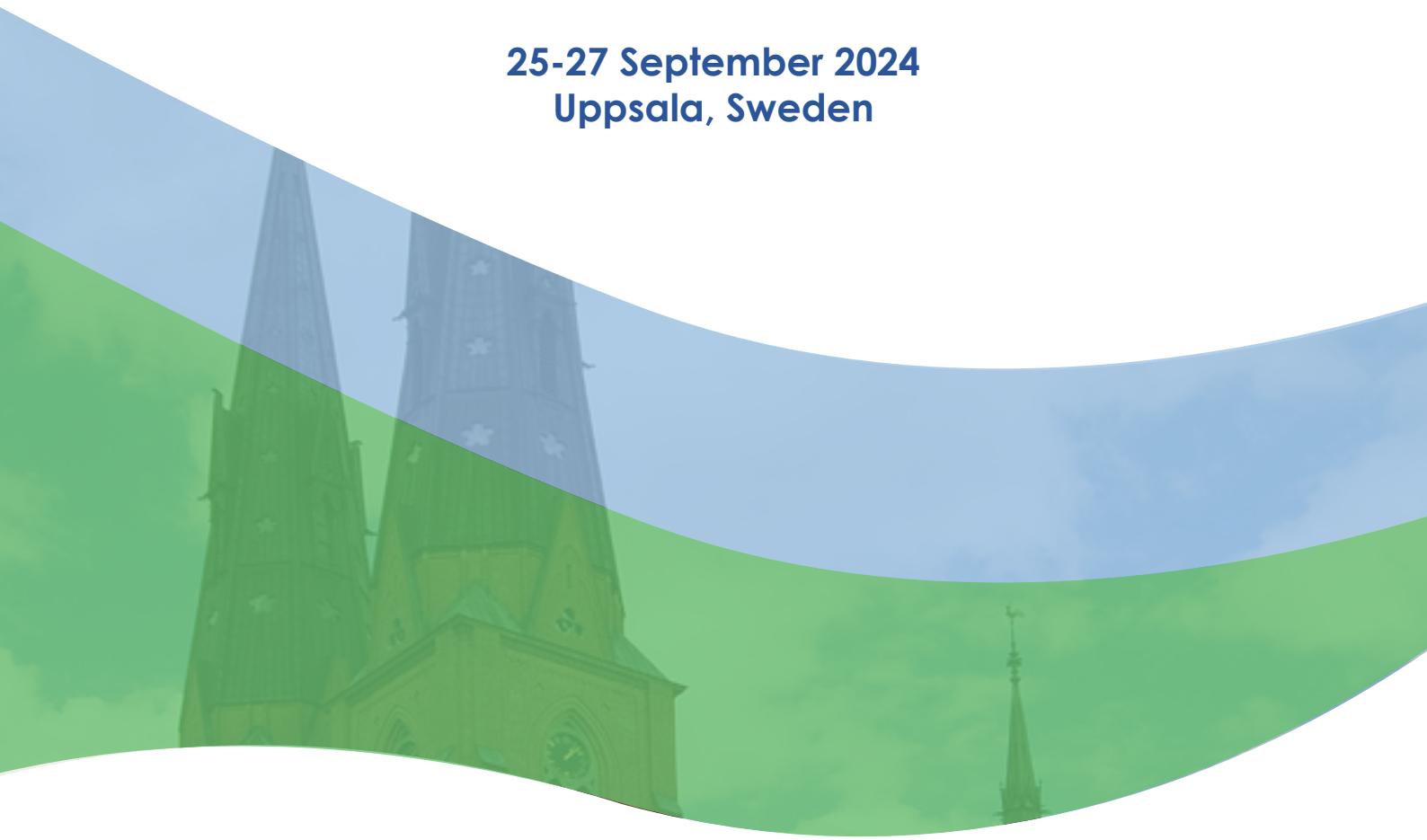
BOOK OF ABSTRACTS

16th EPIZONE Annual Meeting

Viruses, vectors and wildlife

25-27 September 2024
Uppsala, Sweden



Hosted by the Swedish Veterinary Agency (SVA)

SWEDISH VETERINARY AGENCY

WELCOME

Dear EPIZONE friends,

It is our great pleasure to welcome you to the 16th Annual Meeting of EPIZONE, hosted by the Swedish Veterinary Agency (SVA), in Uppsala!

The theme of the 16th Annual Meeting will be 'Viruses, vectors and wildlife', referring to properties of discerned recent epidemics and emerging diseases.

The focus will be on current research efforts in the field of epizootic animal diseases, including the usual EPIZONE themes, with extra attention to the role of vectors and wildlife. African swine fever, Avian influenza and vector-borne diseases will be specifically addressed. A stimulating scientific program will be provided by invited distinguished speakers and selected oral and poster presentations.

The venue will be "Norrlands nation" in Uppsala, owned by a student association since 1887. The city of Uppsala is closely associated with education and research, with the influence of Uppsala University since its foundation in 1477. Veterinary medicine is also tightly linked to Uppsala – the Swedish University of Agricultural Sciences (SLU), located a few kilometres from the city centre, harbours the only veterinary education in Sweden. Three governmental authorities of veterinary importance, the Swedish Food Agency, the Medical Products Agency, and the Swedish Veterinary Agency, are also located in Uppsala. Therefore, it is a fitting location to exchange scientific expertise, and to create and revive connections.

Our organizing and scientific committees developed a program with 8 renowned keynote speakers and more than 150 abstracts presented as talks or posters. The focus of the meeting will be on the EPIZONE partner institutes' recent research in the field of epizootic animal diseases to exchange the latest research information and to establish new contacts and collaborations. The Young EPIZONE group will organize an interesting program for young scientists. This year, there will be an additional exchange from former Young EPIZONE members who will share their experiences on networking and career paths.

Looking at the current challenges to control important epizootic diseases like African swine fever, Bluetongue and Avian Influenza, the collaborations established by and maintained within EPIZONE are as important as ever. With this 16th annual meeting, we hope to foster them further to which the enjoyable social program certainly also will contribute. We are sure that the friendly city of Uppsala, with its long academic history will be a good place for interesting presentation, inspiring discussions, and scientific curiosity.

We wish you a very interesting and fruitful meeting.

Jonas Johansson Wensman
Chair Local Organizing Committee, SVA

Wim van der Poel
Coordinator of EPIZONE

ORGANIZERS

The 16th EPIZONE Annual Meeting is organized by:

Swedish Veterinary Agency, SVA
www.sva.se/en

EPIZONE ERG
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ACKNOWLEDGEMENT

We are very grateful for the following companies supporting the 16th EPIZONE Annual Meeting

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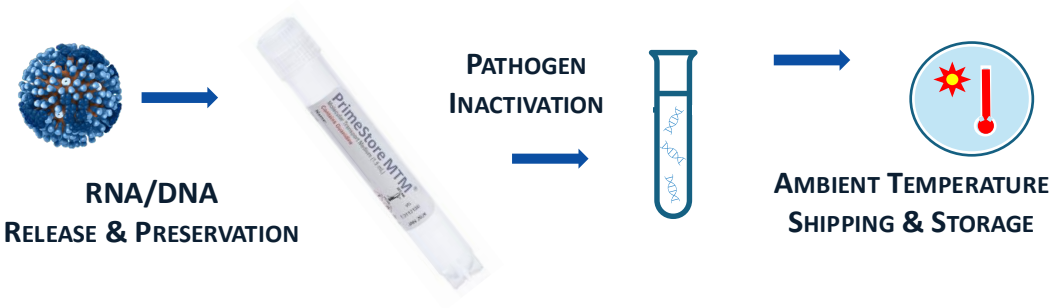

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CONTENT

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- Malin Grant, Swedish Veterinary Agency, Sweden
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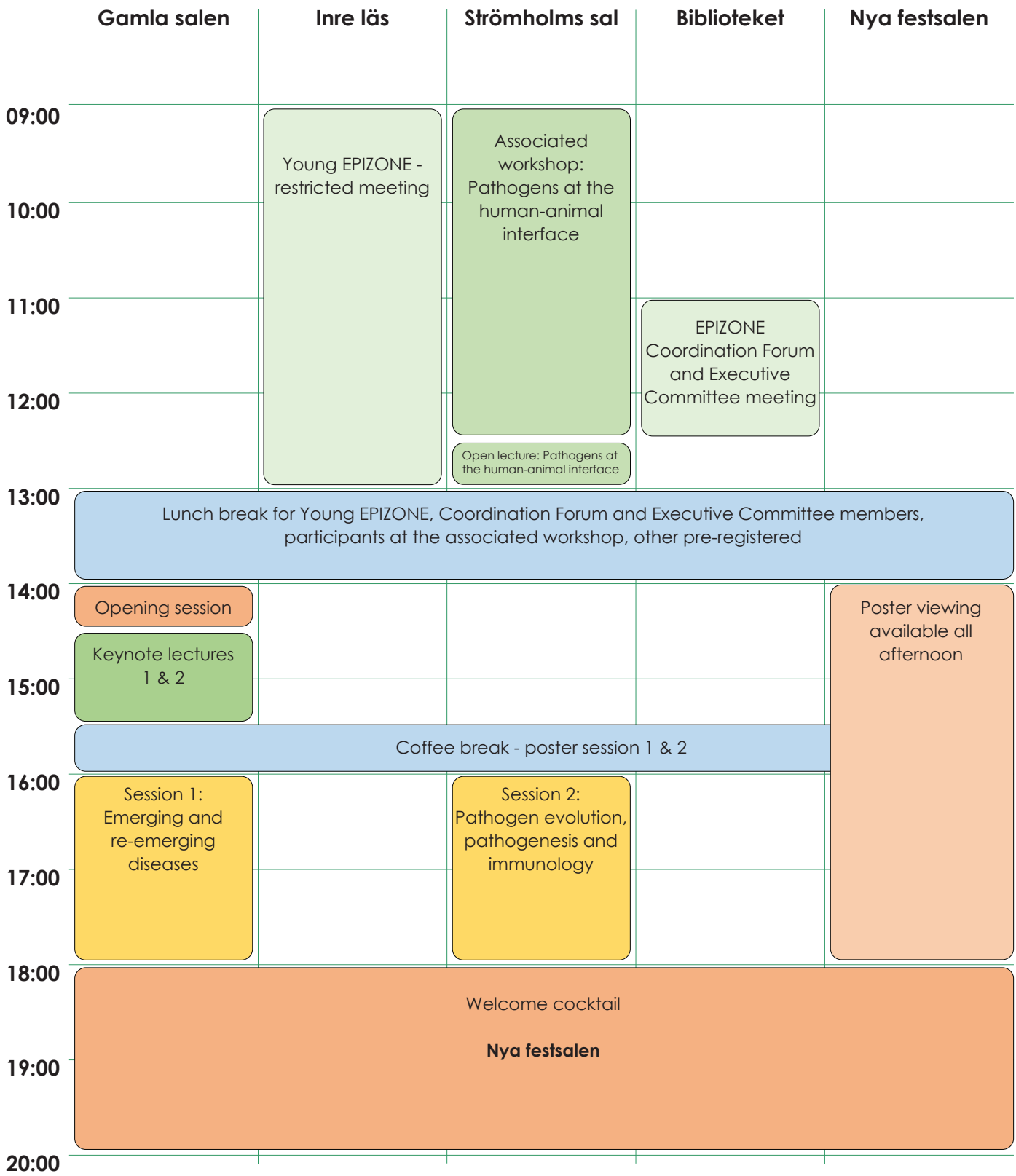
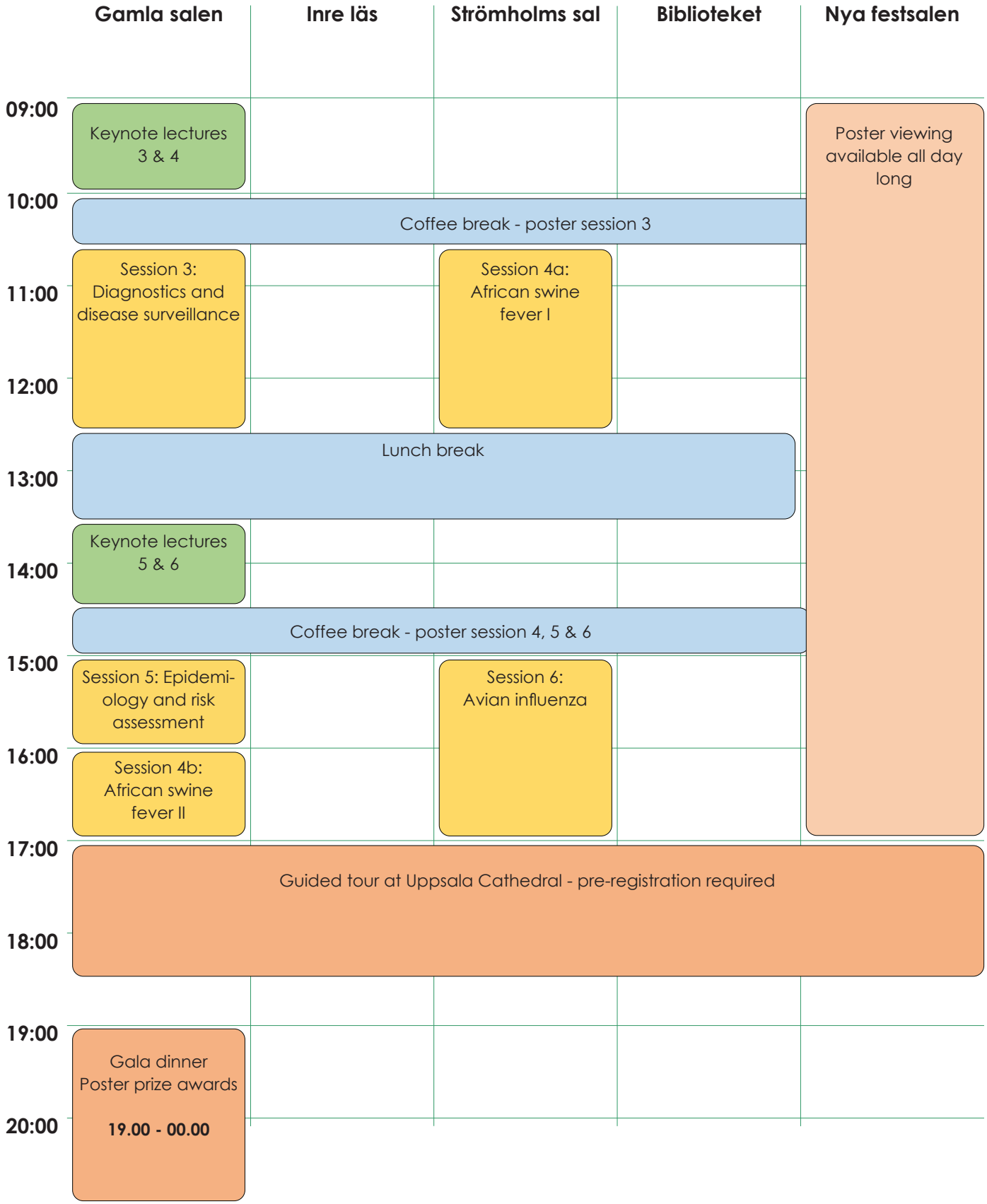
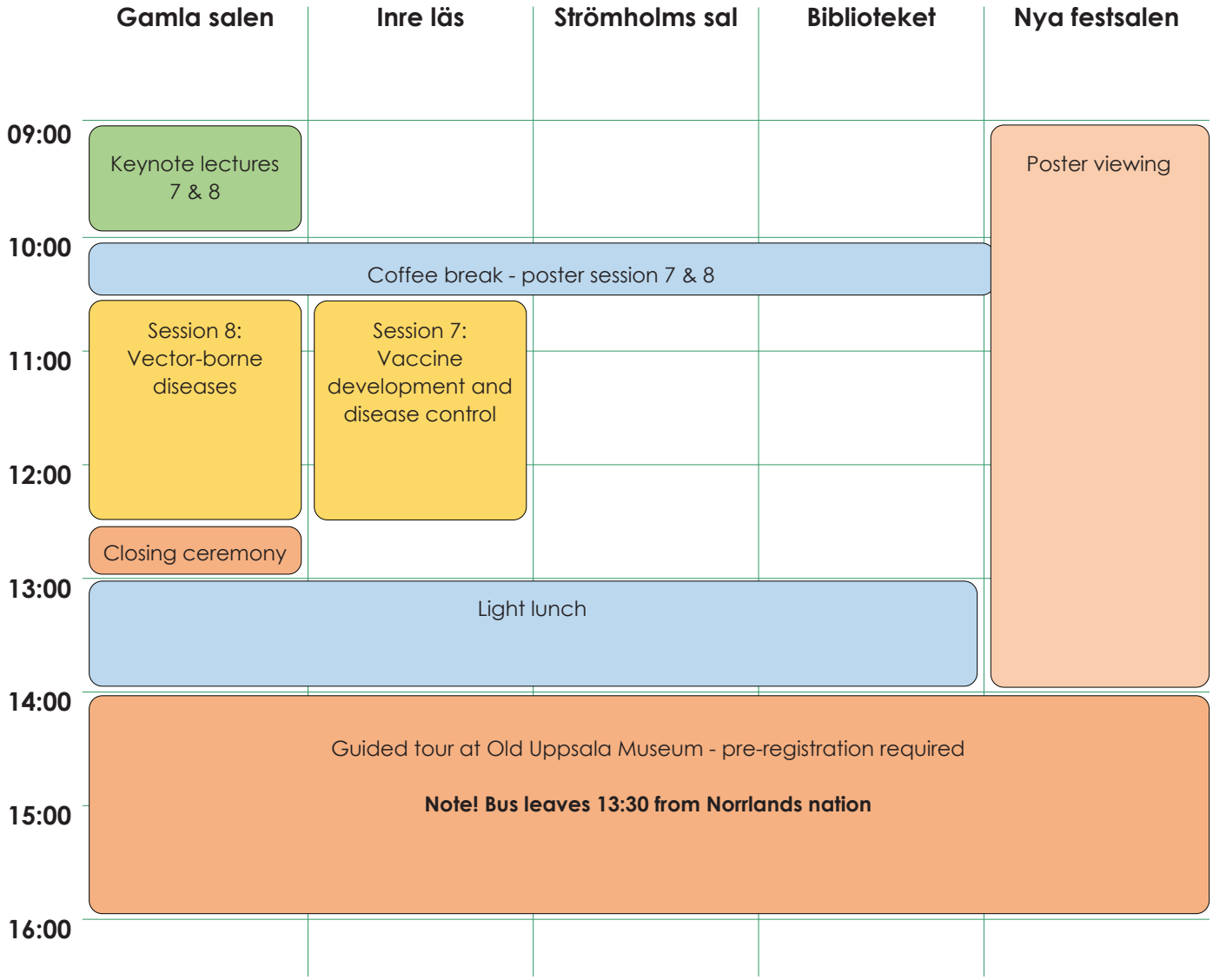
25 SEPTEMBER - PROGRAMME OVERVIEW



26 SEPTEMBER - PROGRAMME OVERVIEW



27 SEPTEMBER - PROGRAMME OVERVIEW



YOUNG EPIZONE MEETING, 25 SEPTEMBER

09:00-09:05 **Welcome and introduction.**

09:05-10:05 *Unfortunately Åsa Burman will be unable to attend the event herself. However, we are excited to announce that her colleague Caroline Uggla will be stepping in!*

"Academic productivity: From efficiency to effectiveness" by Caroline Uggla

Caroline Uggla is Reader (Docent) and a researcher in demography at Stockholm University and Åbo Akademi. She is a workshop leader at Finish on Time. Caroline is passionate about academic writing and productivity, continuously developing our knowledge not only about what we write, but also how we do it. In 2023, she published a popular science book called Familjeformeln (Natur&Kultur) that has gotten extensive media coverage. Caroline will share the productivity tools she used in writing this book, and in her academic research.

10:05-10:25 **Coffee break**

10:25-11:10 **Speed dating**, get to know each other and share joys and challenges of being a PhD student or young researcher.

11:10-11:45 **"Science communication during an infodemic" By Emma Frans**

Emma Frans is an epidemiologist, researcher at the Karolinska Institute and a science writer for Svenska Dagbladet. In social media, she has managed to build a substantial platform by effectively and humorously conveying critical thinking and a scientific approach. She is also a frequent guest on television and radio. Emma is the author of five popular science books and has been awarded numerous prizes and honors, including the Grand Journalist Prize for her pedagogical ability to convey research and science and the King's Medal of the 8th size by the Order of the Seraphim ribbon.

11:45-13:00 **Presentations from the original Young Epizone, by Robert Vrancken, Eefke Weesendorp & Matthijn de Boer**

Presentations from the original Young Epizone by Robert Vrancken PhD, Program Director at ViroVet, YE 2008-2011; Eefke Weesendorp, PhD, Head of department Bacterial surveillance and response, RIVM, YE 2008-2011 and Matthijn de Boer, PhD, Program Director at WBVR, YE 2008-2011. This year is the 16-year anniversary of the first meeting within Young Epizone. The first meeting was in 2008 Italy (Brescia). The founders of Young Epizone will present where their careers have taken them and what the Young Epizone network has meant to them.

DETAILED PROGRAMME

Wednesday 25 September 2024

08:00-18:00 **Registration**, *Reception desk*

09:00-13:00 **Young EPIZONE - restricted meeting**, *Inre läs*

09:00-09:05 Welcome and introduction

09:05-10:05 Academic productivity: From efficiency to effectiveness
Caroline Uggla

10:05-10:25 Coffee break

10:25-11:10 Speed dating, get to know each other and share joys and challenges of being a PhD student or young researcher

11:10-11:45 Science communication during an infodemic
Emma Frans

11:45-13:00 Presentations from the original Young EPIZONE
Robert Vrancken; Eefke Weesendorp; Matthijn de Boer

09:00-12:30 **Associated workshop: Pathogens at the human-animal interface**, *Strömholms sal*

11:00-12:30 **EPIZONE Coordination Forum and Executive Committee meeting**, *Biblioteket*

12:30-13:00 **Open lecture: Pathogens at the human-animal interface**, *Strömholms sal*
Chairs: Johanna LINDAHL; Mahmoud M. Naguib
James Stewart

13:00-14:00 **Lunch break for Young EPIZONE, Coordination Forum and Executive Committee members, participants at the associated workshop, other pre-registered**, *Nya festsalen*

14:00-14:30 **Opening session**, *Gamla salen*
Karl Ståhl; Wim H. M. Van der Poel; Jonas Johansson Wensman

14:00-18:00 **Poster viewing available all afternoon**, *Nya festsalen*

14:30-15:30 **Keynote lectures 1 & 2**, *Gamla salen*
Chairs: Jonas Johansson Wensman; Thomas Bruun Rasmussen

DETAILED PROGRAMME

Wednesday 25 September 2024

14:30-15:00 BTV-3 in the Netherlands: experiences of the first outbreak year.
Inge Santman; *Rene van den Brom; Jet Mars; Katrien van den Brink; Eveline Dijkstra; Lotte Roos*

15:00-15:30 Dissecting protective and detrimental immune responses against African swine fever virus
Artur Summerfield

15:30-16:00 Coffee break - poster session 1 & 2, *Nya festsalen*

16:00-18:00 Session 1: Emerging and re-emerging diseases, *Gamla salen*
Chairs: Jonas Johansson Wensman; Estelle Ågren

16:00-16:15 Evidence of exposure to SARS-CoV-2 and various neutralizing antibody profiles against variants of concerns in companion animals, Luxembourg
Chantal Snoeck; *Regina Sinner; Aurélie Sausy; Olivia Domingues; Francesco Delogu; Carole Meersschaert; Markus Ollert; Judith Hübschen*

16:15-16:30 Detection of Coronaviruses and Reoviruses in European Hedgehogs in Northern Italy
Ana Moreno; *Sabrina Canziani; Tiziana Trogu; Clara Tolini; Maya Carrera; Gabriele Leo; Federica Maccarinelli; Ambra Nucci; Enrica Sozzi; Davide Lelli; Antonio Lavazza*

16:30-16:45 Research despite the war: surveillance of emerging viral and bacterial pathogens in wild birds and animals in Ukraine
Denys Muzyka; *Oleksandr Rula; Nataliia Muzyka; Oleksandr Mezinov; Anton Vlaschenko; Ruslana Echkenko; Anastasiia Popova; Polina Yurko; Oleksandr Gaidash; Mary Pantin-Jackwood; Martin Beer; Jeanne Fair; Jen Owen; Jonas Waldenström*

16:45-17:00 Serologic and knowledge, attitude and practice on prevention of zoonotic diseases of wildlife farmers in Vietnam: results from a mixed-method study
Thi Thanh Ha NGUYEN; *Bernard BETT; Hu Suk LEE; Tien Thang NGUYEN; Hung NGUYEN; Xuan Sinh DANG; Fred UNGER; Nghia Vuong BUI; Duy Tung DAO; Johanna LINDAHL*

17:00-17:15 The first clinical report of Red deerpoxvirus in semi-domesticated reindeer in Sweden
Karin Wallin Philippot; *Mikael Leijon; Tomas Jinnerot; Ingebjørg Helena Nymo; Veronica Lengquist; Ulrika Rockström; Ylva Persson; Jonas Johansson Wensman*

DETAILED PROGRAMME

Wednesday 25 September 2024

17:15-17:30 A new triplex RT-qPCR to detect and differentiate Bluetongue and Epizootic Hemorrhagic Disease in a single well
Adrien LIMOZIN; *Léa DESPOIS; Loïc COMTET; Adrien LIMOZIN; Emilie BIANCHINI*

17:30-17:45 Value of whole genome sequencing in Animal Health - Characterization of Bluetongue & Epizootic Hemorrhagic Disease outbreaks in France 2023
Mathilde Gondard; *Lydie Postic; Mathilde Turpaud; Fabrice Touzain; Yannick BLANCHARD; Fabien Vorimore; Mai-Lan Tran; Grégory CAIGNARD; Damien VITOUR; Stéphan ZIENTARA; Corinne Sailleau; Emmanuel Breard*

17:45-18:00 Evaluation of the pathogenic potential of recent (2021-2022) Spanish West Nile virus strains of lineages 1 and 2 in a mouse model
Francisco Llorente; *Elisa Pérez-Ramírez; Desirée Dafouz-Bustos; Belén Gómez-Martín; Núria Busquets; Miguel Ángel Jiménez-Clavero*

16:00-18:00 Session 2: Pathogen evolution, pathogenesis and immunology, *Strömholms sal*
Chairs: Thomas Bruun Rasmussen; Ylva Lindgren

16:00-16:15 "Not all viruses come alone" The outcome of bluetongue virus coinfections.
Rhiannon Moody; *Caroline Wright; Marc Guimera; Dan Horton; Karin Darpel*

16:15-16:30 Experimental infection study with a recent bluetongue virus serotype 3 (BTV-3/Net2023) strain in sheep
Norbert Stockhofe-Zurwieden; *Rineke de Jong; Mieke Maris-Veldhuis; Rene van Gennip; Sandra van de Water; Piet van Rijn; Rik de Swart; Melle Holwerda*

16:30-16:45 Influence of inoculation dose and route on Epizootic Haemorrhagic Disease Virus pathogenesis and induced immune response in cattle
Ilse De Leeuw; *Rubén Villalba; Montserrat Agüero; Nick De Regge*

16:45-17:00 The leader protein is necessary for the establishment of persistent infection by type O foot-and-mouth disease virus.
Sandra Blaise-Boisseau; *Benedikt Litz; Caroline Michaud; Ignacio Alvarez; Sehl-Ewert Julia; Angele Breithaupt; Anja Landmesser; Aurore Romey; Hélène Huet; Cindy Bernelin; Anne-Laure Salomez; Anthony Relmy; Guillaume Girault; Stéphan ZIENTARA; Florian Pfaff; Labib Bakkali Kassimi; Martin Beer; Sara Häglund; Jean-Francois Valarcher; Michael Eschbaumer*

17:00-17:15 The cell junction and adhesion protein vinculin restricts permissivity to pestiviruses
Elena Leveringhaus; *Paul Becher; Alexander Postel*

DETAILED PROGRAMME

Wednesday 25 September 2024

17:15-17:30 Single-cell RNA-Seq analysis of monocytes from pigs infected with virulent African swine fever virus
Lihong Liu; *Marek Walczak; Katarzyna Podgórska; Daniel Pérez-Núñez; Yolanda Revilla*

17:30-17:45 Evolution and host-adaptation of rabies virus during a historic fox-driven epidemic in Switzerland
Farzane Shams; *Claudia Bachofen; Alex Popinga; Torsten Seuberlich*

17:45-18:00 Final discussion

18:00-20:00 **Welcome cocktail**, *Nya festsalen*

DETAILED PROGRAMME

Thursday 26 September 2024

08:00-17:00 **Registration**, *Reception desk*

09:00-10:00 **Keynote lectures 3 & 4**, *Gamla salen*
Chairs: Anne-Lie Blomström; Erika Chenais

09:00-09:30 Why waste wastewater when it is a gold mine for pathogen surveillance and epidemiology
Maja Malmberg

09:30-10:00 African swine fever and its way through Asia and towards Europe (Ukraine 2016-2024: lessons learned and war influence)
Anton Gerilovych

09:00-17:00 **Poster viewing available all day long**, *Nya festsalen*

10:00-10:30 **Coffee break - poster session 3**, *Nya festsalen*

10:30-12:30 **Session 3: Diagnostics and disease surveillance**, *Gamla salen*
Chairs: Anne-Lie Blomström; Håkan Andersson

10:30-10:45 Antigenic characterisation of Swine influenza H1N2 viruses in Italy
Ana Moreno; *Anna Castelli; Laura Soliani; Tiziana Trogu; Enrica Sozzi; Davide Lelli; Chiara Chiapponi; Alice Prosperi; Silvia Faccini; Carlo Rosignoli; Cristian Salogni; Giovanni Loris Alborali*

10:45-11:00 Blood swabs represent an alternative sample matrix for Classical swine fever antibody ELISAs
Denise Meyer; *Sandra Blome; Lia Ebner; Paul Becher*

11:00-11:15 A novel ambitious approach to targeted surveillance of West Nile Virus in Danish mosquitoes
Louise Lohse; *Ann Sofie Olesen; René Bødker*

11:15-11:30 The conquest of the West by West-Nile and Usutu viruses, two emerging flaviviruses in France and Europe
Mathilde Gondard; *Camille Migné; Marine Dumarest; Tehepuaura Helle; Yannick BLANCHARD; Sylvie Lecollinet; Cécile Beck; Stéphanie Desvaux; Anouk Decors; Nolwenn Dheilly; Christel Marcillaud-Pitel; Coralie Lupo; Pierre Triz; Thierry Petit; Thomas Charpentier; Marianne Depecker; Noémie Chevalier; Fabien Vorimore; Mai-Lan Tran; Stéphan ZIENTARA; Sandra Martin-Latil; Gaëlle Gonzalez*

DETAILED PROGRAMME

Thursday 26 September 2024

11:30-11:45 Virus Infections among Wild Eurasian Tundra Reindeer (Rangifer tarandus tarandus) in Iceland – a different story than in Fennoscandia
Morten Tryland; *Ingebjørg Helena Nymo; Rán Þórarinsdóttir; Javier Sánchez Romano*

11:45-12:00 Characterization of a MERS-like betacoronavirus in Danish brown long-eared bats (Plecotus auritus)
Camille Melissa Johnston; *Vithiagaran Gunalan; Louise Lohse; Thomas Bruun Rasmussen*

12:00-12:15 Probability of freedom from Peste des Petits Ruminants in Georgia and Armenia.
Adam Kotorashvili; *Tengiz Chaligava; Tea Enukidze; Ketevan Goginashvili; Ji-Yeon Hyeon; Natia Kartiskhia; Satenik Kharatyan; Adam Kotorashvili; Tigran Markosyan; Guillermo Risatti; Karl Ståhl; Pertsh Tumanyan; Nino Vepkhvadze; Erika Chenais*

12:15-12:30 Environmental-based surveillance as complementary tool for epidemic monitoring under a One Health approach
Chantal Snoeck; *Manon Chassaing; Cécile Walczak; Aurélie Sausy; Delphine Collard; Gwenaëlle Le Coroller; Joël Mossong; Anne Vergison; Dritan Bejko; Manon Bourg; Judith Hübschen; Henry-Michel Cauchie; Leslie Ogorzaly*

10:30-12:30 Session 4a: African swine fever I, *Strömholms sal*
Chairs: Karl Ståhl; Marylène Tignon

10:30-10:45 First outbreak of African swine fever in Sweden – Local epidemiology, surveillance and eradication strategies
Erika Chenais; *Viktor Ahlberg; Kristofer Andersson; Fereshteh Banihashem; Lars Björk; Maria Cedersmyg; Linda Ernholm; Jenny Frössling; Wiktor Gustafsson; Lena Hellqvist Björnerot; Cecilia Hultén; Hyeyoung Kim; Mikael Leijon; Anders Lindström; Lihong Liu; Anders Nilsson; Maria Nöremark; Karin Olofsson-Sannö; Emelie Pettersson; Thomas Rosendal; Marie Sjölund; Henrik Thurfjell; Stefan Widgren; Emil Wikström-Lassa; Siamak Zohari; Erik Ågren; Estelle Ågren; Karl Ståhl*

10:45-11:00 Perceptions and experiences of hunters involved in the management of the first African swine fever outbreak in Sweden
Hedvig Gröndal; *Hedvig Stenberg; Susanna Sternberg Lewerin; Karl Ståhl; Erika Chenais*

DETAILED PROGRAMME

Thursday 26 September 2024

11:00-11:15 Disease dynamics in wild boar and domestic pigs inoculated intranasally with the virulent African swine fever virus genotype II strain "Armenia 2007"
Pedro Jose Sanchez-Cordon; *Fabian Z. X. Lean; Carrie Batten; Falko Steinbach; Aleksija Neimanis; Marie-Frédérique Le Potier; Emil Wikström-Lassa; Felicity Wynne; Rebecca Strong; Stephen McCleary; Noemi Rayon; Helen Crooke; Dolores Gavier-Widén; Alejandro Núñez*

11:15-11:30 Into the Wild: Exotic Insights into Immunity against African Swine Fever Virus
Alexander Schäfer; *Virginia Friedrichs; Florian Pfaff; Ulrike Blohm; Jörg Beckmann; Martin Beer; Sandra Blome*

11:30-11:45 Early mRNA expression profiles of key innate immunity effectors in pigs experimentally infected with BE18 and E70 ASFV strains
Nadjah Radia Adjadj; *Nadège Balmelle; Brecht Droesbeke; Ann Brigitte Cay; Hans Nauwynck; Marylène Tignon*

11:45-12:00 African swine fever pathology: Applying histologic methods to compare disease progression in wild boar and domestic pigs
Emil Wikström-Lassa; *Pedro Jose Sanchez-Cordon; Aleksija Neimanis; Bjørnar Ytrehus; Karl Ståhl; Dolores Gavier-Widén*

12:00-12:15 Infection of pigs with African swine fever virus following oral or intranasal inoculations using different doses of the virus
Ann Sofie Olesen; *Christina M. Lazov; Thomas Bruun Rasmussen; Anette Bøtner; Graham J. Belsham; Louise Lohse*

12:15-12:30 From Crisis to Control: Insights for African swine fever resilience in red river hogs and warthogs
Virginia Friedrichs; *Alexander Schäfer; Florian Pfaff; Ulrike Blohm; Jörg Beckmann; Martin Beer; Sandra Blome*

12:30-13:30 **Lunch**, *Nya festsalen*

13:30-14:30 **Keynote lectures 5 & 6**, *Gamla salen*
Chairs: Siamak Zohari; Susanna Sternberg Lewerin

13:30-14:00 Living risk assessment of animal disease threats to Europe
Clazien de Vos

14:00-14:30 From the Arctic to the Antarctic – a tale of avian influenza viruses in the wild
Jonas Waldenström

DETAILED PROGRAMME

Thursday 26 September 2024

14:30-15:00 **Coffee break - poster session 4, 5 & 6**, *Nya festsalen*

15:00-16:00 **Session 5: Epidemiology and risk assessment**, *Gamla salen*
Chairs: Susanna Sternberg Lewerin; Cecilia Hultén

15:00-15:15 Lethal Borna disease virus 1 (BoDV-1) infections of humans and animals – in-depth molecular epidemiology and phylogeography
Dennis Rubbenstroth; *Arnt Ebinger; Pauline D. Santos; Florian Pfaff; Ralf Dürrwald; Jolanta Kolodziejek; Kore Schlottau; Viktoria Ruf; Friederike Liesche-Starnecker; Armin Ensser; Klaus Korn; Reiner Ulrich; Jenny Fürstenau; Kaspar Matiasek; Florian Hansmann; Torsten Seuberlich; Daniel Nobach; Matthias Müller; Antonie Neubauer-Juric; Marcel Suchowski; Markus Bauswein; Hans-Helmut Niller; Barbara Schmidt; Dennis Tappe; Daniel Cadar; Timo Homeier-Bachmann; Viola C. Haring; Kirsten Pörtner; Christina Frank; Lars Mundhenk; Bernd Hoffmann; Jochen Herms; Wolfgang Baumgärtner; Norbert Nowotny; Jürgen Schlegel; Rainer G. Ulrich; Martin Beer*

15:15-15:30 Characteristics of the Swedish cattle movement network 2005 - 2022 and implications for disease spread
Ivana R. Ewerlöf; *Jenny Frössling; Stefan Gunnarsson; Emma Hurri; Lena Stengärde; Madeleine Tråven; Stefan Widgren*

15:30-15:45 Citizen science dependent disease surveillance in wildlife
Maria Nöremark; *Aleksija Neimanis; Ellinor Spörndly-Nees; Hyeyoung Kim; Maria Johansson; Åsa Waldo; Henrik Uhlhorn; Erik Ågren*

15:45-16:00 AFRICAN SWINE FEVER: PRESENCE IN SWINE MEAT PRODUCTS SMUGGLED INTO ITALY
MARIA SERENA BEATO; *VINCENZO CAPUTO; SILVIA PAVONE; Cristina Casciari; Michela Pela; Elisabetta Rossi; Roberta Biccheri; GIULIA COSTANTINO; CECILIA RIGHI; Carmen Iscaro; Monica Giammarioli; Francesco Feliziani*

15:00-17:00 **Session 6: Avian influenza**, *Strömholms sal*
Chairs: Mikael Berg; Siamak Zohari; Malin Grant

15:00-15:15 Avian Influenza virus in Egypt: current situation and strains replacement
Mahmoud M. Naguib; *Maram Tawakol; Nehal Nabil; Amany Adel; Nahed Yehia; Marwa Abdelmagid; Fatma Amer; Neveen Rabie; Mohamed Samy; Dalia Said; Eman Fargaly; Ahmed Erfan; Abdullah Selim; Johanna LINDAHL; Åke LUNDKVIST; Momtaz Shahein*

DETAILED PROGRAMME

Thursday 26 September 2024

15:15-15:30 Assessment of air contamination by avian influenza viruses in live poultry markets in Bangladesh
Sukanta Chowdhury; *Mohammad Enayet Hossain; Jiaxin Ling; Mohammed Ziaur Rahman; Mahmoud M. Naguib; Johanna LINDAHL*

15:30-15:45 Exploring the Influence of Environmental and Climatic Factors on Highly Pathogenic Avian Influenza Outbreaks in Italy
Diletta Fornasiero; *Bianca Zecchin; Matteo Mazzucato; Ambra Pastori; Grazia Manca; ISABELLA MONNE; Simon Dellicour; Mariette Ducatez; Paolo Mulatti; Claire Guinat*

15:45-16:00 Farm level risk factors for highly pathogenic avian influenza in Swedish poultry
Malin Grant; *Désirée S. Jansson; Arianna Cormin; Magdalena Jacobson; Maria Nöremark*

16:00-16:15 Zoorganoids Biobank, an animal organoid repository for the study and prevention of epizootic and zoonotic diseases.
Gerardo Ceada; *Ferran Tarrés-Freixas; Nuria Navarro; Marta Pérez-Simó; Noelia Carmona-Vicente; Laura Bonillo-Lopez; Alejandro Moreno; Carlos López; Natàlia Majó; Joaquim Segalés; Karl Kochanowski; Júlia Vergara-Alert*

16:15-16:30 Efficient replication of avian and mammalian influenza A viruses in bovine airway epithelial cells
Ang Su; *Miaomiao Yan; Georg Herrler; Paul Becher*

16:30-17:00 General discussion

16:00-17:00 Session 4b: African swine fever II, *Gamla salen*
Chairs: Marylène Tignon; Erika Chenais

16:00-16:15 Detection of infectious African Swine Fever Virus within spiked materials after incubation at different temperatures for different lengths of time
Christina M. Lazov; *Ann Sofie Olesen; Anette Bøtner; Graham J. Belsham*

16:15-16:30 African swine fever in Northern Uganda: how smallholders understand and deal with the virus
Klara Fischer; ***Gwendolyn Varley***; *Ana Maria Mutis; Karl Ståhl; Erika Chenais*

16:30-16:45 No evidence of undetected infection with African swine fever virus in wild boar tested in Estonia
Imbi Nurmoja; *Annika Vilem; Harles Kaup; Olev Kalda; Arvo Viltrop*

DETAILED PROGRAMME

Thursday 26 September 2024

16:45-17:00 Estimating post mortal interval of wild boar carcasses to establish a timeline in the Swedish ASF outbreak
Karin Olofsson-Sannö; *Emil Wikström-Lassa; Anders Lindström; Erik Ågren; Erika Chenais*

17:00-18:30 **Guided tour at Uppsala Cathedral - pre-registration required**, *Uppsala Cathedral*

19:00-00:00 **Gala dinner**, *Gamla salen*

Announcement of poster prize awards
Poster prizes are sponsored by EPIZONE and Gold Standard Diagnostics

DETAILED PROGRAMME

Friday 27 September 2024

08:00-14:00 **Registration**, *Reception desk*

09:00-10:00 **Keynote lectures 7 & 8**, *Gamla salen*
Chairs: Jean-Francois Valarcher; Jonas Johansson Wensman

09:00-09:30 Vaccination against high pathogenicity avian influenza: first lessons from the French duck vaccination plan
Jean-Luc Guerin

09:30-10:00 Harnessing Satellite Technology for Vector-Borne Disease Surveillance: Insights from Italy and North Africa
Luca Candeloro

09:00-14:00 **Poster viewing**, *Nya festsalen*

10:00-10:30 **Coffee break - poster session 7 & 8**, *Nya festsalen*

10:30-12:30 **Session 8: Vector-borne diseases**, *Gamla salen*
Chairs: Jonas Johansson Wensman; Hedvig Stenberg

10:30-10:45 Early detection and tracking of West Nile virus in Germany by using a functional nationwide wild bird network
Ute Ziegler; *Franziska Schopf; Anne Schwarzer; Felicitas Bergmann; Markus Keller; Christine Fast; Balal Sadeghi; Martin H. Groschup*

10:45-11:00 Culex pipiens from the United Kingdom demonstrate transmission potential for West Nile virus lineage 1 and lineage 2
Karen Mansfield; *Estela González; Sanam Sewgobind; Insiyah Parekh; Luis Hernández-Triana; Nicholas Johnson*

11:00-11:15 Infected Culex pipiens mosquitoes can transmit West Nile virus lineage 1 and 2 after overwintering in temperate winter conditions
Albert Burgas-Pau; *Jaume Gardela; Raquel Rivas; Marta Verdún; Núria Pujol; Carles Aranda; Núria Busquets*

11:15-11:30 Sindbis virus (SINV) in the Netherlands: First proof of local circulation in wild birds and horses
Cora M. Holicki*; *Kiki Streng; Jenny C. Hesson; Heather Graham; Felicity Chandler; Louie Krol; Rody Blom; Emmanuelle Munger; Constantianus J. M. Koenraadt; Maarten Schrama; Åke LUNDKVIST; Marion P. G. Koopmans; Henk van der Jeugd; Wim H. M. Van der Poel; Reina S. Sikkema*

DETAILED PROGRAMME

Friday 27 September 2024

11:30-11:45 A new highly sensitive indirect Enzyme Linked Immuno Sorbent Assay for the detection of antibodies against African Horse Sickness Virus
Loïc COMTET; *Océane MERCIER; Rémy BONJOUR; Alix CARPENTIER; Philippe POURQUIER*

11:45-12:00 Emerging orbivirus preparedness and transmission potential: lessons learned from Schmallenberg virus in Norway 2012 – 2023
Gebbiena Bron; *Marie Myklatun Krosness; Annette Kampen; Petter Hopp; Siv Klevar; Johan Åkerstedt*

12:00-12:15 Emerging zoonotic Wesselsbron flavivirus causes severe hepatitis and is transmitted directly to suckling lambs
Obdulio García-Nicolás; *Marta Zimoch; Llorenç Grau-Roma; Matthias Liniger; Noelle Donzé; Aurélie Godel; Damián Escribano; Paraskevi Pramateftaki; Sergi Torres-Puig; José Joaquín Cerón; Joerg Jores; Artur Summerfield; Nicolas Ruggli; Charaf Benarafa*

12:15-12:30 Evaluation of disease dynamics and immune response in sheep experimentally infected subcutaneously or intranasally with Rift Valley fever virus.
Sara Moran De Bustos; *Iris Sanchez del Pozo; Laura Fernandez del Ama; Antonia Gonzalez-Guirado; Elisabet Fuentes; David Sardon; Miriam Pedrera; Belen Borrego; Alejandro Brun; David Rodriguez-Temporal; Belen Rodriguez-Sanchez; Pedro Jose Sanchez-Cordon*

10:30-12:30 Session 7: Vaccine development and disease control, *Strömholms sal*
Chairs: Jean-Francois Valarcher; Wim H. M. Van der Poel

10:30-10:45 Development of African horse sickness Disabled Infectious Single Animal (DISA)-DIVA vaccines and evaluation of safety and efficacy in IFNAR-/- mice
Piet van Rijn; *Sergio Utrilla-Trigo; Rene van Gennip; Luis Jiménez-Cabello; Eva Calvo-Pinilla; Sunitha Joseph; Ulrich Wernery; Javier Ortego*

10:45-11:00 Safety and immunogenicity of African horse sickness Disabled Infectious Single Animal (DISA)-DIVA vaccines in horses
Piet van Rijn; *David Craig; Christiana Hebel; Rene van Gennip; Sunitha Joseph; Ulrich Wernery*

11:00-11:15 To cast virus before pigs and still not make them sick: The unsuccessful story of intra-oropharyngeal infection with foot-and-mouth disease virus
Saskia Weber; *Constantin Lorenz; Kira Wisnewski; Martin Beer; Michael Eschbaumer*

RECENT EVENT

Friday 27 September 2024

11:15-11:30 FlagT4G vaccine confers protection against transplacental transmission of highly virulent CSFV after single vaccination in pregnant sows
Llilianne Ganges; *Liani Coronado; Adriana Muñoz-Aguiera; Guillermo Cantero; Patricia Martínez; Mònica Alberch; Rosa Rosell; Douglas P. Gladue; Manuel Borca*

11:30-11:45 Cross-protection induced by genotype II modified live African swine fever virus candidate vaccine(s) against genotype I but not against genotype IX.
Anusyah Rathakrishnan; *Hanneke Hemmink; Sam Smith; Ana Reis; Linda Dixon*

11:45-12:00 Safety and efficacy studies of two DIVA African swine fever vaccine candidates in domestic pigs
Nadia Casado; *Carmina Gallardo; Zoltán Zadori; Alejandro Soler; Alicia Simón; Covadonga Pérez; Raquel Nieto; Jovita Fernández-Pinero; Marisa Arias*

12:00-12:15 Enhanced virus-specific T-cell responses correlate with improved performance of adenovirus vectored vaccines against genotype I African swine fever
Chris Netherton; *Raquel Portugal; Priscilla Y.L. Tng; Laila Al-Adwani; Linda Dixon*

12:15-12:30 Vaccination of zoo birds against highly pathogenic avian influenza viruses (H5N1) using propagation-defective RNA replicon particles
Gert Zimmer; *Marion Stettler; Fabia Wyss; Christian Wenker; Elisabeth Heiderich; Karin Darpel; Stefan Hoby*

12:30-13:00 **Closing ceremony**, *Gamla salen*
Chairs: Wim H. M. Van der Poel; Jonas Johansson Wensman

13:00-14:00 **Light lunch**, *Nya festsalen*

14:00-16:00 **Guided tour at Old Uppsala museum**, *Gamla Uppsala*

Keynote lecture

Dr. Inge Santman-Berends

Senior Veterinary Epidemiologist
Royal GD, Deventer, Netherlands

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Lecture: 14.30 - 15.00, 25 September - Gamla salen

Dr. Inge Santman-Berends is employed as Senior Veterinary Epidemiologist and head of the epidemiology group at Royal GD in Deventer the Netherlands. Her tasks and responsibilities include supervising the veterinary epidemiology team of Royal GD, coordinate and being responsible for the epidemiological input in research projects and provide epidemiological and statistical input when needed.

BTV-3 in the Netherlands: experiences of the first outbreak year

On 5 September 2023, bluetonguevirus serotype 3 (BTV-3) was diagnosed for this first time in the Netherlands.

During the subsequent period multiple studies were initiated to obtain insight in the spread and impact of this outbreak.

Immediately after the first incursion, a retrospective antibody screening was conducted of sheep and cattle samples that were submitted to Royal GD in August 2023. The results revealed no massive spread of the virus before the date of its first detection. Between September and December 2023, BTV-3 spread rapidly across the country infecting more than five thousand cattle and sheep farms. Farmers reported massive morbidity and mortality and especially in sheep the impact was enormous. An impact analysis on sheep census data revealed an 55 thousand dead sheep associated with BTV-3 in 2023. A longitudinal study in which five cattle and five sheep farms were followed during a thirteen week period after confirmation of a BTV-3 infection, showed a large variety in the amount and severity of clinical signs. In the five sheep farms, an average case-fatality of 75% was observed. In the five cattle herds mortality remained limited. In the spring of 2024 a large scale BTV antibody screening was conducted on bulk tank milk of all Dutch dairy farms and in serum samples of 390 sheep herds, thirteen sheep per herd. The results showed that on 64% of the dairy cattle farms and 47% of the sheep farms antibody positive animals were present. However, the animal level prevalence was still relatively low with an estimate of 23% in dairy cattle and 10% in sheep. Additionally, farmers were requested to fill in a questionnaire about their farm and grazing management in 2023 and these data were combined with their diagnostic results. A lower herd and animal prevalence was associated with keeping the animals indoors and ensuring air movement by use of large side openings in the stable and ventilation.

The presented data underlined the importance of BTV-3 vaccination before the vector season in 2024 and since its availability in May 2024, approximately 90% of Dutch sheep and 53% of the Dutch dairy farmers decided to vaccinate. Nevertheless, since July, the spread of BTV-3 is enormous again and even though the first impression is that mortality rates in sheep are lower compared to those of 2023, still many farmers report sick and dead sheep resulting in a major impact in the second year.

Keynote lecture

Prof. Artur Summerfield

Head of the Research Department
Institute of Virology and Immunology, Mittelhäusern, Switzerland

artur.summerfield@unibe.ch

Lecture: 15.00 - 15.30, 25 September - Gamla salen

Prof. 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.

Dissecting protective and detrimental immune responses against African swine fever virus

African swine fever virus (ASFV) represents one of the deadliest infectious diseases of swine, and virulent forms of the virus cannot be controlled by the immune system. Instead, ASFV causes an aberrant and sepsis-like immune response, which is very often detrimental for the animals. Live attenuated vaccine can induce protective immunity, but the immunological mechanisms and correlates of protection are not well defined.

By using a systems immunology approach, we have determined the innate and adaptive immune responses that are related to survival of an acute primary infection with a moderately virulent strain of ASFV, as following re-challenge with highly virulent ASFV.

