EPIZONE

BOOK OF ABSTRACTS

EPIZONE Annual Meeting 2026 in Bern, Switzerland, 2-4 September 2026

Hosted by

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Institut für Virologie und Immunologie

UNIVERSITÄT BERN

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Contents

Welcome

Dear EPIZONE members, dear Young EPIZONE members, dear participants,

We are delighted to welcome you in Switzerland for three days of scientific exchange, discussion, and collaboration at a time when preparedness for emerging and re-emerging animal diseases has never been more important.

The ongoing spread and continuous threat of highly contagious animal and vector-borne diseases in Europe is unprecedented. Being prepared for animal health crises is more important than ever. Exchange among research experts through strong networks remains a pillar of preparedness.

The 2026 EPIZONE meeting focuses on exploring how research can better support the necessary preparations for emergencies involving highly contagious animal diseases and zoonoses within a One Health approach. In this context, preparation means understanding the mechanisms of infection transmission, developing solutions to control diseases before they impact the livestock sector, and ensuring the necessary diagnostic capacities and (novel) vaccines and vaccination strategies are developed and in place. The Young EPIZONE session looks at career pathways and provides important insights on communication research findings.

With a carefully selected range of keynote talks representing various stakeholders involved in infectious disease control, along with scientific sessions covering a wide range of topics and presentations from EPIZONE research partners on the latest advances in detection, prevention, and understanding of key pathogens, we aim to facilitate a stimulating, insightful, and collaborative meeting in the heart of Switzerland.

Thank you for being part of EPIZONE 2026.

We wish you an excellent meeting and an enjoyable stay in Bern.

Barbara and Matthijn

Barbara Wieland
Chair Local Organizing Committee, IVI

Matthijn de Boer
Coordinator of EPIZONE

Acknowledgements

We thank the following sponsors for their generous support of the 2026 EPIZONE Annual Meeting:

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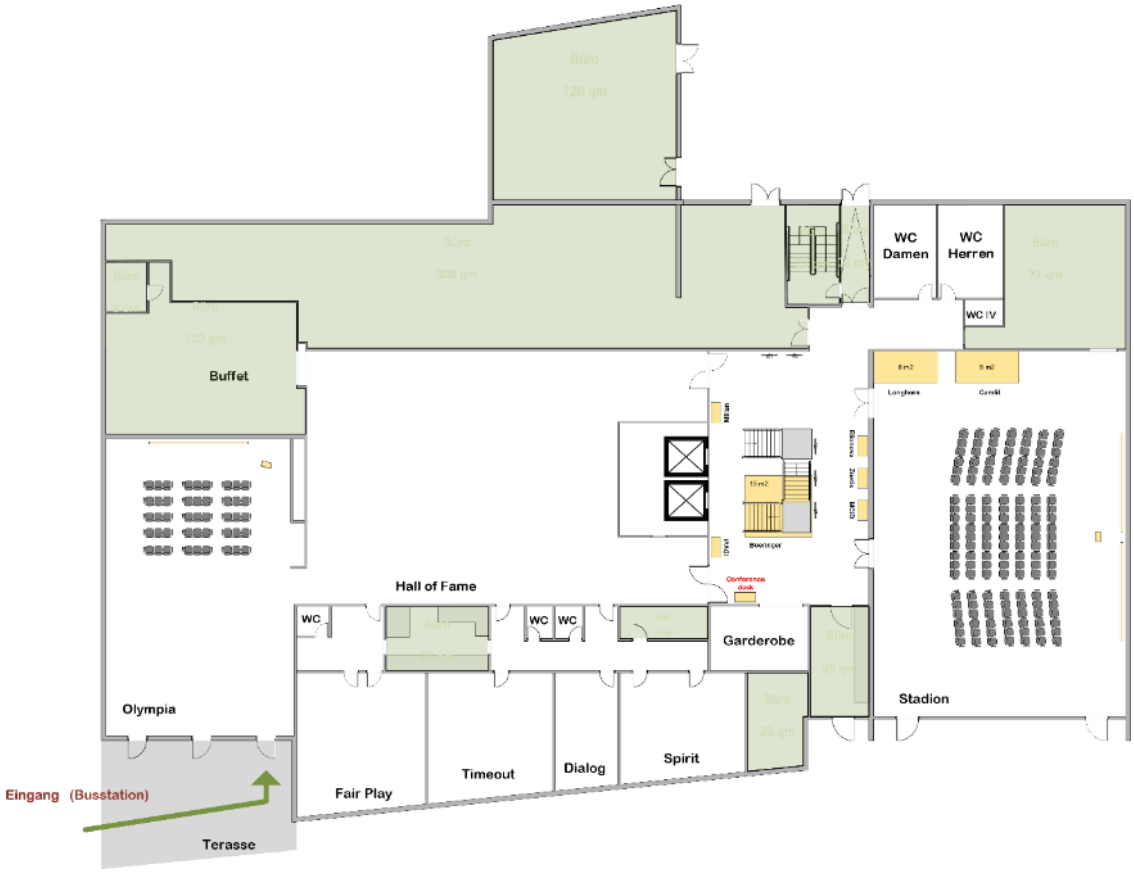
Venue Plan


