infections in pigs. By expressing cell surface receptors, macrophages become viral targets and reservoirs. In the case of upper respiratory tract infections, many viruses target the peripheral nasal mucosa. Recent *in vivo* and *in vitro* (primary nasal cell cultures) porcine reproductive and respiratory syndrome virus (PRRSV) studies demonstrated that several macrophage subsets exist in the porcine nasal mucosa, and that virus strains with different virulence showed altered tropism for these different macrophage populations. To further investigate these macrophage subsets, total RNA sequencing was performed in parallel on two subsets from the nasal mucosa and lung macrophages. Macrophages were isolated by either fluorescent activated cell sorting (FACS; cf. bulk RNAseq) or laser capture microdissection (LCM; cf. LCM RNAseq) in combination with immunofluorescence staining against two macrophage markers, CD163 and Sialoadhesin (Sn). With both RNAseq methods, nasal macrophages showed a different transcriptomic profile compared to lung macrophages. Differentially expressed genes were identified in the two subsets of nasal macrophages. Gene set enrichment analysis on the three macrophage populations showed that GO terms and KEGG pathways on LCM RNAseq data were more specific to the spatial location of macrophage subsets than bulk RNAseq data. Cell type signature analysis revealed that nasal $CD163^{+}Sn^{-}$ cells resemble squamous epithelial cells (LCM RNAseq) or antigen presenting cells (bulk RNAseq) while nasal $CD163^{+}Sn^{+}$ cells are more like fibroblasts/stromal cells (LCM RNAseq) or vascular endothelial cell (bulk RNAseq). Our results confirmed that not only macrophages in different tissues but also macrophages in different areas within the same tissue have different transcriptional programs, suggesting their differential roles in their interaction with pathogens and corresponding immune responses.

O149.

Infectious bronchitis virus and its variants in Canada

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INTRODUCTION

In Canada infection of chickens with infectious bronchitis virus (IBV) was for a long time limited to sporadic outbreaks. Between 2000-2011 IBV-positive samples that were genotyped contained primarily strains related to variant IBVs circulating in the US at that time. However, in early 2012 and in 2013 an increased number of submissions related to IBV infection were reported. These cases involved multiple commodity groups and resulted in increased mortality due to respiratory disease and nephropathogenic involvement in broilers, and/or caused egg-production drops in egg-laying birds. The field situation shifted in 2016 when IBV emerged as the most important viral chicken pathogen causing severe disease and resulted in increased losses affecting all commodity groups.

RESULTS

Based on sequence comparisons of the hypervariable region of the IBV spike protein gene from over 1,500 IBV-positive samples field strains detected in Canada from 2000-2022 could be divided into 5 major groups: 1) vaccine-like, classic viruses, such as Connecticut and Massachusetts; 2) "domestic" Canadian variants not described elsewhere, such as strain Qu_mv; 3) variant viruses related to strains described in the US such as DMV/1639/11, California/1734/04, CU/82792/GA98 and Pennsylvania/Wolg/98; 4) Exotic, non-Canadian, non-US viruses, such as strain 4/91 (793b); 5) "untypable" IBVs not showing significant similarity to previously described IBV strains.

DISCUSSION

Massachusetts-type vaccines are the most common IBV vaccine type used in Canada, occasionally used in combination with Connecticut-derived IBV strains. These two vaccines could provide some, albeit low levels of cross-protection against certain IBV variants, possibly due to cross-reactivity involving cytotoxic T lymphocytes. However, 4/91, California 1734/04 and DMV/1639/11 -like outbreaks were often associated with more severe disease in all chicken commodity groups. Therefore, it appeared that challenge with these IBV strains cannot be adequately mitigated by vaccines available in Canada and alternative vaccination protocols will need to be contemplated.

150.

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

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Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by the Crimean-Congo hemorrhagic fever virus (CCHFV). The virus causes an often-fatal hemorrhagic illness in humans, with up to 50% case fatality rate. On the contrary, animals do not develop clinical symptoms upon infection, but viremia and CCHFV-specific antibodies can be observed. The detection of anti-CCHFV antibodies in livestock is therefore used to reveal CCHFV infection risk areas. CCHFV-specific antibodies can be detected either by viral neutralization, ELISAs or indirect immunofluorescence assays.

Materials and methods

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

For assay validation, 95 cattle and 176 small ruminant sera from animals from CCHF endemic regions served as a CCHFV positive reference serum panel. The CCHF antibody status of the positive reference samples had been previously confirmed by three serological assays (FLI-inhouse ELISA, species adapted VectorBest ELISA and Euroimmune IFA). 402 cattle and 808 small ruminant sera from Germany and France served as negative serum panel, as both countries are considered outside of the CCHFV endemic zone. Moreover, sera from monkeys, camels, rats, ferrets, raccoon dogs, raccoons, foxes, hares, pigs and humans were tested, in order to determine the suitability of this novel ELISA for these species.

Results and discussion

All negative reference sera (n= 2136) were confirmed by the novel CCHF double antigen ELISA indicating a specificity of 100% (CI 95%: 99.8% - 100%). 268 of 271 positive reference sera were tested positive for CCHFV-specific antibodies which means a sensitivity of 98.9% (CI 95%: 96.8% - 99.8%).

151.

A new ASF triplex qPCR, with ambient temperature shipping, offering ultra-rapid results

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Introduction

Control and eradication programs for African Swine Fever (ASF) require accurate and reliable diagnostic tests. IDvet developed a new triplex qPCR kit for ASF diagnosis, ID Gene™ African Swine Fever Triplex (IDASFTRI), which offers ultra-rapid (35 min) or rapid (55 min) protocols, and exogenous and endogenous controls to ensure accurate results. The kit is ready-to-use, and can be shipped at ambient temperature worldwide, reducing shipping costs and the environmental footprint.

Materials and methods

The kit simultaneously amplifies VP72 gene target DNA as well as endogenous and exogenous non-target positive controls.

Analytical specificity was evaluated with 40 reference ASFV DNAs provided by the European Union reference Laboratory (EURL, CISA-INIA, SPAIN) and the National Reference Laboratory (ANSES Ploufragan, France) and 22 other pathogens involved in animal disease.

Analytical sensitivity was evaluated with a synthetic nucleic acid and the limit of detection of the PCR (LDPCR) was determined. The Method Detection Limit (MDL) was determined by using negative swine blood, bone marrow, and oropharyngeal fluids samples spiked with the genotype I ASFV Georgia 2007/1 strain at 107,8 HAU/mL (NRL laboratory for ASF, ANSES Ploufragan Laboratory). The spiked samples were extracted with IDvet's nucleic acid purification columns (SPIN) and magnetic beads (MAGFAST, 20 min).

The performance of the rapid and ultra-rapid amplification programs were compared by FLI, Germany, on 67 characterized field samples, 69 experimental infection samples and 35 ASF strains of different genotypes.

Results and conclusion

The kit successfully detected all isolates and all genotypes tested without cross-reactions with other pathogens, showing 100% inclusivity (97/97) and 100% exclusivity (22/22).

The LDPCR (95%) was established around 5 copies/PCR. The MDL obtained with IDvet's extraction methods for spiked swine oropharyngeal fluids samples was 102.8 HAU/ml, and 103.8 HAU/mL for spiked swine blood and bone marrow samples.

The ultra-rapid and rapid amplification programs show excellent agreement (Kappa=1 ; mean difference $\Delta Cq = 1$).

The kit offers:

- the fastest amplification protocol on the market (35 minutes)
- excellent performance: high inclusivity on all tested ASFV strains; LDPCR = 5 copies / PCR
- Optimal test reliability thanks to endogenous and exogenous controls included in the kit
- Ready-to-use liquid format with economical ambient temperature shipping worldwide

O63.

Frequent infection of cats with SARS-CoV-2 irrespective of pre-existing enzootic coronavirus immunity, Brazil 2020

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Carnivores such as cats and minks are highly susceptible to SARS-CoV-2. Brazil is a global COVID-19 hot spot and several cases of human-to-cat transmission have been documented. We investigated the spread of SARS-CoV-2 by testing 547 domestic cats sampled between July-November 2020 from seven states in southern, southeastern, and northeastern Brazil. Moreover, we investigated whether immune responses elicited by enzootic coronaviruses affect SARS-CoV-2 infection in cats. We found infection with significantly higher neutralizing antibody titers against the Gamma variant of concern, endemic in Brazil during 2020, than against an early SARS-CoV-2 B.1 isolate ($p<0.0001$), validating the use of Gamma for further testing. The overall SARS-CoV-2 seroprevalence in Brazilian cats during late 2020 validated by plaque reduction neutralization test (PRNT90) was 7.3% (95% CI, 5.3-9.8). There was no significant difference in SARS-CoV-2 seroprevalence in cats between Brazilian states, suggesting homogeneous infection levels ranging from 4.6% (95% CI, 2.2-8.4) to 11.4% (95% CI, 6.7-17.4; $p=0.4438$). Seroprevalence of the prototypic cat coronavirus Feline coronavirus (FCoV) in a PRNT90 was high at 33.3% (95% CI, 24.9-42.5) and seroprevalence of Bovine coronavirus (BCoV) was low at 1.7% (95% CI, 0.2-5.9) in a PRNT90. Neutralizing antibody titers were significantly lower for FCoV than for SARS-CoV-2 ($p=0.0001$), consistent with relatively more recent infection of cats with SARS-CoV-2. Neither the magnitude of SARS-CoV-2 antibody titers ($p=0.6390$), nor SARS-CoV-2 infection status were affected by FCoV serostatus ($p=0.8863$). Our data suggest that pre-existing immunity against enzootic coronaviruses neither prevents, nor enhances SARS-CoV-2 infection in cats. High SARS-CoV-2 seroprevalence already during the first year of the pandemic substantiates frequent infection of domestic cats and raises concerns on potential SARS-CoV-2 mutations escaping human immunity upon spillback.

152.

First molecular detection of Equine Herpesvirus type 3 (EHV-3) in Chile

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Equine herpesvirus type 3 (EHV-3) is the etiological agent of the equine coital rash (ECE), a venereal disease of worldwide distribution. In October of 2019, four equines, one male and three females presented genital lesions compatible with EHV-3 infection at the Sector Agua Buena Chica, Bulnes commune, region of Ñuble, central Chile (Latitude: -36.7333 Longitude: -72.3). The samples were taken with a sterile swab from papular or crustal lesions on vulvar or preputial areas (Fig. 1a and 1b), and stored at -4ºC in the Virology Laboratory of the Faculty of Veterinary Sciences of the University de Concepción, Chillán Campus. DNA extraction was carried out using the Dneasy Blood & Tissue kit (Qiagen, Cat. 69506) following the manufacturer's specifications, and the samples stored at -20 °C until use. To detect EHV-3 we implemented a conventional PCR with primers targeting a conserved fragment (520 bp) of the glycoprotein G gene (gG). Positive samples were sequenced in both directions, the sequences edited with BioEdit and an alignment with homologue sequences was constructed with the Clustal W algorithm, and phylogenetic relationships evaluated with the Bayesian method using MrBayes v3.1.2. Through BLASTn analyses we confirmed the first EHV-3 positive case for Chile, since three identical sequences of gG were obtained from three mares. The consensus sequence was 99.7% (428/429 bp) identical to a homologue haplotype from Brazil (GQ336877), and clustered into a monophyletic group with EHV-3 sequences obtained from Japan, Russia and Brazil (Fig. 2). In conclusion, for the first time we confirm the presence of an EHV-3 genomic sequence, in Chile. The recognition of the pathology will allow for the identification of distributional areas and therefore predict reproduction risks in the Chilean equine population.

O32.

Degree of PCV2 uptake by porcine monocytes is strain-dependent and is associated with amino acid characteristics on the outside of the capsid

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Porcine circovirus type 2 (PCV2) is associated with several economically important diseases that are designated as PCV2-associated diseases (PCVADs). PCV2 is replicating in lymphoblasts and PCV2 particles are taken up by monocytes without effective replication or complete degradation. Glycosaminoglycans (GAGs) have been demonstrated to be important receptors for PCV2 binding and entry. The objective of this study was to determine whether differences exist in viral uptake and outcome among six PCV2 strains in primary porcine monocytes: Stoon-1010 (PCV2a; PMWS)), 1121 (PCV2a; abortion), 1147 (PCV2b; PDNS), 09V448 (PCV2d-1; PCVAD), DE222-13 (PCV2d-2; PCVAD), and 19V245 (PCV2d-2; PCVAD). The uptake of PCV2 in peripheral blood monocytes was different between the PCV2 strains. A large number of PCV2 particles were found in the monocytes for Stoon-1010, DE222-13 and 19V245, while a low number were found for 1121, 1147 and 09V448. Competition with and removal of glycosaminoglycans (GAGs) on the cell surface demonstrated an important role of chondroitin sulfate (CS) and dermatan sulfate (DS) on PCV2 entry into monocytes. The mapping of positively/negatively charged amino acids exposed on the surface of PCV2 capsids revealed that their number and distribution may have an impact on the binding of the capsids to GAGs and may influence the PCV2 internalization into monocytes. Based on the distribution of positively charged amino acids on PCV2 capsids, phosphacan was hypothesized and further demonstrated as an effective candidate to mediate virus attachment and internalization to monocytes.

Keywords: Porcine circovirus type 2, PCV2-associated disease (PCVAD), post-weaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephritis syndrome (PDNS), abortion, peripheral blood monocytes, viral capsids, uptake, glycosaminoglycans, receptors, phosphacan

33.

T-lymphoblasts from Landrace pigs are more susceptible to porcine circovirus type 2 (PCV2) than T-lymphoblasts from Piétrain pigs and the replication is strain-dependent

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Porcine circovirus type 2 (PCV2) is the etiological agent of PCV2-associated diseases (PCVAD); it targets mainly lymphoblasts. Pigs suffering from PCVAD display lymphocyte depletion in lymphoid tissues. PCV-2 infected Landrace pigs have a higher susceptibility to PCV2-associated lesions and postweaning multisystemic wasting syndrome (PMWS) development than Piétrain pigs. Our previous studies demonstrated that PCV2 can infect T-lymphoblasts, binds partially to these cells via chondroitin sulfate (CS) and enters via clathrin-mediated endocytosis.
Six PCV2 strains were used in this study. Three Landrace pigs and three Piétrain pigs were used as blood donors. The differences in replication kinetics demonstrated that T-lymphoblasts of Landrace pigs are more susceptible to PCV2 infection than those of Piétrain pigs. Next, a strain-dependent PCV2-replication was found in T-lymphoblasts. As important attachment receptors for initiating viral infection, glycosaminoglycans (GAGs) were also examined. Their expression on the cell surface and the colocalization with PCV2 particles were determined by immunofluorescence staining. HS was not detected in T-lymphoblasts from either pig breed. Landrace pigs exhibited a relatively higher expression of CS and decorin than Piétrain pigs. However, the number of bound viral particles did not show a significant difference between the two breeds. Therefore, the efficiency of viral binding with cell surface GAGs is not the main factor causing the difference in infection. A step further in the replication cycle will be responsible for this.
This study explains the higher PCV2 susceptibility of T-lymphoblasts of Landrace pigs compared to those of Piétrain pigs and reveals a strain-dependent difference in PCV2 replication in T-lymphoblasts.

Keywords: Porcine circovirus type 2 (PCV2), PCV2-associated disease (PCVAD), post-weaning multisystemic wasting syndrome (PMWS), Landrace, Piétrain, T-lymphoblasts, replication, glycosaminoglycans, endosomal-lysosomal system, pH drop, viral escape, viral evolution

101.

Detection of SARS-CoV-2 in a Dog with Hemorrhagic Diarrhea

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Although the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has presumably a zoonotic origin, virus transmission has also occurred in the inverse direction. In fact, SARS-CoV-2 has been detected in several animal's species and the source of infection was mostly humans, particularly their owners or caretakers. Among these species highlight dogs (*Canis lupus familiaris*), which have shown to become infected when it is in close contact with infected humans. In this work, we report a dog from the North African Autonomous Spanish city of Ceuta suffering from hemorrhagic diarrhea which presumably acquired the infection from their owners in early January 2021. For the virus identification, a dog's stool sample was collected. Virus was firstly detected by a two-tube RTqPCR assay which amplified a 397-nucletide region of the spike (*S*) gene. Specifically, this *S* gene region was the central part of the receptor binding domain (RBD), which after being Sanger sequenced showed the potential concerning Ile402Val substitution. Although the low viral load (Ct ≈ 33) presented in the animal, we conducted Next Generation Sequencing (NGS) to obtain the complete genome sequence of the virus, and we achieve to cover more than 90% of its genome. This almost complete virus sequence allowed us to classify it as B.1.177 variant, the most predominant variant in Spain and other European countries during this pandemic period. Even though the Ile402Val substitution is not specific of this variant, phylogenetic analysis confirmed its classification within B.1.177 variant. Therefore, although the virus sequencing was impossible in their owners, this clearly suggests that the animal acquired the infection from them. Moreover, NGS reads mapping to bacterial genomes proved that the dog fecal microbiome fitted best the characteristic microbiome of dog's acute hemorrhagic diarrhea. Thus, our findings further exemplify pets' implication in the pandemic, and stress the possibility that this virus could affect dog's gastrointestinal tract.

O34.