The ability to control acute infection was associated with an early and transient IFN-alpha response, controlled systemic inflammation, dendritic cell activation and an early induction of a T helper cell response within the first week of infection. A transient innate immune system activation was also identified as a correlate of protection during the memory phase (re-challenge). In addition, early ASFV-specific memory helper and cytotoxic T cells detectable in the blood after challenge were associated with protection. Our data also indicate a role for B cells, as a plasma cell response with production of antibodies to certain viral proteins were more pronounced in protected animals.

Defining mechanisms and correlates of protection will help to rationally design more efficacious and safer vaccines against ASFV.

Keynote lecture

Dr. Maja Malmberg

Associate professor in molecular infection biology
Swedish University of Agricultural Sciences, Uppsala, Sweden

maja.malmberg@slu.se

Lecture: 09.00 - 09.30, 26 September - Gamla salen

Dr. Maja Malmberg is an associate professor in molecular infection biology at the section of virology at the Swedish University of Agricultural Sciences (SLU). Her research interest focuses on virus discovery and molecular tools for pathogens surveillance. Since 2020 she has worked with wastewater surveillance of viruses in Sweden, initially focused on SARS-CoV2.She holds a MSc in Engineering (biotechnology and genomics) from Umeå University (Sweden), and a PhD in Medicine from Karolinska Institutet (Sweden).

Why waste wastewater when it is a gold mine for pathogen surveillance and epidemiology

Good surveillance systems are critical to know which pathogens are circulating and to what extent. Prior to the COVID-19 pandemic wastewater-based monitoring of pathogens existed but had not gotten much attention. This quickly changed during the pandemic, when it became evident that SARS-CoV2 RNA was not only possible to detect in wastewater but it was also shown that monitoring of SARS-CoV2 RNA correlated with covid-19 clinical disease, and was a good indicator. With the increased interest in the technology, large improvements were made in the molecular methodologies used, which led to the possibility to also monitor the quick development of novel variants through for example, high-throughput sequencing.

During this keynote presentation I will give some examples of how surveillance of pathogens in wastewater in Sweden is done currently and what could be done in the future.

In Sweden, the work of with SARS-CoV2 surveillance has been done mainly at the Swedish Environmental Epidemiology Center (SEEC), at the Swedish University of Agricultural Sciences (SLU). Currently, in total 43% of the Swedish population is covered by the sampling set-up (19 locations) and the results are used by the Swedish Public Health Agency, and are publicly available every week at www.pathogens.se. The pathogens that are being monitored routinely are SARS-CoV2 (levels and variants), influenza A, influenza B and RS-virus.

In collaboration with the start-up company APLEX Bio, a novel multiplex PCR-based detection method called Hyperplex PCR™ has been evaluated and proven to be fast, sensitive and cost-efficient. The results show great potential for its usage in surveillance of pathogens in wastewater. As an example, it was shown that avian influenza of subtype H5N1 could be detected in Swedish wastewater from a municipality treatment plant in the same area and time as an avian influenza outbreak in birds occurred.

All in all, wastewater is truly a gold mine for pathogen surveillance and epidemiology. Wastewater is a naturally pooled samples which provide population-based information at a ridiculously low price compared to other alternatives. With the advancements in the technologies, it is now a robust surveillance tool that improves society's ability to be prepared for both existing and novel pathogenic threats.

Keynote lecture

Prof. Anton Gerilovych

Director general
One Health Scientific and Research Institute, PSI, Kharkiv, Ukraine

antger2011@gmail.com

Lecture: 09.30 - 10.00, 26 September - Gamla salen/Online

Prof. Anton Gerilovych is an NAAS Corresponding member, President of One Health Institute, NGO, Senior Researcher/Director's advisor for international affairs and development, affairs and development, at the State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise.

African swine fever and its way through Asia and towards Europe (Ukraine 2016-2024: lessons learned and war influence)

African swine fever (ASF) is the biggest threat for European pig farming of nowadays. This is highly contagious disease could be transmitted as by the contacts of the domestic and wild pigs. Also the Ornithodoros ticks demonstrate the potential of horizontal virus transmission. Disease was occurred in Caucasus region (Georgia, Armenia, Azerbaijan) firstly (2007), and has been transmitted transboundary to Russian Federation (2008). The first outbreak of African swine fever was reported in Ukraine (the human-factor associated introduction from Russian Federation) in 2012. The ASF outbreaks were reported in Belarus (2013), Lithuania, Latvia and Poland (2014), Estonia (2015), Germany, Romania, Moldova, Hungary, Slovakia.

About 6000 disease outbreaks were detected in last years on the Eurasian disease areal. Economical losses are grate, and are associated with stamping out, quarantine measures, international trade restrictions etc.

The disease is the significant problem for Ukrainian pig industry and entire economy as Ukraine is agrarian state. Over 700 outbreaks of ASF were described in Ukraine since 2012. Most "domestic" cases were associated with weak level of the biosecurity in small farms and backyards, but also several industrial farms were involved as well. 17 outbreaks were detected in 2024, including 11 regions of Ukraine. Now there exist around 16 compartments of the disease potential risk zones in the North-central, Northeastern and Eastern parts of Ukraine. Some compartment is strongly associated with the zone of russian aggression against Ukraine, located in Lugansk and Donetsk regions of Ukraine, where the epidemiology of disease is unknown, and risks of infected animals' migration is very high (7-9 balls of the risk).
Wild boar factor was the reason of the disease distribution from Russia and Belarus to Ukraine, Baltic States, Poland, and other EU member states, where disease cases has been occurred in the wildlife. Now ASF is the endemic problem in whole territory of Ukraine, mostly associated with human factor and illegal trade, as well as internal populations of wild boar. For disease transmission study on the vector level the Project DTRA-ARS-USDA-STCU P609 related to ASF risk evaluation has been executed. We detected Ornithodorine softticks populations in the South of country. These populations will be analysed deeply to determine the possible role of them in ASF distribution areal development. The "human factor" role study demonstrates its significance (within the DTRA-funded project UP-10). Effective disease management was started to be implemented after development and setting up of the compensation program and depopulation of pigs in private sector. The war in Ukraine, caused by full-scale russian invasion associated with multiple difficulties and losses in the agricultural sector (lack of veterinary surveillance, losses in pig farms, losses of feedstuff etc.), as well as massive migration of human during first half of 2022.

ASF represents high risks for EU member states associated with international trade, wild boars' migration (7-9 balls), and moderate (2-3 balls) risk levels associated with soft ticks' areal enlargement to the North. The disease control requires improvement and support due to the russian aggression and agrarian losses produced by occupants, also it supported disease distribution and preservation.

Keynote lecture

Dr. Clazien de Vos

Risk analyst
Wageningen Bioveterinary Research, Netherlands

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Lecture: 13.30 - 14.00, 26 September - Gamla salen

Dr. Clazien de Vos works at Wageningen Bioveterinary Research in the Netherlands. She is a risk analyst with extensive experience in qualitative, semi-quantitative and quantitative risk assessment in the veterinary field focusing on the introduction and transmission risk of notifiable, vector-borne and zoonotic infections and antimicrobial resistance.

Living risk assesement of animal disease threats to Europe

Safeguarding public and animal health is a moving target, with changes in climate and human activity raising new threats. Preparedness is warranted to prevent outbreaks of emerging and re-emerging diseases or to detect outbreaks in an early stage. Decision support in the context of health management requires a continuous effort to synthesize multiple sources of evidence. Recent advancements in data availability, data handling and computing power have opened up possibilities to automate some of these processes by tools capable of making sense of large volumes of complex and multidisciplinary data. At the same time, these tools must enable decision makers to adjust to rapid changes in the evidence inputs, and constantly evolving knowledge. Over the last decade, several generic risk assessment tools were developed that took up this challenge, addressing the incursion risk of multiple diseases via multiple introduction routes. At European level, a Living One Health Risk Assessment tool (L'ORA) was built, that can assess the risk for zoonotic and animal diseases using a holistic, One Health approach, and allowing for automatic updates of the risk assessment when new evidence is available in the linked source databases.

L'ORA estimates the risk of disease incursion as the resultant of four steps: 1) Disease occurrence in source areas based on the distribution of diseases worldwide; 2) Rate of incursion based on the individuals or products moved from source areas to target areas and their probability of infection or contamination and contact with susceptible hosts in target areas; 3) Extent of disease spread in target areas, considering both domestic and wild animal populations, as well as humans and vectors if relevant; and 4) Impact of the disease outbreak and concurrent control measures on public and animal health, accounting for economic, societal, and environmental impacts.

L'ORA is used to assess the incursion risk of multiple diseases at NUTS2 level in the European Union. Results are now available for ten diseases: African swine fever, West Nile fever, highly pathogenic avian influenza, Rift Valley fever, Japanese encephalitis, rabies, Crimean-Congo hemorrhagic fever, lumpy skin disease, epizootic hemorrhagic disease and equine encephalosis.

L'ORA is a decision support tool that was built as a generic tool comparing the relative risks of multiple diseases across multiple target areas. Results can be used to identify areas of greater risk for each of the diseases to inform risk managers on where to focus attention and resources, and to guide risk-based surveillance.

Keynote lecture

Prof. Jonas Waldenström

Professor in microbiology
Linnaeus University, in Kalmar, Sweden

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Lecture: 14.00 - 14.30, 27 September - Gamla salen

Prof. Jonas Waldenström is professor at Linnaeus University, in Kalmar, Sweden. He is interested in the disease ecology of (mainly) avian pathogens, especially those that have a broad host range and can infect poultry or mammals. Since 2002, his research group carry out active surveillance for avian influenza viruses in wild birds, particularly in migratory waterfowl. By combining disease and pathogen data with movement ecology data from telemetry studies of birds they want to understand how viruses (and other pathogens) travel the world.

From the Arctic to the Antarctic – a tale of avian influenza viruses in the wild

The 2.3.4.4b lineage of the H5N1 highly pathogenic avian influenza virus has made a remarkable journey across the globe, infecting a range of wild and domestic birds in its wake, but also spilling over into mammal – and even human – hosts. Yet this is but one of a multitude of avian influenza viruses that occurs in wild birds, particularly birds associated with wetlands such as ducks and gulls. How did this particular virus become so successful – and so virulent compared to other viruses? In veterinary medicine we tend to focus on the viruses that cause most harm, but there is much to be learned by understanding the ecology, epidemiology, and evolution of low-pathogenic avian influenza viruses in their reservoir hosts.

In this talk, I will present a general overview of avian influenza viruses in wild birds and summarize findings from my research on migratory waterfowl in Europe. By combining disease and pathogen data from low-pathogenic viruses with movement ecology data from telemetry studies of birds we can start to understand how viruses travel the world. My hope is to inspire you to see low-pathogenic viruses as epidemiologically interesting, and not as bycatch when looking for highly pathogenic viruses. Finally, I will present on a newly started European initiative for active surveillance that can help us better understand the risks of spillovers from wild birds to poultry.

Keynote lecture

Prof. Jean-Luc Guerin

DVM, PhD, Dipl. ECPVS
National Veterinary College of Toulouse, France

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Lecture: 09.00 - 09.30, 27 September - Gamla salen

Prof. Jean-Luc Guérin, DVM, PhD, Dipl. ECPVS, is full professor in poultry medicine at the National Veterinary College of Toulouse, France and Director of the "host-pathogens interactions" joint research unit (INRAE-ENVT), covering virology, prions, bacteriology, immunology and epidemiology of infectious diseases in animals, in a One Health perspective.

His own research is focused on viruses of poultry, and for some years, pathobiology of highly pathogenic avian influenza. A priority is the development of innovative approaches of viral detection, including from environmental samples and at the wild birds/poultry interface.

Vaccination against high pathogenicity avian influenza: first lessons from the French duck vaccination plan

High pathogenicity avian influenza viruses (HPAIVs) have caused major epizootics in the last years with devastating consequences for poultry and wildlife worldwide. Domestic and wild ducks can be highly susceptible to HPAIVs, and infection leads to efficient viral replication and massive shedding (i.e., high titres for an extended time), contributing to widespread viral dissemination. Importantly, ducks are known to shed high amounts of virus in the earliest phase of infection, although the dynamics and impact of environmental contamination in the epidemiology of HPAIV outbreaks is still poorly understood. Based on epidemiological modelling at the regional scale, it has been clear that the control of HPAI in the specific sector of duck industry was critically important in France to reach a global control in the other poultry sectors. So far, the main doctrine of HPAI control has been based on early detection of outbreaks and their control through rapid culling, with a sparing use of vaccination. Mass vaccination for HPAI has therefore been only used in some low- and middle-income countries, where biosecurity and early detection could not be applied, with poor or mixed benefits.

Recent advances make now possible to implement vaccination with a strict control: the marketing of next-generation vaccines (RNA-based, sub-units, recombinant herpesviruses, …) opens new avenues to keep under a strict control the potential circulation of wild avian influenza viruses in vaccinated flocks. Molecular testing is now widely available to be implemented at a mass-scale. Altogether, these improvements in vaccines, diagnostic tools and our knowledge make possible a safe and efficient implementation of vaccination in the framework of a global control strategy.

Based on these considerations, a vaccination plan focused on ducks has been prepared and implemented in France since October 2023, after a risk assessment and complementary vaccine trials in field conditions. A comprehensive analysis of the outcomes of this vaccination plan after its first year is important to assess the relevance of this strategy, at least in the specific context of the French poultry production.

On a long-term perspective, we should remember that no single strategy will be sufficient to tackle the threat of HPAI clade 2.3.4.4.b, but fine-tuned combination of the different leverages (biosecurity, regulation of farms density, surveillance) along with vaccination, should make it possible to reach this target.

Keynote lecture

Dr. Luca Candeloro

Statistics and GIS Unit
IZSAM, in Teramo, Italy

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Lecture: 09.30 - 10.00, 27 September - Gamla salen

Dr. Luca Candeloro is a statistician and data scientist with extensive experience in the field of veterinary epidemiology. He currently works at the Department of Statistics and GIS at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM).

He is an expert in advanced statistical analysis, modelling, and social network analysis. Recently, his work has focused on integrating satellite imagery and machine learning techniques to improve the prevention and control of vector-borne diseases. His contributions have been crucial in advancing the field and providing innovative solutions for disease monitoring and public health interventions.

Harnessing Satellite Technology for Vector-Borne Disease Surveillance: Insights from Italy and North Africa

Earth Observation (EO) data are playing a crucial role in the surveillance and prediction of vector-borne diseases (VBDs) under the One Health approach. In Italy, EO data have been utilized to establish an Early Warning System for West Nile virus (WNV), employing satellite imagery to forecast the spatial and temporal dynamics of the virus. Concurrently, in North Africa, the WOAH project "PROVNA" has developed an EO-based system targeting diseases like Rift Valley fever (RVF), focusing on risk-based surveillance.

In Italy, EO data such as Land Surface Temperature and Normalised Difference Vegetation Index were pre-processed and incorporated into a machine learning model (XGBoost) to predict WNV circulation. In North Africa, the study area, covering countries like Algeria, Egypt, and Tunisia, was classified into ecoregions using EO data from 2018-2022. The Self Organising Map (SOM) clustering method was applied to identify ecoregions.

The Italian model successfully predicted WNV circulation, with results accessible through a web application, providing a practical tool for disease control. In North Africa, the defined ecoregions facilitated the monitoring of VBDs, enhancing targeted surveillance efforts.

The integration of EO data into VBD surveillance systems provides critical support for Veterinary Services, enabling more effective and resource-efficient surveillance strategies. The methodologies developed in Italy and North Africa showcase the potential of EO data to strengthen public health interventions across diverse regions and diseases, in line with WOAH's One Health strategy for coordinated VBD control.

RECENT EVENT

Emergence of peste des petits ruminants virus in Romania and Greece - insights from field and genetic investigations

Arnaud Bataille 1,
1 European Union Reference Laboratory for peste des petits ruminants (EURL-PPR), CIRAD

Background and Objectives
Peste des petits ruminants virus has just emerged for the first time in sheep and goat farms in Romania and Greece in July 2024. This is the first emergence of PPR in Europe since reports of outbreaks in Bulgaria in 2018.

Results
In both Romania and Greece, a wide variety of symptoms typical to PPR have been observed in small ruminants infected, including apathy, loss of appetite, nasal and ocular discharges, sudden death and diarrhea. In both countries, there were delays of 2-4 weeks in contacting veterinary authorities and confirming PPR infection, notably due to poor awareness of local veterinarian and misdiagnosis of symptoms observed. From the moment suspicion of PPR infection was raised, the response of Romanian and Greek authorities have been swift to control the disease. At present, outbreaks are still being identified but notifications have slowed down. Field investigations and genetic characterization of the virus strongly suggest a common origin for the two emergences. Full genome sequences were obtained from samples collected in Romania and Greece, showing that the most closely related published strain was one isolated from Georgia in 2016 (98% identity).

Conclusion
Additional sequencing data of recent outbreaks in other countries in the region is needed to further investigate the origin of the outbreak in Romania and Greece. Increased awareness of European veterinarians to PPR threat is needed to increase rapid response in case of new emergence.

Session 1

Emerging and re-emerging diseases

Oral Presentations

A new triplex RT-qPCR to detect and differentiate Bluetongue and Epizootic Hemorrhagic Disease in a single well

Adrien LIMOZIN[1]
Lea DESPOIS[1], Loïc COMTET[1], Philippe POURQUIER[1], Emilie BIANCHINI[1]
[1] Innovative Diagnostics

Background and Objectives: BlueTongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) are Orbiviruses respectively responsible for bluetongue (BT) and Epizootic Hemorrhagic Disease (EHD), which were reported in 2023 across Europe : BTV-3 (NL, BE, UK), new variant of BTV-8 (FR, IT) and EHDV (ES, FR).

They have huge economic impacts, and display similar clinical symptoms, making laboratory testing essential for diagnosis. Innovative Diagnostics offers a new kit, the **ID Gene™ BTV & EHDV Triplex.** It allows, in a single well, for the detection and differentiation of both BTV & EHDV alongside with a sample endogenous control. **Results** can be obtained **in 50 min** with a **rapid amplification program**, **compatible** with **all IDGene™ kits**, making possible to **test** on the **same run** for **different Orbiviruses RT-qPCRs** therefore offering maximum **flexibility** & **testing capacity** by **optimizing lab equipments ressources**.

Material and Methods: Blood RNA purifications were performed with the ID Gene™ Mag Fast magnetic beads (21 min). Diagnostic specificities for BTV and EHDV were assessed with 327 negative samples, respectively. Sensititivies were tested on 70 BTV positive samples and 143 EHDV positive samples. Inclusivity was assessed on 3 reference panels: 13 EHDV RNAs and 36 BTV RNAs (French national reference laboratory for BTV & EHDV, Anses), 7 EHDV RNAs (The Pirbright Institute, UK) and 10 BTV RNAs (FLI, Germany).

Results: The ID Gene™ BTV & EHDV Triplex measured specificities with respect to both BTV and EHDV were 100%, CI95% [99.1-100], n=327. Measured diagnostic sensitivity with respect to BTV and EHDV targets was respectively 100%, IC95% [99.1-100], n=70 and 98.9%, IC95% [98.2-100], n=143. BTV+/EHDV+ were constituted by mixing vol/vol positive samples from each virus. Even for low Cq values, no competition was observed on the qPCR, indicating the ability to detect possibly co-infected animals. All BTV and EHDV strains tested, including the BTV and EHDV strains detected in Europe in 2023, were efficiently detected by the new ID GeneTM kit, indicating a perfect inclusivity.

Conclusion: The new ID Gene™ BTV & EHDV Triplex enables to efficiently detect and differentiate BT and/from EHD in only one reaction. In regions where both viruses can co-circulate, this RT-qPCR is, in complement to the existing IDGene™ qPCRs, the ideal tool for **differential diagnosis testing**, for **disease surveillance** and **testing before animal movements**.

Detection of Coronaviruses and Reoviruses in European Hedgehogs in Northern Italy

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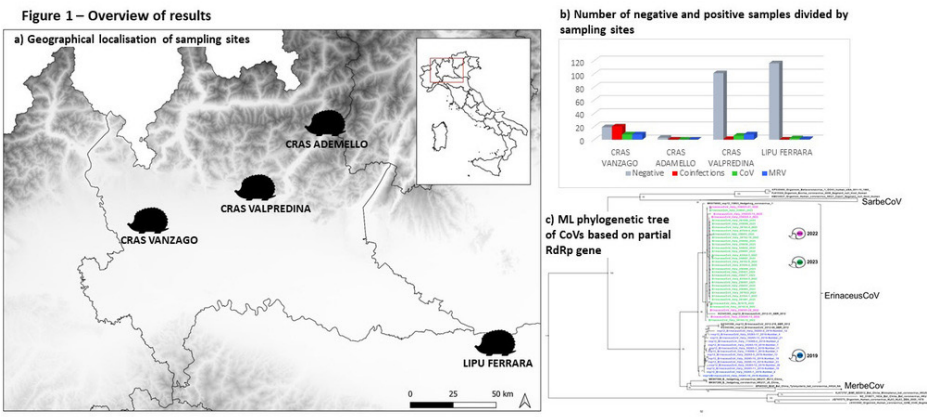
Background and Objectives: The role of wildlife could be predominant in the epidemiology of several pathogens, some of which are of zoonotic interest. Hedgehogs are small, synanthropic mammals widespread throughout Europe and capable of harbouring infectious agents. Possible contact with other domestic and wild animals and humans in urban settings could favour the transmission of such pathogens, highlighting and confirming their role as potential reservoirs not only for parasites and bacteria but also for viral agents. This study aims to investigate the presence of potentially zoonotic viruses in hedgehogs found dead or died in four wildlife rehabilitation centres in Lombardy and Emilia Romagna (Northern Italy).
Material and Methods: In the biennium 2022-2023, a total of 293 hedgehogs were conferred to IZSLER. From each carcass, a pool of organs (intestine and lungs) was tested to detect Coronavirus (CoV) and Mammalian Orthoreovirus (MRV) by specific RT-PCRs targeting in RNA dependent RNA polymerase gene and L segment, respectively. Positive samples to Real-Time RT-PCR for MRV were typed with specific RT-PCR targeting in segment S. All positive samples were sequenced by Sanger to confirm the positivity and were subjected to virological isolation on MARC and VERO cells.
Results: CoV was detected in 34 samples, all belonging to Betacoronavirus Erinaceus/VMC/DEU/2012. Of the 38 MRV-positive samples, 24 were typed as Mammalian Orthoreovirus type 3 (MRV3). MRV type 2 and 3 and Cov were contemporarily identified in one sample. Finally, in 21 samples there was a coinfection of CoV and MRV, most of these concentrated in the same rehabilitation centre. Viral isolation on cell culture was successful for 18 MRVs, whereas all CoV PCR-positive samples were negative in virological examination.
Conclusion: These results confirm the susceptibility of hedgehogs to these viruses. The well-documented presence of CoVs in this species suggests that hedgehogs may probably act as reservoirs for the infection. However, the epidemiological role played in the spread of MRV remains unclear. The moderate occurrence of co-infections in the same centre could be explained by management measures taken by operators that, in certain cases, provide multiple housing for animals of the same species. However, this phenomenon is relevant because it could lead to the formation of new viral lineages.



Funding source/acknowledgements (optional): Next Generation EU-MUR PNRR Extended Partnership Initiative on Emerging Infectious Diseases (project no. PE00000007INF-ACT) and project CCM SURVEID (project n. B93C22001210001)

Evaluation of the pathogenic potential of recent (2021-2022) Spanish West Nile virus strains of lineages 1 and 2 in a mouse model

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Background and Objectives: West Nile virus (WNV) is one of the most widespread emerging arbovirus in the world. Their incidence and spread have remarkably increased in Europe in the last decades with two genetic lineages (L1 and L2) currently circulating. Since its first isolation in 2007, L1 is rapidly spreading in south-western Spain, with clinical cases in horses and humans occurring since 2010, including an unprecedented outbreak in humans in 2020. Meanwhile in Catalonia (north-eastern Spain), L2 has been circulating at least since 2017. Data obtained previously in our laboratory indicated that there were not significant differences in pathogenicity among the L1 strains circulating in Andalusia (southern Spain) during the outbreak in 2020 and other strains detected in the area in previous years. Consequently, the higher incidence observed in 2020 should be attributed to causes other than intrinsic strain virulence, e.g. vector abundance or host competence. In turn, L2 strains from Catalonia showed lower pathogenicity, which could explain the lower incidence in this region in terms of human and equine cases. Since 2020, the incidence of the disease is still relevant and higher than that observed in the previous decade, including the first cases in humans in Catalonia and nearby areas. Therefore, there is a need to continuously evaluate the pathogenicity of the new strains to estimate the risk for human and animal health. To that end, we performed a pathogenicity evaluation using a mouse model assay previously developed for this purpose.
Material and Methods: Groups of twelve four-week-old female Swiss mice were inoculated with 1000 pfu of each viral isolate: four L2 isolates from Catalonia (2021, 2022), one L1 isolate from Andalusia (2021) and a previously evaluated L1 isolate (2020) as control. Mice were monitored daily for symptoms up to 3 weeks after inoculation.
Results: First clinical signs appeared 6-7 days-post-infection (dpi) and mortality started between 6 and 8 dpi. Catalonian L2 isolates showed lower virulence profiles than Andalusian L1 isolates with statistically significant differences in survival curves and median survival times. L1 isolates from Andalusia can be considered highly virulent while L2 isolates from Catalonia showed moderate virulence.
Conclusion: These results confirm that intrinsic pathogenicity of recent (2021-2022) Spanish isolates is similar to that observed for the strains circulating in previous years. Consequently, variations in disease incidence, including the first human cases in Catalonia may respond to other reasons, such as host competence in birds or mosquito abundance.

Funding source/acknowledgements (optional): Study funded by PID2020-116768RR-C2

Evidence of exposure to SARS-CoV-2 and various neutralizing antibody profiles against variants of concerns in companion animals, Luxembourg

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Background and Objectives: Besides highly susceptible farmed minks, the highest number of independent SARS-CoV-2 spillovers is reported in cats and dogs. In addition, some virus variants acquired mutations altering host tropism. We thus assessed the prevalence of SARS-CoV-2 infections in companion animals in Luxembourg and aimed at identifying the factors promoting inter-species transmission to assist authorities in their containment efforts in light of SARS-CoV-2 evolution and variant replacements.
Material and Methods: A total of 220 cats and 264 dogs were recruited in veterinary clinics between October 2020 and April 2021 coinciding with the 3rd (driven by B.1 strains) and 4th (Alpha B.1.1.7 and Beta B.1.351) SARS-CoV-2 waves. Among those, 24.4% of animals were recruited in households with history of SARS-CoV-2 in humans. Serum samples were tested for the presence of anti-SARS-CoV-2 antibodies by a surrogate neutralization assay. Respiratory swabs, rectal swabs and stool samples were tested for the presence of viral RNA by RT-qPCR and positives were sequenced with the Artic protocol on a MinION flow cell. A second cohort of diagnostic leftover samples was collected between April 2020 and March 2022. The cohort included 918 cat and 1572 dog sera tested by a multiplexed surrogate neutralization assay harbouring recombinant spike proteins from several variants.
Results: In the first cohort, 4/711 samples tested positive by RT-qPCR. All positive animals originated from SARS-CoV-2 affected households. Variants B.1.221, B.1.177, B.1 as well as Beta B.1.351 were identified, corresponding to the variants concomitantly circulating in the human population. Prevalence of neutralizing antibodies (NAbs) reached 28.2% (cats: 25.5%; dogs: 30.4%) in SARS-CoV-2 affected households. In these households, we did not identify any factors contributing to an increased risk of transmission, likely due to already intensive contacts. In the second cohort, 4.2% of cats and 3.0% of dogs had NAbs. The majority of animal sera had the highest NAb titers against the ancestral B.1 strain (55%), followed by Delta B.1.617.2 (16%), Alpha B.1.1.7 (14%), Omicron B.1.1.529-BA.1 (6%) and Gamma P.1 (4%). Rolling 3-months seroprevalence rates tended to increase over time, likely resulting from increasing opportunities for exposure to the virus and/or lasting presence of NAbs after infection.
Conclusion: Seroprevalence rates were not consistent with increasing transmission rates of variants of concerns to companion animals or sustained animal-to-animal transmission. This study nevertheless highlighted the high frequency of cross-species transmission events in SARS-CoV-2 affected households, regardless of the variants at play. Clear recommendations to affected owners for minimizing transmission to domestic animals are thus warranted.

Funding source/acknowledgements (optional): This study was supported by Luxembourg National Research Fund (FNR) (grant COVID-19/2020-1/14716613/COLIVE), by the Fondation Rudy von Sternberg, and by Luxembourg Institute of Health and the Ministère de l'Agriculture, de la Viticulture et du Développement rural.

Research despite the war: surveillance of emerging viral and bacterial pathogens in wild birds and animals in Ukraine

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Background and Objectives: Wild birds and animals are host for many pathogens that pose risk to human and domestic animal, such as Influenza A virus, flaviviruses, coronavirus, avian avuloviruses, tick-borne pathogens, and drug-resistant bacteria. Surveillance of such pathogens is vital for predicting and preparing for future outbreaks and pandemics. The war in Ukraine has been creating new challenges for One Health, and extends beyond Ukraine, to all of Europe.
Material and Methods: Research on emerging wildlife pathogens have been conducted in Ukraine from 2009 till 2024. Classical virological, serological methods, PCR and sequencing were used for testing.
Results: Surveillance efforts have been continuing despite war in Ukraine which started in 2014 and full-scale invasion in 2022.

Influenza: Throughout study period there were active circulation of avian influenza viruses in wild birds, with 207 AIVs isolated, mainly low pathogenic viruses but also 20 highly pathogenic viruses. The prevalence range was 0.16-19.65%. Genetic analysis revealed large genetic diversity and phylogenetic association with AIVs reported from Europe, Siberia, Asia. Seropositivity to animal influenza was detected in pet (cats 4.2-10.4%, dogs 6.7-10.4%), pigs (14.8%) horses (15.4-70.0%). In wild animals, 12.9% of red foxes, 66.6% of wild horses had antibody to influenza. Influenza viruses were isolated from domestic pigs, and 6.6% pigs were PCR positive.

Avian avuloviruses (AaV) of different serotype were detected in wild birds. Some of them has not been characterized completely yet. Newcastle disease virus of different genotypes were detected in more than ten species of wild birds.

Coronaviruses (CoV). About 5-57.14% of Ukrainian insectivores bats resulted positive for CoV in urban and natural landscapes.

West Nile Virus. Serological surveillance revealed seropositivity in wild birds (7.4-62.3%), bats (33.3-57.9%), domestic horses (87.0%) and deer (33.3%).

Bacteriological surveillance showed active circulation of multi-drug resistant *E. coli* (prevalence 10.0 - 31.8%) and *Salmonella spp* (1.7 – 50%) in wild birds.
Conclusion: We detected several pathogens of importance to human and animal health in wild and domestic animals in Ukraine, highlighting a potential threat of spillover from wild reservoirs to domestic animals and humans. The ongoing war can have a significant impact on the circulation of pathogens, especially our ability to detect outbreaks in time, and hence epidemiological outcomes could be worsened. Our findings highlight importance of continued surveillance efforts in Ukraine. As disease knows no borders, surveillance in Ukraine is regionally beneficial, including for the European Union.

Funding source/acknowledgements (optional): Research was funded by NAAS, R&D Joint project of MES of Ukraine, NRFU (#2021.01/0006), STCU (P444a-b, P777, P781), U.S. DoD BTRP (UP4, AZDN).

Serologic and knowledge, attitude and practice on prevention of zoonotic diseases of wildlife farmers in Vietnam: results from a mixed-method study

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Background and Objectives: While captive wildlife farming contributes to income generation and food security, it raises concerns about the transmission of zoonoses, which are diseases that can transfer from animals to humans. This study aims to understand the knowledge, attitudes, and practices of wildlife farmers that contribute to zoonotic transmission of various wildlife viral pathogens in two provinces in Vietnam.
Material and Methods: Following a One Health approach, we collaborated with One Health provincial multi-sectoral teams representing animal health, public health, and forestry protection to perform a cross-sectional survey using mixed methods. The study was carried out between October 2023 and March 2024 in Lao Cai Province (northern region) and Dong Nai Province (southern region) in Vietnam. In total, we conducted 206 face-to-face interviews using structured questionnaires, 30 key informant interviews, and 5 focus group discussions with wildlife farmers. Serological testing was performed to detect prior infection with hantavirus and hepatitis E virus.
Results: The overall proportion of individual seropositive to hantavirus and hepatitis E virus were 9.2% (n=19) and 30.6% (n=63) respectively. Quantitative results showed that around 40% of participants had never heard of zoonoses, and 36% of them were unaware that diseases in wildlife can be transmitted to humans. A total of 79% of participants, primarily male (58%), reported consuming wild animals within the past year. While the majority (91%) understood the increased risk of zoonotic disease from undercooked or raw wild meat, 43 consumers still reported eating blood pudding from various wild animals (e.g. bamboo rats, wild boars, snakes). Furthermore, hygienic attitudes and practices were inadequate, 14% of participants believed using personal protective equipment was unnecessary when contacting wildlife; 53% never wore gloves; 83% never wore protective clothing; and 34% never wore masks during cage cleaning or animal contact. Knowledge, attitude, and practices of wildlife farmers did not show a significant association with seropositivity. Moreover, qualitative results also showed that few of the participants possessed sufficient knowledge of zoonotic diseases and the associated transmission risks within the wildlife value chain.
Conclusion: This study highlights a significant knowledge gap and low perceived risk associated with zoonotic disease transmission in the wildlife value chain. These findings also reveal a critical need to further explore zoonotic risks of activities regarding wildlife contact and to better understand risks of wildlife practices and consumption.

Funding source/acknowledgements (optional): CGIAR One Health Initiative

The first clinical report of Red deerpoxvirus in semi-domesticated reindeer in Sweden

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Background and Objectives: Parapoxviruses cause contagious pustular dermatitis and stomatitis in various host species. In semi-domesticated reindeer (*Rangifer tarandus tarandus*), *Parapoxvirus orf* (orf) and *Parapoxvirus pseudocowpox* have previously been described in outbreaks in Fennoscandia, with varying clinical impact. Another known parapoxvirus is the red deerpoxvirus *(Parapoxvirus reddeerpox)*, which was first isolated in red deer in New Zealand in 1995, and has until now not been detected in other species than red deer in natural settings.
Material and Methods: In September 2023 in Norrbotten, five reindeer calves with contagious ecthyma-like lesions and affected eyelids, out of approximately 150 reindeer, were sampled and analyzed for parapoxvirus at the Swedish Veterinary Agency (SVA). The most severely affected calf was positive for parapoxvirus and after sequencing, the virus was characterized as red deerpoxvirus.
Results: The reindeer calf had an affected general appearance at clinical examination and was therefore euthanized and necropsied. Multiple crusts and lesions were detected on the muzzle and eyelids around both eyes, in the oral mucosa, tongue, and gingiva. In addition, one submandibular lymph node was mild-moderately enlarged. The affected eyelids, muzzle, and oral mucosa were sampled by rubbing an eSwab under crusts and over the conjunctival fornix and oral mucosa with lesions. *Post-mortem* serum and tissue samples from eyelids, oral mucosa, the enlarged lymph node, kidney, liver, and spleen were collected and saved (-20°C). The body condition was normal. Nucleic acid from the conjunctival- and oral swabs from the calf was extracted and analyzed for the presence of parapoxvirus, cervidpoxvirus (CPV), cervid herpesvirus 2 (CvHV2), and *Chlamydia* using real-time PCR. The samples came out positive for parapoxvirus. Sequencing of the DNA-polymerase gene revealed 99.7% identity on the amino acid level with red deerpoxvirus strain HL953 (NC_025963.1) from a red deer in Germany in 2013, corresponding to a three amino acid difference. In addition, one of the other affected calves in the group was positive for CPV. Additional analyses will be complemented before the conference.
Conclusion: The clinical signs reported were indistinguishable from previous reports of lesions caused by other poxviruses, like orf and CPV in reindeer, which underlines the difficulties of diagnosing the causative agent based only on clinical observations. To our knowledge, this is the first report of red deerpoxvirus being involved in clinical disease in reindeer. This is also the first finding of red deerpoxvirus in a species other than red deer.

Value of whole genome sequencing in Animal Health - Characterization of Bluetongue & Epizootic Hemorrhagic Disease outbreaks in France 2023

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Background and Objectives: Bluetongue (BTV) and Epizootic Hemorrhagic Disease (EHDV) are two notifiable animal diseases transmitted to ruminants through small biting midges belonging to the Culicoides genus. The two viruses involved, Bluetongue virus (BTV) and Epizootic hemorrhagic disease virus (EHDV) respectively, belong to the Orbivirus genus of the Reoviridae family, which include double-stranded RNA segmented genomes (10 segments). During the second semester of 2023, multiple Orbivirus outbreaks occurred in cattle and sheep from mainland France and Corsica. EHDV entered the country from the south-west leading to multiple EHD outbreaks in the region and animal movement restrictions. In parallel, unexpected severe clinical cases of BT were reported in Aveyron and Corsica in sheep and cattle infected with BTV-8, suggesting an increased virulence of the circulating strain. Finally, BTV-4 outbreaks were also reported in sheep in Corsica. Here, we describe the genomic characterization of the EHDV-8, BTV-8 and BTV-4 strains circulating in France 2023.
Material and Methods: Viral genome sequencing was carried out using a custom *Orbivirus*-focused SISPA approach and the Oxford Nanopore technology. Sequencing were performed on isolated strains and blood samples collected on the field from infected animals and sent to the Reference Laboratory for BT and EHD diagnostic and confirmation
Results: Full genome sequences were recovered from both isolates and field samples. EHDV-8 genomes detected in France were almost identical to those detected in Sardinia and Tunisia since 2021. BTV-8 genomes analysis revealed the circulation of two distinct strains: the enzootic BTV-8 strain and a new strain responsible for the unexpected severe clinical signs reported in sheep and cattle this year and of unknown origin. Finally, BTV-4 genomes analysis revealed that the strain described in 2016 in Corsica is still circulating on the field and was not replaced by the BTV-4 strain detected lately in 2021.
Conclusion: Sequencing viral genomes, particularly from blood samples, enables rapid identification of circulating genotypes in an epizootic context. The exceptional nature of EHD and BT epizootics in France in 2023 underlines the need for epidemiological and genomic research to identify the factors involved in these emergencies.

Session 1

Emerging and re-emerging diseases

Poster Presentations

A new RT-qPCR kit enabling a specific detection of Bluetongue virus serotype 3

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[1] Innovative Diagnostics

Background and Objectives: Bluetongue virus (BTV), belonging to the genus Orbivirus within the family Reoviridae, is responsible for bluetongue (BT), one of the WOAH-listed major diseases of ruminants. Since 1998, serotypes 1, 2, 3, 4, 6, 8, 9, 11, and 16 have been reported in Europe. In september 2023, new BTV-3 outbreaks were reported in sheep farms in The Netherlands. These events were rapidly followed by diffusion to Belgium, Germany and United Kingdom.In this context, Innovative Diagnostics has developed a new molecular diagnostic tool, the ID Gene™ Bluetongue genotype 3 Duplex. It allows specific and exclusive detection of BTV serotype 3, including the recent BTV-3 strain detected in the aformentionned countries (BTV-3/NET-2023), in blood, spleen and organs from aborted animals (spleen, heart and liver). This study present the validation and performance of this new IDGene™ kit.
Material and Methods: Diagnostic specificity was assessed on 115 negative whole blood samples from cattle, from France, confirmed negative with IDGene™ Bluetongue Duplex all serotypes RT-qPCR. Diagnostic sensititivy was tested on 8 BTV-3 positive samples, kindly provided by two NRLs : 5 samples which were tested positive by the French NRL, and 3 RNAs extracted from sheep whole blood samples collected in Belgium. Samples were kindly provided by the respective NRLs. Inclusivity was tested on a panel of BTV-3, including the BTV-3/NET-2023 strains which emerged in the Netherlands in 2023. Exclusivity with respect to other BTV serotypes and to EHDV serotypes was assessed on 3 panels : 38 BTV isolates provided the French NRL for BTV & EHDV and the FLI (Germany) and on 20 EHDV isolates from the French NRL and The Pirbright Institute (TPI, United Kingdom).
Results: The ID Gene™ Bluetongue genotype 3 Duplex measured specificity was 100%, IC95% [96.8-100], n=115. Measured diagnostic sensitivity was 100%, IC95% [67.6-100], n=8. All BTV-3 strains tested, including BTV-3/NET-2023, were detected by the ID Gene™ kit. All other BTV serotypes as well as all EHDV strains tested were not detected, showing the ID Gene™ kit's excellent excluvisity.
Conclusion: The IDGene™ Bluetongue genotype 3 Duplex kit offers a specific detection of bluetongue virus serotype 3, including the BTV-3 strains detected in western Europe in 2023. Results can be obtained in 50 min with a rapid amplification program, compatible with all IDGene™ kits, making possible to test on the same run for different Orbiviruses RT-qPCRs therefore offering maximum flexibility & testing capacity by optimizing lab equipments ressources.

Discospondylitis caused by Brucella canis in an imported dog and testing of contact dogs: a case study

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Background and Objectives: *Brucella canis* is a bacterium that predominantly causes reproductive disorders in dogs, but clinical signs such as arthritis and discospondylitis have also been described. In Sweden, the infection has been confirmed in a handful of cases since the first report in 2011. Majority of suspected and confirmed cases have been breeding dogs. *Brucella canis* is a zoonotic agent.
Material and Methods: Serological methods used were enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA). Culture for *B. canis* was performed in safety laboratory on selective media. Polymerase Chain Reaction (PCR) analysis was performed using two different validated methods.
Results: Case presentation: A seven-months-old intact male dog originating from a dog shelter in Romania presented to a veterinary hospital with back pain and intermittent pyrexia. Diagnostic imaging of the abdomen revealed prostatic changes. Urinary culture was negative. *Brucella canis* antibodies were detected in serum by ELISA and LFIA. The dog was treated with analgetics and antibiotics and improved clinically. However, complete resolution of clinical signs was not achieved, and the dog was later euthanised. Necropsy showed a chronic multifocal prostatitis and a spondylosis-like, ossified change over L1-L2. Cultures of prostate, testes, spleen, and lymph nodes were negative for *B. canis*, but PCR on prostatic sample was positive for *Brucella* spp.

Contact dogs: The dog lived in the same household as three neutered adult dogs from the same shelter in Romania. None of these dogs showed clinical signs of disease. ELISA for *B. canis* were performed twice. One dog was positive on both occasions, with a rise in titre, and the other two only on the second occasion. Urine from all contact dogs was sampled twice and cultured for *B. canis,* with negative results. The dog that was serologically positive on two occasions was euthanised and sent for necropsy. Samples taken from multiple organs for culture and PCR were all negative for *B. canis*.
Conclusion: This is the first described Swedish case of *B. canis* in a non-breeding dog, with a full necropsy in both index case and one contact dog, as well as serological and microbiological examinations in several contact dogs. It highlights the complexity of canine brucellosis, the array of clinical signs and that multiple analytical methods might be necessary to confirm infection. It also supports reports from other European countries emphasising that importing dogs with unknown background comes with a risk of introduction of *B. canis*, as well as other rare infections.

In vivo models for rustrela virus (RusV) infection in potential reservoir and spill-over hosts

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Background and Objectives: Rustrela virus (RusV; species Rubivirus strelense) is a recently discovered relative of the human rubella virus (Rubivirus rubellae) and causes fatal non-suppurative meningoencephalomyelitis in a broad range of mammals, including domestic, wild and zoo animals, in Germany, Austria and Sweden. In domestic cats, RusV was demonstrated to be the causative agent of 'staggering disease', a neuronal disorder that had remained of unknown aetiology for almost five decades. Based on its reportedly broad range of susceptible hosts, a zoonotic potential of RusV cannot be excluded. Apparently healthy yellow-necked field mice (Apodemus flavicollis) and wood mice (Apodemus sylvaticus) were identified as potential reservoir hosts. However, many questions regarding the biology and epidemiology of RusV in reservoir and spill-over hosts remain elusive, such as course of infection, pathogenesis, and transmission routes. First attempts of virus isolation have failed and in vivo infection models have not been established.
Material and Methods: In this study, we experimentally inoculated wood mice, representing potential reservoir hosts, and Lewis rats, potentially representing spill-over hosts, with brain homogenates originating from RusV-infected animals via different inoculation routes. Viral RNA was detected by RusV-specific RT-qPCR and RusV-reactive antibodies by immunofluorescence antibody test.
Results: Inoculation via intracranial as well as via combined intranasal/peroral route resulted in persistent RusV infection in both rodent species. In contrast, combined intramuscular/ subcutaneous injection failed to establish detectable infection. Viral loads were highest in the central nervous system but the virus spread also to peripheral organs, including nose, salivary glands and intestinal tract and urinary bladder. Shedding of viral RNA was frequently detected in oral swabs and environmental samples collected from infected wood mice, but only sparsely from rats. Seroconversion was detectable only in wood mice at 8 to 12 weeks after inoculation. Neither wood mice nor rats developed any clinical signs attributable to the infection during twelve weeks post infection, but RusV-infected rats showed significantly reduced weight gain as compared to non-infected controls and non-suppurative encephalitis was observed in infected rats as early as four weeks post infection.
Conclusion: Our study provides the first infection model for RusV and indicate that natural infection may occur via mucosal rather than parenteral route. Shedding of viral RNA suggests that wood mice may serve as natural reservoir hosts, while the barely detectable viral shedding and the development of subclinical encephalitis in rats may rather represent a spill-over infection, as assumed for diseased zoo animals, cats and other mammals.

Laboratory Investigation of Sheep and Goat Pox Virus in Georgia

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Background and Objectives: Sheep and goat pox (SGP) is a highly contagious viral disease affecting small ruminants, causing significant economic losses worldwide. The causative agents, Sheep Pox Virus (SPPV) and Goat Pox Virus (GTPV), both belong to the genus Capripoxvirus of the Poxviridae family. They are classified on the U.S. Department of Health and Human Services (HHS) and the U.S. Department of Agriculture (USDA) list of select agents and toxins, as well as the World Organisation for Animal Health (WOAH) notifiable diseases list. The disease manifests with fever, characteristic skin lesions, respiratory distress, and generalized lymphadenopathy. Morbidity and mortality rates can significantly increase (100%) depending on the virulence of the isolates, among fully susceptible populations, including neonates and young animals. SGPox spreads through direct contact, fomites, and vectors, persisting in environments and scab debris for prolonged periods. This disease threatens food security and livelihoods, particularly in regions where small ruminants play a vital role in agriculture and rural economies.
Material and Methods: In 2024, the National Food Agency (NFA) received a report from the Dedoplistsqaro, Kakheti, from 2 different farms about a suspected case of sheep and goat pox. 20 samples were promptly delivered to the State Laboratory of Agriculture of Georgia (SLA). Pathological samples including smears, internal organs, tissues, skin nodules, and scabs. The samples were examined using Reverse Transcription Polymerase Chain Reaction - RT-PCR (*ID Gene Capripox viral triplex*).
Results: From the received samples, 4 positive cases from both farms were confirmed in total. The National Food Agency has launched a rapid vaccination program and quarantine measures. Samples are still being sent to a laboratory for disease monitoring but no more positive cases have been reported. Confirmed samples from the State Laboratory of Agriculture were forwarded to the Sciensano laboratory in Belgium for further investigation and sequencing to determine the strain.
Conclusion: Based on the results obtained and considering the continuous import and export of animals between Georgia and neighboring countries where the infection remains prevalent, it can be concluded that the country faces a persistent risk of disease transmission. Therefore, along with the improvement of epidemiological surveillance, the laboratory needs to increase diagnostic capacity and implement modern technologies to be able to distinguish the vaccine strain from the field strain and determine which strain is present in the country using Next-generation sequencing (NGS). Introducing these methods will be also beneficial in diagnosing other pathological agents.

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Newly updated RT-qPCR for an efficient Bluetongue virus type 8 and 4 detection, including BTV-8 strains reported in France and Italy in 2023

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Background and Objectives: Bluetongue virus (BTV), is responsible for bluetongue (BT), one of the WOAH-listed major diseases of ruminants. Since 1998, serotype 1, 2, 3, 4, 6, 8, 9, 11, and 16 have been reported in Europe. During summer 2023, several clinical cases of bluetongue serotype 8 (BTV-8) occurred in cattle and sheep in France and Italy. More clinical signs were observed during that BTV-8 emergence, and the French NRL confirmed the presence of new BTV-8 variants, which were unfortunately giving bad PCR signals or which were not detected by the ID Gene™ Bluetongue genotypes 8 and 4 Triplex. Therefore, Innovative Diagnostics updated its diagnostic tool by modifying the BTV-8 target of the ID Gene™ Bluetongue genotypes 8 and 4 Triplex kit to allow proper detection of the BTV-8 newly identified in France and Italy. This study summarizes validation data of this updated ID Gene™ 2.0 kit.
Material and Methods: Diagnostic specificity was assessed on 115 negative whole blood samples from cattle, from France, confirmed negative with the ID Gene™ Bluetongue Duplex RT-qPCR. Diagnostic sensititivy was tested on 45 BTV-8 positive samples and 15 BTV-4 positive samples. Inclusivity was tested on a panel of BTV-4 and BTV-8 isolates, including the new BTV-8 strains detected in 2023, provided by the French NRL for BTV&EHDV. Exclusivity with respect to other BTV serotypes and to EHDV serotypes was assessed on 38 BTV isolates provided the French NRL for BTV & EHDV and the FLI (Germany) and on 20 EHDV isolates from the French NRL and The Pirbright Institute (TPI, United Kingdom). The WOAH Reference Laboratory for BTV of Teramo (Italy) also conducted a trial of the kit.
Results: The ID Gene™ Bluetongue genotype 8 and 4 Triplex 2.0 measured specificity was 100 %, IC95% [96.8-100], n=115. Measured diagnostic sensitivity with respect to BTV-8 and BTV-4 was respectively 100%, IC95% [92-100], n=45 and 100%, IC95% [80-100], n=15. All BTV-8 strains tested, including the strains detected in France and Italy in 2023, were efficiently detected by the updated ID Gene™ kit, giving a perfect inclusivity. All other BTV serotypes as well as all EHDV strains included in the panels tested were not detected, showing the ID Gene™ kit's excellent excluvisity.
Conclusion: The updated ID Gene™ Bluetongue genotype 8 and 4 Triplex 2.0 kit, offers a specific and exclusive detection of bluetongue type 8 and 4, including the BTV-8 strains reported in France and Italy in 2023.

Novel human derived swine influenza A virus H3N2 genotype circulating in pigs in Northern Italy

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Background and Objectives: The circulation of different influenza A viruses in swine (IAV-S) contributes to the constant generation of novel reassortant strains with different combinations of swine, avian and human genes. In recent years, the four main IAV-S subtypes circulating in pigs in the EU are H1avN1, H1huN2, H1pdmN1 and H3N2. The latter emerged in 1984 from a reassortment event between human-like swine H3N2 and H1avN1. As of now, H3N2 viruses are detected at low levels in pigs in Italy, where the main genotype is represented by Ghent84-derived swine-like H3 and N2, combined with avian-like internal gene cassette. Nevertheless, here we describe three IAV-S H3N2 isolates with a new genotype, first detected in 2021 in Italian pigs, probably originating from a reassortment event between swine and human IAVs.
Material and Methods: IAV real-time RT-PCR screening was performed on swine clinical samples and isolation was attempted. RNA was extracted from isolates, RT-PCR was performed, then DNA libraries were sequenced on a MiSeq instrument. Phylogenetic analyses were performed on a dataset comprising European swine and human HA-H3 and NA-N2 sequences downloaded from NCBI's GenBank database and sequences from our internal database. Sequence alignments were performed with MUSCLE and refined with BioEdit; the Iqtree2 software was used to infer Maximum-Likelihood phylogenetic trees.
Results: From diagnostic pig samples collected from 2021 to 2023 and routinely characterized for IAV-S in Italy, the phylogenetic analysis of the HA-H3 showed that three H3N2 samples clustered closely with human HA-H3 sequences of the 2018-2019 influenza season from strains isolated in Europe. Regarding the NA-N2, it clustered with the NA from H1huN2 IAV-S currently circulating in Italian swine, which originated from an introduction of an N2 of seasonal human origin back in 2000. Lastly, the new H3N2 genotype was characterized by an avian-like internal gene cassette, commonly detected in swine IAVs.
Conclusion: Here we report the detection of three viruses, belonging to a new human/swine reassortant H3N2 IAV-S genotype, identified in 2021, 2022 and 2023. The 2021 sample was from the Piedmont region, while the other two were from the Lombardy region, where most Italian swine farms are located. Further analyses are needed to better investigate how this new genotype emerged. Given the ever evolving and mutating landscape of IAVs, there is a reinforced need to perform continuous surveillance, to better monitor circulating strains and to track the emergence of new variants at the human-animal interface in the pig population.