WC Damen
WC Herren
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Buffet
Hall of Fame
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Garderobe
Olympia
Stadion
Fair Play
Timeout
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Spirit
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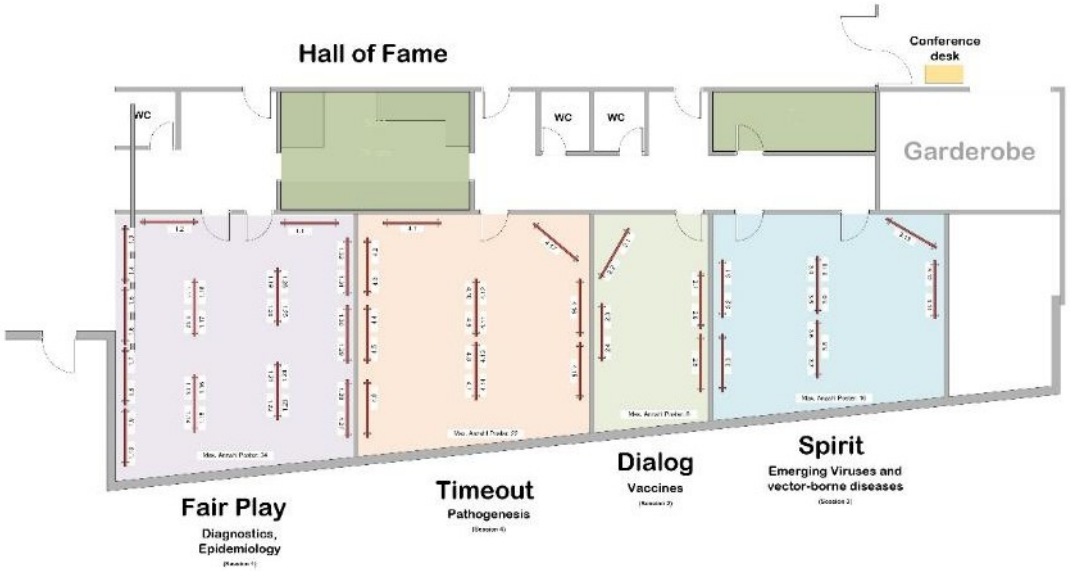
Hall of Fame
Conference desk
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Garderobe
Fair Play
Diagnostics, Epidemiology
Timeout
Pathogenesis
Dialog
Vaccines
Spirit
Emerging Viruses and vector-borne diseases

Program

Young EPIZONE – Wednesday, 2 September 2026

08:30 – 08:40	**Opening & Introduction**
08:40 – 09:00	**Introduction to the Young EPIZONE Network**
09:00 – 10:00	**“Career Reality Check” – Interactive Panel & Live Polling**
10:00 – 10:30	*Coffee Break*
10:30 – 12:00	**Social Media Workshop**
12:00	*Lunch*

“Career Reality Check” Panelists

- **Prof. Dr. Carmen Faso** is a professor at the University of Bern, where she established her own research group with a focus on parasitic protists. She is also co-chair of the Multidisciplinary Center for Infectious Diseases.
- **Dr. Laurent Monnerat** was CEO of Zoetis in Germany and Abaxis Europe, then a cantonal veterinarian in Switzerland, and is now Director of the Swiss Federal Food Safety and Veterinary Office.
- **Dr. Adriano Taddeo** is currently a Product Manager at Miltenyi Biotec, where he bridges cutting-edge immunology with commercial product strategy in the biomedical research field
- **Prof. Dr. Thomas Kaufmann** is an Associate Professor at the Institute of Pharmacology, University of Bern, Switzerland. In 2021 he co-founded ATANIS Biotech AG, a spin-off company of the University of Bern, which specializes in advanced allergy diagnostics.
- **Dr. Büşra Çoban-Poppinga**, neuroscientist and former postdoctoral researcher. After around ten years in academic research, she is now navigating the transition into the life sciences industry.
- **Prof. Dr. Llilianne Ganges**, veterinary virologist, Principal Investigator at the Institute of Agrifood Research and Technology (IRTA-CReSA, Barcelona, Spain), and Coordinator of the WOAH Reference Laboratory for Classical Swine Fever.