Genome-wide CRISPR/Cas9 Knockout Screen in Porcine Cells to Identify Relevant Host Factors for African Swine Fever Virus Replication

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African swine fever virus (ASFV) causes a devastating disease of domestic pigs and wild boar (*Sus scrofa)* which so far cannot be controlled by vaccination or treatment. It currently affects many countries worldwide leading to drastic economic losses. ASFV is a complex DNA virus which enters host cells via endocytic pathways and replicates within the cytoplasm. Its genome encodes up to 167 open reading frames, and more than 134 of the deduced protein products have been identified by mass spectrometry. However, the functions of most ASFV proteins and their interactions with cellular factors during virus replication remain to be elucidated.
To uncover host cellular proteins relevant for ASFV replication a genome-wide CRISPR/Cas9 knockout screen was performed in a permissive wild boar lung cell line (WSL). For this purpose, the porcine CRISPR/Cas9 knockout library SsCRISPRko.v1 was used, which is targeting all known genes of the porcine genome with three to four single guide RNAs (sgRNAs) each. In total the library encoded 83,381 sgRNAs encompassing 1001 non-targeting control and 82,380 gene specific sgRNAs (Hölper et al., 2021, DOI: 10.3390/v13081574).
After lentivirus transduction, WSL cells expressing a puromycin resistance gene, sgRNAs and Cas9 nuclease were selected and infected with ASFV. DNA of the surviving cell population and of uninfected control cells was prepared, and analyzed by comparative next generation (Ion Torrent) sequencing. Changes in the abundance of individual sgRNA genes in the surviving cell pools were recorded. Several sgRNA sets targeting the same cellular genes were found to be significantly enriched in two to four independent infection experiments. Interestingly, many of the corresponding cellular gene products are involved in similar cellular pathways, particularly in endocytosis and in adaptive immunity. Currently the identified hits are verified by targeted knockout and subsequent reconstitution of selected genes in WSL cells. In conclusion, our host genome-wide CRISPR/Cas9 knockout screen seems to be a valuable tool to unravel molecular mechanisms of ASFV replication, and to identify targets for novel antiviral strategies.

O172.

Bartha-K61 vaccine protects against novel Suid herpesvirus 1 strains

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The efficacy Bartha-K61 vaccine strain against two novel Suid herpesvirus 1 (SuHV1) strains has been compared. In negative pressure individual isolators, 45-day old seronegative pigs previously oronasally /intramuscularly vaccinated with the Bartha-K61 strain, along with unvaccinated controls, were challenged either with the Hercules Greek strain (genotype 1) or the HeN1 Chinese strain (genotype 2) of SuHV1, both isolated in 2011. Clinical signs and body temperature were daily observed, and nasal swabs, faeces, blood and bodyweight were collected. In the unvaccinated pigs, a superior virulence of HeN1 strain over Hercules strain (higher mortality and clinical score) had been observed ($p<0.05$). However, vaccination with the Bartha-K61 vaccine, had almost equally protected against both virulent strains as such was evidenced by a drastic reduction in morbidity, mortality, bodyweight loss and virus excretion to almost similar extent ($p<0.05$). Our study showed that despite differences in virulence, the applied vaccination scheme with the Bartha-K61 strain could equally protect nursery pigs against both the European and Chinese strains.

35.

Age-depended Pseudorabies Virus Invasion in Porcine Central Nervous System

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Suid Herpesvirus 1, also known as Aujeszky's Disease Virus or Pseudorabies Virus (PRV) is a member of the genus *Varicellovirus*, subfamily *Alphaherpesvirinae* and family *Herpesviridae*. PRV is the causative agent for pseudorabies, a disease that mainly affects pigs and incidentally other domestic and wild animals. In non-porcine species PRV is almost always fatal causing neurological disease, while in pigs the outcome of the disease depends on the age. More specifically, neurological signs and death are the main clinical manifestations prior weaning, while in fatteners the disease is characterized by the development of respiratory symptoms. In breeding animals the main clinical manifestations are abortion in sows and reversible infertility in boars. One of the main routes that the virus follows on its way to the porcine CNS is the trigeminal nervous pathway. In this study, an attempt was made to investigate the effect of the interaction between the nerve development and the virus infection. For that reason, 42 pigs at the age of one (n=14), three (n=14) and five (n=14) weeks inoculated with 10^7 $TCID_{50}$ of the PRV Kaplan strain, and euthanized at one- or four-days post inoculation (DPI). The tissues of the trigeminal nervous pathway were collected and examined for virus replication in cell cultures, for nerve morphology by light and transmission electron microscopy, and for viral antigen visualization by immunohistochemistry. The results also showed that the virus titres and the clinical symptoms reduced as the age of the pigs increases, while, at the same time the development of the maxillary nerve's myelin and axon ceases. In addition, the level of disruption of the nerve structure was more prominent in one-week-old pigs compared to five-week-old pigs. In conclusion, the results of this study support the correlation between the age-depended PRV neuroinvasion in pigs and the morphological changes of neurons. Our findings may contribute to scientific literature regarding neuropathogenesis of PRV and alphaherpesviruses.

102.

Genetic Diversity of PRRSV in Northern Italy from 2011 to 2021

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the agent of one of the most globally spread pathologies in swine farm, as it causes reproductive failure in saws and severe respiratory symptomatology in fattening pigs. There are two distinct genotypes that share only 50-70% of their genome: PRRSV-1 (or European type) and PRRSV-2 (or American type). The main strategy to contain PRRSV infections is the usage of Modified Live Virus-based vaccines; although they do not give full protection against heterologous strains and reversion of virulence may occur. Being an SSRNA+ virus, PRRSV undergoes frequently through point mutations and recombination events. Therefore, molecular surveillance is fundamental to monitor said continuous modifications.
Here we present a 10-year retrospective study where ORF-7 PRRSV sequences collected in Northern Italy were analyzed to establish this grade of genetic heterogeneity.
Molecular screening through qPCR was conducted on clinical sample from Lombardy and Emilia-Romagna regions. Sanger sequencing of ORF-7 region was performed on positive sample obtaining 5765 sequences from 2011 to 2021. After excluding 100% identical and ambiguous-nucleotides-containing sequences, a refined dataset of 3370 field sequences was obtained and aligned with 37 reference sequences in MEGAX using MUSCLE algorithm. Then Maximum Likelihood tree was calculated using default parameters and 200 bootstrap replications with PRRSV-2 strain VR2332 used as the outgroup.
Phylogenetic analysis showed that most of the sequences (n=3354) belonged to PRRSV-1, as only 16 clustered with PRRSV-2 prototype sequence. Among PRRSV-1 sequences 486 were similar to Lelystad prototype strain while the majority of sequences (n=2876) grouped into a separate Italian-like clade in which at least 3 well-defined subclades can be observed.
These results provide an overall picture of past and current PRRSV strains circulating in swine farms in the North of Italy and confirm a very high genetic heterogeneity as well as notable differences of Italian strains compared with other strains in Europe.

O173.

Characterization of Protective Immune Responses in Domestic Pigs following Intradermal Immunization with the Attenuated African Swine Fever Virus (ASFV) Lv17/WB/Rie1.

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African swine fever (ASF) is a devastating viral disease for domestic pigs and wild boar and a serious global threat for the pig industry. Despite the efforts carried out over the last decades, no licensed vaccines have been approved so far. Live attenuated (LAV) vaccines have been shown to be effective against virulent challenges. However, the immunological mechanisms of protection are poorly understood. We aim to characterize such mechanisms, with special attention to the role of different subsets of T lymphocytes following immunization of domestic pigs with the Lv17/WB/Rie1, a naturally attenuated non-haemadsorbing ASFV genotype II isolate obtained from a wild boar in Latvia in 2017 able to confer protection against the highly virulent ASFV genotype II isolate Arm07. Pigs were immunized intradermally by homologous prime/boost. Thirty-five days after the prime, pigs were challenged with the virulent Arm07 ASFV. Serum and blood samples were collected weekly. Sera were assayed for ASFV-specific antibodies using an indirect immunoperoxidase test (IPT) and for different immunomodulatory cytokines using commercial ELISA kits. Peripheral blood mononuclear cells (PBMC) were isolated from heparin blood and stimulated *ex vivo* with virus or mock to assess longitudinally cytokine responses using an IFN-gamma ELISpot assay. An extensive functional and phenotypic characterization of responder cells was carried out using flow cytometry.
After prime immunization, all pigs developed a strong ASFV antibody response and were protected against virulent challenge. Early induction of moderate peaks of IL-8, IFN-gamma and TNF-alpha was observed in serum after first immunization. Lv17/WB/Rie1 elicited a robust ASFV-specific IFN-gamma T cell response against both viruses. Specific CD8+ (CD3+CD4-CD8^{high}) and CD4+ (CD3+CD4+CD8^{low}) T cell responses were observed against both viruses in the immunized pigs. A proportion of these IFN-gamma responding cells co-expressed TNF-alpha, and a proportion of these responding cells also displayed signs of cytotoxicity with CD107a mobilization and co-expression of TNF-alpha. We will additionally present data on the effect of immunization and challenge on phenotype and/or function of other cell populations.
The results obtained will contribute to understand the mechanisms underlying immune protection conferred by attenuated viruses that will help to develop more effective and safer vaccines against ASFV.

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O36.

Molecular determinants of ASFV hemadsorption and virulence

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Differences in virulence among ASFV strains vary from 100% of mortality in acute infection, to low or no mortality induced by attenuated strains. One of the main features associated with virulence is the hemadsorption (HAD) which induces "rosette" formation around infected cells. However, the molecular mechanisms connecting HAD and virulence are still unknown. Regarding the viral factors involved, ASFV EP153R and CD2v proteins (EP402R) have been previously reported to be responsible of HAD. Here, for the first time, we demonstrate by ectopic expression and ASFV mutants that HAD relied exclusively on CD2v and more specifically on its Nt domain, whereas no role for EP153R has been found. On the other hand, CD2v-Nt is highly glycosylated, and inhibition of glycosylation prevented HAD. Moreover, we have identified several key residues within the multiple predicted glycosylation sites within CD2v-Nt. Interestingly, CD2v-Nt mutants lacking these specific residues did not induce HAD, although the whole molecule still presented a highly glycosylation pattern. Finally, we investigated the CD2v HAD ability of the naturally attenuated, non-HAD NH/P68 strain. We discover, for the first time, that CD2v mRNA is detected during NH/P68 infection, though no HAD was found. To study this controversy, two constructs, NH/P68 CD2v full-length and NH/P68 CD2v-Nt, were generated and transfected in COS cells. In these conditions, none of the expression vectors were able to induce HAD after erythrocytes incubation. Finally, a putatively important role of the signal peptide, which is present in CD2v from virulent strains, but absent in NH/P68, is currently under study. Furthermore, we show that within the CD2-Nt domain from Arm/07 strain, the predicted signal peptide is essential for HAD function. Preliminary results suggest that signal peptide of CD2v may be a signature for virulence.

174.

Evaluation of the protective capacity of passive immunity against *Bovine alphaherpesvirus-1* (BoHV-1) after challenge infection with wild-type (wt) BoHV-1°

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In a previous study (1), two gE-deleted marker vaccines were shown to effectively transfer passive immunity (p.i.) from dams to calves until post-calving days 180. To date, scientific papers on the protective capacity of p.i. against BoHV-1 are limited (2-4). The aim of this study was to investigate the protective capacity of p.i. transferred from dams immunized with inactivated gE-deleted marker vaccine to calves after the challenge infection with wt BoHV-1.
Twelve calves were divided into four groups (A, B, C, D) with three animals each. The A and B groups consisted of 3-month-old calves, while the C and D groups involved of 6-month-old calves. The A and C groups had neutralizing antibodies (NA) with a mean titre of 1.41 $\log_{10}$ and 2.17 $\log_{10}$, respectively. In contrast, the B and D groups represented negative controls.
All animals were subjected to challenge infection with a wt of BoHV-1 (5) using a dose of 5 x $10^{7,50}$ $TCID_{50}$/mL for each calf.
The calves were kept under clinical observation for 28 post-challenge days (PCD), and the rectal temperatures were taken daily. Nasal swabs and serum samples were collected at different time points during the entire experimental period for virus isolation and detection of NA.
After challenge infection, severe clinical signs of Infectious Bovine Rhinotracheitis (IBR) were observed in all animals from 2 to 10 PCD. Moreover, the virus was shed in all groups from 1 to 10 PCD with a mean titre ranging from 10^{-1} to 10^{-5} $TCID_{50}$/mL (2-3).
A progressive increase in the NA titre was detected in the A and C groups with a mean titre of 2.63 $\log_{10}$ and 2.90 $\log_{10}$ on PCD 28, respectively. In B and D groups, NA was detected PCD 14 with a mean titre of 2.32 $\log_{10}$ and 2.63 $\log_{10}$, respectively. These titres increased until the end of the experiments with a mean titre of 2.93 $\log_{10}$ and 2.63 $\log_{10}$ (2-4).
In conclusion, the p.i. investigated in this study does not protect the calves against the wt BoHV-1. Therefore, further studies are needed to investigate the best immunization schemes in calves using marker vaccines.

1) Petrini et al., 2020; 2) Lemaire et al., 1995; 3) Lemaire et al., 1999; 4) Schynts et al., 2001;
5) Petrini et al., 2021.

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103.

Outbreak of Neuroinvasive Strain of Equid Alphaherpesvirus 1 in Serbia

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Equid alphaherpesvirus 1 (formerly equine herpesvirus 1 - EHV-1) is one of the most significant and widespread viral pathogens in horses. The virus primarily causes respiratory disease, but also abortion and neurological symptoms. Recently, infections caused by neuropathogenic strains of EHV-1 that cause equine herpesvirus myeloencephalopathy (EHM) have become more common. The epizootic of the highly pathogenic EHM strain of EHV-1 in Europe, was detected in early February 2021 during a horse race in Valencia, Spain, and spread quickly to many European countries.
The subject of this case report is a health disorder in an equestrian club with about 20 horses. The outbreak of the disease was preceded by the introduction of a new mare into the club on December 15, 2020, which neither then, nor later showed clinical signs of the disease. The first symptoms of the disease were recorded on December 25 in one mare (the mare fell and could not get up, the hind legs were paralyzed. From December 25, daily measurement of body temperature is introduced, and until the end of January 2021, a total of 7 horses had a high temperature. Clinical symptoms also include leaning the head against the wall and circular movements of the animals around the hind legs, and both foals aborted in the last trimester of pregnancy. Three horses died (2 euthanized; the last death was on January 28, 2021).
The presence of EHV1, EHV4, EAV and EIV were examined by real-time RT-PCR method, and the presence of antibodies against these viruses was tested by VNT and HI test. The first samples from December 30, 2020 - nasal swabs of 4 horses with a clinical signs gave a positive result in 2 samples for EHV-1. During January, the EHV-1 was detected in both the aborted fetus and in the cerebrospinal fluid of the euthanized mare, and the virus was also isolated on the RK-13 cell line. A negative result was obtained for all other viruses tested. Serological tests conducted a month after the appearance of the first symptoms determined a high titer of antibodies against EHV-1 in all individuals in the equestrian club.
Occurrence of enzootic EHV-1 EHM virus infection was detected in Serbia at the end of 2020 - the origin of the virus is unknown. During the winter and spring of 2021, several more cases of EHM in horses were detected, but since the spring of 2021, no any later cases have been registered in Serbia.

Keywords: EHV-1, EHM strain, disease outbreak, Serbia

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194.

Detection and Typing of Coronaviruses in Bats in Serbia

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Several species of bats are known as a reservoir of a large number of emergent and highly pathogenic viruses, among which, coronaviruses certainly occupy a significant place. Only during the last 20 years of the 21st century, 3 significant epidemics / pandemics with a severe clinical picture were recorded: coronavirus of severe acute respiratory syndrome (SARS-CoV), Respiratory coronavirus syndrome of the Middle East (MERS-CoV), and the current pandemic of coronavirus disease (COVID-19) caused by the coronavirus of severe acute respiratory syndrome 2 (SARS-CoV-2).
Recent research on the presence of coronavirus in bats in Serbia began during 2016/2017. The feces sample of 142 individuals from 12 localities from different parts of Serbia were collected. During 2020, fecal samples from another 40 bats were collected from 2 localities. The analysis were performed by the conventional RT-PCR method according to the protocol of Rihtaric et al. from 2010. The presence of coronavirus was detected in 24.65% (35/142) of the tested samples from 2016 and 2017 and in 7 of the 12 tested localities, as well as in 27.50% (11/40) of the tested samples from 2020 on both tested localities. Overall, the presence of coronavirus was found in 8 of the 15 bat species analysed (*Myotis nattereri, Myotis capaccinii, Myotis myotis, Myotis blythii, Rhinolophus ferrumequinum, Rhinolophus euryale, Miniopterus schreibersii* and *Rhinolophus hipposideros*). Some of the detected viruses were molecularly typed and characterized as virus strains belong to alpha (most of them) and beta bat coronaviruses similar to those detected in bats in neighbouring countries Bulgaria, Italy, Hungary, Spain and others. This research is still ongoing.
Although it is known that bats can serve as a potential source of viral pathogens, none of the species of bats identified so far in Serbia have been linked to the development of the disease in humans in Serbia. The obtained research results, as well as the current pandemic situation caused by the SARS-CoV-2 virus, indicate the need for much more intensive and coordinated surveillance of zoonotic potential viruses in bats in Serbia and the region based entirely on the postulates of "One Health".

Keywords: coronaviruses, bats, Serbia

Acknowledgments: This work was funded by Ministry of Education, Science and Technological development of Republic of Serbia by the Contract No: 451-03-68/2022-14/200031, and by project “Monitoring of bat roosts and populations in Serbia”, Grant No. 401-00-200/2016-17, co-funded by the Ministry of Environmental Protection of the Republic of Serbia and Natural History Museum in Belgrade.

153.