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Production of monoclonal antibodies against bat-borne emerging zoonotic Issyk-Kul Virus

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Background and Objectives: Issyk-Kul virus (ISKV) belonging to the *Nairoviridae* family, Bunyavirales order, was isolated for the first time in 1970 in Asia from *Nyctalus noctula* bats and bat ticks. It has spread to Europe, firstly in Germany, then in Sweden and recently it was also detected in Italy from an adult female of *Hypsugo savii*. Bats are ISKV reservoir hosts, but sporadic illness (fever, headache and nausea) outbreaks in humans are possible due to virus transmission. Climate changes promote the arbovirus-related zoonosis onset, even of the neglected ones such as ISKV. The need for available diagnostic tests specific for emerging agents is crucial for disease preparedness, management and infection containment. In the present study, we describe the initial characterization of monoclonal antibodies (mAbs) against ISKV produced using hybridoma technology.
Material and Methods: An Italian ISKV isolate was expanded on MARC-145 cells, inactivated, purified by sucrose cushion ultracentrifugation steps and used to immunize two BALB/c mice. The mAbs-producing hybridomas were generated and screened by indirect ELISA and Western Blotting (WB) using the purified virus and by immunoperoxidase (IP) assay on ISKV-infected cells. To better characterize the mAbs, the viral nucleoprotein (NP) was produced in *E.coli* using the sequence of the ISKV Italian isolate (OR583911), with optimization of the codon usage. The recombinant NP (rNP) was obtained under denatured conditions and purified by immobilized metal ion affinity chromatography (IMAC), giving a yield of 2 mg/L. The rNP and the mAbs were evaluated in WB and indirect ELISA.
Results: A total of 69 hybridomas reacted against ISKV in IP and seven recognized a viral protein of 55 kDa, presumably corresponding to the NP, in WB. The rNP was recognized in WB by all the seven mAbs that reacted with the viral NP antigen. Further three mAbs recognized rNP in indirect ELISA, proving to react with conformational epitopes.
Conclusion: Given the importance of the availability of diagnostic tools that can accurately identify the presence or absence of emerging zoonotic pathogens, our preliminary results are encouraging that the produced reagents can be used to develop specific assays. Indeed, as NP is one of the targets of the humoral immune response within the *Nairoviridae* family, the combination of mAbs and recombinant protein is a key factor in producing robust diagnostic assays and overcoming biosecurity issues due to the use of viral antigens. Further characterization of the remaining hybridomas is ongoing.

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Reverse Zoonosis of SARS-CoV-2 Alpha Variant Infection in an African Lion at Colombo Zoo with Respiratory Distress and Complications from septicemia

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Background and Objectives: The global impact of the COVID-19 pandemic has been profound. As of March 3, 2024, Sri Lanka has witnessed 672,750 cases and 16,897 fatalities across three distinct waves. The third wave, which began on April 15, 2021, was characterized by peaks driven by the Alpha, Delta, and Omicron variants. The Alpha variant's higher transmissibility led to an exponential surge in cases and fatalities from April to June 2021. Notably, while other countries reported sporadic COVID-19 cases in animals, Sri Lanka remained free of such cases until June 2021 when a 14-year-old African lion (*Panthera leo*) at the Colombo Zoo exhibited respiratory distress symptoms, prompting microbiological investigations.
Material and Methods: SARS-CoV-2 RT-PCR (Altona kit), routine bacteriology, acid fast bacilli and mycobacterial culture were performed on nasal swabs collected from the lion. A 12-year-old lioness from the same compound, displaying mild symptoms subsequently, was also tested for SARS-CoV-2 RNA. The SARS-CoV-2 genome from the lion's nasal swab PCR extracts and from an infected zoo keeper was sequenced. For the follow up testing, SARS-CoV-2 RT-PCR was conducted on the lion's nasal swabs collected after 10 days. Subsequent blood culture was performed for further investigations and management of the lion's deteriorating condition.
Results: Nasal swabs from both the lion and the lioness were tested positive for SARS-CoV-2 RNA. The routine bacterial culture showed a mixed growth of *Acinetobacter*, Methicillin Resistant *Staphylococcus aureus*, and *Pseudomonas*, but were negative for acid fast bacilli and mycobacterial culture. The SARS-CoV-2 genome sequencing from both the lion and an infected zoo keeper was identified as the SARS CoV-2 Alpha variant. Follow-up Testing with repeat RT-PCR for SARS-CoV-2 became negative after 10 days and the subsequent blood culture confirmed Methicillin Resistant *Staphylococcus aureus* septicemia.
Conclusion: Detection and identification of the SARS-CoV-2 Alpha variant in the lion indicates reverse zoonosis, likely due to spill-over from human infections. This variant was dominant during June 2021, coinciding with the first peak of the third COVID-19 wave in Sri Lanka. The initial detection of Methicillin Resistant *Staphylococcus aureus* from nasal swabs, subsequent deterioration of the lion's condition despite the absence of SARS-CoV-2 after 10 days, and the presence of Methicillin Resistant *Staphylococcus aureus* septicemia suggest a complication of secondary lung infection following Covid-19. Guided antibiotic therapy played a crucial role in saving the lion's life. This report represents the first documented case of reverse zoonosis involving the SARS-CoV-2 Alpha variant in Sri Lanka.

Sustainable Intensification of Zambian Goat Production in a Changing World

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Background and Objectives: Sustainable livestock farming ensures global food security and poverty reduction given the socio-economic and climate variations society is undergoing. Goats have been known to play a crucial role in enhancing livelihoods due to their adaptability and resilience, particularly for low-income farming households. Despite their importance, goats are often overlooked in agricultural policies and development initiatives. This project aims to bridge this gap by recognizing and leveraging goats' unique potential to enhance resilience in these economically disadvantaged farming communities, emphasizing their affordability, rapid reproductive rate, and adaptability to challenging environments.
Material and Methods: The study was conducted to address critical gaps in understanding the prevalence, dynamics, and impact of infectious diseases among goats in Zambia, particularly focusing on both climate-sensitive and intensification-linked diseases. Among the climate-sensitive infectious diseases under scrutiny is Rift Valley fever which is influenced by climatic variations and can pose significant threats to goat health and productivity. Additionally, intensification-linked diseases such as Q-fever and haemonchosis are being investigated due to their association with intensified production systems and the potential for increased prevalence in densely populated goat populations.
Results: By examining a comprehensive range of infectious agents, including those with the potential for emergence or re-emergence, this study aims to contribute significantly to the existing knowledge base on goat health and disease management strategies. Furthermore, the research explores the socioeconomic aspects of goat farming, exploring how disease burdens and management practices intersect with poverty alleviation efforts and investigates goat husbandry practices and trade patterns. Understanding these complex linkages is crucial for designing targeted interventions that not only enhance goat health and productivity but also contribute to the overall resilience and economic well-being of low-income farming households.
Conclusion: Advanced methodologies are employed to hypothesize variations in disease prevalence attributed to climate change and intensified production systems. One key postulation is that climate change and intensified production may lead to shifts in disease prevalence and distribution among goat populations, impacting their health and productivity. The study aims to provide detailed insights into the complex interplay between infectious diseases, climatic dynamics, and farming techniques, guiding targeted interventions for enhancing goat health and productivity. Such insights are pivotal for addressing emerging and re-emerging diseases, thereby contributing to poverty alleviation and reinforcing food security in a dynamic global landscape.

The role of sheep as host of the Epizootic haemorrhagic disease serotype 8 virus strain circulating in Spain during 2022-23

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Background and Objectives: Epizootic hemorrhagic disease is a non-contagious infectious viral disease transmitted through different species of diptera of the genus Culicoides. It affects both domestic and wild ruminants, especially bovines and deer. Its presence in Europe was notified for the first time in Italy in November 2022, and a few days later in Spain where a serotype 8 viral strain spread very rapidly in 2023. Despite the wide circulation of the virus throughout the country, no clinical signs were observed in sheep. The susceptibility of this species and its role in the transmission of the disease may be key in the control of the disease.
Material and Methods: Animals from 11 ovine farms without clinical signs in affected areas were analyzed (n=1754) by ELISA for serogroup specific antibodies detection and/or RT-PCR for serogroup viral genome detection. Additionally, five sheep were experimentally infected with an Epizootic haemorrhagic disease virus serotype 8 strain isolated from an affected bovine in Spain in 2022, and samples analyzed by the same methods.
Results: The results from field samples showed that sheep is a susceptible species, since positive results were detected in 7 farms, although with low prevalences: 13% of the samples were positive by ELISA and 10,7% by RT-PCR. Regarding experimentally infected sheep, no clinical signs or increase in temperature were observed in any animal. Virus genome was detected in EDTA blood from two sheep at 3-5 days post-infection, and three of them seroconverted from day 7 post-infection.
Conclusion: Both field and experimental data confirm a reduced susceptibility of sheep to infection with this strain which suggests that ovine may not be involved in transmission or have a secondary role.

Understanding zoonotic pathogens and risk factors from wildlife in Southeast Asia: A systematic literature review

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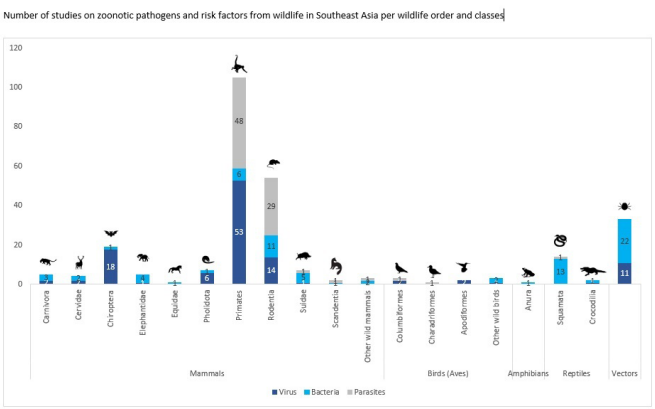
Background and Objectives: The COVID-19 pandemic has once again demonstrated the significance of the human-animal interface in the emergence of zoonotic diseases, with wildlife serving as an important source of infection. A better understanding of the specific pathogens and mechanisms involved is vital to prepare against future outbreaks, especially in Southeast Asia, considered a hotspot for zoonotic diseases. As such, the objective of this paper is to review and synthesize the published literature on wildlife zoonoses in this region.
Material and Methods: Following a systematic search and screening process, we included studies published in a journal between 2012 and 2022 as well as described any aspect of wildlife zoonoses in Southeast Asia.
Results: The results show a diverse range of potential zoonotic pathogens (including viruses, parasites, and bacteria), and the widespread occurrence of zoonotic diseases from wildlife among Southeast Asian countries. Drivers of zoonotic pathogen spillover include (i) ecological factors (e.g. disruption of wild animal habitat, contaminated water/food, climate change), (ii) animal characteristics (e.g. movement patterns, age-related susceptibility), (iii) individual human factors (e.g. age, gender, hygienic practices) and (iv) human-animal interactions (e.g. contact with wild animals, exposure to vectors).
Conclusion: The diverse drivers of zoonoses in Southeast Asia put its communities at risk for infection. To mitigate these risks, global health efforts must adopt a One Health approach to foster collaboration across human animal, and wildlife health sectors. This approach should prioritize educating individuals on safe animal interactions and enhancing disease surveillance mechanisms.



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Session 2

Pathogen evolution, pathogenesis and immunology

Oral Presentations

Evolution and host-adaptation of rabies virus during a historic fox-driven epidemic in Switzerland

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Background and Objectives: Rabies virus (RABV) remains one of the most feared and major threats to public health causing a lethal and currently largely incurable disease, i.e., rabies encephalomyelitis. Switzerland experienced a 30-year-long rabies epidemic between 1967 and 1996. The course of the epidemic suggested that the disease first entered Switzerland across the German boarder and that a second wave originated from France. The epidemic was mainly driven by foxes. However, other mammalian species and humans were affected. This study aims to reconstruct the evolutionary dynamics of rabies virus infections during this epidemic and of viral host adaptations upon transmission to various mammalian species.
Material and Methods: In a first step, 43 RABV-positive samples from 10 different domestic and wild animal species from different regions of Switzerland covering the epidemic years were selected from our Archive. Then, RNA extracts were submitted to high-throughput sequencing. We then analyzed the generated full-coding sequences for phylogenetic relations, evolutionary rates and selection pressures.
Results: Phylogenetic analysis revealed two distinct phylogenetic clades corresponding to the early and late stages of the Swiss epidemic. The preliminary results show the mean rate of evolution to be 5.8 E−4 substitutions/site/year (95% Highest Posterior Density of 3.1–8.6 × E−4 subs/site/year), clearly surpassing the described global rate 2.44 x E-4 substitutions/site/year and warranting further investigation.

Our results indicate also differences in selective pressures acting on different RABV proteins, such as a high rate of synonymous to non-synonymous changes (0.325) in the Phosphoprotein coding region and a low one (0.0295), indicating functional conservation, in the coding region for the Nucleoprotein (N). Additionally, the coding region for the surface Glycoprotein (G), the major target of neutralizing antibodies, exhibited the highest substitution rate, highlighting the importance of monitoring this region, e.g., for estimations on vaccine efficacy.

Subsequent analyses on these and of data from further samples from the Swiss RABV eradication, will focus on the influence of host species, geographic and temporal influences on RABV diversity, e.g., in specific genomic sites.
Conclusion: We expect this study to provide us with a better understanding of the molecular evolution of RABV in an eradication situation which may help to maintain and adapt control strategies towards global rabies eradication.

Experimental infection study with a recent bluetongue virus serotype 3 (BTV-3/Net2023) strain in sheep

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Background and Objectives: In September 2023, sheep with clinical signs of Bluetongue (BT) disease were observed in The Netherlands and laboratory analysis confirmed infection with BTV serotype 3 (Holwerda et al. 2023). The virus quickly spread through the Netherlands and at the end of December 2023 BTV had been diagnosed at > 4.400 farms and approximately 37.000 sheep had succumbed to the disease. Bluetongue virus (BTV) belongs to the genus Orbivirus of the Reoviridae virus family, and is an arbovirus that is transmitted by biting Culicoides-midges. The causative virus of the recent outbreak is designated BTV-3/NET2023. Phylogenetic analyses revealed similarity to recent Italian and Tunisian BTV3 isolates. and less with earlier isolates. To enable future studies investigating the pathogenesis and pathology of this severe (b)ovine disease and to evaluate vaccine efficacy of potential BTV3 vaccines a reliable animal model is developed.
Material and Methods: Two groups of 8 sheep (ewes; Texel/Swifter breed, +/- 9 months of age) were housed in two stables in the high containment (BSL3) facilities and randomly allocated to two treatment groups, which were infected and one treatment group with two ewes in each of the animal rooms, which remained as uninfected control group (treatmetn group 3). In each infection group 6 ewes were subcutaneously inoculated with 2 x 2 ml at a concentration of $1*10^5$ (Low dose; treatment group 1) or $1*10^6$ TCID50/ml (High dose; treatment group 2). The inoculum strain BTV-3/NET2023 had been passaged twice in Culicoides Sonorensis-cells (KC-cells), followed by one passage in Baby Hamster Kidney (BHK)-cells.
Results: All BTV-infected sheep with either high or low dose of virus developed fever, with the majority of animals showing an elevated body temperature at 5 DPI. In the same time period the 24 hour activity measurements revealed a strong and rapid reduction of activity patteren in all infected sheep and the manifestation of clincial signs such depression, loss of appetite, excessive salivation, lameness, shortness of breath and subcutaneous oedema formation appeared in in subsequent days. From 9 DPI onward 11/12 inoculated sheep reached their humane endpoints and had to be euthanized. The top of the viremia measured by PCR was reached on day 5-6 post-inoculation. Viral replication could be measured 1-2 days before the fever was observed in both groups. Seroconversion occurred between day 6 and 10 post-inoculation.
Conclusion: Infection with the current BTV-3 strain induces consistent and severe clincial disease with signs of micravascular injury in various tissues.

Influence of inoculation dose and route on Epizootic Haemorrhagic Disease Virus pathogenesis and induced immune response in cattle

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Background and Objectives: Following the first ever detection of Epizootic Haemorrhagic Disease Virus serotype 8 (EHDV-8) in Europe during late 2022, more precisely in Italy and Spain, the virus quickly spread towards Portugal and reached France in September 2023. This virus poses significant challenges to the European livestock industry due to the absence of vaccines combined with trade restrictions from infected regions. The lack of knowledge about EHDV-8, including its pathogenesis and induced host immune response, complicates the development of control policies, highlighting the urgent need for more research.
Material and Methods: To address this knowledge gap, an EHDV-8 infection study was conducted whereby groups of 3 cattle were inoculated with a Spanish virus isolate at varying doses (high and medium) and via various routes (intradermal and subcutaneous). Cattle were clinically monitored daily for 21 days post inoculation (dpi) and regularly sampled for virological and immunological analyses.
Results: All inoculated cattle developed viremia within 3 dpi, which peaked between 7 to 10 days and remained stable till the end of the study. Elevated body temperatures were measured in all groups after inoculation, although fever spikes up to 40°C were only detected in both high-dose groups. No other clinical symptoms were noted, except in one animal from the high-dose subcutaneously inoculated group. Starting from 10 dpi, this animal exhibited apathy, conjunctivitis, and swollen lymph nodes. Almost no virus excretion, as measured by PCR on nasal and buccal swabs, was observed in any of the animals. At necropsy, higher viral genomic loads were detected in the lymph nodes and internal organs compared to muscles, brain and skin samples. This pattern was similar for all challenged groups. Interestingly, the kidney of the clinical animal displayed petechia with a Ct-value of 20, being an outlier compared to all other organs tested. Where all other inoculated animals had a peak of IFNy release on 5-7 dpi, this animal only showed a peak on 17 dpi by IGRA, indicative of a suppressed cellular immune response. Furthermore, a strong humoral immune response was observed in all groups, with 100% seroconversion by 10 dpi.
Conclusion: The presented data show that blood, lymph nodes and internal organs are the most suitable matrices for diagnostic purposes and that the pathogenesis and induced immune response is little impacted by the inoculation dose and route. This infection model will facilitate further scientific research regarding this disease and could be used to evaluate the safety and efficacy of vaccine candidates.

Single-cell RNA-Seq analysis of monocytes from pigs infected with virulent African swine fever virus

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Background and Objectives: African swine fever virus (ASFV) is the causative agent of a viral hemorrhagic disease of domestic pigs and European wild boar. Pigs often die within 1-2 weeks after infection with a virulent ASFV. As a large double-stranded DNA virus, the ASFV genome encodes all viral components necessary for replication once entering host cells such as monocytes, macrophages, and dendritic cells. The pathogenesis of the disease is complex and has not been fully understood. The objective of this study was to explore host and viral gene expression in monocytes from pigs infected a virulent ASFV
Material and Methods: An experimental infection of pigs (n=6) with the virulent strain Arm/07/CBM/c2 was carried out in a BSL3 animal facility at National Veterinary Research Institute, Pulawy, Poland. All procedures were done according to actual legal regulations. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood, cryopreserved and shipped in dry ice to Swedish Veterinary Agency, Uppsala, Sweden for single-cell RNA-Sequencing (scRNA-Seq) analysis. PBMCs were partitioned into barcoded gel beads-in-emulsion (GEMs) and 3' gene expression libraries were constructed using Chromium Next GEM Single Cell 3' Reagent Kits v3.1 (10x Genomics) and sequenced. Data analysis included demultiplexing, barcode processing, gene (feature) annotation and counting using cellranger (10x Genomics). R package "Seurat" was used for quality control, dimensionality reduction, integration and clustering, cell type identification, differential analysis. Gene set enrichment analysis (GSEA) was done by R package "fgsea" against molecular signature database (MSigDB).
Results: All viral genes were detected in the infected monocytes, and there was a correlation between number of viral genes and expression levels. The infected monocytes contained high percentage of viral genes. Differentiation analysis determined over 2000 differentially expressed genes (DEGs) in the infected monocytes compared to the controls. The majority of DEGs were downregulated, affecting monocyte functionality such as phagocytosis, antigen presentation and cytokine production, as well as ability to differentiate into macrophages. Gene set enrichment analysis (GSEA) showed a strong inhibition of almost all hallmark pathways or gene ontology biological processes. In contrast, the exposed monocytes derived from the infected pigs but with no traces of viral genes presence in the cells, activated IFN-alpha/gamma antiviral responses among other pathways and biological processes.
Conclusion: In conclusion, scRNA-seq analysis revealed a distinct gene expression pattern between the infected and the exposed monocytes from pigs infected with virulent ASFV, providing useful insights into the pathogenesis of African swine fever.

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The cell junction and adhesion protein vinculin restricts permissivity to pestiviruses

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Background and Objectives: The cell junction and adhesion protein vinculin (VCL) is known to play a role in infection with human immunodeficiency virus (HIV), Ebola virus and Rous sarcoma virus. Apart from these, its relevance in viral infections has been little researched to date. However, one study suggested that knockdown of VCL had an inhibitory effect on infection with classical swine fever virus (CSFV). CSFV is a highly relevant pestivirus within the family *Flaviviridae* and related to other important animal pathogens such as bovine viral diarrhea virus (BVDV). In order to determine whether VCL is a host cell factor involved in pestiviral replication, we investigated its impact on infections with different pestiviruses.
Material and Methods: For this purpose, VCL knockout and VCL rescue cell lines based on porcine embryonic kidney (SPEV) cells were engineered by CRISPR/Cas9 gene editing and subsequent trans-complementation. The modified cell lines were characterized genetically and phenotypically with regard to expression of VCL and other junction proteins such as zonula occludens-1 (ZO-1). Then, the permissivity of the modified cell lines to three different CSFV strains and the distinct Bungowannah pestivirus (BuPV) also infecting pigs was investigated. In addition, the impact of VCL on pestiviruses mainly infecting ruminant hosts (BVDV-1, HoBi-like pestivirus and giraffe pestivirus) was studied.
Results: Sequencing as well as Western blotting and immunofluorescence staining of VCL confirmed successful engineering of a VCL knockout and a VCL rescue cell line. Interestingly, VCL knockout led to distortion of the regular ZO-1 pattern that was partly restored by VCL rescue. Unexpectedly, VCL knockout resulted in strongly enhanced infections with CSFV and BuPV which were partly mitigated again by VCL rescue. Furthermore, the low permissivity of naive SPEV cells to ruminant pestiviruses *in vitro* was also increased by VCL knockout and consistently reversed by VCL rescue.
Conclusion: It can be concluded that the presence of VCL has a negative effect on the permissivity of SPEV cells to different porcine and ruminant pestiviruses. Interestingly, knockdown of VCL and other proteins connecting the plasma membrane with the actin cytoskeleton in human cells similarly resulted in increased HIV infection. In this context, this study indicates that VCL might be a cellular restriction factor for replication of very diverse viruses. Further studies will characterize the interaction of the involved cellular and viral proteins in more detail and investigate the role of VCL in infections with other viruses.

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The leader protein is necessary for the establishment of persistent infection by type O foot-and-mouth disease virus.

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Background and Objectives: Foot-and-mouth disease (FMD) is a contagious and devastating viral disease of livestock. The persistence of the virus in ruminants after clinical recovery remains problematic, with underlying mechanisms still poorly understood. A prolonged infection may occur if a virus can counteract the host immune response. For FMD virus (FMDV), the major viral protein known to be involved in innate immune escape during acute infection is the leader protease (Lpro). It was previously shown that type A leaderless FMDV are highly attenuated *in vivo*. Both wild type and leaderless viruses may replicate in the nasopharynx, the primary site of infection, but leaderless viruses failed to cause viremia and generalized disease. It was however not explored if the leaderless FMDV could establish a persistent infection. In the framework of the ICRAD FMDV_PersIstOmics project, we have investigated if a recombinant leaderless FMDV O could persist *in vitro* in multilayer cells from bovine dorsal soft palate (DSP) grown at the air-liquid interface (ALI) and *in vivo* in cattle.
Material and Methods: DSP-ALI cells were inoculated with either recombinant wildtype (wt) FMDV O/FRA/1/2001 and its leaderless derivative. The upper multilayer was washed 6, 12, 24 and 48h post-infection, then each two or three days until 35dpi. In a parallel *in vivo* study, two groups of eight cattle each were inoculated with either wt FMDV or its leaderless derivative by intranasopharyngeal deposition, then observed and sampled for 35 days. Two cattle from each group were euthanized 24 hpi and tissue samples were collected to evaluate early-stage virus distribution.
Results: *In vitro*, live wt virus was isolated from supernatants of 5 of 6 cultures, while no leaderless live virus was detected after 48h post-inoculation of DSP-ALI. Likewise, leaderless viral RNA decreased from 7dpi until 28dpi and was undetectable at 35dpi. *In vivo*, the remaining six cattle in the wt group developed pronounced generalized disease and shed large amounts of virus during the first 10 to 14 days of the experiment. In contrast, leaderless-FMDV-infected cattle did not show any clinical signs of FMD, nor was any virus detected in serum or secretions. Two of six animals in the wt group remained persistently infected until the end of the trial at 35dpi. There was no evidence of persistent infection in leaderless-FMDV-infected cattle.
Conclusion: Overall, our results show that leaderless FMDV O cannot establish a prolonged or persistent infection in DSP-ALI cells and is strongly attenuated in cattle and unable to persist (Litz *et al*, 2024).

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"Not all viruses come alone" The outcome of bluetongue virus coinfections.

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Background and Objectives: Bluetongue virus (BTV) belongs to the genus Orbivirus within the family Sedoreoviridae and is the causative agent of the economically important, haemorrhagic disease of ruminants, bluetongue. BTV is transmitted by Culicoides biting midges and has a ten-segmented double stranded RNA genome and therefore can undergo reassortment, exchanging genome segments with other BTV strains coinfecting the same cell. Reassortment is a major driver of BTV diversity and evolution. One possible outcome of asynchronous BTV coinfection is superinfection exclusion, whereby infection of a cell by one virus blocks co-infection of that same cell by a second virus, and hence constraining genome-segment reassortment. In this study we investigated BTV coinfection and the impact of coinfection on reassortment.
Material and Methods: Bovine (BFA) or Culicoides sonorensis (KC) cells were asynchronously coinfected with BTV serotypes 8 and 1 (BTV-8 and BTV-1). The outcome of coinfection was assessed both in the cell supernatant and the cell pellet using a serotype-specific qRT-PCR and an RNA fluorescent-in-situ-hybridization (FISH), probe-based assay, respectively. Furthermore, reassortment frequencies in the progeny virus were investigated using a segment-specific qRT-PCR assay.
Results: In both mammalian and insect cell culture, with a 24-hour delay between coinfection, BTV-1 fully excludes BTV-8 infection (BTV-8 RNA not detected after 72hrs post infection). Conversely, when the serotypes are reversed under the same conditions,
BTV-8 is unable to exclude BTV-1 infection (ct of 19.7 for BTV-1 RNA at 72hrs post coinfection versus ct of 17.8 in the matched single infection control). This demonstrates for the first time that superinfection exclusion for BTV varies with serotype. Furthermore, the frequency of BTV reassortment was dependent on coinfection condition and cell line. Reassortment was more frequent in a mammalian cell line when compared to the insect cell line (100% of viruses were reassortants under synchronous coinfection conditions within mammalian cell line versus 80% under synchronous coinfection conditions within insect cell line). Under certain coinfection conditions, specific segment combinations preferentially reassorted together, however for some coinfection conditions no trends in segment combinations were observed. Importantly, all segments of the BTV genome were able to and did frequently reassort.
Conclusion: In conclusion, investigating the outcome of segmented virus coinfection furthers our knowledge regarding BTV reassortment. Reassortment leads to viral diversity which consequently facilitates viral evolution and adaptation, which in turn impacts viral fitness, replication and transmission that ultimately can affect disease outcome.

Session 2

Pathogen evolution, pathogenesis and immunology

Poster Presentations

Co-infection of peste des petits ruminants virus (PPRV) and foot and mouth disease virus (FMDV) in sheep

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Background and Objectives: Viral infections in ruminants lead to a significant productivity decline in livestock animals, resulting in important economic losses, which can be aggravated by viral disease co-circulation. *Peste des petits ruminants* virus (PPRV) and Foot and Mouth disease virus (FMDV) are both RNA viruses capable of infecting small ruminants. While FMDV infection is often subclinical in these animals, the virus retains the ability to replicate and transmit to other hosts. Conversely, PPRV induces an acute debilitating disease by suppressing the immune system, often leading to secondary infections. Given the shared geographical distribution of these two viruses, they can serve as an ideal model for studying viral co-infections in small ruminants and their effects in the outcome of the disease.
Material and Methods: To assess the impact of PPRV and FMDV co-infection, four groups of five sheep were infected with FMDV at days 3, 5, 8 and 15 post-PPRV infection, while two control groups of five animals were inoculated solely either with PPRV or FMDV.
Results: All sheep exposed to PPRV exhibited clinical signs of infection (pyrexia, muconasal secretion, rheum, cough), which were exacerbated and prolonged in animals co-infected with FMDV. We observed that in all groups co-infected with both viruses, FMDV viral load was higher compared to the group solely infected with FMDV. This was particularly noticeable in group infected with FMDV at 15 days post-PPRV infection. We found that PPRV viremia was prolonged in the group infected with FMDV at day 5 post-PPRV infection. Moreover, all sheep developed neutralizing antibodies (NAb) against FMDV and PPRV, with NAb production against FMDV occurring more rapidly than against PPRV.
Conclusion: FMDV infection in this experiment was mostly subclinical, and this is confirmed by the low viremia detected by RT-qPCR. We have nonetheless found that in all groups that were previously infected with PPRV, the FMDV load in blood was higher than in the control group only infected with FMDV. PPRV viremia was prolonged in the group infected with FMDV at day 5 post-PPRV infection. This group also developed greater clinical signs when compared to the other groups, suggesting that co-infection within this time window could worsen disease outcome. We will assess in the future T cell responses to FMDV and PPRV using inactivated virus as antigen in ELISPOT assays and in intracellular cytokine staining by flow cytometry.

Contribution of intestinal porcine organoids to the study of host-virus interactions of transmissible gastroenteritis virus

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Background and Objectives: Cellular culture (*In Cellula*) and/or animal experimentation (*In Vivo*) are standard methods for studying host-pathogen interactions. However, both methods have methodological or ethical limitations. Organoids, produced by the differentiation and self-organization of stem cells, recapitulate the epithelial cellular diversity and structural organization of organs from which they are derived and, as such, represent an alternative to the *In Cellula* and *In Vivo* methods.

The Viral Genetic and Biosafety Unit has implemented the production of porcine intestinal organoids (PIO) and developed a protocol for infection based on PIO's monolayer to expose cell receptors to porcine transmissible gastroenteritis (TGEV) alpha-coronaviruses. Two TGEV strains were used for the experiments: one belonging to the Purdue genogroup, adapted to cell culture, and the other belonging to the Miller genogroup, which is poorly adapted to cell culture.
Material and Methods: To evaluate the advantages and limitations of organoids in the study of host-virus interactions, piglets, PIO and ST cells were infected with a comparable viral genomic load of 10E8 N gene copies of the virus. For each model, infection kinetics were performed, and cellular RNAs were harvested for RNA-seq analysis of host-virus interactions.
Results: Comparison of the gene expression between the three models without any infection revealed Pearson's correlation of 0,422 and 0,485 between PIO and piglet jejunum or ST cells, respectively, when this correlation was only 0,229 between piglets jejunum and ST cells. 56% of the genes expressed were common to all the three models. A total of 1103 genes were expressed in both the PIO and piglet jejunum. Gene ontology analysis of these genes revealed enrichment in the cellular components of the brush border, tight junctions, and apical/basal plasma membranes.

The relative expression of the TGEV-N gene displayed differential dynamics depending on the strain. For the Miller strain, N viral gene expression decreased after 9 hpi, in contrast to the Purdue strain, for which N expression increased up to 24h. At the same MOI in organoids, 1000 times more of the viral N gene transcript was found after Purdue infection at 24h compared after Miller infection. *In Vivo*, a 3-log difference in viral N gene expression was observed in the piglet jejunum, in favor of the Miller strain.
Conclusion: In conclusion, the Purdue strain maintained an increased ability to infect PIOs (as for ST cells) compared to the Miller strain, in contrast to the *In Vivo* model. Transcriptomic analysis is currently being performed for these three infected experimental models.

Differential gene expression signatures between high and low pathogenic CSFV using porcine bone marrow-derived antigen presenting cells

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Background and Objectives: Classical swine fever (CSF) is one of the most important transboundary viral diseases affecting domestic and wild pigs. Infection with CSF virus (CSFV) results in a strong immunosuppression accompanied by aberrant pro-inflammatory responses, which is related to disease severity and viral replication.
Material and Methods: To gain a better understanding of CSF pathogenesis, a transcriptomic analysis was performed using porcine bone marrow-derived dendritic cells (poBMDC) infected ex vivo with two different cDNA-derived CSFV, the low-virulence strain Pinar de Rio (vPdR-36U) carrying a poly-U insertion in the 3' untranslated region (UTR) or the lethal double-mutant vPdR-H30K-5U. These two viruses were characterized previously in vivo.
Results: The transcriptomic profile of vPdR-36U- or vPdR-H30K-5U-infected poBMDC versus uninfected poBMDC revealed 946 and 2643 differentially expressed genes (DEGs), respectively. 191 DEGs were observed when the two virus-infected cells were compared. Importantly, poBMDC infected with the lethal CSFV strain overexpressed immune checkpoint molecules limiting the adaptive immune responses and involved in immune exhaustion and immunosuppression mechanisms, in particular PD-L1, CD276 and LAG3, while the CTLA-4 and CD154 genes were down-regulated. In addition, transcription of tristetraprolin (TTP) that plays a role in binding mRNA 3'-UTR AU-rich regions and in mRNA decay was turned-off in the presence of the lethal virus. KEGG pathways analyses showed that innate immunity-related pathways were overexpressed, while adaptive immunity-related pathways were underexpressed in cells infected with the lethal CSFV. The DNA replication pathways and the cellular senescence pathways were overexpressed, and the metabolic pathways and the biosynthesis of amino acid pathways, among others, were underexpressed similarly by the lethal and the non-lethal viruses.
Conclusion: Taken together, these findings shed new light on the mechanisms of viral disease immunopathogenesis and thus represent a basis for the development of tools for the detection of early immune system dysfunction markers due to viral infections.

DOUBLE-STRANDED RNA ORBIVIRUS DISRUPTS THE DNA-SENSING cGAS-STING AXIS TO PREVENT TYPE I INTERFERON INDUCTION

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Background and Objectives: Cyclic GMP-AMP synthase (cGAS) is a DNA sensing cellular receptor that induces IFN-I transcription in response to pathogen and host derived cytosolic DNA and can limit the replication of some RNA viruses. Some viruses have nonetheless evolved mechanisms to antagonize cGAS sensing. In this study, we evaluated the interaction between Bluetongue virus (BTV), the prototypical dsRNA virus of the *Orbivirus* genus and the *Sedoreoviridae* family, and cGAS.
Material and Methods: To study whether BTV infection could result in mitochondrial membrane destabilization and cytosolic DNA accumulation that would induce cGAS activation, we infected ovine ST cells with BTV-8 at MOIs of 0.1, 1 and 5 and assessed mitochondrial membrane potential with JC-1. To evaluate whether BTV is capable of inhibiting IFN-I induction through DNA-sensing pathways, sheep thymus cells were infected with BTV-8 and we evaluated by RT-qPCR transcription levels of Ifna and Isg15 genes after DNA stimulation. To determine whether BTV infection affected the cGAS-STING pathway to decrease the aforementioned DNA-induced IFN-I induction, and cGAS expression was studied via SDS-PAGE followed by Western blot. To assess which cellular pathways are implicated in BTV-mediated degradation of cGAS, expression levels of cGAS were studied in BTV-infected cells treated with proteasome inhibitors MG-132 and lactacystin and autophagy inhibitors 3-MA and BAF-A1.
Results: We found mitochondrial damage and DNA accumulation in the cytoplasm of infected cells. In addition, we show that BTV infection blocks DNA-induced IFN-I transcription and that virus infection prevents DNA sensing by inducing cGAS and STING degradation. We identify BTV-NS3 as the viral protein responsible for cGAS degradation, showing that NS3 physically interacts with cGAS and induces its degradation through an autophagy-dependent mechanism.
Conclusion: Taken together, these findings identify for the first time a mechanism by which a dsRNA virus interferes with a DNA sensing pathway to evade the innate immune response.

Dual Recognition of Type II precursor of Histo-Blood Group Antigens and Subterminal Sialic Acid on Gangliosides by the P[11] Porcine Rotavirus

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Background and Objectives: Rotavirus, one of the most common viral pathogens causing acute gastroenteritis in young children and animals, utilizes glycans as receptors in a genotype-dependent manner. The glycan arrays indicated that VP8 proteins of P[11] genotype human rotavirus N155 and bovine rotavirus B233 bind to precursors of histo-blood group antigens (HBGAs) but not to sialylated glycans, while it is still unknown whether P[11] porcine rotaviruses (PRV) also use precursors of HBGAs or other glycans. Rotavirus attachments on the intestinal epithelial cells have been incompletely elucidated due to the lack of model systems that recapitulate cellular polarity, architecture and functionality of the intestine.
Material and Methods: To analyse whether P[11] PRV 4555 binds to HBGAs or the precursor of HBGAs, a synthetic oligosaccharides binding assay was applied to detect the interactions between VP8 proteins of rotaviruses and A type antigen or lacto-N-neotetraose (LNnT, type II precursor of HBGAs). To analyse PRV infection, we established porcine intestinal epithelial cells derived from enteroids. To analyse whether P[11] PRV 4555 utilizes terminal sialic acids (SA), MA104 cells and porcine intestinal epithelial cells were treated with neuraminidase prior to infections. To analyse whether P[11] PRV 4555 utilizes gangliosides, Cholera Toxin B subunit (CTB) was used to pre-treat MA104 cells to block the endogenous GM1a. A mixture of exogenous gangliosides, individual gangliosides (GM1a, GD1a, GD1b, GT1b, GM3) or asialoglycosphingolipids (GA1, LacCer) were used for pre-incubation of the virus to block ganglioside binding sites of the virus.
Results: Application of specific antibodies for fluorescent staining indicated that 3D enteroids contain multiple intestinal epithelial cell types. A binding assay with synthetic oligosaccharides showed that the VP8 protein of P[11] PRV 4555 binds to type II precursor LNnT, but not A type antigen. Infections of P[11] PRV 4555 were not inhibited by neuraminidase treatment, while they were significantly inhibited by the blocking of endogenous GM1a with CTB and pre-incubation of the virus with exogenous GM1a, suggesting that GM1a was involved in the infection of P[11] PRV 4555. In addition to GM1a, preincubation of the virus with exogenous GD1a, GD1b and GT1b prevented infections as well, whereas exogenous GM3 prevented infections only at an early time point, and exogenous GA1 and LacCer didn't show any inhibition of infections, indicating that P[11] PRV 4555 preferentially utilized gangliosides containing subterminal SA.
Conclusion: Overall, our results proved that P[11] PRV 4555 not only binds to the type II precursor of HBGA, but also utilizes gangliosides containing subterminal SA.

Effects of Peste des petits ruminants virus (PPRV) infection on caprine dendritic cell activation and antigenic presentation

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Background and Objectives: Peste des petits ruminants virus (PPRV) is a morbillivirus of the *Paramyxoviridae* family that infects domestic and wild small ruminants. PPRV infection leads to immunosuppression, which can cause opportunistic infections that eventually result in the animal´s death. PPRV is the causative agent of Peste des petits ruminants (PPR), a WOAH notifiable disease affecting goats and sheep. In general, PPR is more severe in goats than in sheep, but the mechanisms behind this phenomenon are still unknown. Dendritic cells (DCs), which have a relevant role in the immune responses to pathogens, can be targeted by PPRV in sheep. In order to determine whether DC are involved in the differential PPRV pathogenicity between sheep and goats, goat monocyte-derived DCs (MoDC) were infected with PPRV.
Material and Methods: Peripheral blood from donor goats was collected to isolate peripheral blood mononuclear cells (PBMC) by Ficoll gradient separation technique. Once the PBMCs were obtained, monocytes ($CD14^+$ cells) were isolated and then differentiated to monocyte-derived DCs (MoDC).In this study, we were able to establish a protocol to obtain goat MoDC expressing the classical MoDC markers (MHCI, MHCII, CD1, CD1w2, CD80, CD86, CD40, CD209, CD11b, CD11c and CD172a) and typical morphological characteristics. Flow cytometry was used to study apoptosis dynamics, comparing mock- and PPRV-infected iMoDC at 48, and 72 h. MoDC phagocytosis capabilities were quantified by microsphere phagocytosis assays. We also used flow cytometry to perform conjugation and mixed lymphocyte reaction assays.
Results: PPRV infection of monocyte precursors did not prevent their differentiation to DC, however their capacity to capture antigen was severely reduced. This indicates that PPRV can infect caprine monocytes and impair their functional differentiation into antigen presenting cells. When we assessed the effect of the infection on differentiated MoDC, PPRV led to the upregulation of maturation markers CD40, CD80 and CD86. Furthermore, the phagocytic capacity of infected MoDC was decreased, which can indicate that the infection produced MoDC maturation. Conjugation and mixed lymphocyte reaction assays however showed that PPRV infection reduced significally caprine MoDC capacity to stimulate $CD4^+$ and $CD8^+$ T cell proliferation. Therefore, PPRV may target goat DCs to alter their ability to present antigens.
Conclusion: To sum up PPRV is able to infect goat MoDCs, affecting the characterictics of these caprine cells, producing a partial maturation and an inefficient antigenic presentation capacity.

Establishment of prospective animal models to investigate the neurocognitive impacts of the post-COVID-19 condition.

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Background and Objectives: The post-COVID-19 condition (PCC) is a debilitating syndrome affecting at least 10% of people previously exposed to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), characterized by persistent or new multi-systemic symptoms three months after the initial infection. Golden Syrian hamster (GSH) and K18-hACE2 transgenic mouse models may help understanding the neuropathology of PCC, as they reproduce some of the features observed in COVID-19 patients, particularly during the acute phase.
Material and Methods: In the first study (S1), GSH were inoculated with 10^4 $TCID_{50}$/animal of a SARS-CoV-2 D614G variant and animals were followed up to 60 days post-inoculation (dpi). Samples collected at necropsy included oropharyngeal swabs (OS), cerebrospinal fluid, plasma, nasal turbinate, lung, brain, muscle, intestine, spleen, and vagus nerve for virological (RT-qPCR and $TCID_{50}$ assay) and/or pathological (histopathology and immunohistochemistry) analyses. Neurocognitive outcomes were assessed through behavioural tests (nest building, burrowing, elevated zero maze and open field tests). In the second study (S2), K18-hACE2 mice were inoculated with four different doses of SARS-CoV-2 D614G variant: $0.5x10^1$, 10^1, 10^2, and 10^3 $TCID_{50}$/animal, and monitored until 20 dpi. At necropsy, OS, blood, lung, and brain samples were collected for virological and/or pathological analysis.
Results: In S1, all GSH experienced weight loss during the acute phase, which overlapped with viral shedding and the presence of replicating virus, as well as severe lesions in respiratory structures. Although such lesions were not observed after 14 dpi, SARS-CoV-2 RNA was detectable in respiratory organs up to 60 dpi. Additionally, a subset of SARS-CoV-2 inoculated individuals underperformed in the open field and elevated zero maze tests from 25 dpi onwards. In S2, the severity of clinical signs was dose-dependent. The lowest dose prevented the manifestation of clinical signs, while a dosage of 10^1 $TCID_{50}$ resulted in a 50% survival rate; higher doses led to increased mortality rates (75% and 100%, respectively). However, regardless of the dose, viral RNA was detected in the OS and lungs of all mice, and brain lesions were found in all animals that had to be euthanized.
Conclusion: Although fully reproducing PCC is challenging, both GSH and transgenic mice can potentially serve as animal models when inoculated with appropriate SARS-CoV-2 doses. Initial behavioural evaluation of GSH suggested potential neurocognitive effects, warranting further exploration. Given the involvement of various pathophysiological mechanisms in PCC, utilizing two different animal species might help elucidating the pathogenesis of this condition and the development of therapeutic interventions.

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Investigation of type-I interferon response during SARS-CoV-2 infection using a kinetic approach in a mouse model

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Background and Objectives: Type I interferon (IFN-I) is among the first molecular responses of the immune system with an important antiviral effect. However, the role of IFN-I in SARS-CoV-2 infection has proven to be complex. Multiple studies have reported high levels of IFN-I in individuals with severe disease, indicating that IFN may also play a detrimental role in COVID-19. The present study aimed to investigate the importance of the IFN-I response during SARS-CoV-2 infection in a mouse model to evaluate its antiviral effect but also its role in an eventual detrimental inflammatory response, which may be linked to an exaggerated IFN-I production.
Material and Methods: Sv/129 IFNAR(-/-) mice deficient for the IFN-I receptor were infected with a mouse-adapted SARS-CoV-2 strain (MA30, gift from Pr S. Perlman) and compared to wild-type infected (WT) mice at 1, 2, and 4 days post-infection (dpi). Six male mice per group were infected with 5.10^3 $TCID_{50}$ via the intranasal route. Viral replication was quantified in the upper and lower respiratory tracts by measuring viral subgenomic RNA. The expression level of innate immunity and inflammatory markers (TNF-a, IL-6, ISG15, MX2, NCF2, Iba1) were investigated by RT-qPCR.
Results: Both groups exhibited a similar weight loss of around 15% at 4dpi compared with uninfected animals, and in both groups, the mice were clinically highly affected. Virus quantification in the nasal turbinates and lungs was comparable at 1 and 2dpi in both groups, but tended to be higher at 4dpi in the KO mice, although this was not statistically significant. mRNA levels of inflammatory markers in the nasal turbinates of WT mice exhibited a marked increase from 1 dpi onwards, with a peak observed at 4dpi. In the KO group, these markers increased later but reached a level that was similar to the WT group at 4 dpi. Interestingly, the marker of Iba1 cells (resident macrophages present in the nasal cavity) was statistically higher in the KO group from 2dpi to 4dpi, suggesting that the lack of IFN-type I response may be counterbalanced by other pathways to recruit these immune cells.
Conclusion: The results of this kinetic study demonstrate that the early IFN-I response since 1dpi is a determining factor in the outcome of the viral replication observed at later time points. However, as the IFN-I response may also contribute to tissue damage *per se*, further investigations including histological analyses are in progress to better decipher the importance of this key component in the physiopathology of SARS-CoV-2.

Nonstructural viral protein amino acid substitution influencing the outcome of feline panleukopenia observed during the emergence of the disease

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Background and Objectives: Feline panleukopenia virus (FPV) is a major viral pathogen of domestic and wild felids. The disease is characterised by diarrhoea, vomiting, dehydration, leukopenia, and death. Due to the pathogen's wide distribution and resistance to environmental factors, vaccination is still the most effective way of prevention. It is assumed that vaccination is an important driving force for mutations in VP1 viral protein that enables evasion of immunity. In 2023, a surge in the number and severity of FPV cases was observed at the University Hospital (UH) at the Faculty of Veterinary Medicine of Zagreb in Croatia. The positivity rate of received diagnostic samples in the Virology Laboratory went from 37,03% in 2022 to 57,38% in 2023 and 58,33% at the beginning of 2024. The study aimed to investigate possible molecular mechanisms responsible for the emergence of the FPV in the city of Zagreb.
Material and Methods: The pilot study included samples, blood count, biochemistry, and medical history from 89 cats admitted to UH during 2023 and the first three months of 2024 who tested positive for FPV. Complete coding sequences were obtained from 52 samples. The obtained nucleotide sequence analysis showed 17 amino acid substitutions in nonstructural FPV protein NS1, eight in NS2, and 12 in capsid protein VP1.
Results: Overall, 22 substitutions were suitable for the statistical analysis. Possible effects of substitutions on clinical signs, infection/treatment outcome and blood count were tested. Substitution NS1 I/V_{443} was significant for the infection/treatment outcome. The case fatality rate of NS1 V_{443} strains was 66,66%, compared to 26,19% in cats infected with NS1 I_{443} strains. The observed difference was statistically significant (p=0,045). None of the substitutions significantly affected the initial clinical score, blood count, diarrhoea or vomiting. The history of vaccination had no positive effect on survival, but it should be emphasised that most vaccinations were not current.
Conclusion: These results, part of an ongoing study that will include more animals, provide a promising direction for future research. They confirm that FPV and its canine counterpart, canine parvovirus 2 (CPV-2), have different evolutionary strategies. While CPV-2 mutations are primarily concentrated in the viral capsid and are responsible for evading immune response, FPV strains could have different pathogenicity due to substitutions of amino acids in nonstructural proteins. This insight opens up new possibilities for understanding and combating this disease, offering hope for improved prevention and treatment strategies in the future.

Strain-dependent differences in the capacity of peste des petits ruminants virus to infect immune cells

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Background and Objectives: Peste des petits ruminants virus (PPRV) is a morbillivirus of the Paramyxoviridae family that mainly affects goats and sheep, but also suids, camelids and some species of wild Artiodactyles. Widespread in Africa, Asia and the Middle East, PPR is a highly virulent disease with an important impact on global economy and food security. Host susceptibility to PPR virus is highly variable, depending in particular on the species affected, the breed within the species and the virus strain. These differences in virulence appear to be inherent in various factors linked to the host, the viral strain and/or the modulation of the host's response to the strain. Capacity of infection of antigen-presenting cells (APC) may play an important role in determining the infection outcome, as these cells transport the virus from the entry point (respiratory track) to lymph nodes before viral spread to other lymphatic and epithelial tissues. The aim of this study was to assess *in vitro* the replication capacity of different strains of PPRV with known low (strain IC89) and high (strain MA08) virulence to goats, in monocyte-derived macrophage (MoMs) cells and monocyte-derived dendritic cells (MoDCs) of sheep and goats, to assess whether any difference could be observed between these APC.
Material and Methods: First, we isolated CD14+ monocytes from peripheral blood mononuclear cells. These monocytes were derived as macrophages (MoMs) and/or dendritic cells (MoDCs). Then, the cells were infected with either PPRV IC89 or MA08 strains at a Multiplicity of Infection of 0.1 for 24h, 48h, 72h, and 96h.
Results: Flow cytometry and fluorescence microscopy results confirmed that both strains could be detected inside MoMs and MoDCs of sheep and goat from 24h post infection (hpi). However, titration of supernatants at different hpi showed that the capacity of PPRV to be released from infected cells varied between strains in MoMs, with no IC89 virus detected in supernatants of MoMs infection. However, viral genetic material was detected in all supernatants by RT-qPCR.
Conclusion: These results suggest that PPRV strain IC89 cannot complete successfully its replication cycle in MoMs of some hosts, impacting the virulence of this strain. *In vitro* infection of MoMs may be a useful model to assess the susceptibility of different hosts without the need of *in vivo* experiments to PPRV and inform surveillance strategies, notably at the livestock-wildlife interface. The mechanisms leading to PPRV replication failure will be further explored in future studies including transcriptomics and proteomics.