EPIZONE Day 1 – Wednesday, 2 September 2026

13:30 – 14:00	*Doors Opening & Poster Mounting*
14:00 – 15:15	**Plenary Session 1 – Current Disease Challenges** Location: Stadion — Special session: Shamonda virus emergence in Switzerland (Jakub Kubacki, Institute of Virology and Immunology IVI) — Hepatitis E (Wim van der Poel, Wageningen University and Research) & recognition of his longtime contribution to EPIZONE
15:15 – 15:30	**Poster Slam – Round 1** Location: Stadion
15:30 – 16:00	*Poster Viewing & Coffee Break*
16:00 – 17:30	**Parallel Session 1.1 – One Health and Zoonoses** Location: Stadion **Parallel Session 1.2 – Addressing Current Challenges (HPAI, BTV, LSD, FMD, PPR, etc.)** Location: Olympia
17:30 – 19:00	**Poster Session**
17:30 – 20:00	*Welcome Reception*

EPIZONE Day 2 – Thursday, 3 September 2026

08:30 – 10:00	**Plenary Session 2 – Strengthen Preparedness** Location: Stadion — Crisis preparedness from a One Health perspective (Jakob Zinsstag, Swiss TPH) — Mobile labs (Sophie Duraffour, Mobile Laboratory and Outbreak Preparedness and Response OPR) — Responding to multiple outbreaks simultaneously: lessons learned from Greece (Konstantia Tasioudi, Department of Molecular Diagnostics, Veterinary Center of Athens) — Panel discussion: how can we improve preparedness and outbreak response?	
10:00 – 10:15	**Poster Slam – Round 2** Location: Stadion	
10:15 – 11:00	**Poster Session** **& Coffee Break**	
11:00 – 12:30	**Parallel Session 2.1 – Diagnostic Tools (Focus on Current Challenges with Re-emerging Diseases)** Location: Stadion	**Parallel Session 2.2 – African Swine Fever** Location: Olympia
12:30 – 13:30	*Lunch*	

EPIZONE Day 2 – Thursday, 3 September 2026

13:30 – 14:30	**Plenary Session 3 – Advancing Research** Location: Stadion — Beyond animal testing: will organ technologies aid vaccine development? (Dirk Werling, Royal Veterinary College, University of London) — How is AI shaping global health? (Filipa Matos Baptista, Siemens Healthineers)
14:30 – 14:45	**Poster Slam – Round 3** Location: Stadion
14:45 – 15:45	**Poster Session** **& Coffee Break**
15:45 – 17:00	**Parallel Session 3.1 – Vaccine Development** Location: Stadion **Parallel Session 3.2 – Disease Surveillance, Epidemiology, Risk Analysis** Location: Olympia
19:00 – 23:30	*Gala Dinner with a View – Excursion onto the Gurten Mountain*

EPIZONE Day 3 – Friday, 4 September 2026

08:30 – 10:00	**Plenary Session 4 – Coping with Future Threats** Location: Stadion — How can we control vector-borne disease? Experiences from Switzerland (Eva Veronesi, Scuola universitaria professionale della Svizzera italiana SUPSI) — Emerging and neglected threats: the example of Hantavirus in Europe (Martin Eiden, Friedrich-Loeffler-Institut FLI) — Updates from EUPAHW (Nathalie Vanderheijden, Coordinator of the European Partnership on Animal Health and Welfare) — Best Abstract: «Deciphering early virus-host interactions associated with pathogenic and protective immunity against African swine fever virus» (Enrique Ezcurra Zubizarreta, IRTA-CReSA)
10:00 – 10:30	**Poster Session** **& Coffee Break**
10:30 – 12:00	**Parallel Session 4.1 – Vector-borne Diseases** Location: Stadion **Parallel Session 4.2 – Pathogenesis and Virology** Location: Olympia
12:00 – 12:15	*Closing Ceremony*
12:30 – 13:30	*Light Lunch*