Production and characterization of monoclonal antibodies against SARS-CoV-2

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SARS-CoV-2 has been in the last two years the most studied virus all over the world. Its genome encodes for four structural proteins, namely Spike (S), Envelope (E), Matrix/Membrane (M), and Nucleocapsid (N). The S protein, in particular its S1 domain, is the major target of the immune response.
In the present study, we describe the production and characterization of a panel of monoclonal antibodies (MAb) elicited versus two different antigens: a commercial recombinant S1 (Creative Diagnostics) and an inactivated SARS-CoV-2 (Wuhan strain). The MAbs were characterized in indirect ELISA using a commercial recombinant S1 and a homemade recombinant Receptor Binding Domain (RBD) both expressed in mammalian cells, and a recombinant N protein expressed in *E.coli*.
A total of 96 hybridomas were obtained against the recombinant S1 protein: they did not recognize immunogenic or neutralizing epitopes since they do not compete with positive human sera in competitive ELISA nor they do neutralise the virus in serum neutralization assay (SN); 23 out of 96 recognized at least four different regions of the RBD and their target epitope is linear since they reacted in Western Blotting (WB).
A total of 35 hybridomas were obtained against the inactivated SARS-CoV-2 antigen: three reacted against a recombinant N protein, 12 against the recombinant S1 protein, while the target of the remaining 20 MAbs was not detected. None of the MAbs against the S1 domain showed to recognize a neutralizing epitope in the SN even if they showed to compete with positive human serum samples. In addition, their target epitopes were conformational since they were not reactive in WB. By reciprocal competitive ELISA between the 12 MAbs against S1, it has been shown that they recognize three different regions within the S1 protein but they were not reactive against the recombinant RBD.
All the anti-S1 and the anti-RBD MAbs were tested against six SARS-CoV-2 variants in immunofluorescence and Indirect ELISA. Based on their reactivity, it seems that the immunogenic conformational epitopes, even if not neutralizing, are progressively lost in the newest viruses while those nonimmunogenic and linear are more conserved within the viral variants.

O104.

Insights into the evolution and pathogenesis of A3B4 reassortants of infectious bursal disease virus (IBDV)

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Infectious bursal disease virus (IBDV) is a bi-segmented dsRNA virus that causes worldwide serious economic losses in poultry production. Recently, the emergence and circulation of novel reassortants of IBDV (assigned to A3B4 genotype) containing the segment A from very virulent strains and segment B from an unidentified source, was reported in Europe. In the present study, we analysed the evolution and dynamics of infection under *in vivo* conditions compared to a very virulent strain.
As shown by the temporal analysis, tMRCA for segment A and B of the A3B4 IBDV genotype, were determined around 1988 and 1981, respectively, indicating a likely reassortment time in the late 1980s that coincides with the first detected field strain in 1992. Moreover, the estimated evolutionary rates for this lineage is considerably high for both segments, suggesting that these strains are in a fast-rate evolutionary process. In turn, from the population dynamics reconstruction, it was inferred that the reassortants has increased its genetic diversity that could be indicative that the new genetic constellation enhance their genetic fitness. The infection of five-week old SPF chickens with the novel reassortant strain (A3B4) and very virulent isolate (A3B2) caused clinical symptoms, gross lesions and mortality (A3B4: 20% and A3B2: 50%). Both strains replicated in examined lymphoid and non-lymphoid tissues with comparable efficiency, and birds infected with the reassortant strain showed severe and precocious bursa atrophy at 4 dpi, while in birds infected with vvIBDV from 7 dpi onwards. Moreover, reduced weight gains of infected birds from 4 and 7 dpi was observed in the reassortant and very virulent group, respectively.
The results obtained confirm that the acquired viral polymerase (encoded by segment B) of A3B4 IBDV firstly provides greater diversity of this genetic lineage, and secondly does not adversely affect the ability to multiply in the host. These strains are fuelled by efficient replication in lymphoid and non-lymphoid organs, but due to lower mortality rate, the presence of virus may be less pronounced and consequently facilitate their spread. Moreover, the resulting immunosuppression and poor performance indicates that these strains pose a threat for poultry production.

O175.

Virulence properties of GI-23 infectious bronchitis virus isolated in Poland and efficacy of different vaccination strategies

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Infectious bronchitis virus (IBV) is one of the most important poultry pathogens, causing significant economic losses worldwide. IBV is characterised by highly genetic, serotype, and pathotypic variability. Despite extensive immunoprophylaxis strategies, the emergence of new genetic lineages is frequently observed in the field, causing disease control to be more complicated. Over the last years, one of the main health problems due to IBV infections in chicken production in Poland is caused by strains belonging to lineage GI-23 (known as Var2), which were initially found mainly in the Middle East region. In this study, the virulence properties and efficacy of different vaccination programmes were evaluated against infection with the IBV GI-23 strain cgammaCoV/Ck/Poland/G052/2016. The pathogenicity of the Var2 isolate was conducted in one-day-old and three-week-old SPF chickens and showed that the course of the disease is age dependent. To evaluate the effectiveness of specific immunoprophylaxis seven vaccination programmes using Mass, 793B, QX alone or in combination, and Var2 live vaccines were tested against the GI-23 infectious bronchitis virus challenge. Ninety-one-day-old commercial broiler chickens obtained from a commercial producer were equally divided into 9 experimental groups. Groups 1–7 were vaccinated according to the manufacturer's recommendations, group 8 served as a challenge control group (not vaccinated, IBV infected), while chickens from group 9 was not vaccinated and not infected and were used as a negative control group. All groups were scored according to the ciliostasis test at 5 days post challenge. Two immunoprophylaxis strategies generated full protection against gammaCoV/Ck/Poland/G052/2016 infection — Var2 given alone and combination of Mass with QX and 793B vaccine (both with a ciliostasis score of 0 and 100% protection). In conclusion, the current study provides significant insights into the virulence properties and efficacy of different vaccination programmes to infection with newly-emerged GI-23 IBV strains in Poland and will be helpful in compilation an appropriate immunoprophylaxis program in poultry production.

37.

Photoconvertion and proximity biotinylation assays are powerful tools to track intracellular mobility and identify molecular interactants of a viral protein in living cells

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Modern molecular biology provides powerful tools to study in living cells the mobility and the interactants of a protein through its expression as a fusion protein. Here, we used this approach to study the Zalpha domain containing protein ORF112 encoded by Cyprinid herpesvirus 3 (CyHV-3). Recombinant CyHV-3 strains encoding ORF112 as a fusion protein were produced. Two protein tags were used: Dendra2 and Apex2. Dendra2 is a photoconvertible "green to red" monomeric fluorescent proteins derived from corals. Neosynthesized proteins express green fluorescence when excited at 488 nm. Interestingly, its exposure to 405 nm laser light induces irreversible "green to red" fluorescence conversion. Converted molecules emits red fluorescence in response to 568 nm excitation. A CyHV-3 recombinant strain expressing Dendra2 fused to ORF112 replicated comparably to the parental wild type strain and the localization of the fusion protein was identical to ORF112. Using photoconversion in living cells, we were able to demonstrate that ORF112 migrates rapidly between cytoplasmic condensates in infected cells. Next, we produced a recombinant CyHV-3 strain expressing ORF112 fused to Apex2 (enhanced ascorbate peroxidase 2). Proximity labelling using Apex2 is the current gold standard approach to study RNA and protein interactions occurring with a protein of interest. Proximity labelling experiment begins by cellular expression of the protein of interest fused with Apex2. Cells are next incubated with biotin-phenol, then with hydrogen peroxide for only one minute, initiating biotin-phenol free radical generation and labelling of molecules close to Apex2. Remarkably, cells labelled using this procedure can then be used to study the subcellular localisation of labelled molecules using electron microscopy (relying on production of DAP polymers) and to identify after lysis and affinity purification (using streptavidin beads) the sequences of labelled RNA (RNA sequencing) and labelled proteins (using mass spectrometry). The results obtained with these two approaches will be presented. They highlight the interest of these two techniques to study viral proteins in living cells.

64.

Cross-protection among porcine enteric coronavirus

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Porcine epidemic diarrhea virus (PEDV) causes a highly contagious enteric disease. Recently, we have demonstrated that *Swine enteric coronavirus* (SeCoV), a chimeric virus from PEDV and another porcine coronavirus, was circulating in Spain, and that, currently, one of the most PEDV frequent variant is a recombinant virus from PEDV and SeCoV (rPEDV-SeCoV). In farms, PEDV recurrent infections are common, due to 1) Entries of variants/virus other than those detected on the farm, against which there would be no cross-protection or, 2) Existence of negative subpopulations (due to the entry of new animals without immunity, or to the presence of animals that were infected at an early age and developed immunity -or that acquired it via colostrum- and have lost it over time). To assess how long homologous immunity lasts and the degree of heterologous protection among different variants of enteric coronavirus two studies were developed. Clinical signs, viral shedding, and immune responses were evaluated. First Study: 75 piglets were orally inoculated (0 days post-inoculation; dpi) with PEDV. On 154 dpi, animals were homologous challenged. All inoculated animals seroconverted for neutralizing antibodies (NA). Despite that, PEDV was detected in feces of all pigs, indicating that the homologous immunity did not prevent the reinfection. Second study: 48 piglets were orally inoculated (Group 1: control; Group 2: PEDV; Group 3: SeCoV; and Group 4: rPEDV-SeCoV). On 20 dpi, all groups were challenged with rPEDV-SeCoV: Group 1 challenged for first time (control-rPEDV-SeCoV); Group 2 and 3 heterologous challenge (PEDV-rPEDV-SeCoV and SeCoV-rPEDV-SeCoV, respectively): Group 4: homologous challenge (rPEDV-SeCoV-rPEDV-SeCoV). After the first challenge, the three viral strains induced an undistinguishable mild-to-moderate clinical disease. After the second challenge, pigs subjected to homologous challenge did not show clinical signs or lesions and viral shedding was only detected in a single animal. Homologous protection was probably due to NA. In contrast, prior exposition to PEDV or SeCoV only provided partial cross-protection (lower for SeCoV), allowing for rPEDV-SeCoV replication and viral shedding in feces. Taken together, the results indicated that sterilizing immunity exist but in a short term, and that cross-protection did not prevent infection and viral shedding.

154.

Uptake and Persistence of Porcine Respiratory Coronavirus in Mealworm (*Tenebrio molitor*) and Black Soldier Fly (*Hermetia illucens*) Larvae

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Mass production of insects may aid in bioconversion of waste to useful products, with an efficiency estimated as high as 92%. With up to 1.4 billion tons of animal manure potentially available in EU alone, rearing of insects using waste products of animal origin such as slaughter byproducts, meat offal and manure provides opportunities for a sustainable circular economy. However, insect rearing using waste of animal origin, could generate a risk of animal virus transmission to humans or animals, when these insects are used as food or feed. Study of virus uptake of insects, and evaluation of virus survival in insect systems may aid in understanding and provide the basis for risk assessments of such hazards.
In this study, we have evaluated the uptake and survival of porcine respiratory coronavirus (PRCV), in larvae of two widely produced insect species *Tenebrio molitor* and *Hermetia illucens,* to firstly, establish a virus exposure bioassay, and secondly, to obtain knowledge about persistence of PRCV, as a model, within these insect larvae.
For *T. molitor* larvae, oral inoculation of a virus suspension was established by directly feeding PRCV-containing cell culture lysate; while the *H. illucens* larvae were inoculated via feed spiked with the virus. Both species of larvae were sampled at regular intervals for up to 9 days post exposure (PE). The presence of viral RNA, or viable virus in the larvae was determined using quantitative RT-PCR or by infection of ST-117 cells, respectively.
PRCV RNA was detected in *T. molitor* on day 0 (0.5 hr PE) and for up to 3 days PE, and in *H. illucens* on day 0 (6-8 hr PE) and for up to 2 days PE. When larvae containing the highest amounts of viral RNA were tested, infectious PRCV was detected in one *T. molitor* larva sampled at 0 days PE.
Our study showed that PRCV does not survive well in larvae of *T. molitor* or *H. illucens,* indicating a rapid degradation of an animal virus such as PRCV in these systems. This is encouraging for the development of the use of these insect larvae as food or feed.

38.

In vitro replication of the white spot syndrome virus in a cuticular explant model of shrimp (*Litopenaeus Vannamei*)

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Introduction: White spot syndrome virus (WSSV) is one of the most prevalent viruses in penaeid shrimp aquaculture and is responsible for major production losses. Since no cell line exists for shrimp, explant systems and primary cell cultures are important tools to study WSSV pathogenesis in penaeid shrimp.
Aims: The aim of this study is to establish for the first time a cuticular explant model to be used for the WSSV infection study.
Methods: The carapace of *Litopenaeus Vannamei* shrimp was isolated. The exoskeleton was gently removed from the cuticular epithelial cells and washed. Muscles and extra connective tissues were removed as much as possible, and the cuticula with epithelium was cut into 0.5 cm^2 explants. Explants were cultured at 27°C with 5% CO_2. The viability of the explants was evaluated in virus-free medium control, mock, and positive controls using EMA staining at 0, 24, and 48 hours post-infection (hpi). Explants were inoculated with WSSV Thai-1 infected shrimp homogenate for one hour. A healthy shrimp homogenate was used as mock inoculum. After inoculum removal and washing, explants were incubated in a shrimp-specific cell culture medium. Explants were harvested and embedded in Methocel at 0, 24, and 48 hpi and stored at -70°C. Cryosections were made and immediately fixed before being stained with a monoclonal antibody directed against viral protein VP28 and FITC-labelled goat anti-mouse IgG. Finally, the slides were mounted with glycerol mounting medium, containing DABCO, and were analyzed with a fluorescent microscope.
Results: At 0, 24 and 48 hpi, 80.3±11.9%, 65.9±18.1%, and 31.7±15.6% of the cells, respectively, were viable in non-inoculated control explants; 70.9±22.7%, 41.8±9.2%, and 35.3±11.4% in mock-inoculated explants and 54.8±20.1%, 18.9±7.2%, and 5.2±3.8% in WSSV infected explants. At 0, 24 and 48hpi, 0%, 1.2±0.9%, and 11.4±6.3% of the cuticular epithelial cells were infected in the inoculated explants. The non-inoculated and mock-inoculated explants were negative.
Conclusion: The results demonstrated that cuticular epithelial cells are susceptible to WSSV and that the cuticular explant model is a reliable system to study WSSV infection/replication.

O195.

Emergence of SARS-CoV-2 variants in farmed mink during the epidemic in Denmark, June-November 2020

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In June 2020, SARS-CoV-2 infection in farmed mink (*Neovison vison*) occurred in three farms in Northern Jutland, Denmark. A mink variant SARS-CoV-2 strain (lineage B.1.1.298), which had the spike protein amino acid substitution Y453F, appeared among some of the mink in the first infected farm. This change is located in the receptor-binding domain and the same variant was also found in all the tested mink at the two other farms and among some people in the area (Hammer et al. 2021).
After a silent period in July, the fourth case of infected mink was detected in mid-August 2020 (Rasmussen et al. 2021). The virus on this farm was closely related to the virus variant that was previously identified on the first three farms and appeared part of the same transmission chain. In addition to Y453F, the virus on the fourth farm had lost two amino acids in the N-terminal domain of the spike protein (ΔH69/V70) and acquired some additional changes in other parts of the genome.
In August and September, more farms tested positive for SARS-CoV-2 and this was coincident with extensive community spread (Larsen et al. 2021). The variants found in mink in this period all belonged to the B.1.1.298 lineage and were likely descendants from the virus introduced in the mink population in June. Additional spike substitutions emerged and genome phylogenies showed a segregation of the viruses in five clusters, including "Cluster 5", indicating multiple transmission chains. The Cluster 5 variant was only observed in a limited number of farms, all in the first part of September, whereas the other clusters persisted until the cull of all mink in Denmark in November, when the outbreak had reached 290 infected farms.
In October, mink infected with other lineage variants were found on mink farms located in the Southern part of Jutland indicating independent introductions of SARS-CoV-2 into these farms. During the last phase of the mink epidemic in October and November, additional lineages of SARS-CoV-2 were detected in mink, of which some were found on more farms. In total, mink in eighteen infected farms were infected with lineage variants other than B.1.1.298.

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O176.

A Genotype II Live Attenuated Vaccine Candidate for African Swine Fever Based on Multiple Targeted Gene Deletions or Modifications

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African swine fever remains a global threat to the pig industry and food security. The lack of vaccines limits control. We applied targeted gene deletion/ mutagenesis to produce a genotype II live attenuated ASFV vaccine candidate based on the successful approach we used previously for genotype I virus. First, we identified surface residues on the ligand binding domain of the genotype II (Georgia 2007/1) CD2v protein. These were individually mutated to identify mutations that abrogated binding of the transiently expressed CD2v protein to red blood cells. We replaced the wild type CD2v with a mutant non-HAD CD2v gene. The adjacent EP153R gene coding for a C-type lectin containing protein was deleted in the same step. These modifications were made from a Georgia 2007/1 isolate lacking DP148R and K145R genes (Rathakrishnan et al., 2021) This vaccine candidate (GeorgiaΔDP148RΔK145RΔEP153R-CD2v_mut) was attenuated and induced no or mild clinical signs and low or no viremia. It induced a vaccine dose-dependent protection against Georgia 2007/1 virulent challenge ranging from 83 to 100%. These pigs had early strong antibody responses to ASFV P72 and high levels of IFN-γ producing cells were induced, not only to genotype II virus stimulants, but also against genotypes I and X, postulating a potential cross-protective phenomenon.

O65.