The ovine/IT pestivirus (ovIT PeV) cannot be horizontally transmitted to pigs in experimental conditions.

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Background and Objectives: The newly reported ovine pestivirus (ovIT PeV), named *Pestivirus O,* showed the highest homology with *Pestivirus suis* (CSFV), one of the most important pathogens for the swine industry. Previous experimental studies established that piglets infected with the ovIT PeV showed viral replication in the blood and mild clinical signs, e.g., wasting and polyarthritis.

This study aimed to elucidate the capacity of ovIT PeV to be horizontally transmitted to in-contact pigs in experimental trials (Min. Aut. 377/2023-PR 27/04/2023).
Material and Methods: Four-weeks-old piglets were infected either by intranasal (five pigs - group A) or intramuscular (five pigs - group B) route with approximately 2.5 x 10^6 TCID50 ovIT PeV strain (IT/ov/67327-2/2019) dose in a volume of 2mL. Five with group A and five with group B pigs were put in contact for the duration of the trial (50 days). Weekly samplings for both infected and contact pigs were taken. Antibody detection against pestivirus and CSFV using ELISA methods was performed. Genome detection was performed on nasal and rectal swabs, sera, and buffy coat. Throughout the trial, the presence of any clinical signs was assessed, and body temperature was measured up to 28 d.p.i. Finally, at autopsy, the meseraic and mandibular lymph nodes, tonsils, brain, kidney, spleen, lung, and liver were collected.
Results: ovIT PeV generated only mild clinical signs in the piglets, including enteritis (two pigs in contact with group B), cutaneous cyanosis (one intramuscular infected pig), and a slight temperature increase in some infected and in-contact animals. However, the virus was able to replicate, as shown by the RNA levels found in some sera, rectal and nasal swabs and buffy coat in both intranasal and intramuscular infected pigs between 7 and 28 d.p.i.. Additionally, viral RNA was detected sporadically at low levels in some samples from pigs in contact with both groups (all five pigs with group A and 3 with group B) between 3 and 28 d.p.i.. The ovIT PeV infection only induced an antibody response in the infected pigs. In contrast, seroconversion was not shown in in-contact pigs. At the end of the study, ovIT PeV viral RNA was not detected in all organs examined.
Conclusion: While the relatively high dose of ovIT PeV used to infect pigs supports our experimental evidence that ovIT PeV transmission to contact pigs by infected animals may be inefficient, this finding opens the door to cautiously optimistic speculation about the virus's limited potential for cross-species transmission under natural conditions.

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Session 3

Diagnostic tools and disease surveillance

Oral Presentations

A novel ambitious approach to targeted surveillance of West Nile Virus in Danish mosquitoes

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Background and Objectives: West Nile virus (WNV) lineage 2 has expanded dramatically in Europe during recent years, including to Germany neighboring Denmark. In light of WNV circulation in regions close to the Danish southern border, it is relevant to enhance our surveillance in Denmark with specific focus on risk areas. Under the EU4Health project entitled OH4Surveillance 'Setting up a coordinated surveillance under the One Health approach' Statens Serum Institut (SSI) and University of Copenhagen (UCPH) have, along with 10 other partners, been granted funds to scale up surveillance activities on priority pathogens, including WNV. Within the framework of the 3-year OH4Surveillance project, we will increase our targeted surveillance for WNV in mosquitoes collected in the southern parts of Denmark.
Material and Methods: Adult non-bloodfed female Culex spp. and Aedes spp. will be collected from wild, agricultural and suburban areas where the wild reservoir hosts and vectors are abundant. During the project, high risk locations and time periods for WNV transmission in birds will be identified and the mosquitos collected using BG Pro CO2 baited suction traps. In total, we anticipate to obtain 40 trap collections per week during July and September. This will result in approximately 500 individual traps collections (~5000 mosquitoes depending on rainfall) during these three months and for each of the three project years. The collected mosquitoes will be kept on a cool chain until laboratory analysis at SSI, including during transport, species identification and pooling to ensure the most optimal sample quality for virus analyses (RT-qPCR for WNV). Mosquitoes collected during the project will also be made available for other flavivirus surveillance activities. Alongside this project, UCPH aim to develop a risk-based approach based on the identification of high transmission risk, which will hopefully allow us to focus surveillance activities on risk periods during the potential transmission season and to increase surveillance in high risk years.
Results: Preliminary results from the 2024 mosquito season will be presented at the meeting.
Conclusion: It is anticipated that the WNV activities within the OH4Surveillance project, including the enhanced surveillance of WNV in the Danish mosquito population, will aid in a more rapid and effective response to this zoonotic vector-borne disease.

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Antigenic characterisation of Swine influenza H1N2 viruses in Italy

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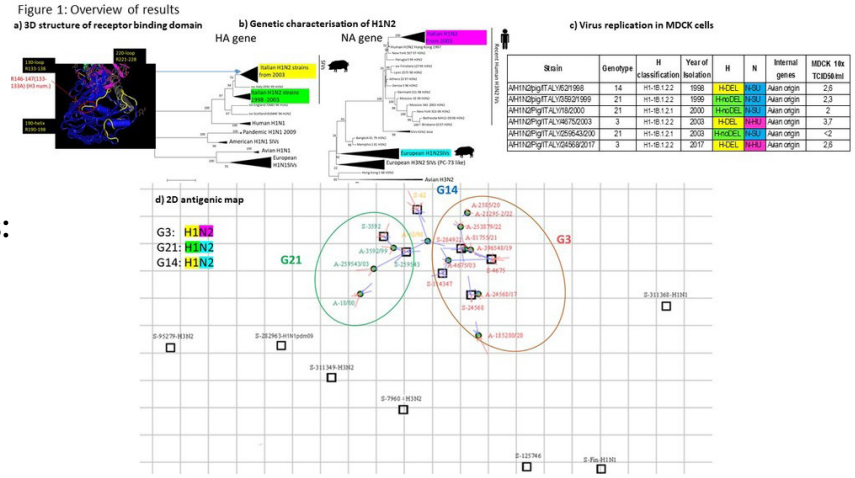
Background and Objectives: The H1N2 subtype, first isolated in Italy in 1998, has progressively increased since the 2000s, becoming one of the most variable and frequently detected subtypes. Within this subtype, a new hemagglutinin (H-1B) characterised by a double amino acid deletion (H1B1.2.2-Hdel) in the receptor binding domain stabilised in the Italian pig population at the end of 2000, combined with a new neuraminidase derived from human seasonal strains (Nhu). The aim of this work is to investigate three H1N2 genotypes characterised by different combinations of H and N (typical of 90's Hnodel/Nsw, new Hdel/Nhu and mixed) through antigenic characterisation studies and evaluation of their replicative capacity in MDCK cells.
Material and Methods: A panel of six H1N2 strains G3:Hdel/Nhu (n. 2), G21:Hnodel/Nsw (n.3) and G14: Hdel/Nsw (n.1) were grown in MDCK cells and titrated in $TCID_{50}$/ml. For antigenic characterisation, 6 additional H1N2 strains of G3 isolated in 2019-22 were included. A panel of 14 pig sera was used, 7 H1N2, 3 H1N1, 1 H1N1pdm09 and 3 H3N2. Hemagglutination inhibition (HI) was performed as described in the WOAH Manual. A 2D-antigen map was generated using the software (https://acmacs-web.antigenic-cartography.org). Virus replication of 6 H1N2 strains was compared in MDCK cells using 100 $TCID_{50}$/50µl virus in 96-well MDCK plates in 10-base serial-dilutions starting from -1 to -8 and immune-histochemical staining (NPA-MAb 5F10) after 4 days of incubation at 37°C.
Results: The HI titres show antigenic differences between different Gs, which are best visualised in the antigenic map where the distance between grids is 1 antigenic unit, corresponding to a 2log HI dilution. The antigenic map shows a differentiation between Gs, G21 (right), G3 (left), including recent H1N2 strains, and G14 (centre). The calculation of the viral titer in $TCID_{50}$/ml after 4 days seems to show a greater replicative capacity of Hdel strains compared to Hnodel strains, which is still one of the predominant Gs among Italian H1N2 infected pigs.
Conclusion: These preliminary data show antigenic differences between the investigated Gs, confirming the genetic differences observed between Hdel and Hnodel. From an epidemiological point of view, G3 has spread in our area, replacing the "old", selectively disadvantaged G21. The double deletion in the RBD could be related to a higher receptor affinity and the combination with Nhu could make these strains more efficient in their dissemination in the pig population. Further studies using strains produced by reverse genetics are ongoing to confirm these results.

Figure 1: Overview of results



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Blood swabs represent an alternative sample matrix for Classical swine fever antibody ELISAs

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Background and Objectives: Classical swine fever (CSF) is a severe systemic disease that effects both domestic pigs and wild boars. In the past, affected wild boar populations have often maintained the infection over months or even years and threatened domestic pig holdings. At present, CSF is not reported within the European Union, but reintroduction is not unlikely due to the increased global exchange. Therefore, targeted surveillance of fallen wild boars is important. As pathological findings in wild boars infected with CSF virus (CSFV) are often comparable to those of animals infected with African swine fever virus (ASFV), differential diagnosis is performed. Previous studies showed that blood swabs represent a pragmatic sampling method that is useful for genome detection of CSFV and ASFV [Petrov et al., 2014; 10.1016/j.vetmic.2014.07.030], as well as for antibody detection of ASFV [Carlson et al., 2017; 10.1111/tbed.12706]. However, its application for the detection of antibodies against CSFV has not been analyzed yet, but would simplify the differential diagnostic analysis of samples collected from fallen wild boars.
Material and Methods: Based on this, two CSFV antibody ELISAs were tested by using swabs soaked with EDTA blood, which was collected from domestic pigs infected with CSFV, as well as from non-infected animals. Pieces of the swab were taken up in sample dilution buffer and were analyzed according to the manufacturers' ELISA protocol for serum. To assess potential species effects, antibody negative EDTA blood samples from domestic pigs, wild boar, red river hogs, and warthogs, were spiked with CSFV antibody positive serum.
Results: The detection of antibodies against CSFV in EDTA swab samples that were collected from domestic pigs ≥ 21 days post infection (dpi) is completely consistent with the results obtained for the corresponding serum samples. Moreover, sample classification of EDTA blood swabs that were collected before 21 dpi was comparable to results obtained for the sera. In addition, all CSFV antibody negative blood swabs scored clearly negative independently whether blood samples from domestic pigs or wild suids were analyzed. Spiked samples of different suids gave comparable results with spiked EDTA blood samples outperforming sera when tested on the swabs.
Conclusion: In conclusion, blood swab samples are not only suitable for the analysis of CSFV genome [1], but also for the detection of antibodies against CSFV using commercially available ELISAs. It is conceivable that one blood swab sample can be applied in CSFV- and ASFV diagnosis, which is advantageous for surveillance in wild boar.

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Characterization of a MERS-like betacoronavirus in Danish brown long-eared bats (Plecotus auritus)

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Background and Objectives: Bats are recognized as natural reservoir hosts for numerous high-impact viruses, with spillover events serving as the foundation for many emerging infectious diseases affecting both animals and humans. Bats are likely the major reservoir of mammalian coronaviruses (CoVs), as the genetic diversity of CoVs in bats far exceeds that known for other hosts, and they are believed to be the evolutionary origin of severe acute respiratory syndrome (SARS) CoV and SARS-CoV-2, and possibly Middle East respiratory syndrome (MERS) CoV. MERS-like betacoronaviruses have been found in bat species from Africa, America, Asia and Europe. In this study, we describe the detection and full genome characterization of a MERS-like betacoronavirus in Danish long-eared bats (*Plecotus auritus*).
Material and Methods: Fecal samples were collected from the bat surveillance program in 2020 from *Plecotus auritus* bats in Almindingen, Bornholm, Denmark, and were screened using pan-CoV RT-PCR assays. Positive samples were sequenced by Illumina MiSeq and assembled into full-length genomes using our metagenomic workflow. Initially, a global phylogenetic tree based on alignment-free genetic distances was used to determine where the positive samples grouped within the *Coronaviridae* family, and subsequently their placement within the subgroups was determined by local maximum likelihood phylogenetic analysis.
Results: Two of the screened fecal samples were positive: BtCoV/16227-9/P.aur/DK/2020 and BtCoV/16227-10/P.aur/DK/2020. Both samples were assembled into full-length genomes after sequencing and phylogenetic analyses revealed that the two coronaviruses belonged to the merbecovirus subgroup within the betacoronaviruses. They form a distinct clade together with MERS-like merbecoviruses isolated from *Eptesicus*, *Hypsugo*, *Ia*, *Pipistrellus*, *Plecotus* and *Vespertilio* spp., all belonging to the *Vespertilionidae* family of microbats, from Western Europe and East Asia.
Conclusion: This is the first case description of MERS-like betacoronaviruses in bats within Denmark.

Funding source/acknowledgements (optional):
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Environmental-based surveillance as complementary tool for epidemic monitoring under a One Health approach

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Background and Objectives: Wastewater-based epidemiology has long been implemented effectively for the surveillance of enteric viruses, such as polio. It however proved particularly successful in monitoring the circulation of SARS-CoV-2 at the community level. The large amount of data from both the population and wastewater highlighted the correlation between cases and viral RNA levels in water. This opened avenues for extending wastewater analysis to a wider range of non-enteric pathogens. Still, surveillance of viruses from animal sources remains largely overlooked, despite being essential for a One Health approach and epidemic preparedness. Our project therefore aimed at implementing a broader environmental surveillance by monitoring wastewater (WW) and surface water (SW) for a panel of viruses impacting both human and animal health.
Material and Methods: The four largest wastewater treatment plants in Luxembourg (covering about 52% of the population) were sampled 1 to 3 times per week for 4 years. Ten additional sites, including ponds, recreational waters, and rivers, were selected across the country to cover wildlife, livestock or mosquito impacted ecosystems and were sampled twice a month for 1 year. Following water concentration steps, nucleic acids were extracted and the presence of a range of RNA viruses (representing the *Orthomyxoviridae, Coronaviridae, Pneumoviridae, Flaviviridae, Caliciviridae and Picornaviridae* families) was assessed by RT-qPCR or RT-ddPCR.
Results: As expected, enteric viruses (Norovirus - NoV, Enterovirus - EntV) were the most frequently detected viruses in WW. Interestingly, influenza A and B, respiratory syncytial virus (RSV) and seasonal human coronaviruses (CoV) were also reliably detected with seasonal variations. For pathogens subject to mandatory notifications to the national authorities, viral RNA levels were significantly correlated with the number of clinical cases. In SW, EntV were detected in all types of collection sites. NoV were particularly present in SW impacted by livestock and to a lesser extent in sites impacted by wildlife. Bovine CoV was seldom detected in rivers close to cattle farms, while influenza A, porcine respiratory CoV, bovine RSV and Usutu virus were not detected, reflecting their lower circulation and/or the lesser applicability of the methods for such viruses.
Conclusion: Our results highlighted the added value of environmental surveillance as a complementary tool for monitoring viral epidemics at the national scale. Our project lays the groundwork for wider implementation and further development, in particular to expand the range of human pathogens and further improve methods for SW surveillance.

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Probability of freedom from Peste des Petits Ruminants in Georgia and Armenia.

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Background and Objectives: Peste des petits ruminants (PPR) is a highly contagious and fatal disease in small ruminants caused by a morbillivirus (PPRV) with significant economic and food security impacts. In January 2016, PPR was detected in Georgia for the first time. This was followed by a targeted vaccination campaign in four affected regions of Georgia. To date, PPR has not been identified in Armenia, however the disease is endemic in neighboring Turkey and Iran. The aims of this project were to assess the epidemiologic situation of PPR in Georgia and Armenia, and establish the probability of freedom from disease.
Material and Methods: A 2-year seroprevalence survey was conducted among sheep and goat herds in both countries. In the absence of PPRV detection, sampling results were used to calculate probability of disease freedom at the municipal, regional, and national level using different assumptions for design prevalence (1% and 5%) and yearly national probability of introduction (1% and 5%). We also computed the equilibrium under the sampling frame from 2021. We calculated the time it would take to reach 90%, 95%, and 99% probability of freedom with a sustained sampling of 25 or 50 animals per municipality, and how this probability would evolve if sampling were discontinued.
Results: In all, 3,279 samples were collected in 22 municipalities of Georgia, and 3,280 samples in 98 municipalities of Armenia. With the assumption of 5% for both design prevalence and probability of introduction, this led to a mean municipality probability of freedom of 94.4% in Georgia and 81.1% in Armenia in 2020. At the national level, for the system under evaluation, this resulted in a probability of freedom of 75.8% in Georgia and 70.6% in Armenia. If 25 animals per municipality were sampled yearly, it would take 3 years in Georgia and 2 years in Armenia to reach 90% freedom, but in both cases equilibrium would be just below 95%.
Conclusion: This highlights that sustained surveillance is necessary to detect disease incursion or prove freedom from disease. The sampling program ended in 2021, however PPR was detected again in Georgia in February 2024, underlining the challenges to maintain disease absence in areas with high risks of introduction.

The conquest of the West by West-Nile and Usutu viruses, two emerging flaviviruses in France and Europe

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Background and Objectives: West-Nile fever virus (WNV) and Usutu virus (USUV) are two neurotropic and zoonotic mosquito-borne viruses belonging to the *Orthoflavivirus* genus of the *Flaviviridae* family. Both viruses are maintained in the wildlife in an enzootic cycle involving a vector, *Culex* spp mosquitoes, and reservoir hosts, various bird species. Humans and horses are considered accidental hosts and epidemiological dead-ends. WNV and USUV spread from Africa where they were first described at the beginning of the twenty century. Nine distinct WNV lineages has been described (WNV-1 to WNV-9), with lineage 1 and 2 considered the most important causative agent of viral encephalitis in human and horses worldwide. In Europe, while WNV-1 and -2 circulation within the avian population is usually asymptomatic, it is a principal concern for human and animal health. Eight lineages of USUV has been described so far, including three African (Africa 1–3) and five European (Europe 1–5). Except for Africa-1, all the USUV lineages were reported in Europe and are responsible for major epizooties in wild and/or captive birds. USUV infections in human, even scarce, have also been reported, raising awareness about the need of USUV surveillance in public health.
Material and Methods: In France, both viruses are present and considered as emerging pathogens due to the extension of their geographical distribution and an increase frequency of the outbreaks. The European and French National Reference laboratory is involved in a multidisciplinary *One Health* surveillance system for WNV and USUV with partners from the medical, veterinary and entomological sectors. The surveillance system in animal includes (i) serological testing of clinically symptomatic horses or captive birds, (ii) molecular detection of viral genomes in targeted organs (brain, spleen and liver) using RT-qPCR and (iii) whole genome sequencing using nanopore technology.
Results: Here we will present the emergence and characterization of WNV in Gironde, a non-endemic region of western France along the Atlantic coast during the season 2022 and 2023. We will also present the molecular epidemiology of avian USUV infections in France since its introduction in 2015.
Conclusion: Both the expansion of WNV geographical distribution and the endemicity of USUV in France underline the need to extend and reinforce the surveillance system over the whole territory. Alerting the authorities at an early stage would enable public decision-makers to better prevent and control virus spread and limit public health consequences especially by securing blood and organ donations.

Virus Infections among Wild Eurasian Tundra Reindeer (Rangifer tarandus tarandus) in Iceland – a different story than in Fennoscandia

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Background and Objectives: In 1787, the fourth attempt to establish a reindeer population in Iceland was successful; 35 semi-domesticated Eurasian tundra reindeer (*Rangifer tarandus tarandus*) was imported from Finnmark County, Norway. The reindeer has been managed as a wild population, now numbering about 4000-5000 animals in North-East Iceland, where they are sympatric grazers with domestic sheep. Previous studies have mainly focused on the reindeer parasitic flora, and little information was available on exposure to viral pathogens. The only known viral disease outbreak was contagious ecthyma caused by parapoxvirus, affecting the udder of females and the muzzle of their calves, in 2016. The aim of the study was to investigate Icelandic reindeer for exposure to viral reindeer pathogens commonly circulating among semi-domesticated reindeer in Fennoscandia and for potentially emerging viral diseases.
Material and Methods: Blood samples and swab samples from the nasal and oral mucosal membranes were obtained during the hunting seasons (July 15th – September 20th), 2017-2019. Serum samples (n=281) were investigated for antibodies against alphaherpesvirus, gammaherpesviruses (malignant catarrhal fever viruses, MCFV), pestivirus, bluetongue virus and Schmallenberg virus, using enzyme-linked immunosorbent assays. DNA from nasal swab samples from 95 adults (2018) and nasal and oral swab samples from 68 adults and 18 calves (2019) were investigated for the presence of parapoxvirus-specific DNA by polymerase chain reaction (PCR) targeting the *B2L* and *GIF* genes.
Results: Two adult reindeer had antibodies against pestivirus (2017) and two adult reindeer had antibodies against MCFV (2018). No antibodies against alphaherpesvirus, bluetongue virus or Schmallenberg virus were detected. DNA specific for parapoxvirus was detected in nasal swab samples from two adult animals (2019).
Conclusion: The results indicate that virus infections commonly found in semi-domesticated reindeer in Fennoscandia, including the donor population in Norway, is present at a low prevalence only and probably do not represent a health threat to these animals. Surprisingly, we did not find evidence of exposure to alphaherpesvirus (i.e., cervid herpesvirus 2; CvHV2) which may have a seroprevalence of 50% or more in herds in Fennoscandia, being a causative agent for infectious keratoconjunctivitis. The finding of parapoxvirus-specific DNA in nasal swabs in apparently healthy reindeer indicates that the virus is circulating, probably with a reservoir in sympatric sheep, and may cause disease in reindeer under the right circumstances. With changing climatic end environmental conditions, the zoo-sanitary situation for reindeer as well as livestock in Iceland may change, necessitating monitoring.

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Session 3

Diagnostic tools and disease surveillance

Poster Presentations

A performant indirect ELISA for the detection of Anti-M. bovis Porcine Antibodies in serum or filter paper samples

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Background and Objectives: The *Mycobacterium tuberculosis* complex bacteria cause Tuberculosis (TB) in various hosts, including wild boar (*Sus scrofa*), a natural carrier of the disease. The ID Screen® Porcine Tuberculosis Indirect ELISA is specifically designed to detect antibodies against *Mycobacterium bovis* in porcine (wild boar and pig) serum and plasma. This flexible new iELISA also offers a performant filter paper sample application, allowing an easy and cost-effective sample collection. The kit includes microplates coated with *Mycobacterium bovis* recombinant protein and an anti-porcine IgG horseradish peroxidase (HRP) conjugate.
Material and Methods: Diagnostic specificity was assessed using 651 pig samples—425 from wild boars and 226 from domestic pigs—originating from Bovine TB-free areas. Diagnostic sensitivity was evaluated on 16 wild boar serum samples, with 2 from France and 14 from Spain. The positive status of the French samples was determined through culture, while those from Spain were confirmed positive using another commercial ELISA (Kit A). Correlation with a commercially available ELISA kit (Kit A) was determined by testing 419 samples from both negative and infected herds. Additionally, 16 samples, comprising highly positive and negative samples, were tested in parallel on serum and filter paper samples (FPS). Threshold samples were included to assess the limit of detection.
Results: ID Screen® Porcine Tuberculosis Indirect measured specificity was 99.7 % $CI_{95\%}$ [98.9 -99.8], n=651. The percentage of correlation between ID Screen® Porcine Tuberculosis Indirect and kit A was greater than 98%, indicated very high correlation with Kit A. Comparable results were obtained for all samples, regardless of the sample type, meaning that serum and FPS can be used equivalently.
Conclusion: The ID Screen® Porcine Tuberculosis Indirect ELISA kit offers high specificity and efficient detection of positive animals, correlating well with other ELISA tests. Results are obtained in just 90 minutes, highlighting its speed and reliability for detecting porcine antibodies against *Mycobacterium bovis*. The kit is compatible with FPS, simplifying and reducing the cost of sample collection. The elution protocol allows fast sample treatment, and results indicate that FPS is a reliable alternative to serum samples for porcine TB detection.

Bat rabies surveillance: Atypical case of EBLV-1 infection in a long distance migrant bat Pipistrellus nathusii

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Background and Objectives: Bats with ~ 1400 species reported in the world have been associated with infectious diseases for centuries. Rabies, the oldest known zoonotic disease was the first recognized bat associated disease. Classified in the family *Rhabdoviridae* and the order *Mononegavirales*, five different viral members are reported in insectivorous bats in Europe: Bokeloh bat lyssavirus (BBLV), European bat 1 lyssavirus (EBLV-1), European bat 2 lyssavirus, Lleida bat lyssavirus (LLEBV) and West Caucasian bat lyssavirus. In France, EBLV-1, BBLV and LLEBV are circulating in some bat species and are associated with *Eptesicus serotinus (n=93 positive cases)*, *Myotis nattereri (n=1)* and *Miniopterus schreibersii* (n=1), respectively. A strong affiliation of EBLV-1 is shown with serotine bats (*Eptesicus serotinus* and *Eptesicus isabellinus*). While few documented cases of EBLV-1 have been reported in other species (n=8), we report here an atypical infection of EBLV-1 in a long distance migrant bat species (*Pipistrellus nathusii)* between summer and winter roosting areas.
Material and Methods: On January 2022, a bat carcass was submitted for rabies testing at the National Reference Laboratory for Rabies in Nancy. Rabies testing was conducting on the brain using the fluorescent antibody test, conventional and real-time pan-lyssavirus SYBR-Green RT-PCR, respectively. The bat was genetically determined by amplification of the partial Cytochrome b gene. The lyssavirus identity was determined by phylogeny on the nearly full genome sequence (11963-bp). Bayesian molecular clock dating of the two lineages a and b of EBLV-1 was also undertaken on a dataset of 60 full genome sequences.
Results: The bat brain was found positive for the presence of antigens and viral RNA. Analysis by molecular clock dating and phylogeny confirmed infection of the bat with the EBLV-1a subtype. A nucleotide similarity of 99.88% was shown between this infected Nathusius' pipistrelle and an infected serotine bat from the Lower Saxony region of Germany reported in 2016 (OU524432).
Conclusion: EBLV-1 is commonly reported in France in serotine bats. While the EBLV-1b subtype is commonly reported from France, Germany and The Netherlands, EBLV-1a is more dispersed and found in a northern west-east axis from France to Ukraine. This report of EBLV-1a in a Nathusius' Pipistrelle, a species which can migrate up to 2000 km, may partially explain the extensive viral movement of EBLV-1a in Europe.

Characterization of Enteric Pathogens in Calves from Central Ethiopia - A Metagenomic Approach

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Background and Objectives: Ethiopia is home to the largest population of livestock in Africa and many people in the country depend on livestock for their livelihood. At the same time the mortality and morbidity among young calves is high, causing major losses to the livestock sector. One of the leading causes behind the high mortality rate is calf diarrhea, a complex disease involving multiple infectious agents of viral, bacterial, and protozoan origin. Previous reports investigating calf diarrhea in Ethiopia have mostly focused on known agents and only a few studies on viruses have been performed. Therefore, we decided to utilize viral metagenomics to characterize the enteric RNA virome of calves in Ethiopia. Furthermore, by using this approach, the detection of yet uncharacterized pathogens that potentially could play a substantial role in calf diarrhea is possible.
Material and Methods: A field study was conducted in January and February of 2023 and fecal samples from diarrheic as well as healthy calves were collected from dairy farms surrounding the capital, Addis Ababa. RNA was extracted from the fecal samples, pooled, and sequenced using Illumina.
Results: Sequencing revealed a large variety of RNA viruses, including known diarrheic viruses such as rotavirus and bovine coronavirus. Interestingly, an unusual rotavirus genotype, G24P[33], was discovered for the first time in Ethiopia, and to our knowledge, for the first time in Africa. In addition, several enteric RNA viruses that have not been detected in cattle in Ethiopia previously, such as norovirus, astrovirus, kobuvirus, enterovirus, bovine picornavirus, picobirnavirus and hunnivirus. Preliminary data shows that a majority of the viruses can be found in calves both with and without diarrhea. Further analysis is currently being done to shed light on any potential differences in virome composition between diarrheic and non-diarrheic calves.
Conclusion: To our knowledge, this the first characterization of the RNA virome in cattle in Ethiopia, revealing a large number of RNA viruses circulating among calves. Next, we are in the process to expand the investigation of the virome in calves by sequencing DNA viruses using Nanopore.

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Complete Diagnostic Solution for Epizootic Hemorrhagic Disease Monitoring

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Background and Objectives: Epizootic hemorrhagic disease (EHD) is a non-contagious disease of wild and domestic ruminant species transmitted by arthropods. EHD in deer often results in high levels of mortality, while in cattle less severe infections are usually observed. The causative agent is the EHD virus, a virus belonging to the same Orbivirus family as bluetongue virus (BTV). EHDV has been detected globally in America, Asia, Africa, Australia, and Middle East. However, it had never been reported in Europe until autumn 2022, when the first cases were identified. In 2023 the number of outbreaks increased, showing an important impact to cattle, and experts suggest this disease might show a similar evolution to BTV in Europe. Due to its high impact to animal wellbeing and to the industry, EHD is listed as notifiable disease to the WOAH. To respond to these outbreaks, we developed a complete diagnostic solution.
Material and Methods: In this work, we show the development of an ELISA for the detection of specific antibodies to EHDV and two lateral flow assays for the detection of antigen and antibodies. The ELISA was developed in a competitive format using VP7-coated plates and a specific monoclonal antibody which was shown to be EHDV-specific. The new ELISA was evaluated with a total of 626 samples, and it was validated to be used with filter-paper blood samples. For LFAs, an analytical evaluation has been performed.
Results: The competitive ELISA has been evaluated with a total of 304 cattle positive samples (blood, serum, and plasma), obtaining a sensitivity of 99.7 %. Additionally, we assayed a total of 81 cattle samples collected in parallel as fresh blood and in filter paper. This study showed the same diagnostic parameters regardless of the sampling method used. To evaluate ELISA's specificity, 98 negative field samples from cattle, deer, sheep, and goat have been evaluated, as well as 24 cattle samples collected from BTV-infected animals. This ELISA exhibited a specificity of 100%. Analytical evaluation of LFAs showed a limit of detection of 5 ng/strip for the antigen-detection LFA and that the antibody-detection LFA detects all the analyzed serotypes (1, 2, 4-6, and 8).
Conclusion: These results indicate that the ELISA is a good tool for monitoring disease evolution, and, thanks to the filter paper blood collection, it can be easily applied to wildlife monitoring. Sample panel will be extended with sheep and deer positive samples in the following months. Moreover, LFAs can help in field investigation of outbreaks.

Coronavirus surveillance in United Kindgom wildlife

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Background and Objectives: Continued surveillance of coronaviruses is important to determine the presence of virus variants circulating amongst wild mammal populations in the UK. Surveillance helps us understand host range and the role of wild species in the transmission and maintenance of coronaviruses, including SARS-CoV-2, and to assess the potential for spillover into humans or other animal populations.
Material and Methods: Through research and surveillance programmes at APHA, and collaborations with wildlife rehabilitators, managers, hunters, and gamekeepers, we have received samples from a range of wild mammals across the UK. Samples were collected during 2021-2023 from deer (sera, n=1,058; swabs and tissues, n=238); badgers (sera, n=328; swabs, n=189); foxes (sera, n=24; swabs, n=1,672); squirrels (sera, n=87; swabs, n=220); and bats (oral and faecal swabs and faeces, n=759). Serum samples were tested for SARS-CoV-2 antibodies by ELISA and virus neutralisation test (VNT). Viral RNA from oral and faecal swabs, faeces and tissue samples were extracted and tested for SARS-CoV-2 RNA by E-gene RRT-PCR, and other coronavirus species by pan-CoV RT-PCR, with positives confirmed by Sanger sequencing and BLAST.
Results: Sera from three fallow deer and seven roe deer were positive for SARS-CoV-2 antibody ELISA and VNT. All deer swab and tissue samples were negative for coronavirus by RT-PCR. Sera from four European badgers were positive by ELISA but negative by VNT. Serum from one badger was positive by VNT with delta and omicron variants (low titre 11.31 IC_{50}) but negative by ELISA. Badger swab samples tested negative for coronavirus by RT-PCR. One red fox was seropositive by ELISA and VNT. Using a pan-CoV RT-PCR, a swab sample collected from a fox in a different region was positive for canine coronavirus, and a swab sample from one grey squirrel was positive for a bovine coronavirus. Swabs and faeces from 13 different native British bat species were screened for coronaviruses of which 32 bats from six species tested positive for alpha (n=26) and betacoronaviruses (n=6). Genetic characterisation of the coronaviruses identified will be presented.
Conclusion: While active SARS-CoV-2 infection was not detected, there is inconclusive evidence to suggest potential exposure to SARS-CoV-2 or an antigenically similar virus in deer, badger and fox populations in the UK. Following the detection of alpha and betacoronaviruses in wildlife, surveillance and further testing of inconclusive samples will continue into 2024 to further identify exposed and/or infected species to inform future disease surveillance and risk management strategies.

Detection and characterization of Bovine Coronavirus and Rotavirus in Calves in Ethiopia

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Background and Objectives: Bovine rotavirus (BRV) and bovine coronavirus (BCoV) are the most common viral enteropathogens in neonatal calf diarrhea, resulting in serious health problems for young calves leading to economic losses in the cattle industry worldwide. The aim of the study was to investigate the prevalence and to genetically characterize BRV and BCoV in dairy calves with and without diarrhea in Ethiopia.
Material and Methods: Stool samples from calves with or without diarrhea, younger than 6 months of age, were collected from dairy farms in Addis Ababa, Ethiopia, between October 2023 and January 2024. The samples were analyzed for the presence of rotavirus using real time RT-PCR targeting the NSP5 gene of BRV. To determine the genotype of BRV, the VP7 and VP4 genes were also analyzed using gene-specific PCRs followed by sequencing and bioinformatic analysis. Additionally, quantitative PCR (q-PCR) targeting the S gene was used to detect BCoV.
Results: Among the total 105 calves, 41 (38.18%) had diarrhea during the survey. Further, the results showed that BRV and BCoV were present in 3.8% (4/105) and 2.85% (3/105) of the calves, respectively. Among the four detected rotaviruses, two are of the G10 genotype associated with diarrheal symptoms. For the non-diarrheal cases, one has the G8 genotype and one remains untyped. All three BCoV were detected in calves with diarrheic symptoms.
Conclusion: In summary, this study highlights the presence of both BRV and BCoV in dairy farms in Addis Ababa, Ethiopia. Further molecular studies are needed to fully characterize and understand the genetic makeup and evolutionary relationships of the circulating viruses.

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Detection of ASFV infection in clinical, environmental and non-invasive samples collected from pigs infected with different infectious doses.

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Background and Objectives: African swine fever virus (ASFV) currently represents the biggest threat to the porcine industry worldwide, with high economic impact and severe animal health and welfare concerns. Outbreaks have occurred in Europe and Asia, since 2007, and, in 2021, ASFV was detected in Hispaniola, Caribbean. Given the lack of a global vaccination strategy against ASFV, control of the virus relies on molecular surveillance. In this work we used the recently minimal equipment colorimetric ASFV LAMP test to determine the kinetics of detection of ASFV Georgia strain in pigs infected using different viral doses
Material and Methods: In parallel, the WOAH recommended qPCR for the ASFV official diagnosis was used. Two groups of 6-week-old pigs, with 20 animals per group, were used (groups A and B). In group A, animals were intranasally inoculated with 10^4 TCID, being the group B intranasally inoculated with $10^{2,5}$ TCID. A large panel of tissues, clinical and environmental samples were collected. Samples that were collected using a non-invasive protocol from the infected animals were also included.
Results: From day 3 and 4 post infection (dpi), almost in the absence of clinical signs, the presence of the ASFV DNA was detected in blood, serum, oral swabs, as well as in different tissues, including bone marrow (of great relevance for the diagnosis of the virus in wild boars) in both experimental groups. On day 5, a strong ASFV DNA load was detected in the air and the walls of the pen, remaining with a high load until 10 dpi. Also, at 7 dpi, a very high viral load was detected in all the samples, except in the feces.
Conclusion: During the presentation we will discuss about the application of this diagnostic tool in a pen-side format and the detection levels per sample will be presented. Special emphasis will be placed on the type of sample that can be collected non-invasively in the infected animals, as well as on the environmental sample collection protocol and its relevance for the fast ASFV diagnosis.

Establishing the Acute Febrile Illness (AFI) Sentinel Surveillance System in the Country of Georgia for Enhanced Public Health Monitoring and Response

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Background and Objectives: Acute febrile illness (AFI) comprises a diverse array of infectious ailments, often presenting overlapping symptoms, posing challenges for diagnosis through clinical observation alone. In August 2021, Georgia initiated AFI sentinel surveillance, acknowledging the significance of probing the root causes of AFI for vital public health insights. The primary aim of this surveillance is to pinpoint the origins and patterns of diseases inducing AFI, whether they are native to Georgia or carry the potential for introduction into the region.
Material and Methods: The initial stages of the research encompassed several key activities, including the development of protocols, identifying sentinel sites for surveillance, and conducting initiation trainings at these sites. A dedicated REDCap cloud server was established for the project, alongside the development, validation, and ongoing implementation of data collection instruments at the sentinel sites. Surveillance efforts include testing for various etiologies, including Leptospira spp., West Nile Virus, Chikungunya virus, Dengue virus, Plasmodium spp., Rickettsia spp. (R. typhi and spotted fever group), Salmonella spp., Leishmania spp., Brucella spp., Borrelia Burgdorferi, Crimean-Congo Hemorrhagic Fever virus (CCHFV), and hantavirus. The AFI surveillance protocol involves characterizing these pathogens using clinical, genetic, and phenotyping methods, employing a variety of techniques such as molecular, serological, and bacterial analyses.
Results: The study has effectively set up six sentinel sites spanning the central and western areas of Georgia. Comprehensive training sessions were conducted for medical staff, covering the epidemiology, clinical symptoms, diagnosis, and preventive measures related to AFI. Standardized algorithms for case and laboratory investigations were formulated. By March 2024, up to 850 patients displaying symptoms of AFI were enrolled. Clinical samples were collected and forwarded for laboratory analysis targeting specific pathogens.
Conclusion: Through this surveillance initiative, Georgia endeavors to delineate the etiological landscape of AFI within its borders. The project seeks to offer guidance on implementing preventive measures in affected areas, drawing insights from its findings. Additionally, it will pinpoint requirements for laboratory diagnostics and suggest policies and interventions to facilitate AFI diagnosis within healthcare facilities.

Funding source/acknowledgements (optional): This study was supported by the US Centers for Disease Control and Prevention (CDC)

Evaluation of the effectiveness and cost-effectiveness of control strategies for the 2018 Rift Valley Fever epidemic in Mayotte.

5. Diagnostic tools and disease surveillance

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Background and Objectives: Rift Valley Fever (RVF) is a vector-borne disease which is endemic in countries across Africa and has seen recent geographical expansions into the Arabian Peninsula. RVF can cause severe infections in both animals and humans. RVF infections in livestock can lead to mass fatalities in young ruminants. In humans the symptoms are non-specific and can often lead to mis-diagnosis. A small proportion progress onto hemorrhagic infection which has a significantly higher mortality. Aims: To evaluate the effectiveness and cost-effectiveness of different control measures for the 2018 Mayotte RVF epidemic.

Material and Methods: To build my framework of models I have used the Metras models (produced in R) (Métras et al., 2016, 2017, 2020) and the EFSA report (produced in MATLAB) (Nielsen et al., 2020) as a template for the Mayotte SEIR livestock and human models.

The recent 2018 RVF outbreak in Mayotte went extinct after nine months. Metras used serological data to fit her models to ensure they behaved correctly. No control strategies were used to control the outbreak. Therefore, by recreating the models we are creating a baseline of the natural course of the RVF outbreak in Mayotte.

I have built a stochastic model fitted to the parameters calculated by Metras. By using Mayotte as a baseline in the model, enables the evaluation of different control strategies e.g., vaccinations or vector control, to establish the most effective control strategy or combination strategies. Once this has been done, I will be able to conduct a cost-effective analysis of these strategies. I will need to work out the best way to do this as the price of the vaccine will vary between local and national levels. One way this could be done would be to find the max cost which is cost effective and use this as a baseline.

Results: I am currently in the process of collecting the results and was hoping to have more information to share with you at this stage but unfortunately, I do not. I am currently running simulations of different vaccination strategies of just livestock vaccination, just human vaccination and a combination of livestock and human vaccination campaigns. Although, there is currently no licenced human vaccines for RVF there is a cross-species vaccine in clinical trials. I also intend to incorporate vector control strategies into the modelling framework.

Conclusion: N/A

Funding source/acknowledgements (optional):

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Evaluation of tuberculosis surveillance in Swedish cattle and farmed deer

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Background and Objectives: Swedish cattle are officially free from tuberculosis (TB; *Mycobacterium bovis* and *M. tuberculosis*), with the last case diagnosed in 1978. Surveillance of TB in animals is challenging as the incubation period is long, clinical signs are unspecific and diagnostic tests in live animals have low sensitivity. Only a few samples are submitted yearly for TB analysis which prompted evaluation of the TB surveillance in Swedish cattle. After many years of freedom from TB, *M. bovis* was detected in farmed deer in 1992. Investigations indicated that it had been introduced and spread in the population after the import of deer in the 1980's. As a result, a compulsory control programme for TB in farmed deer was established. The deer population is now considered free from TB with the last case identified in 1997. Hence, a transition from a control programme to surveillance is desirable.

Material and Methods: Surveillance of TB is mainly conducted via postmortem inspection at slaughter and to a lesser extent through necropsies for both cattle and deer. For the evaluation of the surveillance in both cattle and farmed deer, a scenario-tree-model approach was used. For cattle, the design prevalence was set to match the requirement in the European Animal Health Law (EU 2020/689); i.e. 0.001% (corresponding to a total of 14 animals). For deer, the design prevalence was set at 3% (within-herd) and 0.5% (between-herd). Three scenarios were calculated for each population, with different estimated risks of introduction of TB.

Results: With adjustments to the present surveillance, the probability of freedom was estimated to ~99% for cattle and ~97% for deer, given a low risk of introduction of TB to the country (1 event per 100 years). When a higher risk of introduction was used (1 event per 20 and 33 years respectively) the probability of freedom was estimated to 93-97% for cattle and 88-93% for deer.

Conclusion: To demonstrate a high confidence in freedom from TB in Swedish cattle and farmed deer, a vigilant surveillance is needed, with maintained focus on imports and postmortem examinations. The study results were used to suggest adjustments in the surveillance system for TB in cattle and to guide the design of a surveillance system to replace the current control programme in farmed deer.

First case of porcine circovirus 3 detection in wild boar (Sus scrofa scrofa) in Sweden

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Background and Objectives: Circoviruses, among the smallest known circular single-stranded DNA viruses, with a genome size of approximately 2 kb, have garnered attention for many years. To date, four porcine circovirus (PCV) species have been identified, including PCV3 and PCV4, discovered in 2015 and 2019, respectively. While PCV4 distribution is primarily limited to countries in South-East Asia and India, PCV3 is widespread globally. Of all PCV species, PCV2 causes the most devastating losses in the swine industry through postweaning multisystemic wasting syndrome, porcine dermatitis and nephropathy syndrome (PDNS), respiratory and enteric disease and reproductive failure, all together also known as porcine circovirus-associated disease. PCV3 inherited many characteristic features from its predecessor, PCV2, and is also found in animals diagnosed with PDNS, reproductive failure and respiratory disorders. Like in pigs, PCV3 infection is also widespread in wild boars. The first PCV3 case was reported in 2018, and since then it has been detected in many countries around the world.
Material and Methods: A total of 35 wild boars from 7 counties of Sweden collected from 1996-2022 were analyzed (Table 1). Samples were obtained from the Swedish Veterinary Agency's archival collection. Viral total nucleic acids were extracted from liver, lungs, spleen and tonsils using EZ1 robotic platform (Qiagen, Hilden, Germany), recovered in 60 µl elution buffer and stored at −80 °C until further processing. Duplex real-time PCR was used to screen for PCV3 and assess PCV2 co-infection. Possible impact on animal health was also investigated.
Results: PCV3 also was found in Swedish farmed pigs including one archival sample from 1993 (Ye et al., 2018). Notably, PCV3 infection was previously undocumented in wild boars in Sweden until this study (Table 1). Although PCV3 infection was associated with emaciation and intestinal inflammation in some cases, further investigation is needed to determine causality. Not all PCV3/PCV2 co-infected animals exhibited noticeable health issues, suggesting additional factors may be at play, such as bacterial infections.
Conclusion: This study marks the first documentation of PCV3 infection in wild boar populations in Sweden, shedding light on the potential impacts of circovirus infections in wildlife and underscoring the importance of ongoing research in this area.

Table 1. High prevalence of PCV3 and PCV2 in the wild boar population

	No. of animals	No. of tissues tested	PCV3 positive	PCV2 positive	PCV2/PCV3 coinfection
Wild boar	35	110	6 (17,1%)	27 (77,1%)	5 (14,3%)

Funding source/acknowledgements (optional): This study was supported by the Swedish Research Council (Vetenskapsrådet) and the CoVetLab research project 43136-70094.

INFLUENZA D VIRUS IN SWEDISH CATTLE

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Background and Objectives: Influenza D virus (IDV) has gained attention in recent years for its potential impact on cattle health, particularly associated with respiratory disease. Furthermore, the detection of IDV-specific antibodies in humans suggests possible transmission from cattle to human. We previously detected IDV-antibodies in Swedish cattle, in 35% (282/799) of tested bulk milk samples from dairy farms in 2019 and 2020. However, a significant gap in our understanding of IDV's role in bovine respiratory disease remains. While different phylogenetic lineages of IDV have been identified worldwide, information regarding the circulating strains in Sweden is lacking. Therefore, this study aims to investigate the phylogeny and the presence of IDV among Swedish cattle with bovine respiratory disease.
Material and Methods: We tested 1680 bovine samples received by the Swedish Veterinary Agency (SVA) between 2021 and 2024, including nasal swabs, trachea and lung tissues from clinical cases with respiratory signs. Initially, the samples were screened for other respiratory pathogens as part of routine diagnostics. Samples were first tested for the presence of IDV using rRT-PCR targeting the nucleoprotein (NP) gene, and positive samples were retested using a second rRT-PCR targeting the polymerase basic protein 1(PB1) for confirmation. Samples with low Ct values (Ct<30) were used for whole-genome sequencing and further genetic characterisation.
Results: A total of **21** positive samples were detected, with a mean Ct value of 27(15-37). Whereas IDV was the only respiratory pathogen detected in two samples, the rest of the samples contained several pathogens. *Pasteurella multocida* was the most common IDV-associated pathogen in the majority of cases (17/**21**), followed by bovine coronavirus (13/**21**). Phylogenetic analysis of all seven gene segments revealed that all IDV strains collected in 2021 belonged to the D/OK cluster. In samples collected in 2023, however, reassortants between D/OK and D/660 were detected. The PB1 gene belonged to the D/660 cluster, whereas the rest of the gene constellation belonged to the D/OK gene pool.
Conclusion: This molecular surveillance data confirms the active circulation of IDV in Sweden and reveal a diversity of strains. The high association of IDV with other respiratory pathogens suggests a potential role as a co-pathogen rather than a single pathogen. Although IDV was detected in calves with respiratory symptoms over a wide geographical area, its prevalence remained relatively low. The identification of different clades and reassortment patterns, combined with its ability to infect a wide range of animal species, highlights the need for continued surveillance of this emerging virus.

Multi-pathogen surveillance with next-gen Hyperplex PCR towards rapid same-tube digital quantification and sequencing-grade genotyping

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Background and Objectives: Within the general context of epizootic pathogen diagnostics and surveillance for veterinary and environmental samples, there is an active pursuit for high throughput/high-frequency methods allowing (1) extreme sensitivity down to few copies of starting material, (2) single base pair sequence specificity to identify closely related sequences (e.g. genotyping for strain/variant identification) and (3) simultaneous measurement of tens to hundreds of target sequences in a single sample without cross-interference. To date, the method selection typically demands a juggle between qPCR/dPCR for routine measurement of select few different pathogens and NGS-based methods for discovery of new sequences and monitoring of closely related sequences.
Material and Methods: Hyperplex PCR™ is a next-generation PCR technology developed by APLEX Bio aiming at filling the current gap between PCR and NGS methods by providing a multiplexity of 100+ targets per tube, sequencing-grade specificity, and sensitivity down to a single copy per reaction. These enhancements are achieved by upgrading the standard PCR workflow with padlock probes and APLEX Bio´s proprietary Nanopixel™ probes, resulting in a workflow comparable to a standard qPCR reaction followed by a fluorescence microscopy read-out, in which results can be obtained within one day. More than 100 different Nanopixel™ codes can be distinguished using 4-5 standard fluorescence filters.
Results: As a proof-of-concept within the context of multi-pathogen pathogen surveillance in highly inhibitory and complex samples, we have demonstrated (1) the analysis of wastewater samples using a multiplex panel targeting multiple influenza strains (A: H1N1, H3N2, H5 and B), as well as other viruses such as SARS-CoV-2 and PMMoV and; (2) the absolute quantification of 5 specific antimicrobial resistance genes in wastewater samples, each with concentrations ranging from less than 10 copies/µL up to 10^5 copies/µL in the extracted material. Remarkably, significant signals above the blank could be measured for influenza H5 in wastewater from a municipal treatment plant in the south of Sweden, coinciding with a known avian Influenza outbreak in birds.
Conclusion: Hyperplex PCR™ is herein demonstrated as a powerful tool enabling affordable high-frequency multi-pathogen monitoring by providing: the following key benefits beyond the current state-of-the-art: (1) multiplexity of 100+ targets per assay without sample splitting; (2) panel customization with new probes within 2 weeks without panel re-optimization; (3) quantification of targets spanning 3-6 orders of magnitude; (4) analysis of DNA&RNA targets within the same sample from viral variants to AMR genes (5) dual PCR and RCA amplification allow the simultaneous detection of low and high abundance targets.

Funding source/acknowledgements (optional): For additonal information about Aplex Bio and Hyperplex PCR visit https://www.aplex.bio/.

Patterns and variations in potential exposure to SARS-CoV-2 as detected in companion animals undergoing international travel between 2019 and 2023.

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Background and Objectives: Since the emergence of SARS-CoV-2 in China in December 2019, the virus disseminated worldwide, facilitated by international travel. During its rapid spread in humans, SARS-CoV-2 evolved into independent new forms better adapted for human-to-human transmission. Due to their close proximity to people, companion animals such as dogs and cats can be exposed to infection and may play potential roles in disease outbreaks, representing putative reservoirs for reverse zoonoses and also hosts for recombination events with other circulating coronaviruses. Therefore, evaluation of SARS-CoV-2 in companion animals is important, to better understand exposure patterns and explore possible public health risks.
Material and Methods: To this end, a large study was launched between the end of 2019 and 2023. Nearly 3,000 companion animal sera were received in the ANSES Laboratory for Rabies and Wildlife for rabies serological screening to assess the efficacy of rabies vaccination in order to facilitate international movements. In tandem, the samples were also analysed for the presence of SARS-CoV-2 antibodies using three different serological assays: the ELISA, seroneutralisation test and microsphere immunoassay. In early 2022, a military conflict in Eastern Europe led to the fraught displacement of many Ukraniain civilians to other European countries, some of whom travelled with their companion animals. The ANSES sample set contained a subset of >750 sera from Ukrainian companion animals collected during 2022 and 2023, submitted for rabies serological screening for travel purposes but which were also tested for SARS-CoV-2 antibodies.
Results: No antibodies against SARS-CoV-2 were detected in samples collected in 2019. In samples collected in 2021, a seroprevalence of 3% by ELISA and 1.5% by seroneutralisation test and microsphere immunoassay was obtained. Unexpectedly, the SARS-CoV-2 antibody results obtained in 2022 from the Ukrainian subset (seroprevalence = 15.8%) were significantly different compared to results obtained from other companion animals (seroprevalence = 6.1%), demonstrating an appreciably higher seroprevalence. The comparison of the three different SARS-CoV-2 serological assays and antibody results, including those obtained for the year 2023, will be presented at the conference.
Conclusion: Initial results show that pets, such as dogs and cats, are exposed to the SARS-CoV-2 virus over the years. This exposure seems to be favoured under certain conditions, probably linked to population movements, but this area remains to be investigated.