Keynote Speakers

Prof. Wim van der Poel

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Lecture: September 2, 14:30 – 15:15, Stadion

Wim van der Poel is professor of Emerging and Zoonotic Viruses at Wageningen University and Research, the Netherlands. He is a Doctor in Veterinary Medicine and obtained his PhD in virology at Utrecht University in 1995. In 2018 he joined the European College of Veterinary Microbiology (ECVM). His research interests have focused on detection and characterization of new and emerging viruses, including zoonoses. He has worked on emerging viruses research for more than 30 years and has been involved in many international research projects and networks including the EJP One Health, the Netherlands Center for One Health (NCOH), PREZODE and the Global One Health Research Partnership (GOHRP). Until 2024 he was Coordinator of the EPIZONE European Research Group. Wim van der Poel relies on a vast network of professional experts in the specific areas of emerging and zoonotic viruses; foodborne viruses and One Health.

Abstract – Hepatitis E Virus, an exemplar of One Health Challenges

Hepatitis E virus (HEV), family Hepeviridae, is a non-enveloped RNA virus and a main cause of hepatitis. In industrialized countries this is mainly due to infections of zoonotic origin resulting in single cases worldwide. Within the species Orthohepevirus A, genotypes 3 and 4 have a main reservoir in domestic swine and this leads to contaminations in the food chain. The virus may be transmitted to humans by pork products and by-products and different types of foods like shellfish, fruits, vegetables, water as environmental route may be involved. Recently, a rat HEV (genus *Rocahepevirus*) has been identified showing zoonotic potential.

In immunocompromised and liver disease patients, HEV infections can be very serious and fatal. Strategic antiviral therapy currently is the treatment of choice for patients chronically infected with HEV. In specialised hospitals such treatment is often successful but may be long-term and costly. Bloodborne transmission of the virus has been reported with a significant medical concern, which made a number of European countries decide to test blood donations for HEV positivity.

Control of zoonotic HEVs clearly needs a One Health approach, because of the multiple animal reservoirs, the virus' stability in the environment and the various transmission routes. Focal points for control along the food chain depend on the type of food and the stage in the food production process. Best feasible inactivation methods include heating at plus 71C, chlorine treatment and UV light. There is a need for sensitive and broad testing of food items that may be implicated in acute HEV infection.

Besides control options at the pork retail level and by the consumer it is important to try to reduce HEV in primary production and the whole swine reservoir. Raising the level of infectious disease control management on swine farms is indicated, so farms should review and tighten their biosecurity protocols and animal movement practises, and be especially diligent about visitors and supplies, feed ingredients, food items, etc.

Prof. Dr. Jakob Zinsstag

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Lecture: September 3, 08:30 – 10:00, Stadion

Prof. Dr. Jakob Zinsstag is a veterinarian with a PhD in tropical animal health. He spent eight years in West Africa at the International Trypanotolerance Centre in The Gambia and four years as the director of the Centre Suisse de Recherches Scientifiques in Côte d'Ivoire. Since 1998 he heads a research group on human and animal health at the Swiss Tropical and Public Health Institute. Since 2011 his is deputy head and since 2025 head of department of Epidemiology and Public Health at Swiss TPH. He focuses on the control of zoonoses in developing countries and the provision of health care to mobile pastoralists using a One Health approach. He is past president of the International Association for Ecology and Health and former president of the scientific board of the Transdisciplinary network of the Swiss Academies. He teaches One Health and Trandisciplinary theory and methods and is lead educator of massive open access online courses One Health: Connecting Humans, Animals and the Environment and Partnering for change: Link research to societal challenges . He is editor-in-chief of CABI One Health resources.

He received the Meritorious Award by the World Organization of Animal Health (WOAH) in 2023. He is a member of the One Health High Level Expert Panel (OHHLEP).

Abstract – Crisis Preparedness and One Health

While clinical medicine essentially concentrates on the human body and its parts, public health focuses on the health of human populations and their social and environmental determinants. Integrated approaches to health extend the focus of attention to humans in their socio-cultural and ecological environment and their mutual interdependencies, paying attention to inter-species interdependencies. Since the beginning of the 21st century, ecosystem approaches to health (EcoHealth), One Health and Planetary Health have emerged as integrated approaches that relate to and expand public health and related fields. In this talk we clarify their respective definitions, philosophical foundations and methodological positions. This clarification is important because the way we define integrated approaches to health shapes research, teaching methods and their translation into policy and practice. One Health is currently operationalized at the level of international organizations, regional organizations and national governments. Integrated approaches to health require urgent adoption and implementation for crisis preparedness at the level of integrated surveillance-response systems for pandemic prevention, vector borne zoonoses, biodiversity loss and antimicrobial resistance.