Milk lactose protects against group A rotavirus infection

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Rotavirus A (RVA) is an important pathogen causing acute gastroenteritis in animals and humans. Attachment to the host receptor is a crucial step for virus replication. The VP8* domain is the distal terminal region of the RVA spike protein VP4 (expressed by the P gene) and is important for rotavirus binding and infectivity. Recent studies have indicated a role for non-sialylated glycans, including mucin core 2 and histo-blood group antigens (HBGAs), in the infectivity of human and animal group A rotaviruses. In the present study, we determined if porcine rotavirus-derived recombinant VP8* of the endemic strains 14R103 G5P[6], 13R054 G5P[7], 12R010 G4P[13], 12R046 G9P[23], and 12R022 G2P[27] interact with hitherto uncharacterized glycans. We successfully produced five recombinant GST-VP8 proteins of genotype P[6], P[7], P[13], P[23], and P[27]. The hemagglutination assay showed genotypes P[7] and P[23] hemagglutinate porcine and human red blood cells. In an array screen of >300 glycans, recombinant VP8* of rotavirus genotype P[6], P[7], and P[13] showed specific binding to glycans with a Galβ1-4Glc (β-lactose) motif, which forms the core structure of HBGAs. The specificity of glycan-binding was confirmed through an ELISA-based oligosaccharide binding assay. Further, 13R054 G5P[7] and 12R046 G9P[23] infectivity was significantly reduced by β-lactose in MA104 cells and primary porcine enterocytes. These data suggest that lactose, the main natural sugar in milk, plays an important role in protecting piglets from enteric viral replication and diarrhea.

O177.

Evaluation of PRRS MLV viremia and transmission for a better prevention of recombination between vaccine strains

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Virulent vaccine-derived recombinant Porcine Reproductive and Respiratory Symdrome Virus (PRRSV)-1 strains have been recently identified. Recombination between PRRSV-1 modified-live virus vaccines (MLV1) may occur when pigs, viremic for a first MLV1, are vaccinated or contaminated (through natural transmission) by a second MLV1 strain. To prevent recombination between MLV1s, it is thus essential to know the viremia duration and transmission abilities of the MLV1s. In the present study, we monitored in growing pigs the vaccine viremia and the transmission capacities of four MLV1s and one PRRSV-2 MLV (MLV2) approved in Europe.

Groups of 6 specific-pathogen-free piglets were either vaccinated with Porcilis PRRS (Porci), Unistrain PRRS (Uni), Ingelvac PRRSFlex EU (Flex), Suvaxyn PRRS MLV (Suv) or with Ingelvac PRRS MLV (MLV2). Just after vaccination, contact pigs were added to evaluate the MLV direct transmission. Blood samples were collected twice a week during 8 weeks post-vaccination to monitor each MLV viremia by RT-qPCR. Transmission parameters were estimated by mathematical modeling.

In vaccinated pigs, two profiles for the duration of MLV viremia were identified: a long lasting viremia for Porci, Uni and MLV2 (mean = 50, 53, 50 days, respectively), and a shorter viremia for Suv and Flex (mean = 44 and 39 days, respectively). Regarding the viral loads, MLV2 and Flex groups showed the highest and the lowest viremia, respectively, whereas Porci and Suv showed similar intermediate viremia. The Uni viral load was between that of MLV2 and Porci/Suv groups. The transmission rate (beta) of the vaccines was also strain dependent. While no transmission was observed with Flex (beta = 7.7E-07), Uni and MLV2 showed high beta (4.5E-02 and 3.2E-02, respectively), whereas Porci and Suv showed lower beta (6.1E-03 for both).

This study demonstrates that the MLVs show different profiles of viremia and transmission that should be considered to prevent the recombination between PRRSV vaccine strains.

196.

An Alternative Strategy against Influenza A virus: shRNAs as Antiviral Molecules

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Influenza A virus (AIV) is considered responsible of pandemic events and infects both human and animals. The animal host plays a crucial role in the potential inter-species recombination of the virus and leads to the emergence of extremely virulent biotypes. In this context, RNA interference (RNAi) technology found great interest for the development of a new generation of drugs, able to inhibit viral replication. RNAi is promoted by a chemically synthetized shRNA (short hairpin RNA) that is specific for a selected sequence that corresponds to the target.
The present study aims to evaluate the ability of three shRNAs, designed on the subgenomic segments coding for H1N1 AIV nucleoprotein (NP6, NP7, NP8), to promote 5 human AIV biotypes inhibition in the permissive Madin-Darby-Canine-Kidney cell line.
shRNAs have been generated in *Escherichia coli* and used to transfect MDCK cell culture. Cell clones expressing a specific shRNA have been *in vitro* amplified in order to be used as substrate for viral infection.
Each cell clone was infected *in vitro* with five human AIV biotypes with 7 dilution points (10^{-1}-10^{-7} from 100$TCID_{50}$ viral stock). After 48 h it was evaluated the capacity of AIV biotypes to induce cytopathic effect on cell substrate compared to the wild type MDCK, used as a control.
The cell clones showed a different sensitivity towards the viral biotypes. NP6 clone demonstrated to be resistant to 10$TCID_{50}$ for each viral strain (no ECP observed). NP8 clone has the similar resistant behaviour of NP6, but it showed ECP at 1$TCID_{50}$ of A/PR/79/2014 virus. NP7 provided different results when infected with the AIV biotypes. It showed to be less efficient than NP6 and NP8 clones but more resistant to the infection than MDCK control.
This preliminary *in vitro* study could show the possibility to develop antiviral drugs shRNA-based. Further studies will be necessary to test the *in vivo* efficacy of this agents in chicken model, after administration of the viral preparation via aerosol. The results obtained from this last step will provide important insights into the production of innovative antiviral drugs based on this technology.

66.

Hemadsorption and type I IFN control are determinants for ASFV virulence

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African swine fever virus (ASFV) is a complex cytoplasmic dsDNA virus that causes a serious hemorrhagic fever in domestic pigs and wild boars and is currently expanding throughout the world. Differences in virulence among the different ASFV strains vary from highly virulent strains with 100% of mortality, to attenuated strains inducing low or no mortality. Our group recently described that one of the main features differencing virulent vs. attenuated strains relies on their ability to control IFN-I pathways. Virulent Arm/07 controls IFN-β production through the modulation of the cellular cGAS-STING pathway, and differences observed in STAT1 and STAT2 degradation efficiency between the virulent and the attenuated strains could in part explain the low virulence pattern observed in pigs infected with attenuated ASFV strains. Our present studies show that ASFV ubiquitin conjugase pI215L is involved in the inhibition of the IFN-I signaling pathway by confirming that the negative modulation of JAK/STAT by pI215L depends on its ubiquitin-conjugating activity. On the other hand, here we demonstrate by ectopic expression and ASFV mutant generation that HAD relied exclusively on CD2v gene, and more specifically, on its Nt domain. Finally, we investigated the CD2v HAD ability of the naturally attenuated, non-HAD NH/P68 strain. We discover, for the first time, that CD2v mRNA is detected during NH/P68 infection, though no HAD was found. To study this controversy, two constructs, NH/P68 CD2v full-length and NH/P68 CD2v-Nt, were generated and transfected in COS cells. In these conditions, none of the expression vectors were able to induce HAD after erythrocytes incubation.
Finally, a putatively important role of the signal peptide, which is present in CD2v from virulent strains, but absent in NH/P68, is currently under study. Preliminary results suggest that signal peptide of CD2v may be a signature for virulence.

O39.

The Role of bovine ADAM17 in Pestivirus Infections and its Importance in the Pestivirus Resistance of CRIB-1 cells

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ADAM17 is a cell surface protease that has important roles in the release of proinflammatory cytokines (TNF-α) and hoemostasis. Additionally, it has been identified as a cellular receptor for the pestivirus Classical Swine Fever Virus (CSFV). Pestiviruses are small, enveloped, positive sense RNA viruses within the family *Flaviviridae*. In recent years, several additional pestivirus species, displaying increasing heterogeneity, were discovered. This also raises the question how conserved infection processes are amongst pestiviruses. Through RNAseq experiments, we could determine a reduced abundance of ADAM17 mRNA in an MDBK derived cell line resistant to pestivirus infection (CRIB-1) and establish the absence of functional ADAM17 in CRIB-1 cells. Genetic analysis revealed deleterious defects in both alleles, explaining the lack of ADAM17 expression. Transcomplementation of bovine ADAM17 in CRIB-1 cells restored their susceptibility to a diverse array of pestiviruses (BVDV-1, CSFV, BVDV-3, LindaV). Knock-out of ADAM17 in MDBK cells resulted in a complete loss of susceptibility to the tested pestiviruses and could be completely reversed by providing bovine ADAM17 in trans, with a tendency of higher susceptibility to infection of the transcomplemented cells when compared to the parental MDBK cells. These data provide evidence that ADAM17 is an essential entry factor for several pestivirus species and solve the decade old puzzle of the pestivirus resistance of CRIB-1 cells. Additionally, we will present evidence of downregulation of ADAM17 by pestiviral E2, which further backs its essential role in the life cycle of several pestiviruses.
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197.

Monitoring of Mammalian Orthoreovirus and Torque Teno Sus Virus in Domestic and Wild Animals in Northern Italy

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Mammalian orthoreovirus (MRV) and Torque teno sus virus (TTSuV) are emerging infectious viruses, with likely zoonotic potential, that may affect both domestic and wild animals. MRV is a dsRNA virus that can cause respiratory, central nervous system, and enteric diseases both in human and in other mammalian hosts. TTSuV is a DNA virus that frequently infects swine and have been associated with hepatic, respiratory, and autoimmune disorders. Moreover, due to efficient transmission routes, TTSuVs are ubiquitous and distributed worldwide. This study describes the occurrence and distribution of MRV and TTSuV in domestic and wild animals in the Po Valley in northern Italy, a flatland with high anthropisation and farm densities.
A total of 440 samples were collected between 2019 and 2022. In particular, pig feces (n=100) were taken from farmed animals, while livers of wild boar (n=175) and hare (n=165) livers from hunted animals. TTSuV was detected by end-point PCR, whereas MRV was detected by nested-PCR, using specific primers. Prevalence was calculated with the Blaker's method.
In pigs, MRV and TTSuV prevalence was 1% (CI95% 0.18-5.45%) and 19% (CI95%.12.5-27.8%), respectively. In wild fauna, MRV was found with higher prevalence in hares (49.09%, CI95% 41.65-56.6%) and wild boars (15.83%, CI95%.10.8-21.5%) than in domestic pigs. Also, TTSuV prevalence was higher in hares (40.61%, CI95% 33.4-48.2%) compared to pigs and wild boars (2.86%, CI95% 1.2-6.5%).
The high MRV prevalence observed in hares and wild boars suggested that wildlife may play a role as *reservoirs* for this virus. TTSuV detection was more relevant in hares and pigs than in wild boars result suggests the importance of further studies to evaluate the precise role (reservoir or spillover host?) of domestic pigs in the transmission and diffusion of such agent.
The presence of MRV and TTSuV in the animals of the Po Valley highlighted the need for future monitoring plans, to improve the knowledge of these likely zoonotic emerging viruses, to evaluate their spread and to assess the risk of transmission from animals to humans and vice versa.

67.

A new avian *in vitro* model for the study of Flavivirus infection

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Usutu virus (USUV) is a RNA virus that belongs to the Flaviviridae family and is closely related to the more pathogenic West Nile virus. USUV is transmitted by mosquito bites, affecting mainly wild and domestic birds, in which two phenotypes are observed: species resistant to infection which include birds of the order Galliformes like the domestic chicken (*Gallus gallus*) and species susceptible to infection including birds of the order Passeriformes, especially Eurasian blackbird (*Turdus merula*) where mass deaths are reported. The mechanisms underlying these differences are still unknown. The objective of this work is to develop *in vitro* models for the study of these phenotypic differences. For this purpose, we decided to use tendon-derived mesenchymal stem cells (MSCs) of blackbird and chicken. MSCs can be isolated up to 48h post-mortem and have an expression pattern similar to that of dermal fibroblasts, which play a key role in the pathogenesis of Flaviviruses at the inoculation site. The phenotypic identity of the cells was confirmed by RT-PCR using primers that amplify MSCs markers. To evaluate MSCs as an infection model, blackbird MSCs were compared with Vero E6 cells, commonly used in Flavivirus studies. Both cells showed permissivity to USUV infection as confirmed by immunocytochemistry using a rabbit polyclonal anti-USUV antibody. In addition, MSCs presented replication kinetics similar, albeit slightly lower, to that of Vero E6. Then, to validate MSC as a model for studying susceptibility/resistance phenotypes, USUV binding and replication kinetics were compared in blackbird and chicken MSCs. No significant differences were observed in the binding of USUV to cells from both species, ruling out differences in receptors between species. In terms of replication kinetics, blackbird MSCs were more permissive to USUV infection than chicken MSCs, with higher viral loads, somewhat replicating the susceptibility/resistance phenotypes observed *in vivo*. In conclusion we isolated, characterized and validated avian MSCs as an interesting model for the study of USUV infection. In addition, these cells facilitate access to *in vitro* models in wild animals, which are difficult to sample due to their conservation status.

105.

The Finding of SARS-CoV-2 in a Wild Eurasian River Otter (*Lutra lutra*) Highlights the Need for Viral Surveillance in Wild Mustelids

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Animals have been directly involved in the three known outbreaks of severe acute respiratory syndromes caused by coronaviruses (2005, 2012 and 2019). In fact, SARS-CoV-2 has presumably a zoonotic origin and its infection have been reported in multiple animal species, although in most of the cases, the infection source was related to the closest relationship of these animals to their owners or caretakers. Nevertheless, SARS-CoV-2 have also been detected in wild animals which apparently had no contact with humans, including mustelids, which is considered as one of the most susceptible animals' family to become infected. In this work, we report the first SARS-CoV-2 infection in an Eurasian river otter (*Lutra lutra*), which was found road killed in the countryside of Valencia province (Eastern Spain) in August 2021. The virus was firstly detected using two different one-tube RTqPCR commercial assays on RNA extracted from the nasopharynx (swabbing) and from lung tissue and mediastinal lymph node homogenates. Furthermore, infection was confirmed by three different two-tube RTqPCR assays which targeted specific regions of the spike (*S*), nucleocapsid (*N*), and *ORF10* genes, using the retrotranscribed cDNA from the mediastinal lymph node sample. Amplified cDNA products were Sanger sequenced and revealed some non-synonymous changes in the *N* and *ORF10* partial sequence, relative to the consensus sequence. Although the combination of these changes was unique, they were separately identified in human patient samples, which points to human origin of the virus. Given the high dependence of mustelids on water and together with our previous report of SARS-CoV-2 infection in two feral American mink (*Neovison vison*), which also belongs to this family, our findings suggest wastewater as the source of infection and highlight the need for SARS-CoV-2 surveillance in wild or feral mustelids to evaluate the risk that these animals could become SARS-CoV-2 reservoirs.

106.

Bovine Viral Diarrhea in dairy cows in Croatia – seroprevalence, virus detection and genotyping

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The aim of the research was to confirm Bovine Viral Diarrhea Virus (BVDV) infection in the dairy cows with clinical signs related to BVDV infection and genetically analyse the BVDV type/s circulating in that herd. Serum samples (n=233) were collected from clinically infected cows and tested for the presence of BVDV antibodies by virus neutralization test and Ab-ELISA test, presence of antigens by Ag-ELISA test, and viral nucleic acid by Real-time RT-PCR method. The virus neutralization test showed 83.26% of the tested cows were positive, and by Ab-ELISA test 87.12% them were positive. The presence of antigens was proven with the Ag-ELISA test in 2 out of 233 samoles (0.86%). After RT-PCR and phylogenetic analysis of two BVDV isolates, it was found both isolates belonged to genotype 1, subtype BVDV-1d. This is the first time that the BVDV-1d subtype has been confirmed in Croatia, although the BVDV-1b and BVDV-1f subtypes have been detected previously on Croatian dairy farms.

107.

Case of Bringing Aujeszky's Disease Virus via Feed to Pig Farms

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Introduction. To increase the nutritional value of feed, producers often use meat and bone meal or blood from slaughterhouses. This is especially dangerous when the same species of animal, for which the feed is intended, is used to make the feed. Thus, the role of feed as a factor in pathogen transmission was previously noted for African swine fever virus (ASF) and porcine circovirus type 2 (PCV2). The role of feed in the transmission of other infectious disease pathogens in pigs has not been sufficiently studied. This abstract describes the case of an epidemiological investigation of Aujeszky's disease outbreak in a pig farm in Dnipropetrovsk Oblast of Ukraine.
Methods. Nucleic acid extraction was performed using QIAamp cador Pathogen Mini Kit (Qiagen). PCR was performed using Maxima Hot Start Green PCR Master Mix (Thermo Scientific) and specific primers for the detection of pig DNA and pathogens of swine viral diseases: PCV2, porcine reproductive and respiratory syndrome (PRRS), porcine parvovirus (PPV), and Aujeszky's disease virus (ADV). The purified feed extract was intranasally administered in guinea pigs for the bioassay. Virological tests were performed using immunofluorescence assay.
Results. In a closed-type farm with approximately 2,880 pigs, there were problems with reproduction, stillbirth, and mortality of piglets in the early stages of life. Previously, we and other laboratories found PCV2, PRRS, and PPV circulating on this farm. Subsequently, we detected ADV genetic material. To determine the routes of introduction, we tested semen brought to the farm and it tested negative. We then checked 3 premixed feeds and found that one contained pig DNA. PCR testing revealed the presence of Aujeszky's virus in this feed. In addition, the virus content in this feed was confirmed by bioassay. ADV antigens were detected using immunofluorescence technique in the imprints of the guinea pig brain. Thus, we found a case of infection of pigs with Aujeszky's virus via feed that contained biological material of pigs.
Conclusions. Strict control over the use of additives of animal origin in the manufacture of feed is necessary, as they may be the transmission path of infectious agents.