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PrimeStore® MTM Molecular Transport Medium method for Classical swine fever virus inactivation: Facilitating biosafety and molecular diagnosis

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Background and Objectives: Classical swine fever (CSF) is one of the most important diseases of swine, being notifiable to the World Organization of Animal Health (WOAH). The disease is caused by classical swine fever virus (CSFV) from the Pestivirus genus, Flaviviridae family. Biological safety is important to consider along with rapid and accurate diagnosis for control of this disease. Thus, the exchange of samples and reference material in a biosafe manner is of great relevance. The present study focuses on validation of the PrimeStore® MTM inactivating molecular transport medium (PS-MTM) method for CSFV inactivation.
Material and Methods: CSFV Alfort/187 strain virus and archived, frozen rectal and nasal swab samples and sera collected from pigs infected with the CSFV Catalonia and Margarita strains were used to validate the PS-MTM method. Samples were incubated for inactivation for one hour at room temperature, at a rate of 0.5 mL of sample in 1 ml of PS-MTM. Viral titers were determined using the viral isolation test. For CSFV RNA detection, the WOAH-recommended RT-qPCR protocol was performed.
Results: The titer of the CSFV Alfort/187 strain before inactivation was $10^{6.26}$ /mL. After inactivation, no residual infectivity was found when evaluating 592 replicates at the 10^{-2} dilution. Notably, the Ct value detected in the four tubes analyzed by RT-qPCR after inactivation was 19,13. In the case of the Ct value for the 10^{-2} dilution, the Ct value was 26,25. Before inactivation, viral titers between $10^{6.5}$ and 10^{7} $TCID_{50}$/mL were found in serum samples from pigs infected with the Catalonia strain. After inactivation, the virus was completely inactivated in the 350 replicates tested at the 10^{-2} dilution. In the case of nasal swabs, viral titers were between $10^{2.2}$ to 10^{4} before inactivation, and reduced to zero after inactivation in the 350 replicates tested in the 10^{-2} dilution.
Conclusion: Thus, the PS-MTM product completely inactivated CSFV in the analyzed samples, evidenced from 10^{-2} dilution. CSFV RNA was also detected before and after inactivation, demonstrating the effectiveness of the samples after treatment for molecular diagnosis. Results obtained with samples collected directly in PS-MTM of sera, nasals and rectal swabs from animals infected with the CSFV Margarita strains will be presented. In addition, the CSFV RNA stability detected by RT-qPCR in samples with PS-MTM during three months at 4°C and room temperature also will be discussed during the meeting.

Production of recombinant African horse sickness virus (AHSV) antigens for serological diagnosis

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Background and Objectives: African horse sickness, caused by the African horse sickness virus (AHSV), is a serious disease of equids on the WHOA list of notifiable diseases. Europe is free of the disease, but the risk of introducing AHSV into Europe is increasing due to climate change and animal transport. Nine serotypes of this virus have been described. The AHSV viral protein 2 (VP2), major component of the virus' outer capsid, is the main target of neutralizing antibodies in infected hosts, thus determining the serotype. The presence of the virus can be detected by various diagnostic tools: PCR to detect the viral genome, or ELISA to detect disease-specific antibodies targeting the VP7, protein of the intern capsid. However, there is no ELISA test to identify the serotype, only a seroneutralization test requiring 5 to 6 days of reaction before results are obtained. In the present study, carried out as part of the European SPIDVAC (Safe Priority Infectious Diseases VACcines) project, we aim to develop type-specific ELISA for AHSV.
Material and Methods: We used the Modified vaccinia virus Ankara (MVA) expression system to produce a recombinant antigen of the AHSV-4 VP2. The VP2 coding sequence was inserted into a pVote plasmid by recombination in the Gateway® cloning system. Vaccinia virus (VacV) was used as a vector for the expression of recombinant VP2. After production in BSR cells, recombinant VP2 was purified by immunoprecipitation using a FLAG tag fused to the viral protein sequence, providing highly purified antigens. VP2 recombinant protein was used to develop a dual antigen sandwich ELISA. The protein is used as an antigen for plate coating and as a reporter after HRP conjugation.
Results: The performance of this ELISA was evaluated with more than 600 serum samples collected from AHS negative, vaccinated or infected animals. Results showed no cross-reactivity with the other 8 AHSV serotypes. The specificity and sensitivity of the test were satisfactory. In addition, this type of dual antigen ELISA enables the detection of specific antibodies in any species.
Conclusion: This ELISA could therefore be used as diagnostic tool for rapid identification of the AHSV serotype.

Proficiency Testing on Foot and Mouth Disease in Georgia as an Essential Tool for Laboratory Competence

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Background and Objectives: History of Proficiency Testing (PT) maintains stability of its strategy at its essence. Beginning with the first PT event in 1946, participations in PT enable the laboratories to demonstrate their capabilities, maintain the proficiency skills of personnel, and understand the expectations from the tests leading to successful performance that is invaluable asset for the laboratory competence throughout the history.

State Laboratory of Agriculture participates in PT at international level for many years with great experience and practical skills obtained within the performance activities. SLA uses this platform for participation in FMD PT, the disease that represents the worldwide concern. Foot-and-Mouth Disease PT Scheme was organized by the FAO World Reference Laboratory for Foot-and- Mouth Disease (with support of EuFMD) and the UK Government's Department of Environment, Food & Rural Affairs (DEFRA).
Material and Methods: SLA received PT panel consisting of n=8 samples for virus detection, laboratory utilized Real-Time PCR technique for the detection of viral RNA using the Roche Light Cycler for FMD. The test specifically detects 3D (Callahan et al., 2002) region of the gene.
Results: Samples n=8 have been tested. From 8 investigated samples 4 were positive and 4 were negative on FMDv. Using routine laboratory diagnostic technique PT results have been adequately interpreted as requested and reported within the time-frame. Final assessment of the status of each sample and overall interpretation of the cases included all of the diagnostic data from the panel.
Conclusion: PT organizer provided feedback report and results satisfied all the requirements, laboratory capability is sufficient to support diagnostics as needed. Selection of correct technique, testing of panel samples, interpretation of the cases showed that the used method is valuable for diagnostics as it is essential for obtaining reliable data for making adequate decisions for the management and analysis within routine laboratory diagnostics.

Funding source/acknowledgements (optional): Support provided by Defense Threat Reduction Agency (DTRA)

Study of the potential role of rodents, particularly rats, in the circulation and transmission of the SARS-CoV-2

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Background and Objectives: Since the beginning of the COVID-19 pandemic, the question of contamination of animals close to humans was raised. Various species (mink, hamsters and cats) were found to be receptive to the infection. Furthermore, while it was initially demonstrated that the Murinae subfamily was not susceptible to infection by the original SARS-CoV-2 strain, the emergence of new variants seems to be changing the situation. Indeed, it has been shown experimentally that the B.1.351 and P.1 variants that appeared in 2021 could infect mice. Over time, other variants have appeared and continue to appear, raising the question of the potential receptivity/sensitivity of rodents to these new variants. In addition, exposure of urban rodents (*Rattus* sp.) to SARS-CoV-2 is largely facilitated by the fact that they circulate/live in sewers, where significant quantities of the SARS-CoV-2 genome are found. It therefore seems necessary to assess the risk of wild urban rodents becoming reservoirs for the virus, from which it could evolve and re-infect humans, as has been observed with mink (*Neovison vison*) and white-tailed deer (*Odocoileus virginianus*).
Material and Methods: We carried out a study to assess the receptivity and sensitivity of four rat lines (two laboratory lines: Wistar and Sprague-Dawley, and two wild lines: *Rattus norvegicus* and *Rattus rattus*) to the Omicron BA.5 sub-variant, using the intranasal route. Inoculated animals were killed at 4 and 14 days post-inoculation. Virological (cell culture and molecular biology), haematological and immunological analyses were carried out on various samples. In addition, a large-scale field study was carried out in parallel on urban rodents (*Rattus norvegicus* and *Mus musculus*) to assess the circulation of SARS-CoV-2 in these populations, which may be in contact with humans. Six different sites across France were selected and over 500 animals were trapped. Various tissues were sampled to look for markers of past or present viral infection.
Results: Initial results showed no difference between the weights of uninfected and infected rats in the 4 lines of rats studied, although seroconversion was observed in all 4 lines of rats at D14. No traces of SARS-CoV-2 RNA were found in the lungs collected from the field animals. The other results, currently being analysed, will be presented at the conference.
Conclusion: Initial results seem to show that rats are not receptive to the SARS-CoV-2 virus, either experimentally or in the field, despite the high presence of the SARS-CoV-2 genome in wastewater.

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

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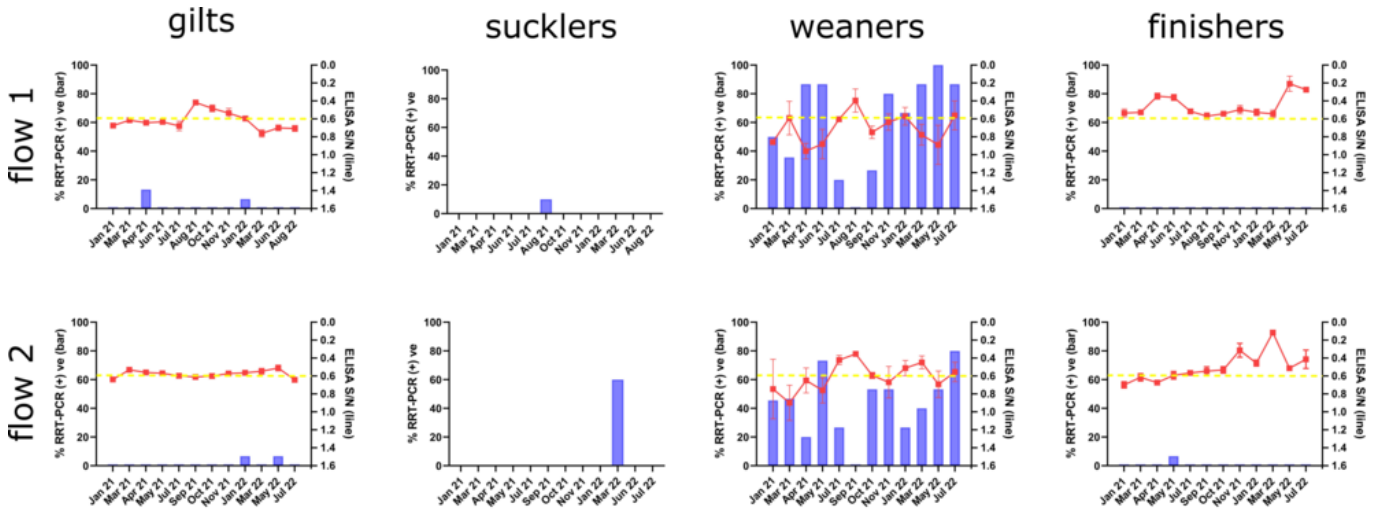
Background and Objectives: Swine influenza A virus is one of the main viral pathogens responsible for respiratory disease in farmed pigs. Whilst outbreaks are often epidemic in nature, increasing reports suggest that continuous, endemic infection of herds is now common. The move towards larger herd sizes and increased intensification in the commercial pig industry may promote endemic infection, however, the impact that intensification has on swine influenza A virus infection dynamics and evolution is unclear.
Material and Methods: We carried out a longitudinal surveillance study over 18 months on two intensive, indoor, multi-site pig production flows. Frequent sampling of all production stages using individual and group sampling methods was performed, followed by virological and immunological testing and whole genome sequencing.
Results: We identified weaned pigs between 4-12 weeks old as the main reservoir of swine influenza A virus in the production flows, with continuous, year-round infection. A single virus subtype was maintained on each farm for the entire duration of the study. Despite the continuous nature of viral circulation, infection levels were not uniform, with increasing exposure at the herd level associated with reduced viral prevalence followed by subsequent rebound infection. Viral evolution was characterised by long periods of stasis punctuated by periods of rapid change coinciding with increasing exposure within the herd. An accumulation of mutations in the surface glycoproteins consistent with antigenic drift was observed, in addition to amino acid substitutions in the internal gene products as well as reassortment exchange of internal gene segments from newly introduced strains.
Conclusion: These data demonstrate that long-term, continuous infection of herds with a single subtype is possible and document the evolutionary mechanisms utilised to achieve this.



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Urine as a potential sample to diagnose West Nile virus in horses

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Background and Objectives: West Nile virus (WNV) is an orthoflavivirus primarily transmitted among wild birds through the bite of infected mosquitoes, and accidentally to humans and horses, which are dead-end hosts. Several studies have investigated the diagnosis of WNV in different human biological samples, including blood, cerebrospinal fluid, and urine. However, the use of urine-based diagnostics for WNV in horses remains unexplored. This study aimed to validate, under laboratory conditions, whether WNV could be detected in horse urine samples, and to assess the feasibility of using urine as a novel diagnostic matrix from WNV-positive horses. The goal of this approach is to improve the diagnosis and surveillance of WNV in horses by isolating and identifying the lineage of the virus in urine.
Material and Methods: Free-catch urine from non-infected horses (n=11) was spiked with WNV lineages 1 and 2. Samples were classified as concentrated, urine specific gravity (USG) >1.010, or diluted, USG <1.010. Additional free-catch urine samples (n=63) were collected between 0 to 95 days from the onset of symptoms (26±25 days, mean±SD) from WNV-positive horses located in different geographical regions of Spain between 2022 and 2024.
Results: The spiked samples demonstrated that the RT-qPCR targeting WNV RNA can detect low concentrations of the viral RNA, although urine can inhibit amplification. Viral cultures showed cytotoxic effects caused by urine components in concentrated samples, while diluted samples showed cytopathic and cytotoxic effects due to WNV replication and urine components, respectively. The analyses revealed absence of detectable WNV RNA in field urine samples across all WNV-positive horses.
Conclusion: Several factors may contribute to the inability to detect WNV from field urine samples. Firstly, the intermittent and transient viremia characteristic of WNV infection in horses might limit the viral load excreted in urine, rendering detection challenging. Additionally, the time elapsed between onset of clinical signs and collection of samples seems critical for WNV detection in horse urine. However, we demonstrated that WNV RNA can be detected and WNV can be isolated in horse urine, even though the presence of inhibitory substances may interfere with nucleic acid amplification and viral propagation. These results underscore the complexity of detecting WNV in urine samples from horses and highlight the need for continued research. Further investigations including samples close to the onset of clinical signs and advanced analytical techniques, such as digital PCR, may provide insights into the kinetics of WNV shedding in urine and improve the surveillance and control of WNV.

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Using viral metagenomics to identify viruses associated with fever in horses in Scandinavia

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Background and Objectives: Viral infections pose a threat to equine welfare and can cause great economical losses for the equine industry. Thus, knowledge about viruses circulating in the equine population with the potential to cause disease is of importance. New technologies such as viral metagenomics combining high-throughput sequencing with bioinformatic analysis allows an unbiased approach to identify all viruses in a sample simultaneously. Therefor in this project we aimed to determine the complete viral composition in samples (nasal swabs and serum/plasma) collected from horses with fever in order to improve the diagnostic of clinical cases with unknown etiology.
Material and Methods: In total sample were collected from horses (>60) in Scandinavia and from these nucleic acid, enriched for viruses through filtration, rRNA depletion and nuclease treatment, was extracted. High-throughput sequencing (Illumina and/or Nanopore) and bioinformatic analysis was used to identify and genetically characterize viruses.
Results: Viruses were found in all investigated sample pools. In the nasal swabs, viruses such as different equine herpes virus types were identified. Equine herpes virus-2 was the most abundant and a near complete genome was obtained. Other viruses were also identified such as e.g., adeno-, papilloma- and picornaviruses. Interestingly, some of these identified viruses showed very low sequence similarity to previous known viruses e.g picornavirus sequences with only around 30% protein identity. On the contrary, in the sera the main viruses identified were Equine pegivirus and Torque teno equus virus 1 (TTeV1). The near complete TTeV1 genome was obtained showing high similarity to a TTeV1 strain from USA.
Conclusion: Thus, in conclusions, this study provides an overview of the different viruses circulating in horses with fever in Scandinavia. It also identifies previously uncharacterized equine viruses whose role in disease is unknown and renders further investigations.

Funding source/acknowledgements (optional): This project was supported by the The Swedish-Norwegian Foundation for Equine Research (project H-20-47-555)

WHOLE GENOME SEQUENCING OF RECENT PARVOVIRUS FROM DOMESTIC AND WILD ANIMALS IN CENTRAL ITALY

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Background and Objectives: Parvoviruses belong to the family *Parvoviridae*, small non-enveloped viruses with linear single-stranded DNA genome. The species *Carnivore protoparvovirus 1* includes two genogroups, Feline Panleukopenia Virus (FPV) and Canine parvovirus (CPV). Both CPV and FPV have been detected in wild carnivores of different genera across the world, with cross-species transmission at the domestic-wildlife interface. In most European countries investigated so far, CPV and FPV strains become endemic in wild reservoirs, although sporadic spillover events are still detected.

In the present study, we characterized viruses of the species *Carnivore protoparvovirus 1* circulating in wild carnivores from central Italy (Umbria and Marche regions), in comparison to virus strains from domestic animals circulating in the same area between 2023 and early 2024.
Material and Methods: To investigate their diversity, we performed genetic and phylogenetic analyses of the whole genome. In order to do this, we used a target PCR approach and Next Generation Sequencing (NGS).
Results: A total of 618 samples were tested for parvovirus collected in 2023 and early 2024 from passive surveillance. In 2023 242/526 samples and in 2024 27/92 resulted positive, respectively. During the study period, 173 samples were from wild animals of which: 24/47 positive from wolves, 15/73 positive from foxes, 6/51 positive from badgers, one from golden jackals and one polecat. The remaining samples were from dogs (n=102) and cats (n=343) of which 52 and 171 positive, respectively. We characterized the complete genome of target positive samples (n=15), including selected cats (n=11) and wildlife strains (n=3 wolves and n=1 fox). All samples from wildlife were collected in 2023. Parvovirus strains from wolves clustered with CPV and the fox strain with FPV. The wolves CPV belonged to CPV2a (n=2) and CPV2b (n=1). Interestingly the CPV2a wolves strains clustered with recently circulating CPV strains detected between 2016 and 2021 in several countries including Italy, China, Vietnam and Nigeria, and the CPV2b with Italian strains detected in 2017 and 2018 in South Italy. The clustering of fox strain with strains from cats may suggest a virus flow between these two species dictated by the predator habit of foxes. Only additional sequence analysis of archived samples and future monitoring may confirm this.
Conclusion: This is the first report of parvovirus circulation in central Italy in wildlife and further studies are necessary to understand the cross-species transmission between domestic and wildlife animals.

Session 4A

African swine fever I

Oral Presentations

First outbreak of African swine fever in Sweden – Local epidemiology, surveillance and eradication strategies

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Background and Objectives: The first case of African swine fever (ASF) was confirmed in Sweden in September 2023. This presentation describes the local epidemiology, including the spatiotemporal dynamics of the outbreak and some of the factors that may have contributed to its apparently successful eradication. Sharing these experiences might serve to guide ASF-free countries in their contingency planning, and shed light on some of the questions that still remain concerning the epidemiology of ASF in the wild boar-habitat cycle.
Material and Methods: Upon detection of the outbreak, strict control measures were put in place in a preliminarily defined infected zone. Carcass search, including geo-localisation, removal, sampling and destruction of found carcasses was initiated and a preliminary core area defined based on the results. Detection of ASFV was performed using real-time PCR. Two taphonomy models were used to estimate the time of death of a selected carcass. Using the geocoordinates indicating the location of each carcass and the average time of death the spatiotemporal evolution of the outbreak was evaluated. An online questionnaire with questions concerning past and present observations of wild boar, hunting ground parameters, hunting bags, trail cameras and wild boar baiting was administred to assess the local wild boar abundance before and during the outbreak.
Results: Six months after confirmation of the first case, 93 wild boar carcasses had been found in the infected zone, of which 62 tested positive for ASF virus (ASFV). All ASFV-positive carcasses were found inside the core area. Based on the taphonomy methods it was assumed that the infection was introduced between early May and late June 2023. The data also indicated that the epidemic curve peaked between mid-August and mid-September, with the last death occurring in late September 2023. Based on the average estimated time of death, geo-localisation of carcasses and two-dimensional kernel density estimation, clustering in space and time was identified. The questionnaire results showed that the wild boar population had increased in the last ten years, but with large variations and geographical heterogeneity in space use.
Conclusion: Disease introduction through natural wild boar movements was excluded and it was assumed that the long-distance translocation of the virus had occurred through human activities. A municipal waste collection centre without wild boar-proof fencing is located close to the epicentre of the outbreak, attracting many wild boar and contributing to the spread of the virus once it had been introduced to the population.

Funding source/acknowledgements (optional):
The authors would like to thank the hunters in Fagersta: for detecting the outbreak in the first place, for the enormous and invaluable efforts invested in carcass search and the control and eradication work, as well for a fantastic collaboration all along the way.

Perceptions and experiences of hunters involved in the management of the first African swine fever outbreak in Sweden

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Background and Objectives: In September 2023, African swine fever was for the first time confirmed in wild boar in Sweden. Local hunters have been key actors both for the detection and the management of the outbreak: the disease was identified when hunters noticed ill and dead boars in the area, reported and sent in samples to the Swedish Veterinary Agency. The hunters were thereafter mobilized to search for wild boar carcasses to identify the extent of the affected area. During the first months of the outbreak, more than 500 local hunters were involved in the search activity, and in total >90 carcasses (or parts thereof) were found. Local hunters were also mobilized for culling of wild boar in the infected zone. This paper presents results from interviews with groups of local hunters in the affected area, who have been involved in the identification and management of African swine fever.
Material and Methods: The interviews were semistructured group interviews and revolved around three themes: perceptions and experiences of African swine fever before, during and after the outbreak. Main themes have been identified.
Results: All the hunters described taking part in managing the outbreak, and that this had been very important to them, especially for the specific area where they usually hunt. The main motivation for their commitment was to make the area free of African swine fever to be able to hunt again. Several interviewees described hunting as extremely important for them - as a lifestyle. Hunting wild boar was described as a major and important part of their hunting, which they before the outbreak maintained through hunting with bait. While the outbreak of African swine fever and the related restrictions were described as stressful and emotionally difficult, the hunters had mainly positive experiences from their activity in managing the outbreak. They described deepening the relations in their own hunting group as well as getting to know other groups of hunters. They also described gaining new knowledge about their hunting land and feeling proud of their commitment. However, some hunters also described feeling stigmatized and viewed as contagious by hunters from other areas, and sometimes being approached by other non-hunting locals as responsible for the outbreak since they had fed the wild boar at the baiting stations.
Conclusion: The interviewed hunters were commited to making the area free of African swine fever in order to hunt again. The hunters had mainly positive experiences from their activity in managing the outbreak.

Funding source/acknowledgements (optional): Formas Akutmedel

Disease dynamics in wild boar and domestic pigs inoculated intranasally with the virulent African swine fever virus genotype II strain "Armenia 2007"

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Background and Objectives: Since the reintroduction of African swine fever virus (ASFV) in Europe in 2007 and its subsequent spread to Asia, wild boar have played a crucial role in maintaining and disseminating the virus. Significant knowledge gaps exist regarding infection dynamics and disease pathogenesis in domestic pigs (DP) and wild boar (WB), particularly at the early infection stage. We aimed to compare DP and WB infected intranasally, to mimic natural infection, with one of the original highly virulent genotype II ASFV strains (Armenia 2007).
Material and Methods: Animals were intranasally inoculated, with a dose of 10^4 HAD_{50}/pig of "Armenia 2007" strain. On days 1, 2, 3, and 5 post-infection (pi), six animals that had been randomly assigned beforehand (comprising 3 DP and 3 WB) were sedated and euthanized by administration of barbiturate each day. The remaining inoculated animals (4 DP and 4 WB) were monitored and euthanized upon reaching a humane endpoints on day 6 (all remaining WB) and day 9 pi (all remaining DP), respectively. Mock-inoculated control animals (3 DP and 3 WB) were euthanized at the conclusion of the experiment (day 12 pi). Parameters assessed include clinical signs, macroscopic lesions, viremia levels, tissue viral load, and virus shedding in nasal and rectal swabs from day 1 pi.
Results: WB exhibited shorter incubation periods, an earlier onset of clinical signs and hyperthermia, as well as more rapid development of severe and extensive haemorrhagic lesions. However, although WB reached a humane endpoint earlier than DP, the macroscopic lesions were comparatively less severe. In addition, WB displayed earlier viremia and an earlier presence of the virus genome in target organs. Notably, lymphoid tissues within the oronasal tract, including the medial retropharyngeal lymph nodes, were identified as key portals for ASFV infection following intranasal exposure in both subspecies. No viral genome was detected in nasal or rectal swabs until shortly before reaching humane endpoint in both DP and WB, suggesting limited virus shedding in acute infections.
Conclusion: These *in vivo* experimental results demonstrated that WB were considerably more susceptible to infection than DP following intranasal exposure to the highly virulent isolate Armenia 2007. Recognising these different host responses in the WB and DP are critical for designing effective control strategies such as vaccine development and understanding the dissemination of ASFV in different host populations.

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Into the Wild: Exotic Insights into Immunity against African Swine Fever Virus

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Background and Objectives: African swine fever (ASF) is a hemorrhagic viral disease caused by the ASF virus (ASFV), with devastating effects and high mortality in Eurasian wild suids and domestic pigs. In contrast, African suids were suspected to be less affected by ASFV infection. To elucidate the underlying mechanisms, we investigated progression and outcome of an ASFV infection in naïve, adult individuals of two highly susceptible European species, domestic pigs (DP) and Eurasian wild boar (WB), and of two African species, Red River hogs (RRH) and ASFV's natural reservoir host species, warthogs (WH).
Material and Methods: The animals were infected intramuscularly with the highly virulent ASFV strain "*Armenia2008*". We analyzed disease progression, viral kinetics, and immune responses in these animals up to 100 days after infection. Flow cytometry, omics platforms, and single-cell RNA-sequencing (sc-RNAseq) were applied to examine ongoing innate and adaptive immune responses in all species.
Results: We found typical clinical manifestation of ASF in DP and WB, while RRH and WH appeared unaffected. Early death in DP and WB correlated with unregulated pro-inflammatory responses and expansion of specific monocyte subsets. These response patterns were not found in African suids. Rather, we found well-orchestrated, non-excessive immune responses in RRH and WH. Omics results indicated that these differences in disease outcome are associated with the immune cell landscape in the blood of these pig species. For example, we found substantial differences in the composition of the myeloid compartment, especially among monocytes, the primary target cells of ASFV, between DP/WB and RRH/WH.
Conclusion: These results indicate that resistance to clinical disease in African suids is characterized by non-inflammatory responses. In contrast, pro-inflammatory cells and pathways were highly elevated in Eurasian pigs, suggesting that a misguided immune response is a contributing factor to lethal disease in these species.

Early mRNA expression profiles of key innate immunity effectors in pigs experimentally infected with BE18 and E70 ASFV strains

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Background and Objectives: African Swine Fever Virus (ASFV) is a pathogen affecting domestic and wild pigs with high fatality rate and causing important economic losses. This DNA virus is transmitted through direct contact with infected animals, ingestion of contaminated feed, indirect contact with contaminated formites or vectored by soft ticks of the Ornithodoros genus. Clinical symptoms include high fever, loss of appetite, haemorrhagic syndrome, skin lesions and respiratory distress. No licenced vaccine or treatment are currently available in the European Union given ASFV's viral complexity, its capacity to evade and modulate the host immune responses. Studies addressing early innate immune responses following *in vivo* ASFV infection are limited.
Material and Methods: In the present study, this aspect was studied after oronasal inoculation of 8 weeks-old pigs with a dose of $10^{5.5}TCID_{50}$ of ASFV BE18 or E70 strains. Three BE18-infected and three E70-infected pigs were euthanized at 24, 48, 72 and 96 hpi. In addition, three uninfected pigs were euthanized at 96 hpi. The mRNA expression profiles of 23 selected cytokines, including pattern recognition receptors, interferons, interferon stimulating genes, chemokines, pro-inflammatory and anti-inflammatory cytokines were analysed at each time point by qPCR in the tonsil of the soft palate, the ventral turbinates of the nasal mucosa, the mediastinal lymph node, the spleen and the diaphragmatic lobes of the lung.
Results: Overall, a higher immune response was observed following infection with E70 compared to BE18 strain. Both strains induced IFN-α, IFN-β and IFN-γ responses in the lymph node and an upregulation of IFN-β in the spleen especially at late time points. The chemokine CXCL10 and CXCL11 and the antiviral proteins ISG15, MX1 and RSAD2 were upregulated in the examined organs, with the most significant increase observed in the lymph nodes, followed by the spleen. Surprisingly, ASFV infection was not associated with a strong inflammatory response except for an upregulation of IL-1β and IL-1α in the lymph nodes and the spleen, respectively. No remarkable expression changes were observed in the tonsils and nasal mucosa. In the lungs, infection led to an upregulation of ISG15, MX1, RSAD2, CXCL11, IFN-β and RIG-I particularly at 96 hpi. Finally, no changes were observed in this study in the levels of the anti-inflammatory cytokines IL-10 and IL-4.
Conclusion: The results suggest that the innate immune response to ASFV infection is influenced by the viral strain and is expressed differently in the infected organs.

African swine fever pathology: Applying histologic methods to compare disease progression in wild boar and domestic pigs

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Background and Objectives: African swine fever (ASF) is one of the biggest threats to global pig production. Wild boar (WB; *Sus scrofa*) plays a significant role in the epidemiology of ASF transmission as a susceptible host and reservoir. However, most data on the host-virus interactions are derived from experimental studies in domestic pigs (DP; *Sus scrofa domesticus*) and comparative pathological studies between WB and DP are limited.

There are indications of differences in pathogenic mechanisms between WB and DP. This study aims to describe the early disease dynamics following experimental intranasal inoculation with a highly virulent ASF virus (ASFV) genotype II isolate (Armenia/07) in WB and DP by histopathologic examination. Additionally, virus antigen distribution in tissues is documented *in situ* using immunohistochemistry (IHC).
Material and Methods: Sixteen WB and sixteen DP were inoculated with virus and additionally three WB and three DP were sham inoculated as controls. On 1-, 2-, 3- and 5-days post-inoculation (dpi), three WB and DP, each, were euthanized and submitted for necropsy. Four WB and four DP were euthanized and necropsied upon reaching a pre-defined humane endpoint.
Results: Both infected WB and DP had similar mild histopathological lesions up to 5 dpi. However, the humane endpoint which corresponded to 6 and 9 dpi for WB and DP, respectively, suggests a higher susceptibility of WB to the clinical impact of ASF. In general, lesions consisted of vascular damage with haemorrhages, especially in lymphoid tissues, destruction of lymphocytes and macrophages and lymphoid depletion. Lesions were more severe in DP than WB at the endpoint.

Using IHC, virus antigen was detected earlier in WB compared to DP. For example, antigen-positive cells were observed in the retropharyngeal and submandibular lymph nodes already at 3 dpi and in 14 out of 21 evaluated organs at 5 dpi in WB. In contrast, viral antigen was only detected in the DP in two organs (bone marrow and retropharyngeal lymph node) by 5 dpi. ASFV antigen primarily was seen in monocytes-macrophages and occasionally lymphocytes, but as the disease progressed, it could also be observed in several different cells including hepatocytes, endothelial, epithelial, and reticular cells.
Conclusion: Our results demonstrated differences in host susceptibility, pathogenic mechanisms, tissue damage and virus dissemination between the two groups and suggests that WB have a higher susceptibility to disease. It challenges the assumption that the pathobiology of ASF in DP can be directly implied for WB and reveals a clear knowledge gap.

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Infection of pigs with African swine fever virus following oral or intranasal inoculations using different doses of the virus

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Background and Objectives: African swine fever virus (ASFV) is known to be a very stable virus within a protein-rich environment and it is believed that indirect transmission of the virus can be mediated via pork products and virus contaminated fomites. ASFV survival and infectiousness in different materials (faeces, straw, insects etc.) that could potentially transmit the virus to pigs via oral uptake has previously been investigated in various studies. However, even though infectious virus is present within such materials, experimental studies in pigs have shown that ASFV infection via the oral route can be difficult to establish. Currently, there is a lack of studies using strict oral inoculations of pigs with different doses of ASFV. Therefore, we aimed to investigate the dose of a European genotype II virus needed to establish oral infection of pigs in our experimental settings.
Material and Methods: In the study, 24 pigs were divided into four groups of 6 pigs each. For three of the groups, pigs were fed soft cakes every second day over a period of four weeks (up to 13 times in total) containing either a low (10^3 $TCID_{50}$), medium (10^4 $TCID_{50}$) or high (10^5 $TCID_{50}$) dose of the ASFV POL/2015/Podlaskie virus. Pigs in the fourth group served as positive controls and were inoculated intranasally at 0 dpi only, using the 10^3 $TCID_{50}$ dose of the virus. Infection with ASFV was demonstrated by presence of clinical signs, fever and by virus detection in blood using qPCR.
Results: The 6 pigs inoculated intranasally with ASFV succumbed to the infection, while only 3 of the 6 pigs that were orally fed the high dose of the virus became infected during the first 2 weeks of the study. Infection was demonstrated by clinical signs, fever and by virus detection in blood using qPCR. None of the 12 pigs that were fed either the medium or low dose of the virus 13 times in total (over a period of 4 weeks) became infected with ASFV.
Conclusion: The results obtained in this study underline that ASFV infection is much easier to establish via the intranasal route when compared to the oral route. The high dose needed in order to establish oral infection could have implications for future strategies using bait vaccines for wild boar.

From Crisis to Control: Insights for African swine fever resilience in red river hogs and warthogs

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Background and Objectives: African swine fever (ASF) is a viral hemorrhagic fever caused by the ASF virus (ASFV), causing high mortality rates in Eurasian wild suids and domestic pigs. In contrast, African suids were suspected to exhibit lower susceptibility to ASFV infection. To uncover the underlying mechanisms, we examined the progression and outcome of an ASFV infection in naïve, adult individuals of two highly susceptible European species, namely domestic pigs (DP) and Eurasian wild boar (WB), as well as two African species, Red River hogs (RRH) and ASFV's natural reservoir host species, the warthog (WH).
Material and Methods: The animals were inoculated intramuscularly with the highly virulent ASFV strain '*Armenia08*'. We evaluated disease manifestation and progression, viral kinetics, and immune responses in these animals for up to 100 days post-infection. To enhance potential insights, we employed a variety of techniques, ranging from conventional virus detection assays such as qPCR and hemadsorption test, to single-cell RNA-sequencing (sc-RNAseq).
Results: We observed typical signs of severe ASF in DP and WB, accompanied by significant viraemia. Both species succumbed to the infection within 7 days. In contrast, RRH and WH displayed no clinical signs and only low viral genome loads in the blood. Moreover, we found high levels of infectious virus in various tissues of DP and WB upon necropsy. In RRH sacrificed at 7 dpi, live virus was only detected in spleen, while multiple organs in WH contained live virus at 7 dpi. The clinical course and viral loads also correlated with the presence of ASFV antigen-positive monocytes in the blood. Furthermore, sc-RNAseq analyses revealed strong inflammatory response patterns in monocytes of DP and WB, whereas responses in RRH and WH were less pronounced.
Conclusion: These results highlighted resistance to clinical disease and a more regulated immune response in African suids, thus expanding our understanding of antiviral responses against this complex DNA virus and contributing to vaccine development strategies.

Session 4A and 4B

African swine fever

Poster Presentations

A novel African Swine Fever DIVA serological assay based on the detection of antibodies against pEP153R and eGFP

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Background and Objectives: African Swine Fever (ASF) is one of the most significant infectious diseases affecting both domestic pig and wild boar populations. Currently, outbreaks have been reported worldwide, and disease management relies on stringent biosecurity measures and surveillance through diagnosis, emphasizing the urgent need for an effective and safe vaccine for ASF control. Thus far, the most promising strategy for vaccine development is based on the modification of the ASF Virus (ASFV) genome to enhance its safety and DIVA characteristics (Differentiation between Infected from Vaccinated Animals). In this context, several promising vaccine candidates based on mutants of Lv17/WB/Rie1, a wild-type live attenuated genotype II ASFV strain, have been generated under VACDIVA project. The objective of the present study was to develop a companion serological assay for some of these vaccine candidates in which the EP153R gene is deleted and replaced by the eGFP reporter gene.
Material and Methods: To achieve this objective, we produced the recombinant pEP153R and eGFP proteins, and demonstrated their immunogenicity in domestic pigs and wild boar. Based on these antigens, we designed and developed a serological ELISA DIVA test, that also includes a highly immunogenic viral protein, p72, as a control.
Results: A first evaluation of the assay was performed using experimental serum samples. A total of 112 samples from 6 domestic pigs (DP) and 87 samples from 8 wild boar (WB), inoculated with the parental virus, were analyzed. The results showed that 100% of the animals seroconverted against p72 and pEP153R, although with a delayed onset between both antibody responses, and all resulted negative against eGFP. On the other hand, 207 samples from 16 DP and 96 samples from 8 WB immunized with VACDIVA candidate vaccines were analyzed. All vaccinated animals were negative against pEP153R, and positive against p72 and eGFP, with a similar seroconversion profile. Currently, serum and alternative samples from field animals are being analyzed for the final validation of the assay.
Conclusion: This DIVA diagnostic assay demonstrates to be a useful companion tool for vaccine candidates based on modified genotype II ASFV strains, in which the EP153R gene has been deleted and/or the eGFP reporter gene has been inserted. This approach could potentially improve surveillance during prospective vaccination campaigns.

Funding source/acknowledgements (optional):
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ASF biosecurity-focused eradication strategy in Sassari province (Sardinia, Italy) in domestic and wild population for disease control purposes

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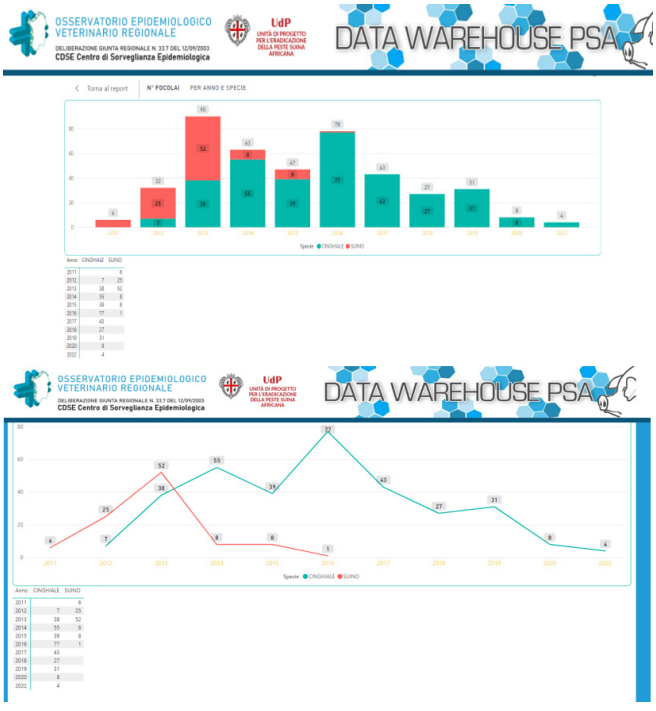
Background and Objectives: African swine fever (ASF) is a devastating infectious disease of domestic pigs and wild boars. Its epidemiological characteristics and the current spread of the disease represent the most serious global animal health emergency. Sardinia is now in the final stage of ASF genotype I eradication after more than 40 years of endemicity. The aim of this work is to show the decrease of both domestic and wild suids ASF cases linked to implementing a new biosecurity-focused eradication strategy.
Material and Methods: In 2013 a recrudescence of ASF was registered in Sardinia, Italy. In province of Sassari, the affected area included 17 Municipalities. The majority of the 35 outbreaks were recorded in the period May-July in only one Municipality. The emergency began in domestic pigs and soon after it was signaled in wild boars. Due to the complex context and the epidemiological situation, in 2015 a new eradication strategy was implemented by the Regional Government. The plan confirmed the banning of free-range pig keeping, historically one of the most important risk factors for ASF persistence, and imposed to increase the farming biosecurity level. Veterinary controls were strengthened along the entire pig production chain in an increasingly rigorous way. Stricter rules were applied to hunting (permitted only to authorised hunting companies), including safe disposal of wild boar offal. Control measures were accompanied by intensive training, awareness and communication activities targeted to farmers, hunters, and the population.
Results: The results are shown in table 1
Conclusion: The new strategy that provided for separation between domestic pig and wild boar population through enhancement of biosecurity in pig farms, together with strong communication and education activities enabled Sardinia to reduce ASF prevalence without any massive wild population culling. Such an ad hoc eradication strategy was fully supported and empowered by all local authorities and stakeholders. The successful and prompt management of the outbreaks indicates the high experience level of Sardinia to face ASF and demonstrates that expertise makes a difference in disease control.



Characterization of a novel African swine fever virus (ASFV) modulator of cytokine responses

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Background and Objectives: The African swine fever virus (ASFV) has now become a worldwide threat for which the only realistic control strategy is the development of a vaccine for immediate global use. Due to the acute nature of the infection and the complexity of the protective porcine anti-ASFV response, development of a virus vaccine attenuated by the deletion of virus host evasion gene(s) inhibiting innate immunity presents a practical solution, and which would stimulate both cellular and serological immunity.

ASFV exhibits a specific tropism for macrophages, which secrete multiple cytokines controlling inflammation and the subsequent adaptive immune response. Through bioinformatic analysis, we have identified a Src Homology 2 (SH2) domain in the ASFV protein DP146L, a member of the multigene family (MGF) 100. Importantly, SH2 domains interact with phosphotyrosine residues which, through reversible tyrosine phosphorylation of intracellular proteins, play a key role in signal transduction pathways stimulated by extracellular ligands such as growth factors and cytokines. Binding to phosphotyrosine-containing peptides involves conserved arginine residues that interact with the negatively charged phosphate on the phosphotyrosine.

The objective of this study was to evaluate the impact of DP146L expression on host signal transduction pathways and the role of the putative SH2 domain on the viral protein function.
Material and Methods: Protein structure homology modelling (Swiss-Model) to identify residues exposed at the DP146L putative phosphate binding pocket, was used. DP146L constructs containing mutations in these residues were synthesised (Genscript) and tested for their ability to inhibit TNFa signalling using a luciferase reporter assay. Briefly, HEK-293T cells were transfected with a NFκB dependent luciferase construct, a β-galactosidase control plasmid and the wild-type DP146L or different DP146L mutants. After 48 hours, cells were stimulated with TNFa for 16 hours and lysed. Luciferase and galactosidase levels were quantified using Bright-Glo™ Luciferase Assay System (Promega) and Galacto-Light™ Reaction Buffer Diluent with Galacton-Plus® Substrate (Life Technologies) respectively.
Results: We showed that the DP146L protein is able to inhibit TNFa induced signalling in transfected cells, suggesting a role for DP146L as a modulator of host pro-inflammatory responses. Surprisingly, none of the mutations in the putative phosphate binding pocket reverted the effect of DP146L, including a mutation in a lysine residue which similarly to arginine is positively charged.
Conclusion: Our data indicates that DP146L inhibits host responses to TNFa but does not support a role for an interaction with tyrosine phosphorylated proteins, as predicted from the presence of a SH2 domain, in this inhibition.

Funding source/acknowledgements (optional): Fundação para a Ciência e a Tecnologia (FCT, Portugal) through the CEECINST/00023/2018 grant, projects 2022.04769.PTDC, UIDB/00276/2020 (CIISA), and LA/P/0059/2020 (AL4AnimalS).

COMPARISON OF DIFFERENT ANTIBODY DETECTION TESTS FOR AFRICAN SWINE FEVER

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Background and Objectives: African swine fever (ASF) stands as a threat to the global swine industry. Its causative agent, the African swine fever virus, has inflicted substantial economic repercussions and necessitated culling of millions of pigs. The ASF has been present in Serbia since 2019 when it was first detected in domestic pigs. In 2023 the biggest recorded epidemic of ASF swept through the domestic pig population in the country resulting in devastating losses to the swine industry. Curbing the spread of ASF relies on the fast detection of infected pigs and the implementation of biosecurity measures. We wanted to compare routine PCR tests and novel lateral flow combo devices which would allow for fast detection of infected pigs and immediate implementation of necessary control measures.
Material and Methods: One hundred blood samples, 65 from ASF-positive domestic pigs and 35 from ASF-negative domestic pigs were analyzed by real-time PCR. PCR Ct values for positive results were recorded. The same samples were also tested with Novel Combo Lateral Flow Assay (combo LFD) which was developed and manufactured by Gold Standard Diagnostics. Antibody-positive samples were tested by confirmatory indirect Immunoperoxidase assay (IPA).
Results: Concerning the combo LFDs, it identified positive antigen signals in 53 samples and positive antibody signals in seven samples. This translates to an antigen sensitivity of 85% and antigen specificity of 100%. Of the seven antibody-positive samples, six were confirmed positive by IPA, while one was identified as a false negative, corroborated by a negative PCR test result. Overall, the sensitivity and specificity of the assay were 91.5% and 100%, respectively. The average Ct values were 23.8 for samples positive for both PCR and antigen and 28 for those positive for antibodies.
Conclusion: In summary, our study provides crucial insights into the comparative efficacy of routine PCR tests and the novel combo LFD in detecting ASF. The results indicated that while real-time PCR remains a highly reliable method for diagnosing ASF the combo LFD offer a valuable and reliable rapid screening tool. Notably, the lower sensitivity of the combo LFD compared to PCR, in terms of antigen detection is improved with an addition of antibody detection which is especially important in later stages of infection when there is a decrease in the viral load. These findings suggest that integrating the combo LFDs into current monitoring programs could enhance the speed of response during ASF outbreaks, potentially curbing the spread by allowing for faster implementation of control measures.

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Comparison of inoculation routes with African Swine Fever Virus Georgia 2007/1

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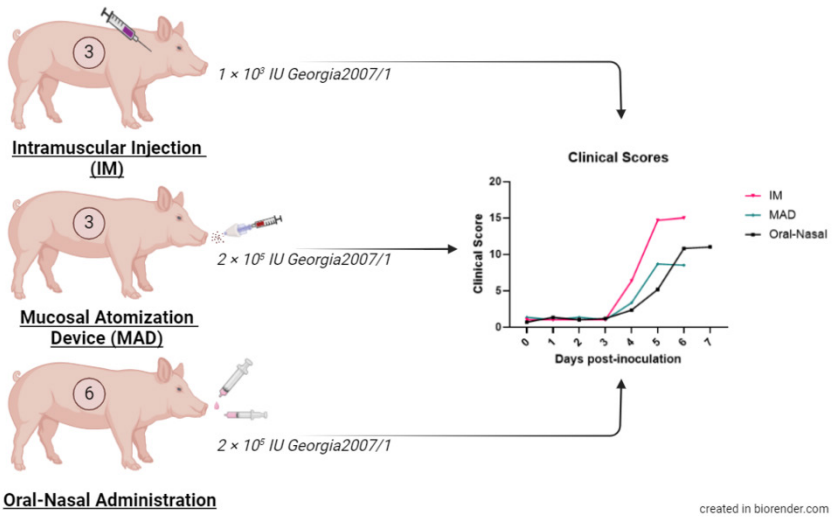
Background and Objectives: African Swine Fever virus (ASFV) is a contagious and lethal disease of domestic pigs and wild boars. The virulent genotype II ASFV is currently spreading throughout Europe and Asia causing economic loss and presenting a threat to food security. Without a widely available vaccine, ASF prevention and control focuses on strict biosecurity and slaughtering of infected herds. Prevalent routes of ASFV transmission include oral transmission through ingestion of infectious virus or contact transmission. Mimicking natural infection routes experimentally is difficult due to variability in the infectious dose, hence intramuscular injection (IM) is commonly used in scientific studies due to its reliability. Oral-nasal inoculation by depositing virus suspension into the nostril and mouth has proven effective, however we hypothesised that Mucosal Atomization Device (MAD) inoculation may more closely resemble a natural route of infection while maintaining the reproducibility of IM inoculation. MAD inoculation uses an intranasal device that atomizes the virus into a fine mist targeting the respiratory tract, however work at our Institute has demonstrated that a significant proportion of MAD inoculum is found in the digestive tract and oropharynx.
Material and Methods: Here we carried out a comparative study with British domestic outbred pigs to assess all three inoculation methods. Twelve, sixteen-week-old pigs (60 – 75 kg) were inoculated with the various methods. For the IM injections, Georgia 2007/1 was grown on porcine bone marrow macrophages, and injected into the neck of three pigs. Both oral-nasal and MAD inoculation used spleen homogenates from animals that had been infected with Georgia 2007/1. Oral-nasal inoculation was carried out on a group of six pigs and MAD inoculation on three pigs.
Results: Each method had slight deviations in the clinical course. Animals inoculated via IM injection developed clinical signs by day 4 and met their end points by day 6. Oral-nasal infection demonstrated slower disease development with humane endpoints being met at day 7 post-inoculation. In comparison, MAD inoculation demonstrated a milder clinical course. This group showed lower average viral titres at termination and humane end points were met with a lower overall clinical score when compared to the other two groups. With all methods of inoculation whole blood viral titres correlated with organ viral titres at termination.
Conclusion: By adopting a more natural route of infection, scientific studies can better mimic the progression of disease as it occurs in the natural environment. Consequently, results of vaccine efficacy studies will closer reflect the outcomes expected in the field.



Detector dogs as a tool to mitigate risk of ASF introduction in Sardinia (Italy): preliminary results

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Background and Objectives: African swine fever (ASF) is a fatal infectious disease that affects wild and domesticated suids. In Sardinia, historically affected by genotype I and in the final stage of eradication, in September 2023, it was detected the first case of ASF genotype II in a domestic pig farm, most likely as a consequence of infected meat products brought from Italy (the outbreak was then declared officially resolved by European Commission).Since the movements of pork meat and products play a key role in ASF introduction and spread, the priority is now to prevent introduction of genotype II in the Island. In 2023 Sardinian Government approved a surveillance and early detection plan that, among other risk mitigation measures, foresees strengthening controls in ports with sniffer dogs. Dogs have an extremely sensitive olfactory system with a limit of detection as low as one part per trillion concentrations, exceeding the instruments currently available. Dogs have been reported to identify distinct volatile organic compounds (VOCs) released by their hosts' metabolic processes in various conditions (ex. viral infections).The aim of this work is to describe the prevention activities with sniffer dogs on passengers arriving in the sea ports of north Sardinia to search meat products possibly contaminated with ASF.
Material and Methods: Official checks took place from November 2023 in Porto Torres and Olbia sea ports respectively 1 time and 2 times a week on a sample of disembarked vehicles, by a team composed of 2 veterinarians, one Prevention technician and 2 dyads (dog and handler, affiliated with Progetto Serena a.p.s.). Dogs were trained to distinguish between pork meat and other meat with an operant conditioning protocol based on previous research. The screening of vehicles and lugagges lasted around 5 minutes and once the dog smelled the target odor, it sat to alert its handler. Products considered at risk were then sampled and sent to the laboratory for ASFV testing.
Results: The results are shown in table 1.
Conclusion: Data evidence that approximately 30% of the examined vehicles carried pork products, highlighting the risk of introduction and spread associated with swine products carried in vehicles and baggage specially from countries affected by ASF. Detector dogs are a quick and reliable method to intercept meat products, making them an effective tool in mitigating risk of introduction. In addition to the checks, it's advisable to raise travelers'awareness with public campaigns. In phase 2 dogs will be trained to find ASF in meat.