Dr. Sophie Duraffour

Bernhard Nocht Institute for Tropical Medicine (BNITM), Germany
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Lecture: September 3, 08:30 – 10:00, Stadion

Dr. Sophie Duraffour completed her PhD in tropical diseases in 2008 in France. Following ten years of research on poxviruses and antivirals at KU Leuven in Belgium, she specialized in viral haemorrhagic fevers and field genomics, with field experience spanning more than ten outbreaks in seven countries. Since 2022, she has led the Outbreak Preparedness and Response (OPR) and Mobile Lab units at the Bernhard Nocht Institute for Tropical Medicine (BNITM) in Hamburg, Germany. Her group develops laboratory capacity in challenging settings to strengthen the detection and genomic surveillance of emerging pathogens, and has contributed to nanopore field pipelines and to key findings on the circulation, emergence and persistence of Ebola virus, among other pathogens. The unit coordinates the European Mobile Laboratory (EMLab), which provides diagnostic capacity during health emergencies. As well as being a WHO/GOARN partner, the EMLab is a certified German asset within the European Union Civil Protection Pool (ECPP).

Dr Sophie Duraffour, Head of Outbreak Preparedness and Response (OPR) & Mobile Lab unit, Department of Virology, Bernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, Germany - Email: sophie.duraffour@bnitm.de

Abstract – One Health, One Lab? Mobile Labs in Human Outbreaks, and Their Potential for Veterinary Response

Mobile laboratories have become a standard component of outbreak response, bringing diagnostic and sequencing capacity directly to affected areas when local infrastructure is absent, overwhelmed, or too distant to support timely case management. Rapid response mobile laboratories (RRMLs) span a range of formats, from truck- or container-based units to lightweight kits deployable within days, each involving trade-offs between capacity, mobility and biosafety. The European Mobile Laboratory (EMLab), coordinated by the Bernhard Nocht Institute for Tropical Medicine, Germany, has been deployed to more than 15 outbreaks, mobilizing over 270 experts, and has provided diagnostics for high-consequence pathogens such as orthoebolaviruses as well as for emerging infections including SARS-CoV-2, alongside on-site genomic surveillance using nanopore field pipelines. This presentation will outline the EMLab concept, what deployment experience has shown about its strengths and limitations, and the conditions under which such a capacity is genuinely useful. It will close by opening the question of whether, and under what conditions, this model might translate to epizootic and transboundary animal disease outbreaks, where sample volumes, biosafety requirements, declaration mechanisms and the economic stakes of a result differ substantially from human outbreak settings.

Dr. Konstantia Tasioudi

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Lecture: September 3, 08:30 – 10:00, Stadion

Konstantia Tasioudi, DVM, MSc Mol Med, MSc SAB, PhD, EMBA is the Head of the Department of Molecular Diagnostics, FMD, Virological, Rickettsial and Exotic Diseases, Athens Veterinary Center, Ministry of Rural Development and Food, which is the NRL for several viral diseases including PPR, FMD, BT, AHS, ASF, CSF, SPPV, LSD.

Abstract – Responding to multiple outbreaks simultaneously: lessons learned from Greece

In recent years, global animal health authorities have faced an increasingly complex epidemiological landscape characterized not by isolated epizootic events, but by overlapping, multi-pathogen threats. Over the last three years, Greece has faced a continuous sequence of concurrent, high-consequence outbreaks—focusing on Peste des Petits Ruminants (PPR), Sheep and Goat Pox (SGP), African Swine Fever (ASF) and Bluetongue (BT) in 2024, SGP, ASF and BT in 2025, and SGP alongside Foot-and-Mouth Disease (FMD) in 2026.
Managing these simultaneous epizootics has placed unprecedented pressure on national veterinary services, Greek National Reference Laboratory (NRL), field personnel, and regulatory frameworks. By evaluating what worked, where bottlenecks occurred, and how emergency response structures adapted in real-time, key messages arise from the experience of the Greek NRL to better respond to future epizootic challenges.