40.

Age-dependent expression of the coronavirus receptors APN, DPP4, ACE2 and TMPRSS2 in different regions of porcine intestines

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Porcine intestines are a major replication site for coronaviruses and rotaviruses resulting in diarrhea and mortality mainly in piglets. The coronavirus spike protein is known to interact with a wide array of host membrane proteins including aminopeptidase N (APN) for transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV), dipeptidylpeptidase 4 (DPP4) for MERS-CoV, angiotensin-coverting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) for SARS-CoV and SARS-CoV2. The expression of these receptors in porcine intestines and their interaction with the most important porcine coronaviruses, TGEV and porcine epidemic diarrhea virus (PEDV) are not well studied. In this study, the age-dependent (3 days, 3 weeks and 3 months) expression of these receptors at different regions of porcine intestines was studied. The expression of APN in the jejunal villi increased from 3 days (42% of the epithelial cells) to 3 weeks (64%) but significantly decreased (34%) at 3 months. The expression in the colon was low with a significant increase in the bottom crypts from 3 days (nill) to 3 weeks (38%) and a significant decrease at 3 months (21%). For DPP4, the expression was only seen in duodenal crypts at 3 months (19%). In the ileal villi of 3-days' old piglets showed the highest expression (69%) when compared to older ages (<45%). In colonic crypts, there was a markedly reduced expression in 3-day pigs (8%) when compared to elder ages (>20%). ACE2 positive cells were dominant in small intestinal crypts, particularly in the ileum (>45%). A significant difference between expression levels in jejunal crypts of the 3-months pigs (8%) was observed when compared to the 3-days pigs (39%). Ileal crypts of 3-days pigs showed a significantly lower expression (21%) in comparison with older ages (>40%). The expression pattern of TMPRSS2 was similar to ACE2 for all ages. In duodenal crypts, the TMPRSS2 positive cells significantly increased from 3 days (13%) to 3 weeks (45%) but decreased again in 3-month old pigs (21%). These results show that the expression pattern of these receptors is highly variable and not correlated with the age-dependent loss of susceptibility; other factors should be considered.

Keywords: APN, DPP4, ACE2, TMPRSS2, coronavirus, porcine intestines

O68.

Interplay between Foot-and-Mouth Disease Virus 3D polymerase and the type I interferon response: a contribution to viral persistence?

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Foot-and-mouth disease (FMD) is a highly contagious viral animal disease with considerable socio-economic impact in case of outbreak. One of the problems associated with this disease is the ability of FMDV (Foot-and-Mouth Disease Virus) to persist for several months in a high proportion of infected animals. While persistence of FMDV has been reported in ruminants, it has not been described in pigs. This differential persistence provides an opportunity to compare virus-host interactions between species, to determine their host specificity and to identify potential links between protein-protein interactions (PPI) and viral persistence. Although the underlying mechanisms remain unknown, we have shown in a previous collaborative project, that persistent infection in primary bovine cells is associated with a long-lasting but attenuated and ineffective innate antiviral response. Modulating this response could thus contribute to the establishment and/or maintenance of a virus-host equilibrium through PPI. In this project we focused on the interplay between FMDV proteins and 16 cellular proteins belonging to the type I interferon (IFN I) pathway, which are described as involved in more than 75% of virus-host PPI. Plasmids expressing these 16 proteins from cattle, pig, sheep and goat, as well as expression vectors for 15 FMDV O-type proteins were constructed. PPI were identified by NanoLuc-2-Hybrid screening. The most promising results concern the 3D polymerase for which no interaction with the IFN pathway has been described so far. This protein was used to screen the cattle, sheep, goat and pig NanoLuc plasmid libraries. When comparing the results of these screens, it appears that most of the interactors are shared with relative strength across species. The FMDV-host PPI, have been confirmed by affinity chromatography GST Pull-Down for the four species and functional validation of these PPI is ongoing. Furthermore, we have demonstrated by luciferase reporter assays the inhibitory effect of 3D polymerase on the induction phase of the IFN response. These combined results strongly suggest that 3D could play an unsuspected part in the escape of FMDV from the host IFN response and potentially in its persistence.

198.

Analysis of the evolutionary profile of RDRP and NSP3 proteins of P Porcine Epidemic Diarrhea Virus (PEDV) of samples obtained from *Tadarida brasiliensis* urban bat

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Active epidemiological surveillance is an important tool to understanding the circulation dynamic of pathogens in the ecosystems. Previous results using this strategy identified several genomic fragments from Coronavirus in swabs obtained from *Tadarida brasiliensis*, a common urban bat specie from America. Since many reads were mapped with PEDV, a critical virus causes of porcine epidemic diarrhea and considering that Brazil is status free for this pathogen, new evaluations were conducted to assembly its genome and establishing the phylogenetic-evolutionary history of this virus. Using the subreads assembling strategy, we assembled six contigs that covering almost the totality of ORF1ab, but it did not assembly only the beginning of nsp1 gene. Phylogenetic analysis using NSP3 and RdRP proteins sequences showed the monophyletic clade between our BATSeq, PEDV and other AlphaCoV already identified in other bats species around the world. Additional analysis showed 100% similarity between BATSeq and NL63-like, a common virus of respiratory infections in humans, already identified in other bats species frugivorous and insectivorous. Therefore, it is possible that there was a possible coevolution between these animals and AlphaCoV.

Keywords: PEDV; urban bats; *Tadarida brasiliensis*; one health; active epidemiological surveillance

O109.

Genetically Identical Strains of Four Different Honeybee Viruses have been Determined in Bumblebee and Honeybee Positive Samples

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In recent years there has been growing evidence that certain types of honeybee viruses could be transmitted between different pollinators. Within a Slovenian voluntary monitoring programme 180 honeybee samples (*Apis mellifera carnica*) were collected from affected apiaries between 2007 and 2018. In addition, from August 2017 to August 2018, a total 148 samples of apparently healthy bumblebees (*Bombus lapidarius, B. pascuorum, B. terrestris, B. lucorum, B. hortorum, B. sylvarum, B. humilis*) were collected at four different locations in Slovenia and all samples were tested for detection of six honeybee viruses by using RT-PCR methods. Direct Sanger's sequencing of a total 158 positive samples (acute bee paralysis virus (ABPV n=33), black queen cell virus (BQCV n=75), sacbrood bee virus (SBV n=25) and Lake Sinai virus (LSV n=25)) was performed from obtained RT-PCR products. The phylogenetic comparison of identified positive samples of bumblebees and detected honeybee field strains of ABPV, BQCV, SBV and LSV has shown from 98.74 to 100 % nucleotide identity between both species. This study not only provides evidence that honeybees and bumblebees are infected with genetically identical or closely related viral strains of four endemically present honeybee viruses but also detects a high diversity of circulating strains in bumblebees, similar as was observed among honeybees. Important new genetic data for endemic strains, circulating in honeybees and bumblebees, are presented.

110.

Determination and Analysis of Twenty-two Complete Genome Sequences of Viruses Found in Diseased and Healthy Honeybee Colonies in Slovenia

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A total 22 new complete genomes of viruses were determined from 19 selected positive samples of diseased and healthy honeybee colonies (*Apis mellifera carnica*) from Slovenia. The viruses were grouped into ten different viral species of mainly honeybee viruses. To provide better coverage of viral complete genomes, the first step was selection of positive samples with high viral loads, previously detected positive by six different quantitative real-time PCR methods, containing one or more honeybee viruses. Selected samples were tested by next generation sequencing (NGS), Illumina platform. The analysis of the data showed four complete genomes of acute bee paralysis virus (ABPV), three complete genomes of black queen cell virus (BQCV), two complete genomes of chronic bee paralysis virus (CBPV), three complete genomes of deformed wing virus A (DWV-A), two complete genomes of deformed wing virus B (DWV-B), four complete genomes of Lake Sinai virus (LSV) and one complete genome of sacbrood bee virus (SBV), Apis rhabdovirus 1 (AR1), Bee Macula-like virus (BeeMLV) and Hubei partiti-like virus 34 (HPLV-34). This study revealed the determination of the first complete genomes of ABPV, BQCV, DWV-A, DWV-B and SBV in Slovenia. In addition, three virus species; AR1, BeeMLV and HPLV-34 were detected for the first time in honeybee samples in Slovenia. Two different variants of DWV virus: DWV-A (strain 341-2/2020) and DWV-B (strain 341-1/2020) with 84,61% nucleotide identity between both complete genomes were determined from the same DWV positive field sample, collected from affected honeybee colony. In addition, two different lineages of LSV3 and LSV4 were determined for the first time. The complete genome of AR1 is the first honeybee virus genome with negative strand RNA molecule described in honeybee samples in Slovenia. BeeMLV genome is one of eight complete genomes of this viral species in the GenBank and HPLV-34 genome is the first complete genome of this viral species detected in the sample of honeybee colonies and there are only two complete genomes of this virus available in the GenBank. NGS provides an excellent new tool for the detection and determination of new viral strains from field samples.

O41.

Evidence of Lumpy skin disease virus transmission from subclinically infected cattle by *Stomoxys calcitrans*

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Lumpy skin disease virus (LSDV) is a vector-transmitted capripox virus that causes disease in cattle. *Stomoxys calcitrans* flies are considered as major vectors and were recently shown to be able to transmit virus from LSDV infected cattle showing typical clinical signs to naïve cattle. Currently no conclusive data is available whether subclinical or preclinical infected cattle play a role in virus transmission. Therefore an in vivo transmission study with 13 donor and 13 acceptor bulls was performed. *S. calcitrans* flies were placed either on subclinical or preclinical infected animals. Subclinical and preclinical lumpy skin disease (LSD) was characterized by the absence of nodules and detection of LSDV genome. LSDV transmission from vectors fed on subclinical donors was evidenced in 2 out of 5 acceptor animals. One of these acceptor animals developed clinical disease while the other remained subclinical. No proof of LSDV transmission from vectors fed on preclinical donors was observed in the 8 acceptor animals. Our results show that subclinical animals can contribute to virus transmission. Therefore, stamping out of only clinical diseased LSD animals seems insufficient and will possibly lead to reappearance of LSD.

O111.

Circulation of Influenza A Virus in Wild Boars in the Emilia-Romagna Region (Northern Italy), between 2017-2022

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Influenza A viruses (IAVs) are single-stranded segmented RNA viruses and are classified into subtypes based on hemagglutinin (HA) and neuraminidase (NA) glycoproteins genetic and antigenic structure. IAVs are widespread agents of respiratory infections both in human and in swine. The *Suidae* population plays a crucial role in IAV ecology, given its role as mixing vessel for viruses of avian and human origin, harboring the potential of generating new pandemic strains, as happended in 2009. While there are many information about IAV dynamics in domestic pigs, only few data are available regarding viral circulation in wild boars and feral pigs. Given that, this study aimed to investigate IAV circulation in Emilia-Romagna wild boars population and to compare it with domestic pigs IAV described situation in an area which is known for its high commercial swine production. Virological surveillance was performed between 2017 and 2022. A total of 4605 wild boars lung samples were screened for IAV with a *real-time* RT-PCR targeting the M gene (matrix). Out of those, 17 samples tested positive (0,36%) (n.1 in 2018, n.4 in 2019, n.9 in 2020 and n.3 in 2021) and 7 were subtyped with RT-PCR. H1N1 was the most frequently detected subtype, with the identification of H1pdm09N1 and H1avN1. Three samples were successfully isolated and sequenced. Whole genome sequencing showed that the isolates belonged to different genotypes: HA-1C-N1av (genotype U), HA-1C-N1av (genotype A) and H1pdm09N1 subtype (HA-1A-N1av) (genotype 31). Genetic sequences of the eight IAV genome segments were compared with IAVs circulating in domestic pigs in the same area and with sequences retrieved from Genbank. In particular, IAV HA gene segments were aligned and phylogenetic tree construction was performed. The results analysis highlighted the simultaneous circulation of the same genotypes in both pigs and wild boars, supporting the hypothesis of SwIAVs spillover events at the wildlife-livestock interface. Given *Sus scrofa*'s crucial role in the development of reassortant strains, these findings highlight the need for a wildlife systematic surveillance against IAV, which poses a biosecurity threat, especially in areas characterized by swine farms high-density.

O42.

Infection kinetics of BTV-X ITL2021 (BTV-32) in small ruminants

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In March 2015, a novel atypical BTV serotype, named BTV-X, was detected in goats in Sardinia, Italy. The same strain remerged in 2021 and, unlike the 2015 strain, was successfully isolated in cell culture. Here, we report the infection kinetics of BTV-X following experimental infection of small ruminants.
Methods. In Trial 1, sheep and goats were inoculated subcutaneously, while in Trial 2 with BTV-X by the intravenous route. Serum and EDTA-blood samples, as well as rectal, nasal, and oral swabs, were collected from all animals 3 times a week. Blood samples and swabs were tested for the presence of BTV-X RNA with a real-time RT-PCR, and positive samples were propagated in cell culture. Serum samples were tested by ELISA, and neutralizing antibodies were titrated by virus-neutralization (VN).
Results. In both trials, animals remained healthy. BTV-X RNA, as well as antibodies, were not detected in any of the subcutaneously inoculated animals (Trial 1).
As to intravenously inoculated animals (Trial 2), BTV RNA was detected only in goats, starting from day post-infection (dpi) 11 and till the end of the trial (dpi135). The virus was isolated from blood samples collected from dpi11 and up to dpi30. Swabs tested negative for BTV RNA at all sampling times. All goats seroconverted but neutralizing antibodies were not revealed by VN.
Discussion. The different kinetics in sheep and goats is suggestive of a strong host tropism of BTV-X, though definitive assumptions cannot be drawn.
The absence of viremia following subcutaneous inoculation may be indicative of vector-free transmission as described for extant atypical BTV serotypes. In this regard, however, BTV-X was not detected in swabs evidence that hinders, apparently, a direct-contact transmission. In this context, more experiments are reasonably warranted.
Goats remained healthy throughout the study period and did not mount a neutralizing immune response despite the long-lived RNAemia, indicating that this species may represent the natural reservoir for BTV-X and BTV-X-like viruses in the field.

O69.

PRRSV-induced CD8 T Cell Responses at the Maternal-Fetal Interface During Late Gestation

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Porcine reproductive and respiratory syndrome (PRRS) negatively affects the global swine industry. It is caused by PRRS-virus (PRRSV), a small positive-stranded enveloped RNA virus. Infection during late gestation causes reproductive failures but *in utero* immune responses remain poorly understood. Our previous findings demonstrated an increase in CD8 T cells with a putative early ($CD8alpha^{+}CD27^{dim}$) effector phenotype at the maternal-fetal interface following PRRSV infection. In a recent PRRSV-infection experiment, CD8 T cell phenotypes and functionality were investigated at the maternal-fetal interface during late gestation. Two groups of pregnant gilts (n = 5) were infected with either the PRRSV field isolate AUT15-33 ($5x10^5$ $TCID_{50}$, intranasal) or sham-inoculated (n = 5) at day 85 of gestation. Twenty-one days post infection, gilts were euthanized and the maternal endometrium (ME) and fetal placenta (FP), from three randomly selected fetuses, were mechanically separated. Collected tissue from the ME and FP was enzymatically digested and mononuclear immune cells were isolated. The phenotype and expansion phase of the CD8 T cells was assessed via the *ex vivo* expression of Ki-67. In addition, the PRRSV-specific response was assessed upon *in vitro* restimulation with PRRSV AUT15-33-based peptide pools and evaluated via intracellular cytokine staining. The fetal preservation and viral loads in the tissues were evaluated based on visual appearance and qRT-PCR, respectively. In infected gilts, we observed a substantial increase in $Ki\text{-}67^{+}perforin^{+}CD27^{dim}$ effector CD8 T cells at both sides of the maternal-fetal interface as compared to the controls. Preliminary analyses suggest that following *in vitro* restimulation with PRRSV AUT15-33-based peptide pools, PRRSV-specific CD8 T cell responses can be identified based on the production of IFN-gamma and TNF-alpha at the maternal-fetal interface. The fetal preservation status in infected gilts was impaired in 39% of the fetuses whereas in control gilts all fetuses were viable and tested negative for viral RNA. The vast majority of fetuses from infected gilts had viral loads in the ME and FP. These data suggest that local T cells, even in the separated FP respond to the PRRSV-infection, but their activation may cause immunopathogenesis. Further functional and *in situ* investigations are required to corroborate this assumption.

O178.

Has the attenuation of CSFV C strain vaccine just resulted in a slow growing virus?

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Classical swine fever (CSF) remains a global problem to pig production and in Europe is an exotic disease and threat. The C strain of CSFV is an extremely efficacious vaccine, but its *modus operandi* remains unresolved. Live vaccines, such as the C strain, were attenuated through multiple passage, often in different hosts. As a consequence, their replication is notably slower than that of pathogenic strains and this applies to C strain both *in vitro* and *in vivo*. Hence it is tempting to speculate that the reduction of replication speed provides the immune system with a head start that allows it to react fast, thereby keeping the vaccine in check and to be prepared for subsequent challenges. In contrast, the immune system cannot hold pace this with pathogenic, fast replicating strains. This would be a conclusion not least from one of our previous studies (doi:10.3389/fimmu.2019.01584), demonstrating a key role for interferon signalling and particularly ISG15 in rapid vaccine protection. We have recently carried out a further study to identify the early events of C strain action on the immune system (doi:10.3390 /ijms22168795). Here, pigs were infected or immunised intranasally with either the pathogenic strain Alfort187 or the C strain vaccine and examined the immune reaction in tonsils over a period of 90 hours. We were able to identify two animals per group that had similar in viral load in the tonsils at 36hpi (Alfort) or 90hpi (C strain) respectively. We here carried out RNASeq analysis using next generation sequencing to determine differentially expressed genes followed by gene set enrichments and pathway analysis. The results demonstrate that C strain and Alfort initiate in fact very different host responses, not only providing C strain with a head start for the immune system but generating a better host reaction overall. In contrast Alfort187 seems to initiate a rather rogue response.