Table 1. Controls with detector dogs

PORT	DAYS	VEHICLES	MEAT OR PDCTS FOUND	% MEAT OR PDCTS
Porto Torres	18	308	76	24,68
Olbia	20	285	86	30,18
TOT	38	593	162	27,3

Developing a complement-dependant cytotoxicity assay to characterise ASFV-specific antibodies

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Background and Objectives: African swine fever virus (ASFV) is a large complex DNA virus that causes African swine fever, a haemorrhagic disease of pigs with up to 100% mortality. Although there are two promising ongoing trials, no widely available commercially licensed vaccine is currently available and immune responses to ASFV are not fully understood. T cells have been shown to play an essential role in protection against ASFV infection, however the roles of B cells are more controversial. Non-neutralising antibody functions such as complement-dependant cytotoxicity (CDC) have been demonstrated previously using ^{51}Cr release assay, but the role they play in ASF infection is not well known.
Material and Methods: This work aims to develop a non-radioactive method to detect and investigate CDC activity of ASFV-specific antibodies. Here we test a number of commonly used and commercially available cytotoxicity assays for this purpose.
Results: Using a real-time fluorescent plate reader, we show that a DNA intercalator is capable of detecting CDC in an influenza HA-expressing cell line and show increasing CDC with increasing anti-HA concentration. We also show that significant ASFV-induced cell death can be detected from 24 to 48 hours post infection. Anti-ASFV antibodies from animals immunised with low virulent ASFV are capable of binding to the surface of ASFV-infected cells at 8 hours post infection, before ASFV-induced death is significant. Our cytotoxicity assay was then used to assess whether such antibodies were capable of mediating CDC and used to compare the CDC function of antibodies raised by a number of different experimental African swine fever vaccines.
Conclusion: This will contribute to the understanding of the B cell responses to ASFV and ASFV vaccines, protective or detrimental, which may inform future vaccine development.

Economics of African swine fever control in Uganda: Expanding perceived impact of quarantine imposition using System Dynamic Approach

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Background and Objectives: Pig production in Uganda is constrained by endemic occurrence of African swine fever (ASF). Current measures taken by the Government of Uganda for controlling ASF outbreaks include quarantines, consisting of trade and livestock movement restrictions. Little is known about the actions taken by, and the impact for, different stakeholders in the pig value chain in response to ASF quarantines. This study describes actions taken by different stakeholders along the pig value chain, the perceived economic impact, and a cost–benefit analysis of different scenarios of ASF quarantine protocols.
Material and Methods: Initial data were collected through a series of ten focus group discussions (FGDs) using participatory epidemiology tools and two key informant interviews (KIIs) with District Veterinary Officers (DVO) of Kisoro and Moyo districts in Uganda. These data formed the basis for implementing a participatory group model building (GMB) approach to parameterize a system dynamics (SD) model of the pig value chain for scenario analysis. This involved guided discussions with 14 stakeholders from the pig value chain to jointly develop models, generate parameters, and validate output of model simulation.
Results: The results from the FGDs and KIIs show that during ASF quarantine, pig value chain actors shifted their activities from formal places such as livestock markets, slaughter slabs, pork butcheries and pork joints to informal places such as farmer homesteads. Farmers were perceived the most economically affected stakeholder group. Implementing biosecurity under different scenarios with the SD model, such as no ASF outbreak, ASF outbreak, and ASF outbreak with quarantine imposition highlights variations in profit margins across stakeholders.
Conclusion: The perceived economic losses provide an insight into the negative economic impact of the quarantine for the different stakeholders.

Funding source/acknowledgements (optional):
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Farmer perceptions and actions following the first outbreak of African Swine Fever in Sweden

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Background and Objectives: In September 2023, the first case of African Swine Fever (ASF) in Swedish wild boar triggered various regional control measures. In addition, a new ASF certification programme for pig herds was initiated in December 2023 to improve biosecurity and proactive disease management. To capture the pig farmers' perceptions about the information they received, the risks to their own herds and the future prospects for their pig production, and what biosecurity measures they had implemented, two surveys were conducted (September 2023 and March 2024).
Material and Methods: Data were collected via online questionnaires distributed to members of the Swedish pig producers' organization.
Results: The first survey received 155 responses (response rate 36%). Most of the respondents had received general information about ASF, and how to protect their farm from ASF introduction. A majority thought the information was easy to understand, that it was relevant and sufficient. If given the necessary resources, 58% of the farmers would like to implement measures such as fencing, and heavily reduce the wild boar population. Two thirds of the farmers had a positive outlook on the future, and 89% did not change their future plans after the ASF outbreak.

The second survey received 113 responses (response rate 27%), with the majority seeing the risk of ASF reappearing in Sweden as high. While many farmers sought biosecurity advice from veterinarians, 43% had not implemented the suggested measures. Very few of the respondents had already signed up for the new ASF certification programme, 13% answered that they will join during 2024, and 19% that they need more time to prepare and will join in 2025. A few answered that they did not plan to join, and 34% that they will join if they end up in an ASF-restricted zone. Of those that did not plan to join the programme, 46% stated that it is too expensive, 19% that the risk of ending up in an infected zone is small, and 19% that they will cease the production if they end up in an infected zone. Reportedly, farmers' discussions concerned their worry about ASF outbreaks, ASF transmission mechanisms, and regulatory compliance.
Conclusion: In conclusion, the farmers were concerned about new ASF outbreaks, and a majority identified cost as a substantial hurdle for improving biosecurity. The results highlight the importance of effective communication and context-specific biosecurity advice and economic support to address the challenges posed by ASF.

Funding source/acknowledgements (optional): Rajala et al 2023 (https://doi.org/10.1186/s13028-023-00722-w) and Rajala et al 2024 (submitted)

Maternal Immunity and African swine fever: Understanding the limits of passive protection

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Background and Objectives: African swine fever (ASF) is an often fatal disease impacting domestic and Eurasian wild pigs, causing severe consequences for swine populations and leading to significant losses for associated economies worldwide. Understanding the role of maternal immunity in ASF pathogenesis is crucial for developing effective control measures. This study aimed to evaluate the potential protective effects of maternal immunity against ASF virus (ASFV) in neonatal piglets.
Material and Methods: Initially, sows were inoculated with the moderately virulent ASFV strain '*Estonia2014*'. Since recent findings demonstrated that sows abort upon the onset of ASFV-induced fever, all pigs underwent synchronization and insemination only after testing negative for ASFV in qPCR. The offspring of two sows that recovered from ASFV infection were sampled once a week after birth to monitor the kinetics of maternal ASFV-specific T cells and antibodies. The offspring of two other sows, in addition to piglets of a naïve control sow without ASFV-specific immunity, were challenged with the highly virulent ASFV strain '*Armenia2008*' on the seventh day of life. To evaluate the impact of ASFV-specific antibodies without ASFV-specific T cells, five piglets from the naïve sow underwent a serum transfer on the fourth day of life.
Results: All sows developed typical signs of disease and viraemia, and five sows recovered from the infection. One sow aborted during late pregnancy, but all other sows gave birth to healthy piglets. Ultimately, one piglet of the naïve sow succumbed due to lethal challenge at 4 dpi, while all other piglets, regardless of receiving a serum transfer, reached the humane endpoint at 6 dpi. The piglets born to immune sows began displaying clinical signs of disease at 5 dpi, and all either succumbed or reached the humane endpoint by 9 dpi. Serology results revealed the presence of antibodies against ASFV-p32 and ASFV-p72 in all piglets born to immune sows. Antibody titers in unchallenged piglets remained stable for at least 60 days after birth. In challenged piglets, those born to immune sows were seropositive before challenge and mostly seronegative after challenge, indicating antibody consumption.
Conclusion: In conclusion, the immunity acquired passively, whether through serum transfer (antibodies) or colostrum (antibodies and T cells), is insufficient to safeguard neonatal pigs from a deadly challenge with highly virulent ASFV.

Perceptions and practices of Swedish wild boar hunters in relation to African swine fever before the first outbreak in Sweden

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Background and Objectives: The first outbreak of African Swine Fever (ASF) in Sweden was detected in 2023 in wild boar. This study was conducted before the first ASF outbreak with the objective of investigating Swedish hunters' perceptions and practices of the disease ahead of any potential future outbreak.
Material and Methods: A mixed-methods interview study with Swedish wild boar hunters, consisting of focus group discussions and a questionnaire, was undertaken between October 2020 and December 2021. Six focus groups were conducted online, and an online questionnaire with questions related to hunting practices, hunting trips and the use of bait was sent to all members of the Swedish Hunting and Wildlife Association. A total of 3244 responses were received.
Results: Three general themes were identified in a thematic analysis of the data from the focus groups: hunters are willing to engage in ASF prevention and control, feasibility is crucial for the implementation of reporting, sampling and control measures, and more information and the greater involvement of the authorities are required in ASF prevention and control. Results from the questionnaire showed that the use of bait was common. Products of animal origin were rarely used for baiting; the most common product used was maize. Hunting trips abroad, especially outside of the Nordic countries, were uncommon.
Conclusion: Hunting tourism and the use of bait do not seem to constitute a major risk for the introduction of ASF to wild boar populations in Sweden. The accessibility of information for everyone and the ease of reporting and sampling are crucial to maintaining the positive engagement of hunters.

The Universe of Transcription Factors Associated with African Swine Fever Virus RNA polymerase

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Background and Objectives: African Swine Fever Virus (ASFV), a nucleo-cytoplasmic large DNA virus (NCLDV), causes a haemorrhagic fever in both wild boar and domesticated pigs. Its rapid spread throughout Eurasia poses a threat to global food security and causes significant economic loss due to the lack of antivirals and vaccines; currently estimated to be over $100bn (USD). The understudied gene expression and transcription mechanisms of ASFV present a crucial knowledge gap. ASFV, similarly to exclusively cytosolic replicating *Poxviridae* species, replicates predominantly within the cytosol and encodes its own RNA polymerase (RNAP), capping- and polyadenylation enzymes.

The ASFV core RNAP comprising 8 subunits has a molecular structure similar to the host RNAPII. Regulatory transcription factors associate with ASFV RNAP and modulate its function, including the temporal control of transcription. Sequence homology with known NCLDV factors can identify some of these factors, including D1133L and G1340L (D6 and A7 in Vaccinia virus respectively) that have been partially characterized and facilitate early transcription. Some late gene factors can be predicted with confidence, other obvious candidates are missing entirely in ASFV; importantly the precise composition of ASFV RNAP complexes changes thereof across the virus life cycle remain unknown.

This project aims to characterise ASFV RNAP-associated factors, including protein and RNA components that associate with the viral RNAP in the virion and the infected cell during early and late infection. Pilotto *et al.* (2024) elucidated the structure of recombinant ASFV RNAP generated in insect cells enabling us to identify areas that can be tagged.
Material and Methods: By generating recombinant ASFV strains encoding affinity-tagged RNAP subunits we have the tool to purify RNAP-containing complexes from virions and infected cells which is currently in progress. We aim to utilise mass spectrometry to identify other factors that associate with the RNAP . We will produce recombinant versions of the identified factors, solve the structures of the complexes they form with the RNAP using cryo-EM, and dissect their function using *in vitro* transcription assays.
Results: Recombinant ASFV viruses with tagged RNAP subunits have been generated, which show localisation within the virus factory of infected cells. These viruses are currently being used to identify factors associated with the viral RNAP via pulldown followed by mass spectrometry.
Conclusion: The identification of transcription factors associated with ASFV RNAP utilising recombinant viruses will fill the current knowledge gap by helping to unravel the mechanisms of ASFV transcription, leading to the potential development of therapeutics targeting this area of the virus lifecycle.

Whole genome sequencing reveals novel large deletion genotype II ASFV strains in Southern Italy in 2023.

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Background and Objectives: Whole-genome sequencing (WGS) plays a crucial role in tracking the African swine fever virus (ASFV) evolution and identifying potential virulence factors or genetic markers associated with different ASFV strains.

After the first introduction in Italy in January 2022, ASF genotype II was detected in wild boars and domestic pigs in May 2023 in Southern Italy (Calabria region).

To further understand the genetic diversity and dissemination of the virus, studies have utilized Illumina WGS technology.
Material and Methods: An analysis was conducted on 24 samples from suspiciuos cases and outbreaks from domestic pigs. We analyzed bone marrow, kidney and spleen collected between May and July 2023 in the province of Reggio Calabria.
Results: All samples confirmed the presence of ASFV genotype II and exhibited a large genomic deletion at the 5' region. This type of deletion spanned 5136 nucleotides, from 10,755 to 15,891 bp, refered to Georgia2007 (FR682468) and involved 12 genes (285L , ASFV G ACD 00160, MGF 110-8L, MGF 100-1R, ASFV G ACD 00190, MGF 110-9L, ASFV G ACD 00210, MGF 110-10-L - MGF110-14L fusion, ASFV G ACD 00240, MGF 110-12L, MGF 110-13La, MGF 110-13Lb).

Furthermore, a second type of variation involved the 3' region resulting in variable truncated ends, approximatively located at position 188,516 to 188,631 nt. It spanned 1,954 to 2,096 nucleotides, and resulted in loss of the following five elements: MGF 360-21R, ASFV G ACD 01980, ASFV G ACD 01990, ITR and DP60R. In particular, two strains (25791_2390/RC/2023, 55135_2737/RC/2023), showed a truncated end at 5' region (position 1-2244 nt), with the loss of DP60L, ITR, ASFV G ACD 01990, MGF360-1La and a 3' extension of 35 nucleotides. Confirmation through PCR and Sanger has been performed. Variable 5' and 3' deletions have been widely described in some genotype II isolates, while the 5' and 3' changes identified in these group of strains are unique.
Conclusion: Despite the apparent temporal stability of the ASFV genome, the study identified major genetic variances amongst the genotype II ASF viruses from Calabria cluster, never describe before.

The data on the genetic variability observed in genotype II ASF strains collected in Calabria cluster need to be further investigated to understand the evolutionary pressure exerted on them by the environment. Phylogenetic study will be carried out in order to investigate the time and place of origin of these strains.

Session 5

Epidemiology and risk assessment

Oral Presentations

AFRICAN SWINE FEVER: PRESENCE IN SWINE MEAT PRODUCTS SMUGGLED INTO ITALY

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Background and Objectives: African Swine Fever (ASF) is a highly contagious virus affecting domestic and wild pigs and a huge threat to the swine industry worldwide. Detected in Georgia in 2007, ASF virus (ASFV) genotype II spread to Asia and Europe and was detected in Italian wild boars in early 2022. Since then, 2,004 wild boar and 21 domestic pig outbreaks, respectively, were recorded. Transmission occurs through direct or indirect contact with fomites or animals, and certain tick species, i.e. Ornithodoros. Feeding pigs with infected pork products contributes to its spread within and across country borders.

Between late 2023 and March 2024, the Italian Ministry of Health (IMH) and the National Extraordinary Commissioner initiated a surveillance program at restaurants, retail establishments, and local markets to investigate the presence of illegally imported pig derived products to assess the risk of ASFV introduction into Italy.
Material and Methods: Confiscated products underwent a PCR-based swine DNA identification and, if positive, a Real Time PCR (RT-PCR) to detect ASFV was performed by the National Reference Laboratory (NRL). All ASFV positive products underwent Central Variable Region (CVR) (B602L gene) Sanger sequencing and viral isolation (VI) on swine-derived peripheral blood mononuclear cells (PBMC) to determine the genotype and presence of infectious virus, respectively.

Thirty ASFV-positive products were administered to four pigs to demonstrate the absence of infectious virus. Pigs were observed daily for clinical signs and rectal temperature for 19 days after feeding (pf). Then, animals were euthanized, and samples of spleen and blood were collected and tested by RT-PCR. The trial was approved by the IMH and performed in accordance with European legislation on the protection of animals used for scientific purposes (Directive 2010/63/EC).
Results: Out of 401 confiscated samples of ascertained swine origin, 104 (25.9%) were ASFV positive, 292 negative, and five inconclusive (1.2%). ASFV-positive products included 58 prepared meals, sixteen cooked meats, thirteen snacks, eight raw and eight cooked pork items, and one ready-to-eat meal. Product origins were not disclosed for the majority of samples, but for many, labelling was in Chinese. All 104 positive samples tested negative by VI.

Genotyping identified genotype II. All pigs fed with ASFV positive products showed no clinical signs and fever, with negative RT-PCR on spleen and blood.
Conclusion: In conclusion, while in vivo testing excluded the infectious virus presence, the monitoring plan revealed that there may exist a risk of ASF spreading through illegally imported pig derived products.

Characteristics of the Swedish cattle movement network 2005 - 2022 and implications for disease spread

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Background and Objectives: Livestock movements between holdings play an important role in the spread of many infectious diseases. Methods of social network analysis are often used in veterinary epidemiology to investigate these movements, which can reveal potential courses of epidemics or help finding holdings with high risk of acquiring or spreading disease. This study explored the network of cattle movements in Sweden from 2005 to 2022.
Material and Methods: All reported cattle events (births, deaths and movements) under the study period were cleaned and analysed through descriptive statistics, social network analysis, trend tests and survival analyses.
Results: Preliminary results showed that the Swedish cattle movement network and population structure changed considerably over the study period. The number of holdings and cattle decreased while the number of reported movements increased (around 50% over the studied period). However, it became less probable for holdings to be connected to a large number of holdings (both direct and indirect contacts). At the same time, the global network metrics revealed a denser network in later years but with more clustering of holdings, a larger average path length between pairs of holdings and a more disassortative network. Altogether, the results indicate that on the recent network diseases might spread locally within clusters of holdings, but more slowly through the whole network and with a smaller potential epidemic size. Results also confirmed that bull calves at holdings with high proportion of females had a high risk to move to another holding, and that bull calves born in later years were more likely to move at an earlier age.
Conclusion: In summary, the study has identified complex patterns and changes that may influence the spread of infectious diseases in cattle. To enable the design of control strategies that take into account both the dynamic nature of the network, and the differences in pathogen behaviour, disease spread modelling might be advised.

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Citizen science dependent disease surveillance in wildlife

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Background and Objectives: Diseases of wildlife can have severe consequences for ecosystems, but also may threaten both livestock and human health. Wildlife disease surveillance programs therefore inform preventive work in the livestock sector in addition to informing wildlife management. Wildlife surveillance programs often depend on voluntary participation of citizens. One example is the Swedish Veterinary Agency's (SVA) general wildlife disease surveillance program in which citizens voluntarily report observations of abnormal or dead wildlife and submit samples or carcasses for examination. This surveillance program plays a pivotal role in transboundary animal disease management. For example, the program detected the outbreak of African swine fever in Sweden 2023 and it enables monitoring of the avian influenza situation in wild birds. Despite the reliance on volunteers in the field, factors that influence reporting and submission of cases are poorly investigated, even though they may have a large impact on the sensitivity of the surveillance program. The aim of this study was to improve understanding of the different components of the surveillance program as a basis for future improvements.
Material and Methods: Cases submitted to SVA's general surveillance program 2010-2020 were investigated using descriptive and spatiotemporal analyses. To capture the volunteer perspective, data were collected through a questionnaire offered to people reporting wildlife cases and focus-group interviews with people who had repeatedly submitted samples or carcasses.
Results: We found strong, uneven spatial distribution. Cases submitted to SVA were highly associated with proximity to SVA. Further, the species composition was skewed, and the size of the animal played a role in type of material submitted (whole carcass versus samples). Legal requirements to report and submit certain species had a strong effect. Among reporters responding to the questionnaire (n=1164), approximately half of the reports of sick or dead wildlife were observed in private gardens and a third of respondents were at home when they observed the case. Half of them were willing to submit the carcass to SVA for analysis, but lack of appropriate storage was the most important factor hindering submission. Both reporters and repeat submitters were primarily autonomously motivated to participate and expressed this in terms of a genuine interest for nature, curiosity and a desire to contribute. Moreover, the opportunity for building competence was highly valued highlighting the importance of feedback and other strategies tailored to encourage and maintain engagement.
Conclusion: The results provide basis for improved wildlife health surveillance in Sweden and elsewhere.

Lethal Borna disease virus 1 (BoDV-1) infections of humans and animals – in-depth molecular epidemiology and phylogeography

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Background and Objectives: Borna disease virus 1 (BoDV-1) is the long-known causative agent of Borna disease, a progressive and mostly fatal neurologic disorder of domestic mammals, resulting from spill-over infection from its natural reservoir host, the bicolored white-toothed shrew (*Crocidura leucodon*). Only in 2018, it has been confirmed that humans may likewise acquire BoDV-1-induced encephalitis with case-fatality rates of more than 90%. The known BoDV-1 endemic area is remarkably restricted to parts of Germany, Austria, Switzerland and the Principality of Liechtenstein.
Material and Methods: To gain comprehensive data on the occurrence of BoDV-1, we analysed current and archived diagnostic material from suspected fatal BoDV-1-induced encephalitis cases in domestic mammals and humans based on clinical and/ or histopathological diagnosis. The samples originated from diagnostic laboratories and pathologies in Germany, Switzerland and Austria. They were analysed by BoDV-1-specific RT-qPCR and sequencing of the BoDV-1 genome was attempted by high throughput and Sanger sequencing for 157 BoDV-1-positive cases.
Results: BoDV-1 infection was confirmed by RT-qPCR in 207 of 231 domestic mammals (89.6%), 28 of 29 humans (96.6%) and seven wild shrews, mainly within the known endemic area. By reporting multiple unpublished cases, this study raises the number of published laboratory-confirmed human BoDV-1 infections to 46 and provides a first comprehensive summary. Generation of 136 complete or partial BoDV-1 genome sequences from animals and humans facilitated an in-depth phylogeographic analysis. Consistent with the low mobility of its shrew reservoir host, BoDV-1 sequences showed a remarkable geographic association, with individual phylogenetic clades occupying distinct and barely overlapping dispersal areas. The closest genetic relatives of most human-derived BoDV-1 sequences were located at distances of less than 40 km from the patient's residence, indicating that spill-over transmission from the natural reservoir usually occurs in the region of the patient´s residence.
Conclusion: In summary, we performed a highly comprehensive phylogeographic analysis of the occurrence of the zoonotic pathogen BoDV-1 in Central Europe. The novel and extended phylogeographic data allow for the definition of risk areas for zoonotic BoDV-1 transmission and facilitate the assessment of geographical sources for individual infection events.

Session 5

Epidemiology and risk assessment

Poster Presentations

Diversity of Kobuvirus in Wild Animal Populations

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Background and Objectives: Kobuvirus is a recently discovered genus of RNA viruses detected in various animal species, including domestic animals and humans. While the prevalence and significance of kobuvirus infections in domestic animals and humans have been increasingly studied, and it's known as an agent causing gastrointestinal illness in humans, limited information is available regarding the presence of kobuvirus in zoo and wild animal populations, which are often in close contact with humans and other animal species, and may serve as reservoirs for various pathogens, including kobuvirus. This study examines the presence of kobuvirus in a wide range of wild animal species using molecular detection techniques.
Material and Methods: A total of 600 fecal samples were collected from a diverse range of wild animal species, including wild canines, wild felines, and rodents, and screened for the presence of Kobuvirus using reverse transcription-polymerase chain reaction (RT-PCR) targeting the 3D polymerase gene of kobuvirus.
Results: Of the 200 wild canine samples analyzed, 25(12.5%) tested positive for kobuvirus. The positive samples were obtained from the following wild canine species: Gray wolves (Canis lupus): 10/50 (20%), Coyotes (Canis latrans): 8/40 (20%), African wild dogs (Lyon Picts): 5/30 (16%), Red foxes (Vulpes): 2/30 (6.7%).

Kobuvirus was detected in 17.5% (35) of the 200 wild feline samples analyzed. The positive samples were obtained from the following wild feline species: Lions (Panthera leo): 12/50 (24%), Tigers (Panther Tigris): 10/40 (25%), Cheetahs (Acronym Justus): 8/30 (26.7%), Jaguars (Panther once): 5/30 (16.7%).

20% of the rodent samples (40 out of 200) tested positive for kobuvirus. The positive samples were obtained from the following rodent species: House mice (Mus musculus): 15/50 (30%) Brown rats (Rattus norvegicus): 12/40 (30%), Wood mice (Apodemus sylvaticus): 8/30 (26.7%), Field voles (Microtus agrestis): 5/30 (16.7%).
Conclusion: Phylogenetic Analysis of the partial polymerase gene sequences revealed that the kobuvirus strains detected in wild canine, felines, and rodents clustered into distinct genetic lineages, suggesting potential host-specific adaptations and evolutionary divergence within kobuvirus populations in these animal groups. These results highlight the broad host range of kobuvirus in zoo and wild animal populations. The findings from this research will contribute to a better understanding of the epidemiology and ecology of kobuvirus infections in animal populations and may have implications for the management and conservation of zoos and wild animals.

Further research is warranted to investigate the transmission dynamics, genetic diversity, and Zoonotic potential of kobuvirus in these animal species for comprehensive disease surveillance and management strategies.

Epidemiological investigation of an African swine fever outbreak on a commercial farm in Uganda

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Background and Objectives: In Uganda, the pig population is increasing and pig production is contributing to food security and household income for many people. The majority of pigs are kept by smallholder farmers, only a few commercial farms exists in the country. African swine fever (ASF) is endemic in Uganda and remains a big threat to the pig industry. This study describes the epidemiological investigation of two ASF outbreaks, in 2021 and in 2022, at a large commercial farm in central Uganda.
Material and Methods: The study comprised farm observation, analysis of farm records, interviews with farm director and veterinary officer to assess the pig movements and biosecurity protocols within the farm, and laboratory testing of biological and feed samples. Samples of whole blood from suspected ASF cases and in-contact pigs, and tissues (spleens and lymph nodes) from dead pigs were collected in recommended sterile containers. Feed samples were collected from trailers while being offloaded into silos and from mills at the feed manufacturing company during loading. Collected samples were transported to Collage of Natural Sciences in Makerere University for laboratory analysis. Using PCR-Real time, genomic DNA was extracted from blood, tissue and feed samples to test for presence of ASFV. We further analyzed the spatial and temporal pattern of ASFV positive samples in the two outbreaks.
Results: The investigations showed that the farm had high levels of external and internal biosecurity. Laboratory results showed 1,121 and 241 biological samples positive for ASF-virus (ASFV) in the 2021 and 2022 ASF outbreaks respectively. Furthermore, 6 feed samples tested positive for ASFV in 2022.
Conclusion: The study underlines the high risk for introduction of ASF at a commerical pig farm in an ASF-endemic setting, and the importance of every-day adhernce to exisiting biosecurity routines.

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Epidemiological investigation of Pestivirus O (ovIT PeV) in ewes and pigs

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Background and Objectives: During 2021-2023, to verify the presence and incidence of a 'new' *Pestivirus O* (ovIT PeV), an epidemiological investigation was carried out on sheep and pig farms located within a 10 km radius around the farm where ovIT PeV was first isolated.
Material and Methods: The serum samples were analysed to determine anti-pestivirus antibodies using a *homemade* competitive ELISA technique and CSFV Antibody Test kit IDEXX. Next, pan-pestivirus real-time RT- PCR was conducted. The sample set was composed as follows: 621 sheep sera from n. six farms; 60 sheep nasal swabs from n. two farms; 1444 pig sera from n. 37 farms in 2021 and 768 pig sera from n. 21 farms in 2022; 488 sera, 340 whole blood and one aborted lamb from the farm of origin of ovIT PeV. Some anti-pestivirus antibody-positive sera were sent to the Italian Reference Centre for Pestiviruses for confirmation and further characterisation by serum cross-neutralization tests, using the viruses ovIT PeV, CSFV, BVDV and BDV. Samples that tested positive in pan-pestivirus real-time RT-PCR were subsequently characterised by Sanger sequencing of a fragment of the 5'UTR region.
Results: Using real-time RT-PCR pan-pestivirus, ovIT PeV was re-detected by identifying 14 sequences in the herd of origin. Moreover, a BVDV-1d strain and a Tunisian sheep-like pestivirus (TSV, *Pestivirus N*) were identified in sheep farm 1, respectively, in 2022 and 2023. The serological tests (Table 1) showed positivity for both pestivirus and CSFV in sera from three farms (Farms 1, 2 and 3). Only two pig sera from two separate herds tested positive for pestivirus. The major cross-reactions were towards the BDV Moredum for samples from farms 2 and 3, towards the CSFV 104 Hannover strain, BDV Moredum and ovIT PeV for samples from farm 1 and the herd of origin.
Conclusion: The virological investigations showed that ovIT PeV was still circulating within the herd of origin. However, several anti-CSFV antibody-positive sera were detected. Indeed, the serum neutralisation tests allowed a better understanding of the epidemiology, and thus, the ovIT PeV circulation in farm 1 was not excluded. It remains to be clarified whether anti-CSFV antibody positivity is due to circulating ovIT PeV or TSV, which is also related to CSFV. Furthermore, detecting a bovine pestivirus (BVDV-1) in a ram confirms the ability of pestiviruses to cross the species barrier and infect a wide range of animals of the order Artyodactila. Finally, of note is also the "incidental" identification of a TSV strain.

sheep		Samples positive for antibodies to pestivirus and CSFV / samples tested						Samples positive for pan-pestivirus real time RT-PCR / samples tested		
		2021		2022		2023		2021	2022	2023
		pestivirus	CSFV	pestivirus	CSFV	pestivirus	CSFV			
Farm 1	serum	101/108	3/108	51/61	11*/61	83/96	35*/96	0/108	1/61 (BVDV 1d)	1/96 (TSV)
	nasal swabs								0/30	
Farm 2	serum	30/82	9/82	26/118	29*/118	0/6	0/6	0/82	0/118	0/6
	nasal swabs								0/30	
Farm 3	serum	3/82	0/82	16/16	3*/16	//	//	0/82	0/16	//
Farm 4	serum	//	//	0/9	0/9	//	//	//	0/9	//
Farm 5	serum	0/11	0/11	0/15	0/15	0/13	0/13	0/11	0/15	0/13
Farm 6	serum	//	//	0/4	0/4	//	//	//	0/4	//

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Farmer logics – motivations and driving forces influencing a sustainable and resilient Swedish dairy production

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Background and Objectives: Over the past forty years, restructuring within the Swedish primary production have resulted in intensive and highly specialized systems dependent on automatic and digital solutions, and fewer but larger production units. Both agriculture and its supportive functions have clustered geographically. This structural rationalisation within the animal production have altered the role of Swedish dairy farmers.

Previous research has identified different farmer logics among primary producers and showed a large variation in drivers and motivators among farmers, affecting decision making and development. The aim of this study was to investigate in what way previous findings on farmer logics apply in the context of Swedish dairy farming, by identifying patterns in the motives and driving forces influencing decision making and the possible effects these patterns may have on robustness and diversity within primary production.
Material and Methods: A survey was conducted and distributed digitally to approximately 9000 registered Swedish cattle farmers in April 2023. The survey included questions about the respondent, the farm, and its production. Active dairy herds were identified among respondents through question branching and presented with Likert scale questions. These respondents were asked to estimate to what extent (scale 1-5) they did or did not agree with 13 statements on attitudes on farmers life, 2 on farmers self-identification and 14 on the respondent views on future farming. The statements used were based on previous findings in research on farmer logics modified to fit the context of Swedish agriculture. The survey rendered 362 responses from the target group of 2600 active dairy cattle farmers, 326 completed the entire questionnaire.
Results: Preliminary results show that respondents were 23-87 years old (median: 55), gender distribution among respondents were 234 male, 77 female and 2 other, and herd size varied between 1-2000 dairy cows (median: 75) from all counties of Sweden.

Ongoing analysis will tell whether there are any detectable patterns among the answers collected. Significant patterns in the data set will be further analysed for possible interconnections and differences and similarities between respondents, farms, and production systems clustered among the answers.
Conclusion: On a systems level, ensuring diversity within the primary production implies finding ways to support farms and farmers with varying geographical distribution, herd size and intensity. Hence, identifying the farmers' views on their production as well as driving forces and motives for establishing, developing, and maintaining the production is essential for building resilience within the food system.

Funding source/acknowledgements (optional): Funded by Formas and Swedish farmers' foundation for agricultural research (SLF)

Impact of Kobuvirus Coinfection on Clinical Course and Mortality in Canine Parvovirus and Feline Panleukopenia Virus Infections

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Background and Objectives: Canine parvovirus (CPV) and Feline panleukopenia virus (FPV) infections remain a significant concern in veterinary medicine due to their high morbidity and mortality rates, particularly in young and unvaccinated populations. Recently, the discovery of Kobuvirus, a novel viral pathogen in dogs and cats, has raised questions about its potential interactions with CPV and FPV and its impact on disease severity and outcomes.

This study aims to investigate the potential synergistic or antagonistic effects of kobuvirus co-infection on the clinical course and mortality rate of CPV and FPV. By elucidating the interaction between these two viral pathogens, we seek to enhance our understanding of the complex dynamics underlying parvovirus infections and identify novel factors that may influence disease progression and outcomes.
Material and Methods: A total of 84 dogs diagnosed with CPV and 100 cats with felinepanleukopenia ,All checked for co-accurrence of kobuvirus infection(confirmed with PCR and RT-pcr method) were included in this study.
Results: The prevalence of kobuvirus coinfection in dogs with CPV was 94.0% (79/84). Also, the prevalence of coinfection with FPV was 80%. This high rate of co-occurrence highlights the potential significance of kobuvirus in the clinical presentation and outcomes of the disease.

The mortality rate was significantly higher in dogs and cats coinfected with kobuvirus compared to those with CPV or FPV alone. In the dogs infected with kobuvirus concurrently, the mortality rate was 75.9% (60/79), while in the CPV-only group, it was 40.0% (2/5). The mortality rate was significantly higher in cats co-infected with kobuvirus (75%) compared to those without kobuvirus coinfection (40%).
Conclusion: Furthermore, Dogs and cats coinfected with kobuvirus presented with more severe clinical signs, including vomiting, diarrhea, dehydration, and lethargy, compared to those with CPV/FPV alone. Additionally, the duration of hospitalization was significantly longer in this group. The viral load of FPV was significantly higher in cats coinfected with kobuvirus compared to those without kobuvirus coinfection.

The high prevalence of kobuvirus coinfection and nearly two-fold increase in mortality rate highlights the potential synergistic or exacerbating effects of kobuvirus coinfection on the severity and prognosis of the disease.

These findings underscore the importance of considering kobuvirus coinfection in the diagnosis and management of panleukopenia virus and canine parvovirus.

Seroprevalence of small ruminant lentiviruses in Swedish sheep and goats

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Background and Objectives: Small ruminant lentiviruses (SRLV) are a group of lentiviruses causing the chronic diseases in sheep and goats known as Maedi/Visna (MV) and Caprine arthritis and encephalitis (CAE). MV is characterised by respiratory signs and wasting whereas CAE mainly manifests as polyarthritis, encephalitis and chronic wasting. The in-herd prevalence is usually high even though only a few animals show clinical signs. The first case of SRLV in Sweden was reported in 1974, and in 1989 a prevalence of 8.2% in the sheep population was estimated. Sweden has 311 638 sheep and 12 685 goats in 12 336 and 2 749 holdings, respectively, according to the yearly sheep and goat count (2023. Since 1993, Sweden has a voluntary control programme for MV, and in 1999 a corresponding programme for CAE started. In 2023, 90% of the 3232 sheep and 274 goat holdings affiliated to the control programme had been declared free from SRLV.
Material and Methods: To investigate the prevalence of SRLV in the Swedish sheep and goat population outside the control programme, 915 sheep and 587 goats from 56 and 41 holdings, respectively, were tested for SRLV. Previously untested holdings were asked to sign up for testing through a web form published in social media. Serum samples from all animals above one year old were collected and analysed with ID Screen® MVV/CAEV Indirect ELISA (Innovative Diagnostics, Grabels, France) and positive results confirmed with ELITEST – MVV/CAEV (Hyphen BioMed, Neuville sur Oise, France).
Results: No sheep tested positive in this study which corresponds to a 95% confidence that the prevalence of SRLV is less than 5%. For goats, test positive animals were found in 7 holdings, which corresponds to 14% (95% CI, 7-26%) of the tested holdings. The proportion of test positive animals within these holdings were 14-85%.
Conclusion: The results indicate that the prevalence of SRLV in Swedish sheep is low, but fairly high in Swedish goats. Strategies and prerequisites for further control and prevention, and a potential future eradication of SRLV needs further investigation. More knowledge is needed about how the disease affects animal welfare, how much it costs both farmers and government, and stakeholders' attitudes towards different control measures.

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Session 6

Avian influenza

Oral Presentations

Assessment of air contamination by avian influenza viruses in live poultry markets in Bangladesh

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Background and Objectives: Low pathogenic (H9N2) and Highly pathogenic (H5N1) avian influenza viruses (AIVs) are endemic in Bangladesh's poultry population since 2007. Live poultry markets (LPMs) are considered as an important source for AIVs transmission and amplification. AIVs have been detected year-round in poultry kept in LPM in Bangladesh. Poultry are also often slaughtered within poultry shops in the LPM, and undergo defeathering process. The trading of live poultry along with current slaughtering methods could contribute to airborne contamination by AIVs. During October 2023-March 2024, we conducted a study in Dhaka and Gazipur city LPMs to assess the risk of air contamination by AIVs.
Material and Methods: A total of 60 air samples were collected from 10 LPMs. The samples were collected for an hour from a randomly selected poultry shop using AeroCollect air collector. Samples were tested at the icddr,b One Health Laboratory to detect AIVs RNA using rRT-PCR (Real-Time Reverse Transcription-Polymerase Chain Reaction).
Results: Of the tested samples, 57 (95%) were tested positive for avian influenza A viral RNA. Among the AIV positive samples, one sample was found positive for H5 subtype, 21 samples positive for H5/H9 co-detection, 31 samples positive for H9 subtype, and 4 samples remained unsubtypeable. Majority (n=49) of the samples revealed a Cq (quantification cycle) value between 30-36 and 8 samples had Cq value <30.The detection of avian influenza viral RNA in air samples at various viral load levels within LPMs highlights the potential cross transmission to LPM workers and visitors, but also shows the importance of the markets for the spread between live birds.
Conclusion: This study emphasized the significance of improving biosecurity measures in LPMs, including the implementation of centrally controlled slaughtering facilities, to mitigate the risk of airborne transmission of AIVs.

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Avian Influenza virus in Egypt: current situation and strains replacement

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Background and Objectives: In Egypt, highly pathogenic avian influenza (HPAI) H5N1 and H5N8 viruses were reported in 2006 and 2016, and resulted in hundreds of outbreaks among different poultry species. In 2011, low pathogenic avian influenza (LPAIV) H9N2 virus was first reported and continue to circulate among the Egyptian poultry populations.
Material and Methods: Here, we bring together data, from our previous/ongoing publications, on different patterns of avian influenza virus (AIV) circulation, evolution, and emergence.
Results: In late 2016, different genotypes of HPAI H5N8 virus of clade 2.3.4.4.b were reported and continue to circulate in different locations in Egypt with an obvious seasonal pattern. Rolling correlation analysis between HPAI H5N1 and HPAI H5N8 revealed a trade-off between the number of positive H5N1 and H5N8 samples around early 2017, which suggest potential virus replacement. Since 2019, no further detection of HPAI H5N1 virus of clade 2.2.1.2 in poultry population in Egypt. In 2021-2022, HPAIV H5N1 virus of clade 2.3.4.4b was found in different wild bird species in Egypt with similar genetic patterns to H5N1 viruses circulating currently in Europe and Africa.

On the other side, low pathogenic avian influenza (LPAI) H9N2 virus continues to circulate among poultry population in Egypt since its first report in 2011. Different genotypes and variants were reported by our group. Furthermore, the pathogenicity of three genetically distinct Egyptian LPAI H9N2 viruses was assessed by experimental infection in chickens. Whole-genome sequencing revealed that H9N2 virus of the Egy-2 G1-B lineage (pigeon-like) became the dominant circulating H9N2 genotype in Egypt since 2016. Considerable variation in virus shedding at day 7 post infections was detected in infected chickens, but no significant difference in pathogenicity was found between the infected groups.
Conclusion: These finding underlines the importance of active surveillance in the timely detection of new AIV subtypes, monitoring virus evolution and have important consequences for effective strategies for disease control. Finally, it is important to reconsider biosecurity measures, live bird trading and marketing practices in Egypt.

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Efficient replication of avian and mammalian influenza A viruses in bovine airway epithelial cells

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Background and Objectives: Unlike pigs, which are well-known to be an important mixing vessel for influenza A viruses (IAV), cattle have historically been of much less concern for interspecies transmission of IAV, despite their close contact with humans. The highly pathogenic avian influenza virus (HPAIV) pandemic, that has emerged in recent years, has been accompanied by an increasing number of transmissions to various mammal species, including marine mammals, carnivores, and, in very rare cases, also humans. The recent transmission and subsequent spread of HPAIV into dairy cattle in several US states has drawn increasing attention to the role of cattle as hosts for influenza viruses and a possible implication of cattle in zoonotic IAV infections.
Material and Methods: To study the susceptibility of bovine airway cells to infection by avian and mammalian IAV, we applied bovine airway epithelial cells for the investigation.
Results: Infection of these cells by different avian, swine and human influenza A virus strains showed efficient replication. Next step, we intend to apply bovine well- differentiated bovine airway cells, an air-liquid-interface (ALI) culture system of filter-grown epithelial cells containing ciliated, mucus-producing cells, basal cells and club cells for the investigation. The release of infecious virus, loss of ciliated cells and the ehickness of epithelial cell layers need to be characterized in more detail.
Conclusion: Taken together, the results of this study show that bovine airway epithelial cells are susceptible to various avian and mammalian influenza A viruses and suggest that the role of cattle as hosts of influenza A viruses and their implication in the transmission of IAV to other animal species and humans should be reconsidered.

Exploring the Influence of Environmental and Climatic Factors on Highly Pathogenic Avian Influenza Outbreaks in Italy

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Background and Objectives: Highly pathogenic avian influenza (HPAI) represents a recurring threat in north-eastern Italy, significantly impacting the poultry sector and wild birds. The variability observed in the recent epidemics prompts questions on the underlying dispersal dynamics of the disease and the environmental factors impacting it. Using a Bayesian phylodynamic framework, this study assesses the impact of key factors on the dispersal history and dynamics of the viral lineages in the region.
Material and Methods: Genomic sequences and epidemiological data from outbreaks in wild birds and poultry in north-eastern Italy, and data including poultry population, elevation, temperature, vegetation, and land cover, were collected since 2016. Continuous phylogeographic analyses using the relaxed random walk diffusion model implemented in BEAST 1.10, and the "seraphim" R package were used to estimate the lineage diffusion velocity and investigate the factors impacting its heterogeneity.
Results: Land cover, elevation and proximity to wetlands resulted associated with the viral lineage dispersal velocities, calling for better insights into wild bird ecology and further investigations of the potential HPAI drivers in the region. However, the observed variations in the dynamics among the considered epidemics suggested the potential presence of additional drivers not included in our analyses.
Conclusion: Our findings help elucidate factors influencing the dispersal dynamics of the HPAI viruses. Nevertheless, other key aspects need to be considered in further research, including wild bird movements, interactions between wild and domestic populations, presence of rodents and/or insects that may mechanically spread HPAI. Furthermore, dynamics at the farm level should be investigated, including biosecurity, vehicle and personnel movements, and farm management practices. Overall, our study illustrates the utility of a multi-faceted approach, integrating genetic and epidemiological data, for a holistic understanding of HPAI ecology. Such comprehensive insights are necessary for designing effective prevention and response strategies, to mitigate the impact of HPAI.

Farm level risk factors for highly pathogenic avian influenza in Swedish poultry

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Background and Objectives: Detections of highly pathogenic avian influenza (HPAI) in wild birds, poultry and mammals have increased globally in recent years. Since 2020, 24 outbreaks were confirmed in Swedish poultry, and 2,385,000 birds died or were depopulated. The outbreaks were caused by indirect transmission from wild birds, but the mechanisms at the wild bird-to-poultry interface are not fully understood. The aim of this study was to investigate farm-level risk factors for introduction of HPAI to Swedish poultry. Additionally, biosecurity practices were assessed to identify challenges and refine recommendations.
Material and Methods: A case-control study was designed, where the case farms had outbreaks of HPAI in November 2020 to December 2022, and matched control farms were located within 10 km of an outbreak farm. The inclusion criteria were to keep more than 2000 birds of either laying hens, pullets, broilers, turkeys, or parent-breeding flocks. This resulted in 18 outbreak farms and 58 non-outbreak farms eligible to be included in the study. All farms were in either Kalmar, Skåne or Östergötland counties. Information on biosecurity practices was gathered using on-farm observations and face-to-face interviews with farmers or staff. The questions and observation points were based on the Biocheck.UGent™ questionnaire (https://biocheckgent.com/) and our own questionnaire focused on HPAI epidemiology. All farms were visited by the first author between May and November 2022, and questions focused on the situation during the months prior to HPAI outbreak on the farm or in the area. Data analysis was performed in R and is still in progress. We first did univariate logistic regressions. Then, we selected variables with p-value < 0.2 and additional variables of interest for multivariate logistic regression. Akaike information criterion (AIC) was used for model selection.
Results: Forty-eight farms participated in the study of which 15 were cases and 33 controls. Variables with p<0.2 in the univariate analysis are available in Table 1. In the preliminary multivariate analysis, the following variables were retained in the model: "ducks, geese or swans noted within 500 m from poultry house", "straw, hay or roughage provided", and "number of poultry houses on the farm". There were no significant differences in total or external score according to Biocheck.UGent™ between cases and controls.
Conclusion: Factors associated with increased risk of outbreaks were identified, however no significant differences in biosecurity practices were noted between cases and controls. Combining our results with other risk factor studies could provide a more complete picture of the relationship between biosecurity and outbreaks.

Table 1: Variables with p<0.2 in univariate logistic regression, based on odds of HPAI outbreak

Variable	OR	95% CI	*p*-value
Ducks, geese or swans noted within 500 m from poultry house	4.69	1.31-18.29	0.02*
Number of poultry houses on the farm	1.30	1.02-1.97	0.09
Two or more languages used in the daily work	2.31	0.67-8.40	0.19
Straw, hay or roughage provided	2.31	0.67-8.40	0.19
Temperature (°C) in the poultry house, minimum level during period	0.89	0.76-1.03	0.12
Netted wall ventilation inlets, without inlet cover or light trap	3.88	0.58-32.32	0.16
Affiliation to biosecurity programme	0.26	0.03-1.74	0.16
Non-heated poultry house	4.0	1.14-15.7	0.04*

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Zoorganoids Biobank, an animal organoid repository for the study and prevention of epizootic and zoonotic diseases.

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Background and Objectives: Animals play a crucial role in the transmission of high-risk pathogens and zoonotic diseases. However, determining the susceptibility of different animal species to various pathogens is challenging, due to limited sample availability and the unpredictable nature of obtaining samples. In this context, organoids offer a promising approach to studying new infectious diseases because of their physiological relevance and long-term maintenance capability. While mouse and human-derived organoids have been extensively used in biomedical research, those from livestock and wildlife species remain largely unexplored. Thus, we aimed to generate a comprehensive repository of organoids from various animal species, to serve as experimental models for infectious disease research.
Material and Methods: We collected tissue samples from the respiratory and intestinal tracts of different livestock and wildlife animals (free-ranging and captive) in Catalonia, Spain. Tissues underwent enzymatic digestion or calcium chelation, and epithelial cells were then embedded in Matrigel to generate organoids. Organoids were seeded as monolayers on Matrigel-coated plates or transwells to access to the apical surface of the cells. These monolayers were further characterized and infected with various highly pathogenic viruses, including influenza viruses and different coronaviruses.
Results: We successfully generated organoids from different parts of the respiratory and intestinal tracts of numerous animal species (i.e., pig, chicken, mink, buzzard, alpaca, red panda, gazella, monkey, roe deer or wolf). These organoids accurately represent the cellular diversity and organization of their corresponding in vivo tissue. By infecting the organoids with different pathogens, we identified variations in susceptibility to highly pathogenic and zoonotic viruses, such as influenza and SARS-CoV-2, across different animal species.
Conclusion: Here we report the establishment of a comprehensive biobank of respiratory and gastrointestinal organoids derived from livestock and wildlife animals. Our organoid biobank serves as a valuable resource for the detection, prevention, and treatment of current and emerging infectious diseases.

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Session 6

Avian influenza

Poster Presentations

A Scoping Review and Narrative Synthesis on Environmental Sampling for Avian Influenza Surveillance in Wild and Domestic Settings and their Interface

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Background and Objectives: The use of environmental sampling for surveillance of pathogens at a population level has become increasingly attractive for their ease-of-use, flexibility, cost-effectiveness. The present study originates from a wider research project on innovative surveillance methods for avian influenza viruses (AIVs) in different environments: wild, domestic and their interface. The development of environmental sampling strategies to complement standard animal sampling might contribute to early detecting AIVs and to the following epidemiological investigations. Our aim is to review the existing literature on environmental sampling in surveillance for AIVs in wild and domestic birds, to improve sampling protocols considering different application scenarios.
Material and Methods: Following 2020 PRISMA guidelines, a scoping review was carried out between February and March 2024. The research was conducted on 3 different databases: Web of Science, Pubmed, and Scopus. All the relevant information were reported in a table and the data were extracted based on: environment (i.e, wild, domestic, interface and experimental); matrix, year, geographic area, AIV pathotype and subtype, and the analytical methods.
Results: A total of 242 scientific papers were screened by title and abstract. After the screening process, 67 documents and 20 additional papers, included through citation chasing, were selected for full text reading. The most common environmental matrices for early detection of AIVs in domestic settings (e.g. poultry farms) are air, dust, feathers and faeces. Water is the most frequent matrix when AI is investigated in wild bird environment (n=37/87 papers), with diverse sampling designs developed to identify AIVs also in biotic matrices (e.g. aquatic organisms and insects). Positive AIV detections by molecular methods were indeed reported in shrimps, crayfish and molluscs, as well as in aquatic plants, mosquitoes and blowflies. AIV genome was found in natural freshwater, brackish and wastewater. The persistence of AIV in water sources was reported to be influenced by several environmental factors, including temperature, pH, salinity. The predominant use of real-time RT-PCR as the primary detection method confirms its effectiveness in research related to influenza in aquatic environments.
Conclusion: The current study highlights the relevance of environmental sampling in complementing surveillance of AI on different matrices while identifying key areas that warrant detailed investigation, including water and biotic matrices. However, the lack of standardization of sampling and diagnostic protocols poses challenges to their wider implementation, data interpretation and comparison. We recommend the elaboration of internationally agreed guidelines on standardized sampling and diagnostics protocols to promote innovative surveillance methods for avian influenza surveillance.

AVIAN INFLUENZA - HIGHLY PATHOGENIC VIRAL SEROTYPES CIRCULATING IN EUROPE, DURING 01.01.2019 – 31.12.2023

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Background and Objectives: A large number of bird species as well as other domestic and wild mammals, including humans, are susceptible to avian influenza virus (AIV) infection. The natural reservoir of the virus is represented by wild aquatic birds that play a decisive role in its circulation. The increased reassortment capacity of avian influenza viruses, when they co-infect the same cell in the host organism, causes the appearance of new viruses that can present pandemic potential. Highly pathogenic viral serotypes (HPAIV) H5 and H7 possess a number of polybasic amino acids at the hemagglutinin cleavage site cleaved by ubiquitous proteases, leading to systemic viral multiplication. The weakly pathogenic viral serotypes (LPAIV) possess a number of monobasic amino acids at the hemagglutinin cleavage site recognized by proteases located in some mucosal tissues and viral replication is limited to the respiratory and intestinal tracts. From the analysis of the data on the confirmed, reported and registered cases in Europe between 01.01.2019 and 31.12.2023, it follows that more than 10,500 events caused by AIV infection were registered, most of them caused by HPAI viral serotypes. In 2019, the circulating serotype was represented by HPAI H5N8, H5 and H5N6; in the year 2020 the predominant circulating serotype was HPAI H5N8 and few events determined by the HPAI H5N1 serotype, in the year 2021 the circulating serotypes HPAI H5N1 and H5N8 determined the majority of events; in 2022 and 2023, the frequency of events caused by HPAIV remained high in Europe, the predominant viral serotypes were HPAI H5N1.