43.

Hepatitis E Virus Oral Infection of Pigs

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Hepatitis E is liver disease of humans, caused by the hepatitis E virus (HEV). Pigs are considered a HEV reservoir and recognised as a source of zoonotic transmission via the consumption of pork meat from infected pigs. Several *in vivo* studies have been performed to understand HEV infection in pigs and how HEV enters the food chain. Many of these studies inoculated intravenously or via oesophageal catheters which isn't reflective of natural (faecal-oral) transmission route. Accordingly, the primary aim was to establish a HEV oral inoculation model in pigs.
For both experiments twelve 10-week-old pigs in groups of four were monitored for ~ 1 month post inoculation with regular sampling for the detection of HEV RNA and antibodies. At post-mortem a selection of tissues was analysed. In Experiment 1, pigs were inoculated with either 10^7, 10^8 or 10^9 GE (genome equivalents) of the NLSWIE3 strain which had been passaged *in vitro*. In the second experiment, two groups were inoculated with 10^7 or 10^8 GE of a recently isolated field strain, ENG/2013/022, and a third group was inoculated with NLSWIE3 at 10^8 GE.
In the first experiment, 67% of the pigs shed HEV RNA in the faeces, 25% exhibited viraemia, 58% were positive in the bile, and 25% were positive in the liver. 10^7 GE seemed optimal for infection as this group recorded the most positive results. However, at the start of the experiment pigs which received doses 10^8 and 10^9 GE of NLSWIE3 possessed some maternal antibody titres which may have skewed the data, accordingly, the experiment was refined via pre-selecting the animals for Experiment 2. In Experiment 2, 100% of the pigs shed HEV RNA in the faeces, and 100% of pigs in the 10^8 GE (ENG/2013/022) group exhibited viraemia. At post-mortem, 75% of pigs were positive in the bile and 42% were positive in the liver.
Oral infection of pigs is reliably possible and a model for HEV which represents the natural route of infection in pigs was established with two different HEV strains. The analysis also demonstrates that viruses of different pathogenicity exist.

199.

SARS-CoV-2 Infection in Captive Animals at Zagreb Zoo

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Less than three years after the first description of coronavirus disease 2019 (COVID-19) in humans, it is clear that SARS-CoV-2 has a broad spectrum of potential hosts. The infection has been confirmed in different species of domestic, captive and wild animals.
On November 12th, 2021, a 16-year-old lioness (lion A) at Zagreb zoo developed signs of respiratory disease. She was one of the four lions kept in the same confinement. By November 21st, gagging, coughing, and dyspnea would last up to 30 minutes, repeating almost every hour, and the lioness struggled to take a breath. The second 16-year-old lioness (lion B) and 15-year-old male (lion C) showed milder clinical signs on November 21st. Both lions would experience gagging cough and dyspnea episodes every half an hour. The five-year-old female (lion D) had the mildest clinical presentation. On November 24th, one episode of laboured breathing was observed. Like lion A, none of the remaining lions showed loss of appetite, change of behaviour or extra-respiratory clinical signs. On November 17th, the caretaker noticed rhinorrhea. The next day at-home antigen COVID test turned out positive. The SARS-CoV-2 infection was confirmed by qRT-PCR on November 22nd.
Nasal swabs and faeces from lions and all animals that have been in close contact with the infected caretaker were sent to the Faculty of the Veterinary Medicine University of Zagreb.
Nasal swabs and faeces from all four lions and Eurasian lynx *(Lynx lynx*) tested positive by real-time RT-PCR for SARS-CoV-2. The nearly whole-genome sequences were identical in all animals to sequences of the delta variant circulating in Croatia at that time.
This is the first report of SARS-CoV-2 infection in captive animals in Croatia and the first description of infection in Eurasian lynx. All lions have recovered, and the Eurasian lynx infection was asymptomatic, but the clinical picture in lion A was severe and needed medical intervention. While SARS-CoV-2 is becoming endemic in most of the world, our experience has shown the danger of spillover of the infection to a captive and the wildlife and its potentially detrimental effect on their health.

O45.

Subclinical PRRSV type 1 infection aggravates clinical course of *Streptococcus suis* infections in pigs

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Streptococcus suis (S.suis) is a major porcine pathogen affecting young pigs mostly between 4 and 10 weeks of age. Although the endemic character with a high prevalence of infection between and in-between farms, is the occurrence of disease (arthritis, meningoencephalitis, serositis) limited to about 3 to 5% of pigs and the reasons for the development of disease are unknown. Epidemiological studies point to a role of co-infections with PRRSV, which is also endemic in the European pig population. The role of a subclinical PRRSV type 1 infection on the clinical outcome of a S. suis infection was elucidated in an animal study. Six week old pigs were allocated to four groups, i. e. group 1) intranasally infected with PRRSV type 1 (strain 07V063) and followed for two or three weeks, group 2) intranasally infected with PRRSV and two weeks later intranasally infected with *S. suis* serotype 2 (strain 10), group 3) with *S. suis* alone and group 4) not infected controls. Pigs were followed clinically and the study was finished one week after *S. suis* infection and pathology performed. Additionally, pigs infected with PRRSV only and control pigs were euthanized at two weeks after start of study and lung tissue was used in a precision cut lung slice (PCLS) assay to study effects of ex-vivo infections with various *S.suis* serotypes in dependence of a pre-existing PRRSV infection. Although PRRSV did not result in clinical disease signs in two weeks after PRRSV infection, led the co-infection to more clinical signs and a higher mortality than the mono-infection with *S. suis*. At 14 days after infection PRRSV viraemia was decreasing, but an increased in systemic viral load two days after S. suis infection was observed. Re-isolation of *S. suis* from organs was much more frequent in the co-infected pigs than in mono-infected pigs. The ex-vivo studies in PCLS assays in lungs from PRRSV-infected did not show differences in transcriptome cytokine profiles (IFN-b, IL-8, IL-6, IL-1b, TNFa) after in-vitro infection with different *S.suis* serotypes. In conclusion, subacute infection with PRRSV type 1, i.e. 14 days post infection enhances disease susceptibility to *S. suis*.

155.

Poly(sodium-4-styrene sulfonate) is a promising drug candidate for feline calicivirus and feline herpesvirus type 1 infections

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Feline herpesvirus type 1 (FHV-1) and feline calicivirus (FCV) are considered to be etiological factors for feline upper respiratory tract disease (URTD), colloquially named 'cat flu'. The most common clinical manifestations of the disease include oral/nasal discharges, gingivitis, conjunctivitis, and sneezing. While the disease is rarely severe, bacterial superinfections pose a danger for young or immunocompromised cats and may be fatal if not managed appropriately. Aciclovir derivatives are widely used as antiherpetic drugs, and penciclovir offers good effectiveness and safety profile in the case of FHV-infected cats. However, there is no specific drug administered in cats in case of FCV infections, and there is no compound active against both pathogens.
In this study, we show that poly(sodium-4-styrene sulfonate) (PSSNa) is a safe and promising antiviral candidate that inhibits the replication of both FCV and FHV-1. PSSNa interacts with FHV-1 virion and blocks its interaction with the host's cell, preventing virus transmission. Interestingly, the polymer exhibits a different mode of action during FCV infection, as it hampers mainly virus replication, inhibiting virus entry only to a certain level. As the compound is highly effective *in vitro*, it is safe in other target species, and no adverse events were observed in our *in vivo* study; an effort was made to validate its efficacy *in clinic*.
Our research provides the basis for further development of PSSNa as an effective and safe antiviral for topical use in cats suffering from the URTD, despite the etiological agent.

44.

Feline herpesvirus type 1: Trails to enter the host cell

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Feline herpesvirus type 1 (FHV-1) is an enveloped dsDNA virus and belongs to the *Herpesviridae* family. It is considered to be one of two main viral etiological factors of feline upper respiratory tract disease. Interestingly, it is estimated that 90% of cats are seropositive to this virus, whereas about 80% of infected animals establish lifelong lasting latency. The most common clinical manifestation of FHV-1 infections includes rhinotracheitis, oral or nasal discharges, conjunctivitis, and sneezing. While the disease is rarely severe, bacterial or viral superinfections pose a danger for young or immunocompromised cats and may be fatal if not managed appropriately. Importantly, the currently available vaccine reduces the severity of the disease; however, it does not prevent the infection or shedding the virus to other susceptible cats. While approved treatment strategies primarily focus on preventing superinfections and supportive therapy, herpesviral infections may also be treated with nucleoside analogs. Penciclovir (PCV) appears to be a potent and safe inhibitor of FHV-1 replication *in vitro,* and its prodrug famciclovir administered to cats is reported to be safe and effective also *in clinic*. However, the problem of antiviral resistance is already reported for herpesviruses, and drugs targeting different molecular targets are needed or will be needed shortly. Amongst different inhibitors, agents blocking the cell entry seem promising, especially considering the possibility of topical application. However, understanding the virus' biology and cellular interactome is essential for this.
The entry of FHV-1 is relatively poorly understood. The virus utilizes proteoglycans of heparan sulfate to adhere to the target cells. However, the fusion site and activating molecules are to be identified. In this study, different experimental approaches have been applied to identify the interactome of the virus during the entry using the fetal feline lung cell line. Moreover, the obtained results were verified using confocal microscopy, as we have tracked the fate of single viral particles entering the cell in the presence of different chemical inhibitors. While the cell line is a relatively poor model to study the virus-host interaction, the results are to be compared and validated using the primary feline fibroblasts.

112.

First Isolation of the Influenza A Virus H16N3 in Ukraine

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Introduction. Wild waterfowl and semiaquatic birds from the order of Anseriformes and Charadriiformes are natural reservoirs of avian influenza viruses (AIVs) and play an important role in the circulation of low pathogenic avian influenza viruses (LPAIVs). AIV includes 16 subtypes of hemagglutinin and 9 subtypes of neuraminidases, which are combined in different variations. H13 and H16 subtypes are associated with gulls and represent a unique gene pool of LPAIVs that differs from that of waterfowl. According to GeneBank, there are 330 characterized AIV of H16 subtype, isolated in 17 countries. The spatial isolation of host populations in the western and eastern hemispheres has led to the evolution of unique gene pools of viruses that have evolved into Eurasian and American genetic lines. However, some pelagic gull populations make intercontinental seasonal migrations, which may contribute to the rapid spread of LPAIV genomes over long distances.
Methods. Biological material was sampled as part of active monitoring of influenza among wild birds in Ukraine in 2010-2015. Serological tests and PCR were conducted in 2011-2012 in the Department of Avian Diseases of the NSC IECVM according to standard methods.
Sequencing was performed at the Friedrich-Loeffler-Institute in 2012.
Results. The hemagglutinating isolate was received from cloacal swab of an adult clinically healthy black-headed gull (*Larus ridibundus*). Serological tests classified the isolate as H16 subtype. Hemagglutinating activity amounted to 7-9 $\log_2$ in Hemagglutination test (HAT). Based on neuraminidase inhibition assays, 3 subtypes of neuraminidase were identified. Thus, viral isolate A/black-headed gull/Utluk/2-2-08/2011 is classified as AIV H16N3. This virus belongs to the unique AIV, which is very rarely isolated. It is low pathogenic to birds, so it may be suitable for the manufacture of veterinary immunobiological drugs. Phylogenetic analysis has shown that the virus has close links with influenza viruses of this subtype from Mongolia A/black-headed gull/Mongolia/1756/2006(H16N3) and KazakhstanA/herring gull/Atyrau/2216/2007(H16N3).
Conclusions. As of today, this is the only H16 subtype virus isolated in Ukraine. Further research is needed to better understand the evolution and ecology of this subtype of viruses.

O179.

Protection conferred by a DNA vaccine against highly pathogenic avian influenza in chickens : effect of vaccine schedule

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Clade 2.3.4.4 highly pathogenic avian influenza (HPAI) H5 viruses of the Asian lineage HPAI H5N1 virus A/goose/Guangdong/1/96 have spread worldwide through wild bird migration in two major waves: in 2014/2015 (clade 2.3.4.4a), and since 2016 up to now (clade 2.3.4.4b). Due to the increasing risk of these HPAI H5 viruses to establish and persist in the wild bird population, implementing vaccination in certain sensitive areas could be a complementary measure to the disease control strategies already applied.

In this study, the efficacy of a novel DNA vaccine, encoding a H5 gene (*A/gyrfalcon/Washington/41088-6/2014* strain) of clade 2.3.4.4a was evaluated in specific pathogen-free (SPF) white leghorn chickens against a homologous and heterologous HPAIH5 viruses. A preliminary analysis of different vaccination schemes allowed the following to be selected for further investigation: a single vaccination at 2 weeks old (1 dose), and a vaccination at 2 weeks old, boosted at 4 weeks (2 doses), with or without adjuvant. The groups that received 1 dose with adjuvant or without adjuvant as well as 2 doses with adjuvant demonstrated full clinical protection and a significant or complete reduction of viral shedding against clade 2.3.4.4a homologous challenge at 6- and 25-week-old. The clade 2.3.4.4b heterologous challenge of 6-week-old chickens vaccinated with 2 doses with or without adjuvant showed similar results, indicating good cross-protection induced by the DNA vaccine. Long lasting humoral immunity was observed in vaccinated chickens up to 18 or 24 weeks of age, depending on the vaccination schedule. The analysis of viral transmission after homologous challenge showed that sentinels vaccinated with 2 doses with adjuvant were fully protected against morbidity and mortality with no excretion detected. This study of H5 DNA vaccine efficacy confirmed the important role that this type of so-called third-generation vaccine could play in the fight against HPAIH5 viruses.

156.

Advances in the molecular biology diagnosis for the Equine Infectious Anemia Virus. Application of targeted sequence enrichment and long reads next-generation sequencing protocols

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Equine infectious anemia virus (EIAV) shares more of its clinical signs as fever, anemia, thrombocytopenia, and anorexia in equids with other equine diseases. It is difficult for the veterinaries to identify this disease and rapid diagnostic tests are necessarily to help in the treatment of the infected horses. Unfortunately, the high mutation rate in the equine infectious anemia virus (EIAV) genome is one if not the main reason why we have not been able to develop a molecular biology diagnostic test for this disease. In addition to that, the low number of full genome sequences available for our scientific community is a limit to the development of those diagnostic tests. In our study, we have taken advantage of the new technologies of sequencing to explore a new approach using the SureSelect target enrichment system coupled with Illumina Next-Generation Sequencing. This approach has been used to characterize the proviral DNA of Equine Infectious Anemia Virus (EIAV) from asymptomatic horses. This approach allowed us to direct sequencing of the EIAV whole genome without cloning or amplification steps. We could then obtain the complete genomic DNA sequences of French EIAV strains for the first time. In addition to that, we also have used another approach using the oxford nanopore technology and obtained long NGS reads of genomic DNA from equine cells infected with EIAV (in-vitro), where we have identified read that contains the complete sequence of the EIAV virus in one read. Those excellent results and the combination of both approaches open to us explore the application of an NGS hybrid protocol. This protocol will help us to identify first I) the EIAV on infected equids even before the seroconversion step and second II) will help us to improve the quality of the EIAV sequences characterized by our lab. Finally, the characterized sequences will contribute to developing a diagnostic test for EIAV based on molecular biology techniques as the RT-qPCR in the near future.

200.

Vector competence of Belgian *Anopheles plumbeus* and *Culex pipiens* mosquitoes for Japanese encephalitis virus

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Japanese encephalitis virus (JEV) is a mosquito-borne virus that is considered an emerging disease. It is therefore important to know if indigenous mosquitoes would be able to spread JEV upon introduction in a new area. Here we determined the vector competence of Belgian field-collected *Anopheles plumbeus* and *Culex pipiens* mosquitoes for JEV. Mosquitoes were orally exposed to an infectious blood meal containing $10^{6.39}$ TCID50/ml JEV (Nakayama strain), followed by a 14-day incubation period at 25°C. Infection and dissemination of JEV was investigated by detection of viral RNA by qRT-PCR in the abdomen and legs/wings/heads, respectively. In *Culex pipiens*, infection and dissemination rates of 34.7 and 21.3 % were found by qPCR while this was 13.8 and 13.8 % for *Anopheles plumbeus*. The majority of qPCR positive samples were confirmed positive in virus isolation. These findings suggest the existence of a stronger midgut infection barrier for JEV in *Anopheles plumbeus* than in *Culex pipien*s. The latter species also seems to have a midgut escape barrier for JEV. Saliva is currently being tested to determine the transmission ratio and the effect of temperature on the vector competence will be studied in the coming months.

O113.