The objective of the study: to sketch an overview of the evolution of circulating AIV HPAI strains and the affected species, which will help us to better understand the viral ecology, to predict possible developments regarding the spread of the disease in certain geographical areas and to identify and apply measures early prevention of contamination, to control threats to animal health, including humans.
Material and Methods: The material is represented by avian influenza events confirmed and registered in the empres-i database, using descriptive statistics and approaches regarding the time and space evolution of AIV HPAI strains.
Results: In the second part of the analyzed period, the viral serotype HPAI H5N1 determined the majority of events.
Conclusion: Forecasting the possible evolution of diseases caused by AIV can help to identify and take early measures to limit the spread of the virus and to help control the risk caused by the dissemination of AIV.

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High seroprevalence of antibodies to Avian Influenza viruses among wild waterfowl in country of Georgia

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Background and Objectives: Serology surveillance of pathogens provides information about the immune response, aiding in understanding the dynamics of virus circulation in birds. In this study we estimated the prevalence (presence of detectable antibodies in serum) of avian influenza viruses (AIV) among waterfowls in different region of Georgia.
Material and Methods: A total 222 serum samples were obtained from wild birds during Spring and Autumn of 2022-2023 in the most important wetland in the country of Georgia, Javakheti uplands and the Black Sea coast. For the detection of antibodies to the nucleoprotein of the Influenza A virus in multiple species an individual serum samples were screened in duplicate by using a commercial ID Screen Influenza an Antibody Competition Multi-Species Competitive ELISA kit (Sensitivity - 100% and Specificity - 96%). Testing was conducted at the R. Lugar's Center for Public Health Research, at the laboratory of the National Center for Disease Control and Public Health of Georgia
Results: As a result, the majority of the samples from 222 mallard and sentinel ducks - 167 (75%) were positive for ID Screen Influenza A Antibody. In the same duck samples positive results on the M gene were obtained just from 128 samples by RT-PCR. Our results, in combination with molecular methods, confirms, that the number of birds that have experienced previous AIV infections is much higher than that indicated by current prevalence rates, those based on RT-PCR from rectal and tracheal swab samples.
Conclusion: Our preliminary findings indicating low current infection rates but high seroprevalences imply that the majority of infections in our sample's birds happened at different periods of the year. With our results, further research is warranted because species with low shedding rates but high seroprevalence may be potential carriers of HPAIV.

Funding source/acknowledgements (optional): The project - "The Mediterranean and Black Sea Flyway: Transboundary Determinants of Avian Zoonotic Infectious Diseases" funding by the Defense Threat Reduction Agency (DTRA)

Investigating the Genetic Diversity of Avian Influenza Viruses in the Northern Irish Wild Birds from 2017-2024

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Background and Objectives: Passive surveillance of wild bird species for avian influenza virus (AIV) is routinely conducted in Northern Ireland as a means of assessing threat to the poultry industry. Poultry farming in Northern Ireland is the largest independent employer in the province which supplies upwards of 30% of all poultry meat consumed within the United Kingdom. This is a measure of the threat posed by wild bird AIV reservoirs to an ultra-high-density poultry farming industry.
Material and Methods: Using a highly multiplexed RT-PCR-based library prep, for rapid and unbiased influenza virus sequencing, we have characterized the genetic evolution of AIV in wild birds in Northern Ireland from 2017 onwards.
Results: Detections of AIV in wild bird species have slowly escalated since 2017. AIV wild bird detections culminated with a major low pathogenicity (H6N1) AIV incursion in Northern Ireland (extending to Great Britain and the Republic of Ireland) in early 2020 followed by the first recorded detections of highly pathogenic (HPAIV) in Northern Ireland, H5N8 and the subsequent H5N1 epizootic. Full genetic assessment during the epizootic was focussed on detections in commercial and backyard poultry. Significant genetic analysis of AIV in wild birds was not conducted and as such, details on genotypes prior to and during the epizootic were not catalogued.
Conclusion: Here, we perform a retrospective analysis of AIV genetic diversity during this period to add regional context to incursions. For the first time we detail a snapshot of the AIV genetic diversity circulating in wild birds in the province prior to and during the HPAIV H5N1 epizootics across 2021 and 2022.

Funding source/acknowledgements (optional):
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Poultry vector vaccines: innovative serological assays for vaccination monitoring and DIVA testing for H5 avian Influenza A.

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Background and Objectives: Influenza viruses belong to the family Orthomyxoviridae. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further divided into subtypes based on their Hemagglutinin and Neuraminidase antigens. Some subtypes containing H5 or H7 are associated with highly pathogenic forms of the disease and high rate of mortality. A current H5 HPAI lineage has been circulating world-wide since 2004 and has been responsible for important poultry losses. To control poultry disease, vaccination is more and more used, especially with recombinant vaccine technology. In the last 5 years, successive waves of H5 Influenza in Europe pushed the health authorities to review their vaccination strategy concerning this virus. Given the need for rapid and reliable serological tools for monitoring of vaccination, IDvet has developed unique indirect ELISAs: one, based on H5 recombinant protein, for the monitoring of recombinant vaccines, and one, based on NP protein, for DIVA strategy (differentiated Infected from Vaccinated Animals).
Material and Methods: Difference species of ducks (France)vaccinated with an in-activated sub-unit AIV-H5 vaccine were tested.

Antibody titers for H5 were evaluated using IDvet optimised H5 iELISA. Samples were also tested with the NP iELISA to monitor field challenge. For each tested flock, the following parameters were measured: mean titers, minimum, maximum, and CV%. All samples with titers higher than 732 for H5 iELISA, and higher than 668 for NP iELISA, were considered positive.
Results: All the flocks vaccinated with H5 vaccines were found positive with the H5 iELISA. Therefore, the positivity of the H5 iELISA, belonging to negative flocks with the NP iELISA, demonstrated the detection of seroconversion induced by vaccines. A preliminary baseline for inactivated sub-unit AIV-H5 vaccination was established thanks to these results.
Conclusion: The H5 indirect ELISA presented is the only quantitative test for the specific detection of H5 antibodies which allows for H5 vaccination monitoring. This H5 iELISA is suitable for the monitoring of inactivated sub-unit AIV-H5 vaccine in ducks. The NP indirect ELISA is an excellent tool for the detection of wild virus in populations vaccinated with recombinant H5 vaccine.

Results of avian influenza surveillance in the Vojvodina Province of Serbia in 2023

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Background and Objectives: Avian influenza (AI) is a highly contagious disease affecting birds, leading to significant health and economic losses in the poultry industry. As a zoonotic disease, it poses an ongoing threat to human health. Additionally, there have been increasing reports of AI infections in carnivores, and recently the virus has caused outbreaks on several cow farms.
Material and Methods: In Serbia, continuous passive surveillance for AI, as mandated by the Animal Health Measures Program, involves examining wild birds found dead and domestic poultry flocks with increased mortality rates for the presence of the virus. An active AI surveillance program was also conducted in 2023 across Serbia, testing cloacal swabs from domestic poultry in household backyards (free range animal holdings) and small village markets, as well as from trapped or hunted wild birds and their feces. Detection and subtyping of AI virus isolates were performed using molecular diagnostic methods, including real-time RT-PCR, conventional RT-PCR and genome sequencing.
Results: In Vojvodina, a province of Serbia, 840 pooled samples of cloacal swabs from backyard poultry across 360 locations were tested during active surveillance. Additionally, 61 pooled samples of cloacal swabs from backyard poultry and ornamental birds collected at local bird markets, as well as 120 fresh fecal samples and 198 cloacal swabs from wild birds from 30 locations across all 7 districts of Vojvodina Province were tested. All samples from backyard poultry tested negative for AI. However, HPAI H5N1 was detected in cloacal swabs from one wild goose, one mallard, two common pochards, and three river gulls. Additionally, AI virus, not of the H5 or H7 subtype, was found in fecal samples from one wild goose. During passive surveillance, 61 carcasses suspected of having AI were tested, with 30 found positive for HPAI H5N1. Among these, four swans and six seagulls from the South Bačka District, three swans from the Srem District, two swans, two backyard chickens, and seven common cranes from the Central Bačka District and six common cranes from the North Banat District were confirmed positive. There was an HPAI H5N1 outbreak in the Central Banat and North Banat Districts, where common cranes were most affected. In 2023, no large poultry farms were infected, resulting in no significant economic losses.
Conclusion: Given these findings and the ongoing unfavorable AI situation across Europe, it is essential to continue implementing a program-based surveillance and early detection system for influenza viruses in Serbia.

Funding source/acknowledgements (optional): This work was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2024, Contract No: 451-03-66/2024-03/200031.African swine fever in Northern Uganda: how smallholders understand and deal

Session 4B

African swine fever II

Oral Presentations

Detection of infectious African Swine Fever Virus within spiked materials after incubation at different temperatures for different lengths of time

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Background and Objectives: Introduction of African Swine Fever Virus (ASFV) into a herd can (among other routes) happen via contaminated fomites. In order to prevent further spread of ASFV worldwide, risk assessments must be done based on the available data for virus survival. Therefore, it is important to collect datasets for virus survival in various potential fomites and to develop tools to easily generate these datasets.

ASFV is known to retain infectiousness and viability for extended amounts of time, but these depend on the type of material and environmental factors such as heat and moisture. The currently available assays to detect infectious virus (cell-culture and qPCR) have inherent disadvantages. Cell culture-based assays – i.e. primary cell culture using porcine lung macrophages (L-PPAM), are sensitive to contaminants and disturbances in cell homeostasis, which can arise from incubation with cell-toxic (but not necessarily virus-toxic) components present in the assayed materials. An alternative method is to use qPCR, which will detect ASFV DNA with high sensitivity and specificity, but does not necessarily correspond to the presence of infectious virus.
Material and Methods: In this study, four different materials present on a pig farm, i.e. faeces, straw, wood shavings and mixed feed, were investigated for their impact on ASFV viability when spiked and incubated with the virus for different times at different temperatures in a humid environment and in the presence of porcine serum. ASFV infectiousness in the samples was investigated using the currently available methods of IPT/PLA test (a staining method using L-PPAM) and qPCR.
Results: Our results showed that infectious virus in the spiked materials could be detected after incubation for up to ten days at the lower temperature range (4°, 22° and 37° C), for up to a few hours at 50° or 60° C, but only for a few minutes at 70° C.

In the positive control (virus in medium with serum), infectious virus was detected up until the end of the experiments, i.e. sixty days at 4°, 22° and 37° C and for two days at 50° C and one day at 60° C. In the experiment performed at 70° C, no infectious virus was found after the five minutes sampling time.
Conclusion: The samples generated and tested in this study will be available for future analysis with new potential qPCR assays for ASFV viability that are currently being developed.

Funding source/acknowledgements (optional): This work was supported by the Danish Swine Levy Foundation (Svineafgiftsfonden).

African swine fever in Northern Uganda: how smallholders understand and deal with the virus

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Background and Objectives: Globally, domestic pigs in smallholder settings account for most of the spread of African Swine Fever (ASF), and it is in these systems that the disease has proven most difficult to control. In situations of chronic poverty, which is the reality for many smallholders, low investments in pig farming leads to low levels of biosecurity, which in turn leads to high risk of spreading and attaining pig diseases, resulting in low and insecure income and livelihood shocks. In addition, lack of and inconsistent advice from veterinarians and other actors is an important component for limited local understanding of how the disease spreads and how to reduce risks for spread. Poverty and marginalization are thus both important consequences of animal disease and reasons why smallholders fail to implement preventive and control recommendations.

In the present study we wanted to dig further into 1) how smallholders understand ASF and relate it to other challenges in pig production, and 2) why pigs are important to smallholders in the study region and what it is that makes people persistently restock pigs despite the challenges with repeated ASF outbreaks.
Material and Methods: This presentation reports on a study with smallholder pig farmers in five sub-villages of one village in Northern Uganda where ASF is endemic. The study is based on semi-structured interviews with 36 farmers who had or had until recently had pigs, two focus groups discussing challenges in pig farming and solutions proposed by farmers, and a short survey with 99 smallholders who lost pigs in an outbreak of ASF in the village that erupted just after the semi-structured interviews and focus group discussions had been conducted. The data was collected between January and May 2024.
Results: Previous research from the area concludes that: smallholders have experienced repeated ASF outbreaks, biosecurity measures are rarely implemented, access to veterinary advice is limited and most smallholders do not have contact with a person with veterinary training. The present study will contribute knowledge about smallholders understanding of ASF and their motivations for having pigs.
Conclusion: We believe that deeper understanding about these issues might lead to better communication about ASF with smallholder farmers and that smallholders might be better supported in preventing future outbreaks by building on their own motivation to succeed in pig production. We are in the process of analysing data and look forward to presenting some preliminary findings at the conference.

Estimating post mortal interval of wild boar carcasses to establish a timeline in the Swedish ASF outbreak

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Background and Objectives: African swine fever (ASF) is a viral hemorrhagic, often fatal disease with high global economic consequences. Sweden was free from ASF until September 6th, 2023 when a dead wild boar was sampled in the passive surveillance and confirmed positive for ASF virus. After a thorough search of the outbreak area, a core area was fenced to restrict wild boar movement. Estimating the time of death (TOD) of positive carcasses in an outbreak can give information that helps to understand the outbreak epidemiology. The purpose of this study was to estimate the time elapsed since death for wild boar carcasses from a ASF outbreak to discern the outbreak timeline and epidemiology.
Material and Methods: Local hunters searching for carcasses were instructed to take photographs of the carcass before it was removed from the forest, sampled, and then incinerated. Information about the habitat, stage of carcass decomposition, presence of maggots and signs of scavengers was collected when possible, using a checklist. A photo evaluation was made for all carcasses found up until October 31st. In addition, a selected carcass in advanced decomposition was evaluated using a more advanced human taphonomy model that calculated day-degrees needed to reach a certain stage of decomposition. Weather data was received from Swedish Metrological and Hydrological Institute (SMHI). Using these methods, a TOD was estimated for each carcass.
Results: Using the photo evaluation, the oldest carcass was estimated to have died between May 17th and July 7th which corresponded well to the more advanced taphonomy model, which for the same carcass indicated an interval from May 8th to June 28th. All evaluated carcasses had an estimated TOD from May to late September. No indication of active disease spread has been noted after September 2023. Using photographs of carcasses is an easy yet reliable way to estimate an approximate TOD in wild boar carcasses, if reference material is available. Severely decomposed carcasses render a wider margin of error, whereas fresh cadavers give a narrower margin of error. However, the methods used gave an estimation of time of introduction of ASF in Sweden and when active spread of disease ceased.
Conclusion: This epidemiologic information helps managers to adjust disease control efforts, to adapt applied restrictions, and can be used as support for the future declaration of freedom from disease.

No evidence of undetected infection with African swine fever virus in wild boar tested in Estonia

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Background and Objectives: African swine fever (ASF) was first detected in Estonia in September 2014, and now, ten years later the country still experiences findings of the virus in wild boar population. During these years, the pattern of the ASF epidemic has been very different, e.g. the number of cases, seroprevalence and geographical features. The peak of the ASF epidemic in Estonia was the period 2015-2017, while 9,0-11,4% of all investigated wild boar were ASFV-positive, observed mortality amongst wild boar was high and most of the regions suffered because of the disease. The same period, appeared a slight increase of seroprevalence in wild boar, from 1,0% in 2015 to 4,6% in 2018.

Despite of all efforts to eradicate the disease from the wild boar population, the country is still not free from the disease. During 18-month period, from February 2019 to August 2020, there were no ASF virus findings neither in wild boar nor in domestic pigs in Estonia. However, in August 2020, a dead wild boar in the central part of the country in a remote forest area away from the larger roads was found infected. In December 2020, the second foci of re-emergency was discovered 150 km North-East from the first, in a region where virus-positive wild boar were not detected for three years. These re-emergencies of the ASFV-positive wild boar have raised a question of the source of infection and the role of survivors, since the only evidence of the infection in these two areas were the findings of antibody-positive wild boar.
Material and Methods: In the present study, we invited hunters on voluntary bases to collect in addition to obligatory serum samples also samples from spleen. The aim was to find wild boar with the virus presence in tissues (spleen) but not circulating in blood. In the period from June 2018 to January 2021, samples from 568 wild boar were collected and investigated. The study included the virus detection from serum and spleen by RT-PCR and antibody detection from serum by ELISA/IPT.
Results: All investigated wild boar (n=568) were PCR-negative to ASFV (from both serum and spleen). Ten animals (1.76%) appeared to be survivor animals (antibody-positive by ELSA and IPT, but PCR-negative).
Conclusion: Thus, we found no evidence of animals carrying the virus only in tissues (spleen), and thus support the long-term hidden circulation of the virus in the environment.

A new highly sensitive indirect Enzyme Linked Immuno Sorbent Assay for the detection of antibodies against African Horse Sickness Virus

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Background and Objectives: African horse sickness (AHS) is an infectious, but non-contagious vector-borne disease of equids caused by the Orbivirus AHSV (family Reoviridae). The main vector are *Culicoïdes* midges. AHS is endemic in sub-Saharan Africa, but outbreaks occurred in many countries surrounding the Mediterranean Sea (Morocco, Spain, Portugal,…), in the Middle East and Asia. AHS is listed by the WOAH; guidelines for official recognition of disease-free status must be applied for international movements. Vaccination is prohibited in officially free countries.The ID Screen® African Horse Sickness Indirect ELISA kit has been developed to detect antibodies against the VP7 protein, which is conserved among all 9 AHSV serotypes. The aim of the study was the evaluation of specificity and sensitivity of the ELISA.
Material and Methods: Diagnostic specificity and sensitivity were evaluated on 1015 sera from non-infected animals (France, Brazil, Argentina, Iceland) and 26 positive sera from different reference laboratories (EU/WOAH Reference Laboratory for AHS,, Spain; the Pirbright Institute, UK; Friedrich-Loeffler-Institut, Germany). Serum samples from ruminants infected with Blue Tongue Virus or Epizootic Hemorrhagic disease Virus were tested. Results were compared to those obtained with another commercial ELISA "Kit A"
Results: The observed specificity of the ID Screen® ELISA was 100% (95%CI [99.6, 100.0], n=1015) and the observed diagnostic sensitivity was 100% (95%CI [87.13, 100.0], n=26). Sera tested in serial dilutions revealed an excellent analytical sensitivity. No-cross reaction was observed with BTV and EHDV positive sera. Evaluation of the ID Screen® ELISA was done by the EU/WOAH RL and it confirmed excellent performances of the new kit. The ID Screen® ELISA demonstrated good correlation with kit A with better analytical sensitivity.
Conclusion: The ID Screen® ELISA is a reliable and easy-to-use test for the detection of AHSV VP7 antibodies. It shows very high specificity and an excellent diagnostic and analytical sensitivity. VP7 antibody detection is the prescribed WOAH method to prove freedom of infection, estimation of prevalence and surveillance of a disease-free status.

Session 7

Vaccine development and disease control

Oral Presentations

Cross-protection induced by genotype II modified live African swine fever virus candidate vaccine(s) against genotype I but not against genotype IX.

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Background and Objectives: African swine fever (ASF) is an economically important pig disease that has led to devastation of the livelihoods of many small-holder and commercial farmers. As pork accounts for 35% of global meat intake, ASF is a threat to food security. The absence of universally approved and licensed vaccines limits control. We previously described modified live vaccine (MLV) candidates for ASF, based on genotype II, in which K145R, EP153R and/or DP148R genes are deleted and the gene coding for CD2v protein, is modified to prevent binding of the virus particle and infected cells to red blood cells (non-haemadsorbing). This reduces virus titre and persistence in blood. We showed this MLV induced dose dependent protection (83 – 100%) against homologous genotype II ASFV challenge (doi: 10.1080/22221751.2023.2265661). In Africa, twenty-four ASFV genotypes are present but information on the cross-genotype protective ability of vaccines is limited. Here, we test our genotype II MLVs ability to induce cross-protection against other ASFV genotypes.
Material and Methods: In this study, the MLV candidates were used in 2 cross-protection experiments. In the first experiment, pigs were immunized and boosted with GeorgiaΔK145RΔEP153R-CD2v_Q96R/K108D intranasally and subsequently challenged with a genotype I ASFV isolate, Benin 97/1. In the second study, pigs were immunized and boosted intramuscularly with GeorgiaΔDP148RΔK145RΔEP153R-CD2v_Q96R/K108D and subsequently challenged with either a genotype I, Benin 97/1 isolate or a genotype IX ASFV, Kenya 1033. Pigs were observed daily, and any clinical signs were scored.
Results: In the first experiment, 83% of pigs immunized intranasally with GeorgiaΔK145RΔEP153R-CD2v_Q96R/K108D, were protected against the genotype I ASFV challenge, the main circulating ASFV in west and central Africa. In the 2nd study, up to 70% of pigs immunized intramuscularly with GeorgiaΔDP148RΔK145RΔEP153R-CD2v_Q96R/K108D were protected against genotype I ASFV. However, the MLV failed to protect pigs challenged with the genotype IX isolate.
Conclusion: In summary, these results show our MLV offered cross-protection against genotype I ASFV but not against genotype IX. In ongoing research, we have constructed similar genotype IX MLVs to test protection against virulent genotype IX. Correlates for cross-protection are also under investigation.

Development of African horse sickness Disabled Infectious Single Animal (DISA)-DIVA vaccines and evaluation of safety and efficacy in IFNAR-/- mice

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Background and Objectives: African Horse Sickness (AHS) is a highly severe disease of equids (WOAH-notifiable disease, category A) caused by AHS-virus (AHSV). AHSV forms a distinct virus species within the genus *Orbivirus* (family *Sedoreoviridae*) of which bluetongue virus is the prototype orbivirus. AHSV contains a segmented genome (Seg-1 to 10) and consists of nine serotypes showing limited cross-neutralization. Marketed live-attenuated vaccines (LAVs) have multiple point mutations in several segments. Their safety is debatable due to residual virulence, reversion to virulence, and reassortment events leading to virulent AHSV-variants. Furthermore, these LAVs cannot be distinguished from field virus.
Material and Methods: An AHS vaccine platform was developed using reverse genetics according to the principles of "Disabled Infectious Single Animal (DISA)" and "Differentiating Infected from VAccinated (DIVA) as has been shown for bluetongue virus.

A cocktail of equal amounts of all nine DISA vaccines (cocktail DISA1-9) was studied in the artificial IFNAR-/- mouse model.
Results: The AHS DISA-DIVA vaccine platform is based on LAV with an in-frame deletion of 231 nucleotides (77 amino acid codons) in Seg-10 encoding dispensable NS3/NS3a protein. Exchange of Seg-2 and Seg-6 encoding serotype determining outer shell proteins VP2 and VP5 resulted in DISA-DIVA vaccine candidates for all nine AHSV serotypes, shortly named DISA1 to DISA9. The accompanying DIVA PCR-test targeting Seg-10 was also developed and differentiates all AHSV-variants from DISA-vaccines.

Mice did not develop PCR-positivity nor clinical signs after prime or prime-boost DISA-vaccination but seroconverted, including for serotype specific neutralizing antibodies. Mice were challenged with virulent AHSV4 or virulent rAHSV5. Control groups showed clinical signs, i.e. ruffled fur and weight loss, and developed viremia. AHSV4-controls also showed ocular discharge and strong reduction in mobility and were euthanized due to ethical reasons at seven days post challenge. DISA-vaccinated mice did not develop clinical signs and survived AHSV challenge. Further, all DISA-vaccinated groups (prime and prime-boost) showed extremely high Ct values (low PCR-positivity) after AHSV challenge but viremia was negative.
Conclusion: In conclusion, DISA-DIVA vaccine candidates for all nine AHSV serotypes are stably attenuated due to the common LAV-backbone and the significant deletion in Seg-10. Cocktail DISA1-9 as well as individual DISA vaccines are completely safe. DISA1-9 is protective as studied for two representative virulent AHSV serotypes. Neutralizing antibody titers indicate protection against more AHSV serotypes. Viremia in infected equids can be differentially detected by the accompanying DIVA PCR-test, irrespective of the vaccination status. Now, DISA1-9 should be studied on safety and efficacy in the equine host.

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Enhanced virus-specific T-cell responses correlate with improved performance of adenovirus vectored vaccines against genotype I African swine fever

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Background and Objectives: The ongoing African swine fever epizootic has now spread across the globe with outbreaks reported on all continents except for South America and Antarctica. Vaccines against the disease are an important control tool and modified live virus vaccines that are effective against genotype II African swine fever virus have proven to be both safe and effective in field trials in southeast Asia. However, genetically and immunologically distinct isolates circulate in Africa and the recent emergence of a hybrid recombinant virus in Asia means new vaccines are still needed.

Subunit vaccines represent an attractive approach due to their inherent safety and the ability to easily differentiate infected from vaccinated animals (DIVA). Development of such vaccines has been slow due to the complexity of the virus itself, a poor understanding of the protective immune response and a lack of non-modified live virus vaccine models for probing protective immunity. Our previous work identified a pool of African swine fever virus antigens that when delivered using replication deficient adenoviruses could protect against severe disease caused by virulent genotype I African swine fever virus. Immunisation with this pool induced African swine fever virus specific antibody and CD8 T-cell responses, but not CD4 T-cell responses, suggesting a possible route to improve vaccine performance.
Material and Methods: Virus and antigen specific immune responses were measured from biobanked samples by interferon gamma enzyme linked immunospot assays and flow cytometry to identify viral proteins that could be used to augment our current pools of antigens. These were then tested in immunisation and challenge experiments in pigs. Cellular immune responses were measured by interferon gamma enzyme linked immunospot assays and flow cytometry recall assays and antigen-specific antibody responses were determined using luciferase-linked antibody capture assays. Viral load in blood and tissues after challenge with genotype I African swine fever virus were determined using quantitative PCR.
Results: A new pool of antigens was identified that when combined with our existing pools led to enhanced CD8 responses and the induction of virus specific CD4 responses. These immune responses correlated to much improved vaccine performance after challenge, with minimal clinical signs and an absence of detectable virus in some animals.
Conclusion: The immune responses generated by these vectors represent a valuable tool to explore the protective immune response to African swine fever and provide the basis for driving vaccine development against genotype I African swine fever virus, as well as other circulating genotypes and immunotypes.

Funding source/acknowledgements (optional): Department for Environment, Food and Rural Affairs

FlagT4G vaccine confers protection against transplacental transmission of highly virulent CSFV after single vaccination in pregnant sows

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Background and Objectives: The transplacental transmission of classical swine fever virus (CSFV) and the resulting congenital persistent infection in newborn piglets have been abundantly described to happens in pregnant sows suffering virus infection. Importantly, the availability of safe commercial vaccines with proven efficacy in pregnant sows to prevent the generation of congenital and postnatal persistent infections are critical tools in controlling the disease in CSF endemic areas
Material and Methods: In this study, six pregnant sows at 44 days of gestation were used Four of them were vaccinated with the FlagT4G vaccine. At 65 days of gestation, 21 days after vaccination, pregnant sows were challenged with 10^5 TCID50 /mL of the highly virulent Margarita strain of CSFV.
Results: Here, it is shown the high efficacy of a single dose of the recombinant FlagT4G vaccine to provide solid protection in pregnant sows against transplacental transmission of a highly virulent CSFV. Pregnant sows vaccinated with FlagT4G at 44 days of gestation elicited strong CSFV-specific antibody response, with neutralizing antibody levels above those required for protection against CSFV. Notably, all foetuses obtained from FlagT4G vaccinated sows after the challenged with a virulent field isolate, lack CSF macroscopic lesions and show a complete absence of the challenge virus in their internal organs
Conclusion: Therefore, pregnant sows, safely vaccinated with FlagT4G without affecting reproductive efficacy, are efficaciously protected, along with their foetuses, against the infection and disease caused by a virulent field strain.

Safety and efficacy studies of two DIVA African swine fever vaccine candidates in domestic pigs

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Background and Objectives: African swine fever (ASF) is a devastating disease present in 77 countries and territories on five continents. There is no vaccine on the market for Europe. The VACDIVA project is working to develop a safe and effective vaccine based on the attenuated natural Lv17/WB/Rie1 (genotype II) LAV candidate. To improve safety and DIVA characteristics, two deletion mutants (DM) ASFV Lv17/WB/Rie1-ΔCD and Lv17/WB/Rie1-ΔCD-UK were made by ZZ's group at VMRI. The Lv17/WB/Rie1-ΔCD has had two genes deleted and is carrying an eGFP instead. The ASFV Lv17/WB/Rie1-ΔCD-UK was created from Lv17/WB/Rie1-ΔCD by deleting the UK gene and substituting with a dsRed marker.
Material and Methods: Both DM were tested *in vivo* in domestic pigs. Eighteen 12-week-old pigs were used. Five animals each in groups 1, 2, and 3 received 100 $TCID_{50}$ per animal of the vaccine via the intramuscular (IM) route. Group 1 was immunised with DM-ΔCD-UK, group 2 with DM-ΔCD, and group 3 with the parental vaccine strain Lv17/WB/Rie1. Three animals in group 4 served as unvaccinated controls. At 30 dpi, animals in all the four groups were challenged with 100 HAD_{50} of Armenia/07 (Arm07) via the IM route.
Results: SAFETY parameters (post-vaccination), were assessed by daily rectal temperature and clinical scoring of eight parameters (ranging from 0, 1 mild, 2 moderate, 3, severe). The Results showed a significant improvement in safety compared with the attenuated parental vaccine prototype. No fever or anorexia was observed in either mutant, and other measured symptoms such as recumbency, respiratory distress, gastrointestinal disturbances or joint swelling were virtually absent (clinical score media less than 0.5). All domestic pigs seroconverted on day 7 (parental) and between 7-14 (mutants) with high antibody titres. The use of DM-ΔCD induced a lower and shorter viremic response than DM-ΔCD-UK. EFFICACY (post-challenge): All pigs vaccinated with DM-ΔCD and DM-ΔCD-UK were fully protected against the Arm07 ASFV strain by 28 days post-vaccination, showing no fever or significant clinical signs following the challenge. Mild to moderate virus shedding was observed in a few animals. Post-mortem examinations at 30-31 days post-challenge showed no pathological changes, although mild congestion was observed in some lymph nodes and organs of several pigs. In contrast, the unvaccinated control pigs, exposed to the same virulent strain, either died or were euthanised due to severe acute clinical signs by 6 and 7 dpi, in accordance with the endpoint criteria
Conclusion: This study identified two promising DIVA ASF vaccine candidates from naturally attenuated genotype II strain

Funding source/acknowledgements (optional): This study was funded by the Horizon 2020 -862874 EU research project "VACDIVA" and the EU Reference Laboratory for ASF (EC contract, CSIC-DG SANTÉ).

Safety and immunogenicity of African horse sickness Disabled Infectious Single Animal (DISA)-DIVA vaccines in horses

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Background and Objectives: African Horse Sickness (AHS) is a highly severe disease of equids (WOAH-notifiable disease, category A) caused by nine serotypes of AHS-virus (AHSV) showing limited cross-neutralization. Marketed live-attenuated vaccines (LAVs) have multiple point mutations in several segments. Therefore, their safety is debatable due to residual virulence, reversion to virulence, uncontrolled reassortment events. Further, AHSV-infected horses cannot be differentiated from LAV-vaccinated horses (lack of DIVA). There is a desperate need for safe, efficacious, broad protective AHS DIVA-vaccine to control AHS and to improve safety of international equine trade. AHS Disabled Infectious Single Animal (DISA) DIVA vaccines for all nine serotypes, shortly named DISA1 to DISA9, are developed and a cocktail (DISA1-9) is successfully studied in the artificial IFNAR -/- mice model.
Material and Methods: DISA-vaccines were studied at CVRL in the equine target host. Ten horses were intramuscularly immunized twice at a 4-weeks interval with cocktail DISA1-9 and immunized again after one year. DISA1-9 was also studied in a field trial in Kenya.

Results: Horses did not show any adverse reaction after immunizations and remained PCR-negative. ELISA-positivity was observed in four horses as early as two weeks post prime-immunization. One week after booster immunization, all horses showed maximal blocking which slowly declined but remained ELISA-positive (mean blocking of 63%) after one year. Blocking was maximal again after re-immunization. Neutralizing antibody titers (nAbs) varied between individual horses and serotypes, however, all horses except one were positive for all serotypes at eight weeks post boost immunization. nAbs slowly declined and increased after re-immunization.

28 horses (Group 1) without known previous vaccinations and ELISA-negative (<50% blocking) were intramuscularly vaccinated twice (four weeks apart) with DISA1-9. 143 horses (Group 2) previously vaccinated with a cocktail of nine inactivated AHSV serotypes were ELISA-positive. These horses were intramuscularly vaccinated once with DISA1-9. No side effects were reported for both groups. Group 1 showed increased % of blocking after prime-vaccination (24±12 to 79±16) which not further increased after boost-vaccination (79±8). Group 2 showed no significantly increase % of blocking after vaccination (79±8 to 87±5). One previously vaccinated horse in Group 2, despite complete seroconversion by ELISA by inactivated vaccine and booster with DISA1-9, became infected by AHSV-9, developed AHS fever but survived.
Conclusion: In conclusion, DISA1 to DISA9 and the DISA1-9 cocktail vaccine are completely safe and induce nAbs against respective AHSV serotypes suggesting broad protection by DISA1-9. Hence, efficacy of DISA1 to DISA9 should now be studied by vaccination-challenge trials in horses.

Funding source/acknowledgements (optional): Central Veterinary Research Laboratory, Dubai, United Arab Emirates
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To cast virus before pigs and still not make them sick: The unsuccessful story of intra-oropharyngeal infection with foot-and-mouth disease virus

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Background and Objectives: Foot-and-mouth disease virus (FMDV) causes vesicular disease in cloven-hoofed animals. Experimental FMDV challenge in swine typically involves intradermal injection into the heel bulb (IDHB) or contact exposure. Contact exposure models mimic natural infection but lacks dose control. While IDHB ensures repeatability, it bypasses mucosal immunity. Standardized models for inoculation of swine with FMDV are crucial for research and vaccine testing, but require consistency and a reproduction of natural infection dynamics. During the development of an orally administered live-attenuated vaccine, pigs were exposed to a genetically engineered infectious FMDV clone by intra-oropharyngeal inoculation (IOP), a simulated natural route previously described by Stenfeldt et al. 2014, in comparison to the standard IDHB method.
Material and Methods: Pigs were inoculated with 10^3 to 10^6 $TCID_{50}$ of FMDV (as determined on LFBK cells). Sedated pigs were placed in dorsal recumbency and 2 ml of inoculum was directly deposited onto the tonsil of the soft palate with a blunt cannula. As a control, pigs were inoculated by IDHB with 10^3 and 10^5 $TCID_{50}$. Swabs and serum samples were taken regularly to document the course of infection. Pigs were euthanized at the onset of clinical FMD or at the end of the experiment 10 days after infection.
Results: While the first IDHB pigs infected with 10^3 $TCID_{50}$ developed fulminant clinical FMD after 48 hours, no clinical disease was found in the IOP groups at doses of 10^3 to 10^5 $TCID_{50}$. Swab samples were negative over the entire trial and neither FMDV RNAemia nor specific antibodies were detected. One IOP pig infected with a dose of 10^6 $TCID_{50}$ showed first clinical signs of FMD 4 days after infection; the other 5 pigs in the group did not develop FMD lesions until at least two days later. As all animals were kept in the same pen, horizontal transmission is likely and the staggered onset of disease suggests that only the first was actually infected by the IOP inoculation itself. Thus, IOP inoculation was both less consistent than IDHB and required a 1000-fold higher dose of virus.
Conclusion: Our findings do not support intra-oropharyngeal infection as a viable alternative to the established IDHB method, at least not for a virus strain not adapted to pigs. Following tests must show whether a pig-adapted strain is better able to overcome the mucosal barrier.

Funding source/acknowledgements (optional):
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Vaccination of zoo birds against highly pathogenic avian influenza viruses (H5N1) using propagation-defective RNA replicon particles

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Background and Objectives: The recent panzootic by highly pathogenic avian influenza viruses (HPAIV) of subtype H5N1 (clade 2.3.4.4b) posed a serious threat to wild bird populations as well as to domestic poultry. Several European zoos reported H5N1 infections of captive birds which were kept in natural pond systems frequently visited by wild birds. The prolonged mandated quarantine or confinement of the zoo birds often resulted in serious health problems. Here, we report on the experimental vaccination of captive wild birds using a propagation-defective vesicular stomatitis virus (VSV) vector.
Material and Methods: We generated a recombinant VSV vector in which the essential VSV glycoprotein (G) gene was replaced with the modified hemagglutinin (H5) gene derived from A/Pelican/Bern/1/2022 (H5N1). The resulting VSVΔG(H5) vector was unable to replicate in an autonomous fashion and was therefore produced on helper cells providing the VSV G protein *in trans*. In total, 317 birds at Bern Animal Park and Zoo Basel, representing 24 different species including Greater flamingos, Dalmatian and Eastern white pelicans, and African penguins, were vaccinated twice with VSVΔG(H5) by intramuscular injection. Immune sera were analysed by ELISA and virus neutralisation test using a surrogate reporter virus that was handled under biosafety level 2 conditions.
Results: No adverse effects directly related to the vaccination were observed indicating that the vaccine was well tolerated. RT-qPCR analysis of pond water samples did not reveal any evidence for shedding of the vector vaccine into the environment. Immunized animals were serologically differentiated from infected ones using a standard NP-ELISA. Birds without previous contact to H5Nx viruses produced high to very high H5Nx-neutralizing antibody titers following the second immunization, while birds showing H5-specific antibodies prior to vaccination, already developed high antibody titers after a single immunization. One year after the first immunization, the immunized animals will be sampled again in order to figure out for how long the neutralizing antibody titers last. At present, we are investigating whether intramuscular and/or mucosal immunization with VSVΔG(H5) will protect chickens against infection with a lethal dose of A/Pelican/Bern/1/2022 (H5N1), and whether the immunized animals will still excrete challenge virus.
Conclusion: In summary, our results indicate that VSVΔG(H5) is an extraordinary safe and highly efficacious marker vaccine for the protection of various bird species against HPAIV. Further development of this prototype vaccine in particular with respect to mucosal administration routes may allow mass application of this vaccine in future.

Funding source/acknowledgements (optional):
Foundation Animal Hospital Basel

Session 7

Vaccine development and disease control

Poster Presentations

Assessing the efficacy of a simplified vaccination approach against Newcastle disease in Danish commercial layers

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Background and Objectives: Newcastle disease is a notifiable avian disease responsible for several panzootics, which has resulted in the establishment of mandatory vaccination programs against the virus in several countries including Denmark. This study aimed to compare the immune response elicited by the Danish mandatory vaccination program with a simplified version of the vaccination program. The two vaccination programs were compared according to level of protection, antibody titers, onset of immunity and duration of immunity.
Material and Methods: A commercial flock of layers was followed from hatching to culling. The flock was divided into two groups according to vaccination programs and kept in separate housing on the same farm. One group was immunized with the standard program of the farm, based on the mandatory program; a vector vaccine (as day-old), a live vaccine (administered twice; at 3 and 9 weeks of age) and an inactivated vaccine (at 17 weeks of age). The other group was immunized with a newly marketed vector vaccine (as day-old) and the same inactivated vaccine as the first group (at 17 weeks of age). Blood samples were collected from 30 randomly selected layers in each flock at 5, 8, 11, 14, 17, 24, 44, 64 and 77 (culling) weeks of age for ELISA and hemagglutination inhibition tests.
Results: This study is blinded to conceal which group received which vaccination program with the blinding set to be lifted when all data analyses are final, which is well before the start of the Epizone conference. The blinded results show a difference in the onset of immunity between the groups with one group demonstrating detectable immunity at 5 weeks of age (97-100% protection), while it took 14 weeks of age for the other group to reach a similar level of protection. Significant differences in antibody titers between the two groups were observed at most sampling time points, and for both groups, antibody levels began to decline after 24 weeks of age.
Conclusion: While the immune responses of the two groups were very comparable and both elicited strong immune responses lasting until at least 77 weeks of age, significant differences were observed in the onset of immunity and in antibody titers at several time points. For both groups the antibody titers peaked at 24 weeks of age followed by a steady decline until culling.

Funding source/acknowledgements (optional):
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High efficacy in Cattle of Bultavo 3 vaccine against BlueTongue virus serotype 3

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Background and Objectives: The Blue Tongue Virus (BTV) is the causative agent of a severe seasonal transboundary diseases affecting ruminants. The recent BTV serotype 3 (BTV3) outbreak, rapidly spreading through Europe, has a significant impact on animal health and is causing severe losses for farmers. In the absence of cross-protection by existing vaccines, a BTV3 vaccine strain entered quickly in development phase to respond to this emergency. This new vaccine has been industrialized for mass production and tested to confirm efficacy against the currently circulating BTV3 strain.
Material and Methods: BTV3 inactivated vaccines were administered to 2 groups of 6 calves with 2-shots, 21 days apart. The vaccines were formulated at low payload with 2 different antigen batches and adjuvanted with aluminum hydroxide and saponin. One group of 6 non-vaccinated animals served as control. All animals were challenged 21 days after vaccination with a virulent BTV3 heterologous strain, isolated from the 2023 outbreak in The Netherlands. The rectal temperature and clinical signs were monitored daily for 14 and 21 days, respectively, in all animals and viraemia was measured by a BTV3 specific qRT-PCR until 21 days post-challenge.
Results: Clinical signs of the BTV disease were observed following challenge in 4 out of 6 control group animals. All 12 vaccinated animals were protected from development of clinical signs of BTV3 disease. Presence of BTV3 RNA was detected in blood samples from all 6 control animals. In the 12 vaccinated calves, no viremia was detected in any animal.
Conclusion: In conclusion, the efficacy of this new vaccine against BTV3 was successfully demonstrated after challenge in calves. Bultavo 3, with a 2-shots regimen for cattle vaccination, is suitable for active immunization of target species older than 23 days of age and resulted in a complete prevention of clinical signs and viraemia. Vaccination with Bultavo 3 fully prevents the risk of disease transmission through midge bites. With this new vaccine, farmers can not only protect their herds but also prevent the BTV3 outbreak to further progress. Indeed, the prevention of viraemia in cattle is the cornerstone to block the transmission cycle of the virus.

High efficacy in Sheep of Bultavo 3, a new vaccine against BlueTongue virus serotype 3

Jiří Nezval[1]
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Background and Objectives: The Blue Tongue Virus (BTV) is the causative agent of a severe seasonal transboundary diseases affecting ruminants. The recent BTV serotype 3 (BTV3) outbreak, rapidly spreading through Europe, has a significant impact on animal health and is causing severe losses for farmers. In the absence of crossprotection by existing vaccines, a BTV3 vaccine strain entered quickly in development phase to respond to this emergency. This new vaccine has been industrialized for mass production and tested to confirm efficacy against the currently circulating BTV3 strain.
Material and Methods: BTV3 inactivated vaccines were administered to 3 groups of 6 lambs (26 to 31 days old). The vaccines contained 3 different antigen payloads and were adjuvanted with aluminum hydroxide and saponin. One group of 6 non-vaccinated animals served as control. Vaccinated animals were monitored for possible local and systemic reactions. All animals were challenged 21 days after vaccination with a virulent BTV3 heterologous strain, isolated from the 2023 outbreak in The Netherlands. The rectal temperature and clinical signs were monitored daily for 16 days in all animals and viraemia was measured by a BTV3 specific qRT-PCR.
Results: No vaccinated animal displayed local or systemic reactions after the vaccination in any of the 3 vaccinated group during the observation period. Following challenge, clinical signs of the BTV disease were observed in all control group animals. One animal of the control group had to be euthanized on ethical grounds. All vaccinated animals were protected from development of clinical signs of BTV3 disease, regardless of the administered dose. Presence of BTV3 RNA was detected in blood samples from all control animals and in two animals from the low-dose vaccinated group and in one animal from the mid-dose vaccinated group. In the group vaccinated with the highest antigen content, no viremia was detected in any animal.
Conclusion: In conclusion, the safety and efficacy of this new vaccine against BTV3 was successfully demonstrated after challenge. Bultavo 3 is suitable for active immunization of target species older than 21 days of age and is indicated for the prevention of clinical signs and mortality and for reduction of circulating virus in the blood of vaccinated animals. Vaccination with Bultavo 3 significantly reduces the risk of disease transmission through midge bites. With this new vaccine, BTV3 outbreaks can be prevented, and farmers can protect not only their herds, but also their livelihoods.

New Infectious Bronchitis ELISA, based on well conserved protein, for improved detection of live vaccines and challenge including variant strains.

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Background and Objectives: Avian infectious bronchitis virus (IBV) is a coronavirus, which infects poultry and causes infectious bronchitis. It is a highly infectious avian pathogen, which affects the respiratory tract, gut, kidney and reproductive systems of chickens. Important mutations could be observed for avian Coronavirus which lead to the appearance of new variants worldwide. To control the disease, vaccination is largely used and based on classic or circulating variant strains. Thus, diagnosis and monitoring of vaccination require laboratory testing, and ELISA may be used for monitoring serum antibody responses.
Material and Methods: The new ID Screen® Infectious Bronchitis Indirect ELISA, based on well conserved recombinant protein, allows for the detection of IBV antibodies in samples.
Results: Different samples coming from vaccinated flocks were tested with this indirect ELISA. For each tested flock, the following parameters were measured: mean titers, minimum, maximum, and CV%. The indirect ELISA was able to monitor the vaccination uptake for all vaccines (including variants strains).
Conclusion: This kit is specifically used for the detection of antibody response after IBV vaccination (including variants: 4/91, 793B, QX, Italy 02, BR1…), and improve detection of challenge in vaccinated flocks (by classic or variants strains).

SPIDVAC – Safe Priority Infectious Diseases VACcines

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Background and Objectives: African Horse Sickness (AHS), Peste des Petits Ruminants (PPR) and Foot-and-mouth disease (FMD) are three priority animal diseases included in the list of notifiable animal diseases of the World Organization for Animal Health (WOAH). The current vaccines for these diseases have critical shortcomings, leaving the EU vulnerable to these transboundary animal diseases. Public rejection of control strategies reliant solely on culling is rising. Moreover, the risk to introduce these diseases is increasing due to climate change, global trade and travel. The SPIDVAC consortium (including 13 partners) aims to develop innovative vaccines against AHS, PPR and FMD. Companion diagnostics will also be developed to differentiate between infected and vaccinated animals. The consortium, including industry partners, will ensure technological readiness and market relevance, aiding policymakers, veterinary services and businesses to reduce infectious animal disease burdens, sustain livestock industries and promote public and animal health.
Material and Methods: Vaccine candidates will be evaluated for safety, efficacy and DIVA capability in small-animal models and target species. The AHS vaccines, which have already been successfully tested in mice, need to be proven in equids before steps towards large-scale production and commercialization can proceed. For PPR, research is focusing on new ways to develop a DIVA-capable product based on established vaccines. FMD prototype vaccines are being assessed for oral administration to pigs. This method could extend to FMDV-susceptible wildlife but requires additional safety studies beyond this project for field use confirmation.
Results: Significant progress was already made in developing these new vaccines. Two AHS vaccine prototypes were completed and successfully tested in mice. Developing PPR vaccines with DIVA capability proved challenging, but solutions are underway and the methodology for the socio-economic study on their acceptance in Senegal was refined. Although the original approach to an attenuated FMD vaccine proved unfeasible, mitigation strategies were prepared, and construction of new FMD vaccine prototypes is ongoing.
Conclusion: By evaluating the safety and efficacy of the vaccine candidates, we contribute to the development of more effective and safe solutions for controlling priority animal diseases. Reliable distinction between vaccinated and infected animals can ease trade and movement restrictions while reducing indiscriminate culling after an outbreak.

Funding source/acknowledgements (optional):
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Survival of Lyssaviruses in the environment

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Background and Objectives: Rabies is a fatal disease caused by the rabies virus and related lyssaviruses. While bites are the recognised transmission route, potential alternative pathways remain a concern. This study investigated the persistence of three lyssaviruses (rabies strain CVS-11, European bat lyssaviruses (EBLV) 1 and 2) on commonly used materials in quarantine facilities and laboratories. These materials (metals, plastics, and straw bedding) together with fur, and water can become contaminated with virus-laden saliva.
Material and Methods: Experiments were conducted at room temperature (22°C) for all conditions, additionally at 4°C for fur as the cooler conditions are relevant for infected animals during winter. Viruses with known concentrations were applied to test surfaces and water. After set incubation periods up to 14 days, samples were frozen at -80°C until analysis. A sensitive Rabies Tissue Culture Infection Test (RTCIT) with two passages was used to detect replicating viruses in all samples. Additionally, viral titres were quantified (TCID50) in selected samples.
Results: On fur, the titres of all viruses decreased over time at both temperatures. Notably, survival on mouse fur at 4°C was at least twice as long compared to 22°C (up to 6 days). Conversely, all three viruses remained stable in water throughout the 14-day study period. On metal and plastic surfaces, all viruses exhibited a gradual decline in viral titres, likely due to dehydration. Importantly, no viable virus was recovered from bedding samples after overnight incubation at room temperature, suggesting absorption by the material potentially reduces transmission risk.
Conclusion: The extended survival in water poses a potential risk factor for humans and animals encountering contaminated water sources. It is important to acknowledge these experiments were conducted under laboratory conditions, excluding factors like sunlight, evaporation and microorganisms that could degrade viruses in real-world settings. Further studies are ongoing to assess the practical implications of virus persistence for transmission.

Funding source/acknowledgements (optional): Department for Environment, Food and Rural Affairs (Defra) through research project SE0433

Session 8

Vector-borne diseases

Oral Presentations

Culex pipiens from the United Kingdom demonstrate transmission potential for West Nile virus lineage 1 and lineage 2

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Background and Objectives: West Nile virus (WNV) is an imminent threat to public and livestock health in the United Kingdom (UK) due to its geographical spread throughout mainland Europe, and the risk of virus incursion via migratory birds. Both lineage 1 and lineage 2 WNV circulate in mainland Europe, although lineage 2 now predominates. Additionally, climate changes and increasing temperatures are affecting mosquito population dynamics, including a longer active season. Native UK *Culex pipiens* s.l. mosquitoes were investigated for their ability to vector and transmit WNV (lineages 1 and 2), assessing the risk for establishment in native mosquito populations following a virus incursion, and the impact of temperature variation on infection, dissemination, and transmission rates.
Material and Methods: In the first baseline experiment, colonised female mosquitoes were provided with a bloodmeal containing WNV lineage 2 at 10^6 or 10^7 plaque forming units (PFU)/ml. Engorged mosquitoes were transferred to experimental cages at 25°C, for sampling at 10- and 14-days post-infection (DPI). In the second experiment, female mosquitoes were provided with a bloodmeal containing WNV lineage 1 (2.6 x 10^6 PFU/ml), and engorged females incubated at 20°C or 25°C for sampling at 7- and 14-DPI. At each time-point, mosquitoes were anaesthetised and dissected to collect wings and legs, body and saliva. Tissues were processed and RNA extracted for molecular analysis, with positive qRT-PCR results providing evidence for infection (body), dissemination (legs and wings) and transmission potential (saliva). Additionally, intact mosquitoes underwent immunohistochemical (IHC) analysis.
Results: For lineage 2 WNV at 25°C, viral RNA in mosquito bodies and legs and wings at both 10- and 14-DPI confirmed infection and virus dissemination within UK *C. pipiens*, supported through positive IHC detection of WNV antigen in mosquito midgut epithelium cells. Viral RNA was detected in saliva at 10-DPI and 14-DPI, providing evidence of WNV transmission potential. For lineage 1 WNV, viral RNA in mosquito bodies (7- and 14-DPI) confirmed that UK *C. pipiens* can become infected at both 20°C or 25°C. Viral RNA in saliva at 7-and 14-DPI suggested WNV lineage 1 transmission potential at both temperatures, even at current average UK summer temperatures (~20°C). Increasing the temperature to 25°C, reflecting recent elevated UK summer temperatures, increased mosquito infection rates and potential for transmission of lineage 1 WNV, along with potential for transmission of lineage 2 WNV.
Conclusion: These data highlight the impact of temperature through climate changes on vector-borne disease transmission, and reaffirm the importance of surveillance and preparedness platforms.