Third generation nanopore sequencing of honeybee hemolymph as a new screening tool for honeybee viruses

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Introduction: Viruses are an important contributor to high honeybee mortality rates. These viruses are mostly picornaviruses but some have not yet been classified such as chronic bee paralysis virus. Historically, the prevalence of these viruses has been determined via PCR at one timepoint. This way of working has different disadvantages. Only the predetermined viruses are screened, which poses the risk of missing important viruses. By only sampling at one timepoint, virus kinetics cannot be determined but these are highly relevant to determine their importance.
Aims and methods: Third generation nanopore sequencing can solve these problems. By using this new technique, all viruses and other pathogens can be screened. Young and old bees (nurses and foragers) were sampled every two weeks in April and May, 2021. Their hemolymph was collected and processed for nanopore sequencing.
Results: A clear difference in viral load can be seen between young and old bees. The most prominent one is Lake Sinai virus which is present at high levels in foragers but absent in nurses. This clear age specificity gives more insights in the still totally unknown pathogenesis of this virus. Sacbrood virus and chronic bee paralysis virus were detected in both age groups. Chronic bee paralysis virus is known to be present in the hemolymph but Sacbrood virus should be prominently located in the head, according to literature. This stresses the opportunity of using hemolymph for virus screening, as most viruses pass through the hemolymph to reach different organs.
Conclusion: Third generation sequencing of honeybee hemolymph is a promising technique to analyze honeybee viral loads and kinetics of infection. Characterization of the microbiome present in honeybee hemolymph in function of time will allow us to study which pathogens are causing problems in vivo.

O114.

An Equine Coronavirus Associated Epidemic of Infectious Pyrexia in Iceland

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An epidemic of infectious pyrexia of unknown aetiology swept through the isolated, native Icelandic horse population in 1998. Despite extensive restrictions, the disease spread from the primary foci all over the country during February – October 1998. Most horses were mildly affected with slightly elevated body temperature and reduced appetite. However, some horses had a temperature up to 42°C and showed anorexia for some days. Approximately 0.02% of the population of 80,000 horses, died due to complications like severe colic and eclampsia in mares at parturition or early lactation. Clinical signs and negative bacterial isolation, together with orofecal transmission strongly pointed toward a viral infection. Despite extensive investigations, no viruses known at that time to infect horses, could be connected to the disease. To determine if this epidemic of infectious pyrexia in horses was caused by equine coronavirus (ECoV) a retrospective, immuno-epidemiological study was conducted. Serum samples from the Icelandic Horse Biobank, collected in the period 1990-2020 were tested, employing a recently developed ELISA for detection of antibodies to equine ECoV together with an ECoV virus neutralisation test (VNT). ECoV antibody seroprevalences in sample sets retrieved before, during and after the epidemic showed a strong increase in seroprevalence in 1998. Antibody responses against ECoV in paired serum samples collected from 18 affected horses during the epidemic revealed both in ELISA and VNT seroconversions or highly significant increases in antibody levels. Paired serum samples collected in 2021 provided evidence for ECoV still circulating in the population and causing infectious pyrexia. This study strongly indicates that the epidemic of infectious pyrexia in native Icelandic horses in 1998 was caused by introduction of ECoV into an immunologically naïve population. The current seroprevalence of 40-60% seems to prevent serious outbreaks.

O157.

Development and Validation of Four Duplex Real-Time PCR Assays for Sensitive Detection of Pathogens Associated with Equine Diarrhoea

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Diarrhoea is a major problem in young and adult horses. Apart from non-infectious causes for diarrhoea such as foal heat diarrhoea, dietary imbalance, lactose intolerance and parasitic infections several bacteria and viruses are associated with diarrhoea. To monitor and detect these infections we developed four duplex PCRs for detection of six viral and bacterial pathogens: equine coronavirus (ECoV), equine rotavirus (ERV), *Lawsonia intracellularis*, toxinogenic *Clostridium difficile* species (*TcdB* gene), toxinogenic *Clostridium perfringens* type A and type C species (CPA, CPB and CPB2 genes) and *Salmonella* species. The foal and yearling diarrhoea PCR panel included all PCRs, whereas in the diarrhoea PCR panel for adult horses ERV and *Lawsonia intracellularis* were excluded. DNA/RNA isolation was carried out with the MagMax-96 Total Pathogen Isolation kit. The extraction platform used was the KingFisher 96 Magnetic Particle processor. For each PCR the AgPath-ID One-Step RT-PCR Kit was used. Primer and probe sets were selected from the literature and specificity and inclusivity were analysed *in silico*. In every duplex PCR one probe was labelled with FAM and one probe was labelled with Cy5. For each pathogen a well-defined strain with known titre in pfu or $TCID_{50}$ per ml was used, and decimal dilution series in PBS and in pooled faecal Eswab suspensions were made in threefold and were tested in three different test runs. The undiluted samples were also used to check for cross-reactivity in a panel of 20 different gastrointestinal viruses and bacteria. Detection limits for the viruses and bacteria ranged from 0.01 – 1 $TCID_{50}$/PCR reaction and from 1-10 cfu/PCR reaction, respectively. All PCRs showed a linear range of at least 4 log10 values, and efficiency of all PCRs was between 90-110%, allowing reliable quantification. Repeatability and reproducibility were well within limits with variation coefficients of < 5% and < 7%, respectively. Although Ct-values of undiluted strains were low in the homologous PCRs, no cross-reactivity was observed with other pathogens represented in the inclusivity/exclusivity panel. These duplex PCR assays form a rapid, sensitive and cost-effective assay to diagnose and quantify simultaneously the main pathogens involved in equine infectious diarrhoea.

158.

African Swine Fever Diagnostics by Molecular Biology Methods Based on Enhanced rules of Bio-Safety and Quality Standards in Georgia

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African swine fever (ASF) is an exotic and especially dangerous disease. Spread of this infection is a global concern and may reoccur in Georgia. The outbreak in Georgia was caused by genotype II (L. Dixson *et al*., 2008). Molecular and genetic study (VP72-based) revealed 23 genotypes of the virus. According to the current data, outbreaks are usually caused by genotypes I and II, which have a high potential of spread. One of the main methods of diagnostics is PCR that represents the main priority to identify disease on the early stage that will provide capability to conduct preventive measures against the disease, to manage it and have accurate diagnostic results. Comprehensive appropriate diagnostic tools and methods have been developed at SLA. The laboratory is BSL-2 level, certified by ISO 9001:2015 and accredited by ISO/IEC 17025:2017. All diagnostic procedures are performed adhering biosafety rules and International Standards.

At present the modified diagnostic methods are established at SLA which provide the opportunity to conduct laboratory tests based on molecular and genetic study. In case of recurrence of the disease, laboratory will be able to diagnose ASFv by qRT-PCR and in case of POS results, laboratory in parallel can determine the genotype of the circulating virus and be able to identify any changes in the virus by ASFv genotyping method.

There are several approved diagnostic methods used at SLA for testing ASFv by virus specific real time polymerase chain reaction (qRT-PCR) and polymerase chain reaction (PCR) for genotyping, where three loci of the ASFv genome are used: 1) C-terminus of *B646L,* which encodes protein p72 (Bastos *et al*., 2003); 2) *E183L* which encodes protein p54 (Gallardo *et al*., 2009); and 3) central variable region primers.

For this reason, n=50 ASFv-containing test samples were selected, collected in different regions of Georgia in 2007-2009. As expected, POS samples by RT-PCR were POS, in parallel performed genotyping and band sizes in the positive samples were typical for genotype II viruses.

This research allowed us to conduct laboratory-based studies to identify molecular and genetic characteristics of ASFv. If necessary, SLA will conduct similar studies in the future in Georgia**.**

O115.

Intra- and Inter-Cattery Epidemiology of Feline Coronavirus in Belgium between 2018 and 2021

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Feline Coronavirus (FCoV) is associated with enteric and systemic infections in cats. While enteric manifestations of the virus are generally asymptomatic, mild to severe enteritis can occur. As enzootic pathogen in all cat populations, Feline Enteric Coronavirus (FECV) can persist for several months up to years within the intestinal epithelium, facilitating *in-host* evolution. The latter poses a potential threat since a shift in viral tropism to monocytes and macrophages is thought to result in Feline Infectious Peritonitis (FIP), a chronic and lethal systemic infection. While studies indicated the importance of some mutations, the exact cause and mechanism of FCoV-to-FIPV conversion are still not well understood. As such this work focused on establishing a feces-to-whole-genome-sequence workflow to evaluate the intra- and inter-cattery epidemiology of Belgian FCoV strains.
Fecal samples from various catteries (n=12) across Belgium were collected between 2018 and 2021. After RT-qPCR confirmation, a targeted FCoV amplicon-tiling protocol was used to generate near-complete (90-100% completeness) FCoV genomes using long-read nanopore sequencing at PathoSense BV. Curated genomes (n=157) were used to study Belgian FCoV diversity, intra- and inter-cattery epidemiology, but also allowed to investigate evolutionary rates and traces (*e.g.* mutations) within the 30 kbp genome. While each cattery represented a unique FCoV clade, intra-cattery epidemiology showed cat-to-cat transmissions over time and a clear association of specific FCoV strains with the introduction of new (foreign) cats. The dataset also allowed to estimate whole genome and spike mutational rates to be $1.44x10^{-3}$ and $4.1x10^{-3}$ nucleotide substitutions per site per year, respectively, suggesting a rather low evolutionary level as compared to other RNA viruses.
Current data represents the biggest FCoV whole genome dataset from a single country to date. A wide diversity of FCoV strains is circulating in Belgium, linking unique strains to each cattery. Also, estimated evolutionary measures could be established for FCoV evolution. Our targeted FCoV whole genome sequencing protocol is promising to allow comparative and comprehensive studies of genetic differences between FECV and FIPV cases. It will also aid to shed light on unanswered research questions on FCoV-to-FIPV conversion and will facilitate the search for new diagnostic tools and cures.

O116.

Molecular Epidemiology of Porcine Parvovirus Type 1 (PPV1) and the Reactivity of Vaccine-induced Antisera against Historical and current PPV1 strains

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Porcine Parvovirus Type 1 (PPV1) contributes to important losses in the swine industry worldwide. During a PPV1 infection, embryos and fetuses are targeted, resulting in stillbirth, mummification, embryonic death, and infertility (SMEDI syndrome). Even though vaccination is common in gilts and sows, strains mainly belonging to the 27a-like group have been spreading in Europe since early 2000s, resulting in SMEDI problems, requiring in-depth studies into the molecular epidemiology and vaccination efficacy of commercial vaccines.
Whole genome sequences from PPV1 cases and ancient viral stocks (1963-2021) from France (n=7), The Netherlands (n=7), Germany (n=4), Belgium (n=2), America (n=2), United Kingdom (n=1) were generated at PathoSense BV. Also 64 Danish VP1/2/3 sequences were included originating from field samples, collected between 2006 and 2021. Using new and existing VP1 data, an extensive BEAST analysis was performed. In addition, vaccination-induced antisera originating form three commercial vaccines (Porcilis© Ery/Parvo/Lepto, Eryseng© Parvo, or ReproCyc© ParvoFLEX) were tested for antibody titers against various field strains using an ImmunoPeroxidase Monolayer (IPMA), Haemagglutinin Inhibition (HI), and Serum Neutralization (SN) assays. Here, we showed that PPV1 has evolved since 1855 [1737, 1933] at a rate of 4.71×10^{-5} nucleotide substitutions per site per year. Extensive sequencing allowed to evaluate and reassess current PPV1 VP1-based classifications, providing evidence for the existence of four relevant phylogenetic groups. While most European strains belong to the PPV1a (G1) or PPV1b (G2 or 27a-like) group, most Asian and American G2 strains and some European strains were divided into a virulent PPV1c (*e.g.* NADL-8) and attenuated PPV1d (*e.g.* NADL-2) group. The increase of the swine population, vaccination degree, and herd management (biosafety and gilt acclimatization) influenced the evolution of PPV1. The reactivity of anti-PPV1 antibodies from sows vaccinated with Porcilis© Parvo, Eryseng© Parvo, or ReproCyc© ParvoFLEX against different PPV1 field strains was the highest upon vaccination with ReproCyc© ParvoFLEX, followed by Eryseng© Parvo, and Porcilis© Parvo. Our findings contribute to the evaluation of the immunogenicity of existing vaccines and support the development of new vaccines. Finally, the potential role of cluster-specific hallmark amino acids in elevated pathogenicity and viral entry are discussed.

117.

Success of Influenza A Virus Whole Genome Sequencing from Oral Fluids Depends on Sample Storage and Sequencing Protocol

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Influenza A Virus (IAV) is a single-stranded negative-sense RNA virus and common cause of seasonal flu. The virus genome comprises 8 RNA segments facilitating genetic reassortments, resulting in a wide variety of IAV strains. To study these processes, its epidemiology and (reverse) zoonotic potential, the genetic code of each segment should be unraveled. Fortunately, numerous sequencing approaches (*e.g.* Oxford Nanopore Technologies) allow cost-efficient sequencing of full-length IAV segments. Importantly, to obtain complete IAV segment sequences, various factors should be considered. These include proper sample collection, storage, and processing. Hence, this work focused on comparing two IAV whole genome sequencing protocols and assessed the influence of sample storage.
Oral Fluids (OFs; n=35) were collected from 21 Polish pig farms between 2017 and 2020. Samples from 2017 were stored at -22°C and transfered to -80°C in 2019. All other samples were immediately stored at -80°C. Prior to storage, RNA was isolated and viral loads were determined by RT-qPCR. Subsequently in 2022, RNA was extracted again and submitted to RT-qPCR viral load assessment and IAV whole genome sequencing long-read sequencing (ONT). Sequencing success of two sequencing protocols (King *et al.,* 2020 and Van Poelvoorde *et al.,* 2021) was determined based on genome completeness. This allowed to recommend guidelines for IAV whole genome sequencing to increase sequencing success from OF field samples. The resulting data showed a significant storage-associated loss of IAV viral load with ΔCt ($Ct_{prior\ storage} - Ct_{post\ storage}$) values of 6.3 (±4.9) and 0.6 (±2.3) for samples stored at -22°C and -80°C, respectively. Even though, samples stored at -22°C for 2 years showed reduced viral loads, some samples (4/13 from 2017) remained stable. While protocol 1 showed a 31% whole genome recovery success, 83% of samples showed 8 complete IAV segments using protocol 2. Thus, targeted IAV whole genome sequencing success from OF samples highly depends on sample storage conditions, suggesting immediate long-term storage at -80°C or RNA isolation. Though, still other factors (*e.g.* sample impurities) should be considered. In addition, care should be taken in choosing the optimal IAV whole genome sequencing protocol when studying field samples using nanopore sequencing.

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O118.

An Epidemiology Model of Influenza A Virus in Wild Birds Based on Surveillance of Black-headed Gulls (*Chroicocephalus ridibundus*)

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Wild aquatic birds are a natural reservoir for low pathogenic avian influenza viruses (LPAIV). The vast majority of influenza A virus (IAV) subtypes (H1–H12, H14-H15) are maintained by birds of the Anatidae family (ducks, geese and waterfowl), while the occurrence of AIV H13 and H16 subtypes depends on birds of the Laridae family (gulls, terns and skimmers), in particular gulls. Annual AIV epidemics occur in gulls in late summer or early autumn in predominantly juvenile birds and are combined with a year-round enzootic state of virus persistence in temperate climates in the northern hemisphere. Infection dynamics of AIVs in wild aquatic birds have been described extensively, yet studies on the mechanisms underlying the epidemiology of multiple AIV subtypes in wild birds are scarce. Here we analyze the year-round population infection dynamics of H13 and H16 AIVs in the population of juvenile, subadult and adult Black-headed gulls (BHGU, *Chroicocephalus ridibundus*) by using a Susceptible Infected Recovered Susceptible (SIRS) model. Demographic values were derived from bird population counts in The Netherlands. Furthermore, model parameters were estimated based on wild bird surveillance and experimental infection of BHGU with H13 and H16 LPAIVs. Our model demonstrated two LPAIV infection peaks during the year: one peak after fledging and one peak late autumn, and varying co-circulation dynamics of H13 and H16 LPAIVs. We anticipate our model to contribute to insights also regarding the population dynamics of the multiple LPAIVs that are circulating among birds of the Anatidae family.

O70.

Alphaherpesvirus-Induced Inhibition of the m6A Writer Complex and Degradation of m6A-Methylated Transcripts

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Over the past years, it has become clear that the so-called epitranscriptome, consisting of chemical modifications in mRNA that affect mRNA translation and processing, serves as an important additional layer in post-transcriptional regulation of gene expression. N6-methyladenosine (m6A) represents the most abundant internal chemical modification in mRNA and is a dynamic modification that is generated via a multi-subunit m6A methyltransferase complex (the m6A writer complex) and can be removed by m6A eraser proteins. M6A-marked mRNA can be recognized by so-called m6A reader proteins, including YTHDF proteins, which may affect mRNA degradation and translation. It has become clear that several dynamic immune-related transcripts, including interferon-related transcripts, are m6A-methylated.

Herpesviruses display a multitude of mechanisms to suppress the host immune response, allowing them to cause lifelong persistent infections. Alphaherpesviruses represent the largest subfamily of the herpesviruses, and include herpes simplex virus (HSV) in man and pseudorabies virus (PRV) in pigs. Currently, little is known about the potential interaction of alphaherpesviruses with the epitranscriptome.

We report that alphaherpesvirus infection of host cells leads to a dramatic reduction in levels of m6A-methylated mRNA, and that these viruses make use of at least two strategies to interfere with the m6A epitranscriptome. A first strategy consists of an inhibition of the m6A writer complex, thereby preventing addition of new m6A modifications to transcripts. This inhibition depends on the viral US3 protein kinase, which triggers phosphorylation of several components of the m6A writer complex and detachment of the complex from the chromatin. These findings provide the first evidence of phosphorylation that is associated with inactivation of the m6A methyltransferase complex, in this case mediated by the viral US3 protein. A second strategy consists of a virus-induced preferential degradation of m6A-containing transcripts, which is triggered via the YTHDF m6A reader proteins. Our data suggest that the dramatic impact of alphaherpesvirus infection on the m6A epitranscriptome may contribute to evasion of the antiviral interferon response, as knockdown of YTHDF results in strongly increased expression of interferon stimulated genes (ISG). Together, these findings provide an entirely novel aspect of alphaherpesvirus-host interactions, indicating that alphaherpesvirus infection dramatically affects the host epitranscriptome.

O119.

Large Scale Cross-Sectional Serosurvey of Hepatitis E Virus Infection in Belgian Pig Farms and Identification of Risk Factors for Herd Infection

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Hepatitis E virus (HEV) is the causative agent of hepatitis E disease in humans. In industrialized countries, sporadic cases of hepatitis E are usually asymptomatic and self-limiting. However, immunocompromised individuals may develop chronic forms of hepatitis E, leading to liver cirrhosis. These cases are due to HEV genotypes 3 or 4, for which pigs are now considered a major animal reservoir of human infection. However, the burden that the porcine HEV reservoir imposes on public health is still unclear. Here, we investigate HEV prevalence in Belgian pigs through a large scale cross-sectional serological survey. Results show the widespread distribution of HEV in Belgian pigs, with a herd seroprevalence of 80.45% ($CI_{95\%}$ = 75.88% - 85.03%). Pigs are infected at a young age and show increasing levels of antibodies with age, leading to individual seroprevalences of 47.86% ($CI_{95\%}$ = 45.93% - 49.78%) and 80.26% ($CI_{95\%}$ = 77.12% - 83.40%) overall and in adult sows, respectively. Depending on herd characteristics, such as size and type, HEV herd statutes differ significantly (p-values = 0.0062 and 0.0002, respectively). Herd seropositivity increases with the size of the herd, from an apparent prevalence of 70.91% ($CI_{95\%}$ = 58.91% - 82.91%) for herds housing between 101 and 500 pigs to 94.12% ($CI_{95\%}$ = 88.53% - 99.71%) for herds housing more than 2000 pigs. HEV prevalences are 73.28% ($CI_{95\%}$ = 65.71% - 80.86%), 95.83% ($CI_{95\%}$ = 90.18% - 100.00%) and 89.23% ($CI_{95\%}$ = 81.69% - 96.77%) for slaughter-, closed and mixed farrow-to-finish pig herds, respectively. All HEV found by nested RT-PCR in sera from pigs weighing less than 40 kg belonged to HEV genotype 3. Finally, the results were coupled to a recently made mandatory questionnaire regarding general biosecurity measures undertaken in the farms. After multivariate analysis, several risk factors of HEV herd-seropositivity were identified: herd type, absence of hand washing between rearing compartments, practice of piglet adoption, absence of a hygiene lock. These results set a baseline for future studies aiming to unravel the dynamic of HEV infection in pigs, evaluate the impact of HEV on public health, and for the potential development of mitigating measures.

121.

Detection of influenza A virus (IAV) in nasal swabs and oral fluids cross-sectionally collected in 35 Polish swine herds

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Influenza A virus (IAV) plays a significant role as a primary and secondary pathogen of respiratory tract in swine. Diagnosis of influenza has to involve laboratory detection of IAV, as the clinical symptoms are not pathognomonic. The detection of the virus, its RNA or proteins in nasal swabs, provides definitive evidence of the infection. It was also shown that IAV can be detected in oral fluids of pigs even at 21 days post infection, which can assist in the detection of subclinical infections. Thus, the aim of the study was to compare usefulness of testing nasal swabs (NS) and oral fluids (OF) for IAV surveillance program.
The study was performed on 35 Polish pig farms reporting influenza-like clinical signs. NS and OF were collected cross-sectionally from 5-20-week-old-pigs, irrespective of their clinical status. RNA from NS pooled by age group and individual OFs was extracted and tested using *virotype* Influenza A RT-PCR kit (Indical).
71.4% tested farms were IAV-positive. The virus prevalence in OF was higher than in NS (43.1% and 23.4%, respectively). The virus was detected most often in NS of nursery pigs while its detection in OFs was possible in all age groups. The viruses detected in 5 NS pools and 6 OFs from 5 farms were subtyped with triplex real-time RT-PCR (Henritzi et al., 2016) and were found to be H1avN2 subtype. In 2 OFs from a single farm H1pdmN1pdm subtype was detected. Although IAV detection in NS could be interpreted as ongoing infection in the respiratory tract, the interpretation of the virus detection solely in OF is more difficult. The finding of IAV only in OF samples, in the absence of the virus detection in NS from six tested farms, may be interpreted as the environmental contamination (after the past infections), or as a proof of a very low prevalence of ongoing infection, which the employed protocol of NS collection and testing was not able to detect. Nevertheless, testing OF samples can be a useful tool for IAV surveillance programs

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O120.

Detection of porcine parainfluenza virus 1 (PPIV1): genetic diversity and co-infections with influenza A virus (IAV) and porcine reproductive and respiratory syndrome virus (PRRSV)

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Porcine parainfluenza virus 1 (PPIV1) has been detected in Asia, the Americas and Europe, but knowledge on its epidemiology and genetic diversity remains very limited. In order to assess the virus prevalence in Poland, nasal swabs and oral fluids collected from pigs from 30 farms were examined with RT real-time PCR. Influenza A virus (IAV) and porcine reproductive and respiratory syndrome virus (PRRSV) infection statuses of PPIV1-positive samples were also tested. Additionally, the complete nucleotide sequences of the fusion (F)-protein gene obtained from samples from 12 Polish herds were analyzed and compared to other available genetic data.
The results showed that 76.7% farms were PPIV1-positive. Different patterns of PPIV1 circulation in herds with mild–moderate respiratory disease were observed. Co-infections with IAV and PRRSV were infrequent and detected in 8 (23.5%) and 4 (11.8%) out of 34 PPIV1-positive nasal swab pools from pens of diseased pigs, respectively. In one pen PPIV1, IAV, and PRRSV were detected at the same time. Interestingly, PPIV1 mean Ct value in samples with a single PPIV1 infection was significantly higher (32.5±3.6) than in samples with co-infections (29.8±3.1) ($p < 0.05$). The virus detection in pig populations exhibiting respiratory clinical signs, negative for PRRSV and IAV, suggests that PPIV1 should be involved in differential diagnosis of respiratory problems.
All Polish sequences were clustered with other European sequences, and with one Hong Kong sequence (clade 1). The sequences from the USA and the remaining from Asia were clustered separately (clade 2), which indicated that both groups may have evolved independently.
More studies are necessary to understand the virus' emergence, epidemiology, and it's role for pigs health.

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159.

Development of a highly sensitive point-of-care test for African swine fever that combines EZ-Fast DNA extraction with LAMP detection: evaluation using naturally infected swine whole blood samples from Vietnam

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While early detection and early containment are key to controlling the African swine fever (ASF) pandemic, the lack of practical testing methods for use in the field are a major barrier to achieving this feat. Here, we describe the development of a rapid and sensitive point-of-care test (POCT), and its evaluation using clinical samples. The POCT enabled crude DNA to be extracted from swine whole blood samples within 10 min at extremely low cost and with relative ease. When combined with LAMP analysis, the entire POCT required a maximum of 40 min from the beginning of DNA extraction to final judgment. Compared to a conventional real-time PCR detection, the POCT showed a 1 log reduction in detection performance, but comparable diagnostic sensitivity of 96.4% (53/55) and diagnostic specificity of 100% (33/33) when evaluated on 89 swine whole blood samples collected from Vietnamese swine farms. The POCT is quicker and easier to perform and does not require special equipment. We validated a sensitive POCT for ASF in swine whole blood samples for field settings. This POCT is expected to facilitate early diagnosis and containment of ASF invasion into both regions in which it is endemic and eradicated.

201.

Studies with Airway Organoids reveal that the respiratory Epithelium of Bats is susceptible to Infection by Influenza Viruses of other Species

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Bats are a natural reservoir for many viruses and therefore important in the interspecies transmission of viral pathogens. To find out whether bat airway cells can be infected by viruses of other mammalian species we developed an organoid culture model derived from the respiratory tract of *Carollia perspicillata*. The cell composition of organoids resembled that of bat trachea and lungs as determined by immunofluorescent staining. Infection studies revealed that bat airway organoids (AOs) from either the trachea or lung, respectively, are susceptible to infection by two different swine influenza A viruses. The bat AOs were also used to develop an air-liquid-interface (ALI) culture system of filter-grown epithelial cells. Infection of these cells by the two swine influenza viruses was as efficient as the infection of porcine ALI cultures. Bat airway cells were found to contain only a low amount of alpha 2,6-linked sialic acids, the preferred receptor determinant for mammalian influenza A viruses. By contrast, alpha 2,3-linked sialic acid, the receptor determinant for avian influenza viruses, is abundantly present on bat cells. Therefore, bat airway cells are expected to be highly susceptible not only to mammalian but also to avian influenza viruses. Our culture models can be extended to other parts of the airways and to other species and thus provide a promising tool to analyze the transmission of viruses both from bats to other species and from other species to bats.

O180.

The telomeric repeats of Marek's disease virus vaccines are required for viral integration and genome maintenance

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Marek's disease virus (MDV) is an alphaherpesvirus that causes deadly lymphomas in chickens. Oncogenic MDV strains have been shown to integrate their genome into the telomeres of host chromosomes in latently infected T cells as well as in tumor cells. Telomeric repeats (TMRs) present at the ends of the MDV genome facilitate the integration into host telomeres. Intriguingly, the integration of oncogenic MDV strains into the host genome is a prerequisite for lymphomagenesis. Three commercially available live-attenuated MDV vaccines, CVI988, SB-1, and HVT, also harbor TMRs in their genome. However, the role of these TMRs in MDV vaccine viruses remains unknown. In this study, we set to determine whether MDV vaccine strains can integrate their genome into the host telomeres. We first assessed the integration properties of MDV vaccine strains during latency in chicken T cells and detected efficient viral genome maintenance and integration in latently infected T cells. Next, we investigated the role of TMRs in viral integration by generating MDV vaccine TMR deletion mutants. We then assessed their replication properties, genome maintenance, and integration. All TMR deletion viruses replicated efficiently in culture, indicating that the TMRs are dispensable for viral replication. However, the absence of the TMRs significantly impaired virus integration and maintenance of the viral genome in infected T cells compared to the wild-type strains. Taken together, our data provide first evidence that the TMRs of commonly used MDV vaccine strains facilitate efficient viral integration, allowing these viruses to maintain their genome during latency.

O181.

High *in vitro* ASFV recombination rate in porcine alveolar macrophages

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In the 21st century, African swine fever virus (ASFV) has become the biggest animal health and economic threat to the swine industry. Despite more than five decades of intensive research efforts there is still no effective vaccine on the market. According to present consensus the development of modified live vaccines (MLV) may provide the earliest solution to this pressing matter. The recombination ability of ASFV may have a serious effect on the stability and therefore the usefulness of MLVs, among other factors. Publications about *in silico* investigations of ASFV isolates suggest a major role of recombination in ASFV evolution, however, experimental evidence is very scarce or non-existent.

As ASFV MLV developers, we want to know more about ASFV recombination, therefore we developed a method for the *in vitro* study of ASFV recombination in one of their main target cells, porcine alveolar macrophage. Using this method, we detected high frequency homologous recombination between different ASFV strains. Isolation and whole genome sequencing of some of the progeny viruses confirmed the generation of recombinant viruses. In addition to the parental marker mutations, a few point mutations could be identified in some of the genomes of the recombinant viruses. The significance of these mutations is unknown, but their emergence is most probably rather the consequence of serial passaging and the altered biology of the recombinants than the recombination event itself.

After simultaneous coinfection, recombination between two genetic loci seemed reciprocal, generating two kinds of progeny viral genomes with allele combinations different from the parental strains and from each other in approximately equal rate. The recombination frequency was proportional to locus distances and reproducible when applying the same infection conditions.

The significance and the potential danger of the high recombination rate to ASFV MLV development and their future application will be discussed.

182.

Serological and *in silico* comparison of 27a viruses with other PPVs

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Perhaps early studies on PPV-27a strains had the highest impact on PPV research in the last two decades, claiming that highly virulent 27a viruses makeup a new cluster among PPVs and cannot be effectively neutralized either by homologous or heterologous sera. They revitalized PPV immunological and vaccine research and resulted in the publication of some very interesting papers. One of the main effects of these studies was raising doubt about the uniform immunological status of PPV species and the effectiveness of existing commercial PPV vaccines. However, later immunological investigations could not always confirm the "uniqueness" of 27a strains.
To gather additional evidence and to clarify issues of dispute we have reinvestigated the phylogenic status of 27a viruses and their relationship with other PPV strains.
Based on our phylogenetic trees and alignments, the members of 27a cluster are distinguished by the presence of the following, collectively occurring genetic markers in the VP2 protein: C261 G682 T1240 C1255 A1306. The occurrence of these five single nucleotide mutations seems to be linked, they can be hardly found alone or in combinations in lesser numbers. The comparison of the VP2 protein of 113 isolates revealed that the highest distance between the two most divergent VP2s is only 32 aa. Structural comparison of the 27a VP2 protein to that of the NADL-2, Kresse, and the vaccine strain 014 found only 8, 4 and 11 amino acid difference on the capsid surface, respectively. Regarding vaccine strains, much more divergent PPVs exist than 27a, but their highest distance from them is not more than 29 aa (~5% of the 579 aa of VP2).
We also studied the reactivity of 27a viruses with homologous and heterologous PPV positive sera from different geographic origins, and compared them with that of the "classic" PPV strains (NADL-2, Kresse, 014) in a virus neutralization assay. No significant difference was found among the different strains.
Based on our investigation and recent publications it is not very probable that 27a serological differences would be the main cause of vaccine failures experienced with this strain. Other possibilities will be discussed.

183

Development of Stable Synthetic Vaccines for Mucosal-based Protection Against Bovine Respiratory Disease

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Respiratory infections in cattle are the most frequently reported cause of death in young calves. Amongst these, Bovine Respiratory Syncytial Virus (BRSV) is a causative agent of pneumonia and high morbidity rates, with Bovine Parainfluenza-3 (BPI-3) virus associated with cough, fever and nasal discharge. Whilst vaccination against these endemic infections and other pathogenic agents is a common approach to disease control and prevention, the use of conventional whole virus vaccines has some limitations. Alternative subunit-type vaccines offer potential advantages, containing only those immunogenic viral proteins necessary to develop protective immunity and negating viral replication in host cells offer an improved safety profile. The delivery and protection of subunit antigens within vaccine administrations to facilitate establishment of effective primary immune responses is essential, and liposomal encapsulation has been used as a delivery system offering additional adjuvant properties. This work examined the potential for recombinantly expressed viral subunit antigens consisting of BRSV glycoprotein F (gF) and BPI-3 hemagglutinin neuraminidase (HN) glycoproteins to be efficiently encapsulated within liposomal particle formulations as a means to establish required immune responses for intranasal-focused immunisations. BRSV gF and BPI-3 HN consensus protein sequences were expressed within baculovirus and *E. coli* expression systems and purified with ammonium sulphate precipitation and ion exchange chromatography. Immunoreactivity of recombinant antigens was confirmed through differential interaction to viral specific sera. Freeze-dried liposome preparations containing BRSV gF and BPI-3 HN antigens were made using soy phosphatidyl choline (SPC), dimethyl dioctadecyl ammonium bromide (DDAB) and D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) using a thin film hydration technique followed by sonication for size reduction. Liposomal formulations were found to contain less than 3% moisture, with glass transition temperatures (Tg) above 40°C. Physico-chemical characterisation of antigen containing liposomes showed the average size to range from 165-185 nm and comparable to that of virus particles. *In vitro* profiling of responses of cellular exposure to liposome formulations demonstrated no negative effect on cell functioning and potential for stimulation of inflammatory cytokine responses in target cells. Ongoing work will further assess the potential of developed formulations to elicit an immuno-protective response via vaccination.

O71.

Fast Isolation of Non-adherent, Lymphocyte-like Haemocytes in Shrimp for Immunological Studies during a WSSV Infection

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Invertebrates only have an innate immunity in which haemocytes play an important role in the immune system. Similar to the variety of cells in the blood of vertebrates, haemocytes in the haemolymph of invertebrate can also be subdivided in different cell types. In our lab, 5 subpopulations of haemocytes were identified in the past by an iodixanol density gradient: hyalinocytes, granulocytes, semi-granulocytes and two subpopulations of non-phagocytic cells. For the two latter subpopulations, the haemocytes have small cytoplasm rims, do not adhere to the plastic cell-culture plates and present folds in the nucleus. These characteristics are similar to those of mammalian lymphocytes. Therefore, they were designated lymphocyte-like haemocytes. Although little is known about their function, we hypothesize, based on their morphology, that they may have a cytotoxic activity. First, a fast isolation technique was developed to separate the non-adherent haemocytes from the adherent haemocytes. After 60 min incubation on cell-culture plates, the non-adherent haemocytes were collected. The purity reached 93% as demonstrated by flow cytometry and light microscopy upon a Hematoxylin and Eosin staining. Cytotoxicity by lymphocytes is mediated by molecules such as perforin and granzymes and therefore, we searched for their genes in the shrimp genome. Genes coding for a torso-like protein, granzyme B and granzyme G were identified. Primers were designed and RT-PCR/RT-qPCR assays were developed. The results demonstrated that torso-like protein, granzyme B and granzyme G were mainly expressed in non-adherent haemocytes. In a 72-hour *in vivo* White Spot Syndrome Virus (WSSV) infection challenge, the mRNA expression of shrimp torso-like protein, granzyme B and granzyme G in haemocytes was increasing over time, which indicated that torso-like protein, granzyme B and granzyme G of shrimp haemocytes are involved in the immune response during a viral infection. In the future, antibodies will be raised against these proteins for more in-depth functional analyses, and more specifically to demonstrate the antiviral activity of shrimp non-adherent haemocytes.

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