Funding source/acknowledgements (optional): Department for Environment, Food and Rural Affairs (DEFRA), the Scottish Government and Welsh Government, grant number SE0576.

Early detection and tracking of West Nile virus in Germany by using a functional nationwide wild bird network

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Background and Objectives: One consequence of climate change is an expansion of various arboviruses such as West Nile virus (WNV) to the Northern Hemisphere. Wild birds play a significant role in this infection cycle as reservoir hosts or amplifiers. Therefore, it is important to monitor them. In this context, the German wild bird network is a nationwide, long existing, and well-functioning surveillance installation for arboviruses which involves various cooperation partners from the avifaunists. This network was a helpful basis for the early detection of WNV introduction in 2018.
Material and Methods: Since then, more than 1,000 avian blood samples from different bird species and samples from over 1,000 deceased birds have been analysed every year. The presence of WNV-specific RNAs in blood and tissues was determined by RT-qPCR methods, followed by Oxford Nanopore whole genome sequencing. Blood sera were screened serologically for flavivirus antibodies using a blocking ELISA with subsequent confirmation of reactive samples by differentiating VNTs. Moreover, an indirect ELISA based on enhanced E-proteins and an in-house developed Luminex assay were used.
Results: WNV RNA positive samples were mainly detected in birds from eastern regions of Germany where WNV circulates endemically. Phylogenetic analyses revealed a low genetic diversity, which is primarily characterized by the enzootic maintenance of one dominant WNV lineage 2 subcluster. This clustering pattern suggests a high degree of genetic relatedness among the circulating strains and indicates the predominance of the specific viral subcluster. Indeed, the genetic composition of the circulating strains remained largely consistent (95%) with previously identified subclusters. However, it is worth noting that three WNV cases were identified which represented other subclusters, indicating some level of genetic diversity within the circulating WNV strains, in the absence of new introduction events from 2021-2022.

Interestingly, there is serological evidence for silent circulation of WNV in non-endemic areas and a noticeable tendency for a westward and southward expansion in Germany has been determined. Nevertheless, the rate of spread is low, probably because many birds are cross-protected by the presence of antibodies against the closely related Usutu virus. This is clearly visible in zoo birds, which often do not suffer severely after WNV infection and show high antibody titres against both viruses.
Conclusion: The "bird´s-eye view" of WNV and other arbovirus circulation provided by the network is a valuable contribution to preparedness in order to protect human and animal health.

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Emerging orbivirus preparedness and transmission potential: lessons learned from Schmallenberg virus in Norway 2012 – 2023

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Background and Objectives: The geographical ranges of vector-borne diseases are changing. Milder and warmer temperatures facilitate changing distributions and phenology of vectors, pathogen replication in the vector and new interactions between vectors and hosts. In this context, mosquito and tick-borne diseases are frequently studied due to their impact on human health. In contrast, midge-borne pathogens affecting animal health, like bluetongue virus (BTV), Schmallenberg virus (SBV) and epizootic haemorrhagic disease virus (EHDV), are often overlooked until an outbreak occurs. The objective of this work is to use SBV, first detected in Norway in 2012, as a case study to estimate the current geographical range, and explore underlying drivers, of orbivirus transmission in Norway to contribute to midge-borne disease preparedness.
Material and Methods: In Norway SBV and BTV surveillance have been conducted bi-annually since 2016 and annually since 2009, respectively, on a changing subset (~500) of dairy farms in southern Norway. Bulk milk samples are tested by ID-screen ELISAs and the results have been communicated in the Norwegian Veterinary Institute's report series (ISSN 1890-3290). In addition to bulk milk surveillance, blood samples submitted in the *Brucella abortus* surveillance programme are tested for SBV. In this study, we complement the ongoing surveillance programmes with nationwide testing of bulk milk samples for anti-SBV antibodies collected from ~1300 dairy farms and 168 dairy goat farms in the fall of 2023. We plan to explore which factors are associated with positive farms, e.g., type of farm, geographic location, nearest neighbour, weather variables and landscape classification.
Results: No BTV antibodies have been detected since 2009. However, 8, 17 and 6% of bulk tank milk samples were positive for SBV antibodies in 2016, 2018 and 2020, respectively, increasing to 62% in 2022 (306 of 503 herds sampled). In addition, locally acquired SBV was detected in Trøndelag county in 2023. That year, 3 of 23 herds (110 animals tested) tested positive for anti-SBV antibodies in the *B. abortus* programme. The results of the nationwide screening and exploration of associated factors are pending.
Conclusion: The surveillance data provide evidence of SBV transmission in areas much further north than the current surveillance program reaches. The data furthermore illustrate that midge-borne virus transmission in Norway occurs, but whether these transmission events are new introductions or re-emerging enzootic circulation remains uncertain. This work further illustrates the importance of better understanding transmission dynamics of orbiviruses and the possible impact of infection on wild and domestic ruminants.

Emerging zoonotic Wesselsbron flavivirus causes severe hepatitis and is transmitted directly to suckling lambs

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Background and Objectives: Wesselsbron virus (WSLV) is a mosquito-borne flavivirus endemic to sub-Saharan Africa. It appears to be circulating in a variety of mammals, with small ruminants such as sheep and goats acting as amplifying hosts in an epizootic cycle. Despite WSLV being mostly transmitted by Aedes spp. mosquitoes, several human cases have been reported to be infected in the absence of mosquitoes by manipulating infected animals or their samples.
Material and Methods: For the present study, the WSLV SA999 strain was reconstructed by reverse genetics using a yeast-based synthetic genomics platform. Lactating ewes were intravenously inoculated with 10^5 $TCID_{50}$/animal of WSLV SA999 strain (clade I) or WSLV SAH177 strain (clade II).
Results: Ewes developed clinical signs of the disease, including high body temperature, a viremia phase lasting up to 7 days, high levels of viral RNA in nasal, ocular, oral, and rectal swabs, and very high levels of RNA and infectious WSLV particles in milk. We detected a significant reduction in the body weight gain of the lambs from ewes infected with the SA999 strain. Importantly, ewes with higher levels of viral RNA in milk transmitted the viral infection to their lambs (40% transmission rate for both WSLV clades). These lambs developed high levels of viremia and prolonged oronasal viral shedding. At 12 days post-infection, viral levels remained very high in organs, especially in the liver, of all infected animals, despite the presence of high levels of neutralizing antibodies. Moreover, immunohistochemistry analysis revealed that tissues positive by RT-qPCR were negative for the viral NS1 protein, except in the liver, suggesting that WSLV is still replicating in hepatocytes. The SA999 strain caused more pronounced lesions in the livers of infected animals, whereas only animals infected with the SAH177 strain had mild neurological lesions. Clinical biochemistry analysis of sera revealed that WSLV causes evident hepatitis, characterized by high levels of AST, bilirubin, biliary acids, adenosine deaminase, urea, cholesterol, and glucose. The viral hepatitis was more severe in SA999-infected animals.
Conclusion: Our results could have a significant impact on the ecology of WSLV in endemic areas where it is overlooked. In animals, the clinical signs of the disease are very similar to those of the Rift Valley fever virus, while in humans, it might be confused with the serologically-related Yellow Fever virus.

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Evaluation of disease dynamics and immune response in sheep experimentally infected subcutaneously or intranasally with Rift Valley fever virus.

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Background and Objectives: Rift Valley fever (RVF) is an arthropod-borne disease caused by an RNA virus that causes abortions and deaths in livestock and wild ruminants, and affects humans. Sheep have been pointed as an attractive experimental model to evaluate pathogenesis and immune mechanisms, as well as to mimic the disease described in other livestock species and humans. It is unclear how the route of infection may influence disease development and subsequent virus transmission, and there are conflicting studies on the role of horizontal transmission in animal infection. Our objective was to evaluate the effect of different routes of inoculation (intranasal vs. subcutaneous) of RVF virus (RVFV) on disease dynamics, immune response and virus transmission.
Material and Methods: Two groups of ten Castilian sheep (Spanish native breed) of both sexes, 9 to 10 weeks of age were used. In each group seven animals were inoculated subcutaneously (SC) or intranasally (IN) with 1 ml containing a high dose of 56/74 strain (5 x 10^6 pfu), while three animals remained as uninoculated contacts. The animals were daily monitored and blood samples were taken at different times until the end of the experiment on day 28 post-infection (pi). Anti-RVFV IgG and IgM antibodies were evaluated by ELISA.
Results: Apart from a transient increase in rectal temperature in inoculated sheep that peaked in both groups on day 2 pi (above 41.5°C), the animals showed no other clinical signs. In addition, contact animals of the SC inoculated group showed a delayed hyperthermia that peaked on day 4 pi, whereas contact animals in the IN inoculated group showed no such rise. As early as day 4 pi, some SC inoculated animals showed a moderate increase of virus-specific IgG and IgM in serum, while by day 7 pi all sheep (inoculated and contacts) had seroconverted. However, IgG and IgM responses were not observed on day 4 pi in the IN inoculated group, although all inoculated sheep, but not contact animals, had also seroconverted by day 7 pi.
Conclusion: In conclusion, both routes of infection induced similar transient subclinical forms. Unlike contact sheep in the SC inoculated group, none of the contact animals of the IN inoculated group showed hyperthermia or RVFV-antibodies, which would confirm virus transmission in the SC inoculated group. Mechanisms involved in the delay of the antibody response in IN inoculated sheep and the non-transmission of the virus to contact animals in this group require further study.

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Infected Culex pipiens mosquitoes can transmit West Nile virus lineage 1 and 2 after overwintering in temperate winter conditions

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Background and Objectives: West Nile virus (WNV) is kept in an enzootic cycle mainly between birds and mosquitoes. Occasionally, it can infect mammals such as equids and humans causing encephalopathies. *Culex pipiens* (Linnaeus, 1758) is considered one of the main vectors of WNV. The yearly persistence of WNV lineages 1 and 2 in temperate regions points out an ability of the virus to overwinter and establish enzootic cycles. The exact mechanism of overwintering remains still unknown. Here, we assessed the ability of infected *Cx. pipiens* to transmit WNV lineages 1 and 2 after overwintering in temperate winter conditions.
Material and Methods: Two groups of 3-to-5 days old *Cx. pipiens* females were inoculated intrathoracically with WNV lineages 1 (WNV-1) and 2 (WNV-2) (6 $\log_{10}$ $TCID_{50}$/ml), respectively. These mosquitoes were maintained in different monthly environmental conditions (temperature, humidity and photoperiod), mimicking the registered ones from October 2022 to May 2023 in a temperate area where WNV circulation had been repetitively reported in the previous years. Moreover, fresh chicken blood was offered every month to test their biting appetite. Females that survived the whole winter period were sacrificed at different time-points, extracting their saliva to assess the ability to transmit the virus by virus titration, their bodies to determine WNV infection in different tissues by immunohistochemistry and their legs and wings to characterize their ecotype molecularly.
Results: *Cx. pipiens* females inoculated with WNV-1 and WNV-2 were able to transmit both lineages after the winter period, as infectious WNV was detected in their saliva. Furthermore, one female inoculated with WNV-2 and another inoculated with WNV-1 fed on blood in January and March, respectively. Immunohistochemistry assays showed infected tissues in inoculated females, including midgut, hindgut, salivary glands and neuronal ganglia.
Conclusion: In the present study, circulating WNV lineages 1 and 2 persisted in several mosquito tissues in infected *Cx. pipiens* mosquitoes kept in temperate winter conditions. We report the ability of infected *Cx. pipiens* females with both WNV lineages 1 and 2 to feed on blood and transmit WNV during a low mosquito activity season. Overall, these findings highlight the risk of triggering an early WNV transmission season in temperate climate regions thought mosquito vectors infected in the previous season. Therefore, in locations where WNV has previously circulated, WNV surveillance programs should enhance vector surveillance early in the mosquito season.

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Sindbis virus (SINV) in the Netherlands: First proof of local circulation in wild birds and horses

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Background and Objectives: Sindbis virus (SINV; *Togaviridae*, Alphavirus) is an arbovirus circulating endemically between ornithophilic mosquitoes (predominantly of the genera *Culex* and *Culiseta*) and wild birds in Eurasia, Africa, and Oceania. Spillover may occur to mammals, such as humans and horses. Clinical manifestations in humans, predominantly in the form of polyarthritis, rash, and fever, have been commonly reported in Northern Europe (Finland and Sweden). The aim of this study was to investigate the presence of SINV in the Netherlands.
Material and Methods: From 2020 to 2022, mosquitoes (n = 12,884; majority belonging to *Cx. pipiens/torrentium*) were trapped throughout the country. An additional PCR-screening of wild birds (n = 10,983) was performed from 2021 to 2022. To further unravel the endemicity of SINV in the country, horses (n = 368) were tested from May 2021 to May 2022 for neutralizing antibodies (nAbs) using a specific plaque reduction neutralization test (PRNT).
Results: All mosquitoes were tested negative for SINV RNA by PCR. One of the tested wild birds was PCR-positive, resulting in the generation of a SINV partial genome sequence from the feathers of a European robin (*Erithacus rubecula*; partially resident) from North Holland. In 2021, SINV nAbs were detected in three horses (without international travel history) from three different provinces. SINV nAbs were also detected in 12 wild birds (12/110; mainly Eurasian blackbirds; *Turdus merula*) in 2021 and 2022, sampled in a 16 km radius around the three seropositive horses and the PCR-positive bird. Based on sampling moment and ringing history, six out of the 12 birds can be considered residents.
Conclusion: This study therefore provides the first evidence for SINV circulation in the avifauna and amongst equine populations in the Netherlands. It also highlights the need for SINV surveillance within Europe and increased awareness amongst veterinarians and health practitioners for this pathogen.

* These authors contributed equally to this article

Session 8

Vector-borne diseases

Poster Presentations

Atypical gastrointestinal lishmaniosis

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Background and Objectives: Leishmaniosis, a zoonotic disease caused by the protozoan parasite Leishmania, is endemic in various regions worldwide, affecting both humans and animals. The disease typically presents with cutaneous and visceral manifestations; however, atypical presentations can pose diagnostic challenges to clinicians. In veterinary medicine, gastrointestinal symptoms in dogs with leishmaniosis are considered rare occurrences, rendering the case of a canine patient exhibiting gaseous stomach and megacolon particularly intriguing and worthy of further investigation.
Material and Methods: This case report aims to document the clinical presentation, diagnostic workup, and treatment approach employed in managing a canine patient with atypical gastrointestinal symptoms associated with leishmaniosis. A comprehensive physical examination, laboratory tests including serology and PCR for Leishmania detection, imaging studies, and histopathological analysis were conducted to confirm the diagnosis and assess the extent of organ involvement. The diagnostic protocol followed established guidelines for the diagnosis of canine leishmaniosis, ensuring a systematic and evidence-based approach to case management.
Results: The results of the diagnostic investigations, including serological tests and PCR analysis, confirmed the presence of Leishmania parasites in the dog's tissues, establishing the diagnosis of atypical leishmaniosis. Imaging studies, such as radiography and ultrasonography, demonstrated abnormalities consistent with gastrointestinal involvement, including megacolon.
Conclusion: This case report underscores the importance of considering atypical presentations of leishmaniosis in veterinary practice, particularly when gastrointestinal symptoms are the predominant clinical manifestation. The successful management of this case emphasizes the significance of early and accurate diagnosis, appropriate treatment, and close monitoring in improving outcomes for dogs with uncommon manifestations of this parasitic disease. The findings of this case report contribute to the limited body of knowledge regarding gastrointestinal involvement in canine leishmaniosis and may aid in the development of more comprehensive diagnostic and treatment protocols for such cases. Further research is warranted to better understand the pathogenesis and optimal management strategies for atypical presentations of leishmaniosis in dogs.

Batai virus infection and vector virus tolerance: virus-derived DNA production in Aedes albopictus cells

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Background and Objectives: Batai virus (BATV) is a tri-segmented negative-sense single-stranded RNA virus belonging to the *Orthobunyavirus* genus. BATV has been shown to infect multiple animal species, with seroprevalence in European livestock (cattle, sheep and goat) reaching up to 46%. Additionally, humans can also be infected. BATV is an arbovirus transmitted by a wide range of mosquito species. Beyond Europe, BATV has been detected in Asia, Africa and in Australia. There is a need to identify novel approaches to control the spread of mosquito-borne arboviruses one such way could be to reduce the circulation of virus in the mosquito population. Recently, it has been proposed that viral-derived DNA (vDNA) produced in mosquitoes upon arboviral infection play an essential role in vector survival and virus tolerance.
This study aims to assess the vDNA production by *Aedes albopictus* cells (U4.4) after infection with BATV.
Material and Methods: The U4.4 cells were infected with BATV (MOI 0.1) and three biological replicates were used for the infected group as well as for the mock-infected group. At 24h, 48h, 72h and 96h after infection, the vDNA production was assessed using PCR. Primers targeting all three viral segments (S, M and L) were used as well as a housekeeping gene (RPS17). The amplicons were sequenced and the correct amplification was confirmed using BLASTn. Additionally, in the supernatant the virus presence was confirmed using a two-step real-time PCR SYBR Green based assay, targeting the S segment.
Results: Our preliminary results show that the BATV infected U4.4 cells produce vDNA originating from the S and M segments. However, there was no production of vDNA linked to the L segment. Additionally, the respective supernatants tested positive for BATV. The mock-infected cells did not produce vDNA and the respective supernatants tested negative for BATV. Moreover, no cytopathic effects were observed in the cells. The results were consistent in all biological replicates, for each experimental condition at 48h, 72h and 96h after infection.
Conclusion: We conclude that BATV infection induce production of vDNA in *Ae. albopictus* cells. Further studies investigating the effect of vDNA on BATV infection through vDNA inhibition as well as *in vivo* studies are ongoing. Through these studies we hope to determine the possible role that vDNA play in the antiviral immune response to orthobunyaviruses. This knowledge could then be utilized to develop novel control strategies.

Development of a reverse genetic system of the recently emerged Epizootic Hemorrhagic Disease virus serotype 8.

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Background and Objectives: The Orbivirus Epizootic Hemorrhagic Disease Virus (EHDV) is an arbovirus transmitted via competent *Culicoides*-midges and can cause disease in wild and domesticated ruminants. The virus has a double stranded RNA genomic structure composed of 10 individual segments. Primarily deer and cattle are susceptible for infection with EHDV which could lead to high fever, oedema, teat erosion or even death. Recently, in 2022, EHDV serotype 8 was introduced into Italy and Spain and quickly spread further northwards from there. At this moment, there is no safe and efficacious vaccine available against EHDV that could contribute in controlling the further spread of the virus. Of the other Orbiviruses Bluetongue and African horse sickness are Disabled Infectious Single Animal (DISA) vaccine platforms available, based on a deletion in the tenth segment (NS3/NS3A), which can be produced with a reverse genetics system. Therefore, the aim of this study is to develop a reverse genetics system of EHDV-8 to allow for the genetic modification of the virus for the production of DISA-vaccines.
Material and Methods: Initially, the full genomic sequence of a cattle-derived virus isolate (EHDV-8/FR2023/10247) was determined and ordered with an additional T7-RNA polymerase promotor and flanked by a restriction enzyme site on the 3'-terminus. Next, the rescue protocol was optimized with an established reverse genetics system of EHDV-2/Ibaraki using capped *in vitro* synthesized transcripts (IVT). Monolayers of BSR-cells were transfected twice, first with 6 expression plasmids encoding the replication machinery of EHDV-2/Ibaraki and second with all 10 IVTs of either EHDV-2/Ibaraki, EHDV-8/FR2023 or EHDV-X/Alpaca. Successful rescue were confirmed with staining of plaques using VP5-specific antibodies.
Results: The first rescues of EHDV-2, -8 an -X with individually generated IVTs were unsuccessful, however, when all segments were synthesized in one IVT-reaction, positive plaques were observed. The successful rescue shows that the replication machinery EHDV-2 is compatible with EHDV-8 and EHDV-X and the generated virus were partially confirmed via Sanger sequencing. Next, both EHDV-8 & X were successfully rescued with silent mutations in segment 10 encoding for different restriction enzyme sites, showing that the generated viruses are recombinant. Finally, viral rescues were performed with an NS3-deletion, and a Green Fluorescent Protein or luciferase reporter were introduced in segment 5 in EHDV-8.
Conclusion: To conclude, a reverse genetics system was successfully developed of both EHDV-8/FR2023 and EHDV-X/Alpaca allowing for the genetic modification of the virus to create DISA-vaccines.

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Emergence and disappearance of Schmallenberg Virus Infected Calves and Lambs in Northern Ireland 2013-2024

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Background and Objectives: Schmallenberg virus (SBV) was first detected in Northern Ireland in stillborn ruminants in 2013 by real time RT-PCR. Offspring of infected ruminants, many of which were stillborn, displayed congenital malformations including, but not limited to, scoliosis, arthrogyposis, hydrocephalus, torticollis and cerebellar hypoplasia. Following the first detection in Northern Ireland in 2013 and the initial spike in surveillance that paralleled concerns associated with what the infection meant for livestock, SBV was not detected again until 2017 in an isolated case. From the beginning of 2018 onwards, there was a marked increase in positive cases of SBV in stillborn lambs tested by RT-PCR in Northern Ireland only for positive detections to stop abruptly in February 2019.
Material and Methods: Viral RNA was extracted from a range of tissues, from selected cases and analysed by real-time RT-PCR with an SBV S-segment specific assay. Partial sequencing of the M-segment was carried out on multiple selected cases. Selected calves and lambs in Northern Ireland between 2013-2018 were also subject to detailed pathological and viral immunohistochemical analysis.
Results: Submission levels of SBV suspects diminished February 2019 and significantly further during and after the global COVID pandemic. There has recently been a resurgence in submissions and SBV has seen renewed interest due to requested inclusion in the differential diagnosis of bluetongue virus and epizootic haemorrhagic disease virus affected suspects, especially in association with stillbirths.
Conclusion: Although detections of SBV have seen a notable upturn in Great Britain in 2023-24, a similar re-emergence in Northern Ireland remains undetected despite enhanced surveillance due to the increased threat from other Culicoides species vectored viruses. Here we retrospectively consider the emergence and re-emergence of SBV in Northern Irish livestock in the past decade and speculate as to the reasons why it remains elusive in spite of recent positive detections elsewhere in the British Isles. Additionally, we detail the findings from the pathology and viral immunohistochemistry of SBV infected calves and lambs in Northern Ireland up to and including 2018 and demonstrate the association between semi-quantitative detection by real-time RT-PCR and anti-SBV nucleoprotein immunohistochemistry in selected tissues.

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Leishmaniasis-Associated Renal Failure in Dogs and its Implications for Canine and Human Health

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Background and Objectives: Leishmaniasis, caused by Leishmania infantum, is a significant vector-borne disease affecting dogs in endemic regions worldwide. Renal failure is a complication of canine leishmaniasis, presenting a diagnostic challenge when clinical signs are absent. This report aims to investigate the clinical presentation, laboratory findings, and diagnostic challenges in 21 dogs presenting with renal failure, all diagnosed with leishmaniasis despite the absence of typical clinical signs.
Material and Methods: This retrospective case series study was conducted at a single veterinary clinic, where 21 dogs presented with renal failure within a day. Medical records were reviewed to collect data on signalment, clinical signs, laboratory results, and diagnostic imaging findings. Diagnostic criteria for renal failure and leishmaniasis were established based on standard guidelines.

Information on age, sex, breed, clinical signs, complete blood count (CBC), serum biochemistry profile, urinalysis, radiographic findings, and leishmaniasis diagnosis was recorded . The A/G ratio was calculated from the serum biochemistry profile as an additional parameter.
Results: All 21 dogs presented with renal failure as the primary clinical sign, with no overt signs of leishmaniasis. Common symptoms included weight loss, vomiting, and lethargy.

Blood tests revealed elevated BUN and creatinine levels, electrolyte imbalances, anemia, and a decreased A/G ratio indicative of proteinuria and inflammation associated with leishmaniasis.

Radiographs showed small, irregular kidneys in most dogs, consistent with advanced chronic renal failure, and some dogs had radiopaque stones suggestive of nephrolithiasis.
Conclusion: The findings of this study underscore the importance of considering leishmaniasis as a differential diagnosis in dogs presenting with renal failure, especially in endemic areas. Leishmaniasis, caused by the Leishmania infantum parasite, poses a significant danger to human health as well. Dogs infected with leishmaniasis can serve as reservoirs for the parasite, potentially leading to transmission to humans through sand fly vectors. The zoonotic nature of leishmaniasis highlights the critical need for early detection and management in dogs to prevent human infections.

The study emphasizes the importance of diagnosing leishmaniasis in dogs with renal failure, particularly in endemic areas. The parasite, caused by the Leishmania infantum, can be transmitted to humans through sand fly vectors. Early detection and management are crucial for improving canine health and controlling the spread of the disease. Further research is needed to understand the complex interplay between renal failure and leishmaniasis in dogs and its implications for human health.

Lumpy skin disease virus (LSD), as a vector borne diseases, new challenge in Georgia

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Background and Objectives: Lumpy skin disease, a member of the Capripoxvirus genus of the Poxviridae is a pathogen that affects cattle. It is transmitted by blood-feeding insects, such as certain species of flies and mosquitoes, or ticks.

These diseases are agents of agroterrorism and are listed as diseases since they cause serious economic losses.

Lumpy skin disease is a recent disease in Georgia. In July 2015, this disease was discovered on the border of Georgia and Azerbaijan. Georgia saw its first two outbreaks of lumpy skin disease (LSD) in late 2016, when 8 infected cows were found in the Racha region. The outbreak was resolved. In 2017, disease also occurred in Samtskhe Javakheti, the border region and Adjara, the Black Sea region. Almost all regions of Georgia were covered. This was the first time in history that the virus had emerged in Europe.
Material and Methods: To prevent further spread of the virus, the Ministry of Agriculture of Georgia, the National Food Agency, and the Food and Agriculture Organization (FAO) are providing prevention and control of the virus. Topics to be covered include risk communication and awareness, vaccination plans, and proper laboratory testing methods. Within the framework of the FAO project, training on the development of a new method was conducted in the Tbilisi, Kutaisi and Akhaltsikhe laboratories. Polymerase chain reaction (PCR) is the most economical and quickest method for the detection of LSDV.
Results: The State laboratory of Agriculture (SLA) diagnosed 24 positive cases of lumpy skin disease by PCR research in 2016, 11 positive cases in 2016, and 13 positive cases in 2018. The National Food Agency vaccinated the cattle in the risky regions along the country's border surrounding the outbreaks. In 2015–2022, 2538053 cattle were vaccinated, 80% of the registered cattle.
Conclusion: The effectiveness of the vaccination campaign is confirmed by the fact that in 2019–2023, there were no recorded cases of LSDV. The reemergence of cattle necessitates vaccination, accurate and timely diagnosis, cattle movement and vector controls, as well as keeping up the awareness campaign and surveillance programs. Research on vector-borne diseases and vectors is underway at the State Agricultural Laboratory with the assistance of a Defense Threat Reduction Agency project.

Funding source/acknowledgements (optional): Defense Threat Reduction Agency (DTRA)

Molecular characterisation of West Nile virus strains circulating in Romania, in 2016-2022

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Background and Objectives: West Nile virus (WNV) is a widespread arbovirus worldwide. Circulation of WNV is endemic in south-eastern Romania, including the capital city of Bucharest, where significant outbreaks of WNV infections have been occurring in human population at least since 1996. Genetic lineage 2 of WNV was first recorded as being responsible for the second WNV outbreak, which occurred in Romania in 2010, and is a WNV strain belonging to East-European clade, similar to Volgograd-2007 isolate. In 2016 the third outbreak with 93 WNV neuroinvasive infections occurred and was determined by a WNV strain, also of genetic lineage 2, but of the central-southern European clade. In 2018 year, the fourth outbreak occurred and 277 neuroinvasive WNV infections were recorded. In the following years, 2019-2022 the number of WNV cases decreased.
Material and Methods: The aim of this study is to provide molecular characterization of WNV strains circulating in Romania during 2016-2022 transmission seasons, as detected in human samples derived from WNV neuroinfections, and mosquito-vectors collected from Bucharest and Tulcea county. A Real-time-PCR screening test (Virus Real-TM, Sacace Biotechnologies) was used for detection of WNV genome in human and female mosquito pools samples. Partial NS5 sequences were obtained by Sanger sequencing (Applied Biosystems) and were used for genotyping and phylogeny studies.
Results: The WNV positive pools contained Culex pipiens specimens collected yearly during June-August. This species has already been documented as a WNV vector in south-eastern Romania. The phylogenetic analyses showed that WNV isolates detected in mosquito pools as well as in human samples during 2016-2022 transmission seasons belong to the genetic lineage 2 central-southern European clade.
Conclusion: A new WNV strain belonging to the genetic lineage 2, central-southern European clade was introduced in 2015 in Romania. This virus strain was detected both in mosquito vectors from Bucharest and Tulcea, as well as in patients during 2016-2022 transmission seasons. This strain was co-circulating in 2015 together with the old strain, Volgograd-2007 from the East-European clade, and was replaced in 2016 by a WNV strain from the Central-Southern European clade. We confirm the circulation and maintenance in the period 2016-2022 of WNV strains belonging to the central-southern European clade of lineage 2 both in human and mosquito samples. This new WNV strain from the Central-Southern European clade was responsible for the second, third and fourth WNV outbreak which occured in 2020, 2016 and 2018 in Romania.

Monitoring of Schmallenberg virus and ruminant-infecting orbiviruses in biting midges in Germany 2019 - 2021

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Background and Objectives: The orthobunyavirus Schmallenberg virus (SBV) is transmitted by biting midges of the genus *Culicoides*. It was first detected in 2011 in Germany and in the following years rapidly spread across many other European countries. Today SBV has an enzootic status in central Europe and causes premature birth, stillbirth, abortions and foetal malformations after the infection of naïve pregnant ruminants during a critical phase in early gestation. Biting midges belong to the smallest haematophagous flies in the world and have a high abundancy near livestock farms. Besides SBV, the midges are considered vectors of viruses like bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV), that are relevant for European livestock. The monitoring of the midges and transmitted viruses is of veterinary importance because the resulting diseases may cause animal suffering and entail economic losses due to management and control measures such as vaccination or trade restrictions.
Material and Methods: To gain an overview of the prevalence of the viruses in the putative *Culicoides* vectors in Germany, a monitoring programme had been established in 2018. From 2019 to 2021 altogether 12,965 pools, containing from 1 to 50 midges, were caught at 57 sites throughout the country. The extracted RNA from the midges was tested in real-time RT-PCRs for the genomes of SBV, EHDV and BTV.
Results: Whereas no EHDV and BTV could be found, 180 (1.4%) pools tested positive for SBV. With 3.9%, the highest prevalence of SBV in biting midges was found in the federal state of Baden-Wuerttemberg, followed by Bavaria (2.4%) and Lower Saxony (1.7%). The regions where SBV was detected often matched with places where SBV-infections in ruminants had been reported at the time of biting midge collection. Over 95% of the SBV-positive pools were caught in the months from May to October. The earliest catch of an SBV-positive pool was in April and the latest in November.
Conclusion: The results from the monitoring programme confirms an enzootic circulation of SBV in the German biting midge population during summer and autumn.

Mosquito Species Composition and Flavivirus-associated Disease Risks at Live and Wet Markets in Laos

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Background and Objectives: Live animal and wet markets (LWMs) are common in many tropical low- and middle-income countries.In these markets, there is frequent standing water, and the presence of humans, live domestic, and wild animals, such as pigs and birds, provides both breeding grounds and ample feed sources for mosquitoes,increasing risks of mosquito-borne diseases in southeast Asia.
Material and Methods: To bridge the knowledge gap of vector-borne disease risks in such settings, we examined mosquitoes and larvae composition at 15 LMWs by using CDC light traps and a hand net between Jan to March in 2023, in Laos. The collected mosquitoes were then identified morphologically at Institut Pasteur du Laos. The mosquito larvae and adults were later pooled according to species/genus and gender. The pools were proceeded for flavivirus screening using a Pan-Flavi RT-PCR assay and the samples of interest were sent for sequencing.
Results: Although the study period was during the dry season, a total of 1119 adult mosquitoes, 159 larvae, and 14 pupa were collected from these LWMs. Specifically, we found adult mosquitoes of five genera present in the LWMs, with *Culex quinquefasciatus* (85.7%, n=959) being the most abundant species and followed by *C. hutchinosoni* (11.3%, n=126), *C. vishnui* (2.0%, n=22), and the other species were *C.gelidus* (n = 5), *C. fuscocephala* (n = 4), *C.sitiens* (n = 2), *Armigeres subalbatus* (n = 4), *Anopheles aconitus* (n = 2), *Aedes vexans* (n = 2), *Aedes albopictus* (n =1), and *Mansonia spp* (n = 1), while all 159 mosquito larvae and 14 pupa belong to the *Culex* mosquito genus. We found that stagnant wastewater caused by the markets was the most common larval habitat (58.4%, n=101), as compared to the other ecological habitat types including running water or vegetation such as ponds, ditches, or puddles at markets. A total of 284 pools were analyzed by the Pan-Flavi RT-PCR assay. We did not find pathogenic flaviviruses from collected mosquitoes but insect-specific viruses have been found.
Conclusion: Considering that *C.quinquefasciatus* is the most abundant species present in LWMs, there might be a risk of disease transmission at interfaces between live animals and humans, for instance, Japanese encephalitis virus which requires both pigs and competent vector *C.quinquefasciatus* to infect humans, or possibly other mosquito-borne diseases. Our study also suggested that stagnant water caused by LWMs could be a hotspot for mosquito breeding, which can further provide information on wastewater management in LWMs for mosquito control.

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Role of rodent reservoirs for Wesselsbron, a neglected zoonotic virus

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Background and Objectives: The Wesselsbron virus (WSLV) is a neglected mosquito-borne flavivirus endemic to sub-Saharan Africa. Despite its prevalence, the specific routes of viral transmission remain unclear and several potential reservoirs have been proposed, including sheep, goats, cattle, horses, and rodents. WSLV infection in adult ruminants is mainly asymptomatic and self-limiting, whereas in pregnant animals it can lead to abortions and fetal malformations. In newborns, viral infection can result in high fever and lethargy; in severe cases, it can be fatal. Importantly, WSLV has zoonotic potential, and acute cases of human infections with Dengue-like symptoms have been reported
Material and Methods: In this study, WT-mice, indluding BALB/c, C57BL/BJ, 129/Sv and CAST/Ei, and immunocomromised Ifnar1 mice were subcutaneusly infected with 102-10^6 $TCID_{50}$ of WSLV, strain SA999 or SAH177.
Results: Wild-type mice showed no signs of disease after infection with WSLV; only C57BL/6J mice displayed susceptibility to infection, with transient viremia and detectable levels of the virus in various organs 4-10 days post-infection. In contrast, immunocompromised Ifnar1 mice experienced severe disease onset within 4-6 days post infection, with viral accumulation in multiple tissues and swelling at the site of infection in some cases.
Conclusion: Taken together, our preliminary rodent studies showed that WSLV replication in mice was dependent on the genetic background and age of the infected animals, providing a basis for further studies in rodents as potential hosts for WSLV, with a special focus on cellular tropism and vector-free transmission studies.

Screening of viruses in ticks collected from wild birds in Slovakia

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Background and Objectives: The relationship between birds and the spread of disease is a well-documented phenomenon. As global temperatures continue to increase, the understanding of tick-borne diseases, their prevalence, and their association with birds, becomes more relevant. Climate change has affected the migration habits of many bird species, and with these changes, the spread of tick-borne viruses follows. In northern latitudes, isolated pockets of ticks could be observed due to bird migration. But with milder winters, ticks are able to establish permanent populations in these areas. The goal of this study was to collect ticks from wild birds, then process and analyse the genetic material from ticks with special emphsis on PCR detection of rna viruses.
Material and Methods: The ticks were collected at the Bird Ringing Station Drienovec, in Slovakia, close to the city of Kosice. The birds were captured and handled by a licenced ornithologist (ĽK) under the Permission No. 3320/2019-6.3 from the Act. No. 543/2002 of the code on nature and landscape protection, granted by the Ministry of Environment of the Slovak Republic. The collection of ticks was conducted between the 22nd of March and the 3rd of September 2019. The prevalence of particular tick species and identification the bird species the ticks were collected from was performed. RNA was isolated from each individual tick by NucleoSpin RNA kit and the classic PCR and subsequent sequencing were performed.
Results: The results of the 145 ticks collected and processed and show that two pools were positive for Orbivirus. Of the ticks collected, 135 were identified as *Ixodes ricinus*, two specimens were identified as *Ixodes ventalloi*, one as *Ixodes frontalis*, two as *Heamaphysalis concinna*, and five ticks were identified as *Ixodes* spp. Of the 145 ticks collected, 36 larvae, 107 nymphs and two female ticks were collected.
Conclusion: The screening of ticks for pathogens is a valuable tool in both the spread of knowledge to the public, and the scientific community as a whole. With both recent and historic pandemics in mind, many of these pathogens have their origins in wildlife. It is in our best interest to continue screening wildlife for current and potential pathogens. Our increased knowledge of the prevalence of potential pathogens may one day prove invaluable in the prevention of another pandemic.

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Seroprevalence of Tick-Borne Encephalitis Virus (TBEV) in free-living herbivores in a nature reserve in the Netherlands

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Background and Objectives: The prevalence of tick-borne encephalitis virus (TBEV) has increased and spread geographically in recent decades. TBEV, among other tick-borne pathogens, is important to both veterinary and public health. It circulates in ticks and small mammals, but large herbivores which are introduced in nature areas, are also important hosts for virus transmission. At the Oostvaardersplassen (OVP) nature reserve in the Netherlands, the presence of pathogens in free-living large herbivores is actively monitored. However, the prevalence of tick-borne pathogens has not yet been assessed. This is important, as for example nature rangers and visitors could potentially be exposed to TBEV. This emphasizes the importance of approaching the control of TBEV and tick-borne pathogens within a One Health framework. Our study aimed to estimate the seroprevalence of TBEV in free-living large herbivores present at the OVP nature reserve.
Material and Methods: We conducted a seroprevalence study of TBEV in red deer, Konik horses and Heck cattle at the OVP nature reserve between September 2023 and March 2024. In addition, available samples from Konik horses from 2019 and 2020 were included. Serum samples were first screened using a commercially available TBEV multi-species competitive ELISA. Borderline and positive samples were then confirmed by a virus neutralisation test (VNT).
Results: Preliminary findings indicate exposure to TBEV in the area. During the sampling period of 2023-2024, forty-seven (47/153) red deer, two (2/47) Konik horses, and no (0/26) Heck cattle had a borderline or positive result in the ELISA. Following VNT, no antibodies were detected in the horse samples, and 11 red deer tested positive, indicating a sero-prevalence of 7.57% (95% CI 3.94-12.94). Additionally, in Konik horse samples of 2019 and 2020, twenty-two (22/181) and five (5/83) samples were borderline or positive in ELISA. Confirmatory VNT detected 1 and 2 positive horses, respectively, indicating a seroprevalence of 0.58% (95% CI 0.025-2.99) and 2.57% (95% CI 0.45-8.55).
Conclusion: Our results indicate past TBEV infections in free-living herbivores at the nature reserve OVP. These new insights can better inform the management of the area and highlight the importance of (re)emerging pathogen surveillance, utilizing wild animals as sentinel species. Further screening for other tick-borne pathogens with zoonotic characteristics is ongoing. This aims to further contribute to the knowledge of pathogen prevalence in nature areas, which gives emphasis to the One Health aspect of the current research.

Surveillance of the dog tick Dermacentor reticulatus in an urban area

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Background and Objectives: Urban environments, which include green spaces, support a variety of ecosystems where ticks and their hosts flourish, presenting risks to public health. It is essential to comprehend the presence of ticks in urban areas for effective management.
Material and Methods: A study spanning three years (2021-2023) was carried out in the city of Košice, Slovakia, focusing on ticks in urban green spaces. Ticks were collected using flagging techniques in wooded and scrubby vegetation areas within the city limits.
Results: *Dermacentor reticulatus*, typically found in rural settings, was identified within the city center where its usual habitat is absent. Significantly different tick abundances were noted between scrubby and wooded areas, with *D. reticulatus* primarily inhabiting the former. Monthly variations in tick density were observed across the years, with *D. reticulatus* activity commencing as early as February.
Conclusion: Our results underscore the significance of accounting for geographical and ecological factors in studies on tick distribution, particularly in urban contexts. Effective public health management strategies should integrate an understanding of tick presence and behavior in urban surroundings, emphasizing the importance of monitoring and implementing measures such as vegetation upkeep to reduce tick-related risks in urban areas.

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The role of rodents as vectors of swine diseases in swine farms in Poland

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Background and Objectives: Rodents are known reservoirs for pathogens that cause infectious diseases in animals. The aim of this study was to investigate swine pathogens in pigs and rodents caught on farms in Poland.
Material and Methods: The analysis was conducted in three Polish swine farms; Farm A maintained 50 sows under an "all-in-all-out" system, Farm B, housing 400 sows, operated a full production cycle and Farm C was a fattening unit with 500 pigs. Epidemiological status of farms was evaluated using ELISAs for porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae*. For *Leptospira spp.,* ELISA and microscopic agglutination test (MAT) were performed. Swine faeces were tested by real-time PCRs for *Lawsonia intracellularis*, *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli* and *Brachy-spira hampsonii*. Rodent tissue samples were analysed by PCR for *L. intracellularis*, and real-time PCR for *B. hyodysenteriae* and *Leptospira spp*. The biosecurity level of the farms was evaluated. Statistical analysis was performed using multiple correspondence analysis (MCA).
Results: All pigs tested were negative for PRRS, *B. hyodysenteriae*, *B. pilosicoli* and *B. hampsonii*. All rodents belonged to the house mouse (*Mus musculus*) species. MCA results indicated a positive correlation between the number of caught mice and detection of *B. hyodysenteriae* in mice, as well as occurrence of *L .intracellularis* in pigs (Farm C). The closed cycle Farm B, which had a lower number of rodents caught, showed higher frequency of *Leptospira spp*. detection in pigs compared to the other farms. *B. hyodysenteriae* infection was not observed in any of the mice tested on Farm B. In Farm A, having a lower biosecurity level, closed production cycle and occasional presence of rodents, the mice were highly positive (above 44.0%) for *B. hyodysenteriae*, which did not correlate with any of the risk factors.
Conclusion: The findings suggest that the epidemiological status of pigs on farms with varying characteristics and the threat posed by infected rodents should be considered in the context of biosecurity measures' effectiveness. Ensuring animal welfare and optimal production organization is paramount. The presence of a higher number of rodents, larger farm size, and semi-open production cycles may contribute to the deterioration of pigs' health despite high biosecurity levels. This study indicates that the occasional presence of rodents, harbouring pathogens that cause disease in pigs, does not significantly affect pigs' health compared to other factors, such as optimal herd size and a closed production cycle, which are crucial for effective swine health management.

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The Socioeconomic impacts of Rift Valley Fever: A Rapid Review

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Background and Objectives: Rift Valley Fever (RVF) is a neglected vector-borne disease which is endemic in many countries across Africa and has seen recent geographical expansions into the Arabian Peninsula. RVF can cause severe infections in both animals and humans. RVF infections in livestock can lead to mass fatalities. In humans the symptoms are non-specific and can often lead to misdiagnosis. However, a small proportion progresses to haemorrhagic infection with a significantly higher mortality rate. The culmination of this can cause severe socio-economic impacts.

Aims: This review aims to identify the main socio-economic impacts caused by RVF outbreaks as well as existing knowledge gaps.
Material and Methods: The review analyses RVF's socioeconomic impact using studies published until 2023.
Results: Ninety-three academic and grey papers were selected, covering 19 countries and 10 methodological approaches.

A variety of socio-economic impacts were found across all levels of society: Livestock trade disruptions to control RVF outbreaks consequently impacted local food security, local and national economies.

Most livestock farmers in endemic countries are subsistence farmers and so rely on their livestock for sustenance and income. RVF outbreaks resulted in a variety of socioeconomic impacts e.g., the inability to pay for school fees. Main barriers to vaccine uptake in communities were lack of access, funds, interest along with other social aspects. The occupational risks for women (and pregnant women) are largely unknown.

Vaccination strategies evaluated through mathematical modelling suggest seasonality and the movement of high concentrations of livestock (for religious events) play key roles in the dissemination of RVF into susceptible populations.

To our knowledge this is the first review on RVF to highlight the clear knowledge gap surrounding the gender differences on risks of RVF exposure, as well as differences on occupational health risk in pastoral communities. Further work is required to fill the gaps identified in this review and inform control policies.
Conclusion: The evidence suggests that policies implemented for the control of RVF are designed without the consideration of farmers needs and motivations, therefore unlikely to be complied with. Greater incorporation of different stakeholders in policy development is required to inform control strategies. There is a clear knowledge gap surrounding the risks of RVF exposure to women. Urgent work is required to fill these gaps and inform control policies.

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Use of the yeast two-hybrid approach for the identification of cellular interactors of AHSV: on the road to understanding virulence mechanisms

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Background and Objectives: African Horse Sickness (**AHS**) is a devastating disease of equids caused by AHS virus (AHSV), an arbovirus of the *Sedoreoviridae* family, genus *Orbivirus*. There are 9 described AHSV serotypes transmitted by haematophagous *Culicoides* midges. The horse infection leads to severe respiratory and circulatory impairment and is often fatal. AHSV is endemic in tropical and subtropical Africa areas but has repeatedly spread outside Africa causing severe outbreaks in Europe and Thailand. These emergences (partly driven by climate changes) in previously free regions argues that this is an active and **ongoing global threat** to the horse industry. AHSV is an obligate intracellular pathogen whose replication cycle requires cellular host reprogramming processes. Viral hijacking of cell signaling pathways is largely driven by virus-host protein-protein interactions (PPIs) but so far, no PPIs study has been conducted for AHSV.
Material and Methods: Thus, to identify virus-host PPIs related to virulence, we have compared the interactome of a AHSV-5 virulent strain and of a AHSV-4 low pathogenic strain with a **yeast two-hybrid (Y2H) system.**
Results: Among the 2,826 interactions highlighted by these experiences, 83 interactors were identified. Interestingly, some interactors seem to be restricted to the virulent serotype like those of VP2. Bio-informatic analyses established interactome maps and identified the biological functions of each cellular partner to reveal AHSV targeted cellular pathways. Preliminary data indicate that the major biological functions hijacked by AHSV are the **host-virus interaction**, **protein transport** and **RNA-binding**. Again interestingly, some viral proteins like the NS3 seem to interact with certain biological functions preferentially like the immune process pathway.
Conclusion: Unravelling the functions of viral proteins and their interactions with host proteins is a prerequisite for the development of new therapeutic and prophylactic solutions. Within this context, this study will also allow us to identify crucial interactors which could play a role in **AHSV virulence mechanisms**.

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Visceral Leishmaniasis Seroprevalence in Dogs in Tehran, Iran: A Cross-Sectional Study in 2023

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Background and Objectives: Visceral leishmaniasis (VL), which is caused by Leishmania infantum, is prevalent in subtropical regions. In Iran, VL primarily affects people in rural areas, with children under 10 years of age being particularly susceptible to VL. Dogs serve as the main reservoirs for transmitting the parasite through sandfly bites. Identifying asymptomatic infected dogs is crucial for controlling the spread of the disease and for preventing human infections. Early detection and management of infected dogs can help mitigate the risk of VL transmission to humans and contribute to overall efforts to control the disease.
Material and Methods: This study, conducted between January and October of 2023, collected data from 429 dogs examined in veterinary hospitals. Permission was acquired from the dog owners. We employed a Direct Agglutination Test to detect and evaluate the presence of anti-Leishmania antibodies. We used PCR for ITS2 to accurately identify species.
Results: It was found that 2.79% of the patients tested positive for leishmaniasis. In addition, the sequence of all isolates was 95% similar to that of L. infantum via the Nested-PCR assay. The highest frequency of positive cases was in the 0–4-year age group and among males, which was statistically significant. (P>0.05). This study highlights the significant role of dogs as the primary reservoir of Leishmania since infected dogs can spread Leishmania parasites for their entire life, without exhibiting any symptoms. The prevalence of visceral leishmaniasis (VL) in dogs in Tehran, Iran is 2.79%, which is comparatively lower than the rates observed in other areas. This indicates that several factors, such as climate, geography, sand fly activity, and socioeconomic situation, can play a role in causing these variations. Regular examination of dogs in high-risk areas is essential to control VL.
Conclusion: This study also highlights the importance of using simple, inexpensive, and reliable diagnostic methods, such as DAT, for VL detection. These findings call for increased awareness and monitoring of VL and the implementation of prevention measures, especially in areas with high populations of dogs and humans.

		Dogs tested (N)	Positive	Odds Ratio ((95% CI)
Sex	Female	81	3(3.70%)	male/female
	Male	347	9(2.59%)	1.423(0.498–4.065)
Age	0-4	265	7(2.64%)	≥4 / 0-4
	≥4	164	5(3.05%)	1.154(0.364–3.660)
Total		429	12(2.79%)	

WNV SURVEILLANCE PROGRAM IN SERBIA IN 2023: THE RESULTS IN VOJVODINA PROVINCE

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Background and Objectives: The concept of the national integrated surveillance program for the West Nile virus (WNV) conducted in 2023 in Serbia and the obtained results of the program on the territory of the Vojvodina Province of Serbia were presented. The WNV surveillance program, funded by the Veterinary Directorate of the Ministry of Agriculture, Forestry and Water Management, was carried out by the veterinary service in cooperation with entomologists and ornithologists. The aim of the program was early detection of WNV circulation in the environment and timely reporting to the public health service and local authorities to increase preparedness for clinical and mosquito control.
Material and Methods: The program was based on the detection of the presence of WNV by molecular mehods in wild birds as natural hosts and mosquitoes as virus vectors, and on serological testing of sentinel horses and young cattle calved after the previous vector season for the presence of WNV-specific IgM or IgG antibodies by ELISA test, respectively.
Results: WNV circulation was detected in all districts of the Province of Vojvodina from the end of June / beginning of July to September 2023. The integrated WNV surveillance program in 2023 detected a lower intensity of WNV circulation compared to 2022, and approximately similar to the level of virus circulation in 2021. The presence of WNV IgM antibodies in the blood serum of horses was detected in 1.81% (10/554) of the tested animals, while the presence of WNV IgG antibodies in the blood serum of young cattle was detected in 14.20% (161/1134) of the tested animals. Some number of WNV IgG positive samples were confirmed by VNT. The presence of WNV was detected in 7.42% (2/27), 5.56% (2/36) and 0% (0/4) of samples of dead, live captured and shot wild birds, as well as in 5.71 % (18/315) of pooled samples of *Culex pipiens* mosquitoes.
Conclusion: The WNV surveillance program in 2023 showed satisfactory results in terms of capacity to indicate the spatial distribution of the risk of human infection.

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