Prof. Dirk Werling

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Lecture: September 3, 13:30 – 14:00, Stadion

After gaining a BSc.VetMed. (Veterinary University Hannover), my Dr.Med.Vet. thesis at the ETH Zuerich/Faculty of Veterinary Medicine, University of Zuerich, examined the impact of Bovine Leukaemia Virus infection on bovine macrophages. After stipends by the Swiss National Science Foundation (SNF), the German Research Foundation (DFG) and a Marie Curie Research Fellowship of the EU, I moved back to the ETH Zuerich as a Senior Scientist (Oberassistent). In 2001, I accepted an Assistant Professorship (Tenure Track) at the Institute of Virology (University of Berne), in the group of Thomas Jungi. In 2003, I accepted a Senior Lectureship at the Royal Veterinary College, and was promoted to Full Professor in 2007. The work my group is currently pursuing relates to mucosal immune systems, esp the innate immune side, the impact of microorganism on the development of the immune system, and how this knowledge can be used to enhance veterinary vaccines.

Abstract – Beyond animal testing: will organ technologies aid vaccine development?

Animal experiments date back to ancient Egypt and Greece and became central to physiological and pharmacological research during the 19th century. By the 20th century, they were systematically used in drug development, with agencies such as the FDA and EMA establishing preclinical testing guidelines following disasters including the 1937 sulfanilamide poisonings and the thalidomide tragedy. Animal testing has since remained the gold standard for assessing drug safety and efficacy. However, there are growing reasons to reduce animal experimentation. Preclinical testing can cost hundreds of thousands to millions of dollars per compound, while biological differences between humans and animals can limit the translation of findings to clinical settings. In some cases, alternative methods may outperform animal models. For example, in silico trials using human cardiomyocyte models predicted clinical arrhythmia with 89% accuracy, compared with 75% for animal models. Ethical concerns have also encouraged alternatives under the Three Rs principle: Replacement, Reduction, and Refinement. These include cell-based assays, organoids, 3D-bioprinted tissues, organ-on-chip systems, and computer modelling using artificial intelligence and machine learning. In my seminar, I will show some examples of where we stand with these technologies in veterinary medicine, how these can be used to develop “strategies” quicker, and what will be needed to obtain comparable data sets between different institutions.

Prof. Filipa Matos Baptista

Siemens Healthineers, Portugal
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Lecture: September 3, 14:00 – 14:30, Stadion

Filipa Matos Baptista, DVM, PhD, MBA, is an Assistant Professor at Nova SBE and Chief of Staff to the Managing Board at Siemens Healthineers, global med tech company. With over 15 years of leadership across health technology, corporate strategy, and academia, her work bridges digital health innovation, executive management, and scalable artificial intelligence applications in global healthcare.

Abstract – How Artificial Intelligence is Redefining Research, Diagnostics, and One Health

Artificial Intelligence is driving a fundamental shift in global health, enabling a transition from reactive measures to proactive prediction. This keynote explores emerging how AI architectures, from 3D molecular folding engines to agentic surveillance networks, are accelerating pathogen discovery, vaccine design, and point-of-care diagnostics. It highlights the role of multimodal data integration across human, animal, and environmental domains in advancing One Health security. Finally, it addresses critical operational and ethical challenges, outlining strategic frameworks required to transition AI innovations into scalable, trusted real-world health interventions.

Dr. Eva Veronesi

Institute of Microbiology, Department of Environment, Constructions, and Design, University of Applied Sciences and Arts of Southern Switzerland (SUPSI)
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Lecture: September 4, 08:30 – 09:00, Stadion

I am a medical and veterinary entomologist with 30 years of experience in the control and preparedness of vector-borne diseases. My field of expertise is surveillance and control of local and invasive mosquitoes. In the last twenty years, I have focused my research on the interaction between pathogens and vectors, investigating transmission mechanisms and aspects of vector competence for several viruses (dengue, Zika, chikungunya, west Nile, bluetongue, African horse sickness, lumpy skin, epizootic haemorragic disease) and vectors (mosquitoes, Culicoides). In my career, I have held several roles, including project manager, group leader, student and staff supervisor, private consultant, reviewer of publications, theses, and scientific reports, and coordinator of clinical trials for vaccine efficacy tests for government, research institutes, universities, NGOs, and private industries. I served as a consultant, project evaluator, and advisor for several organisations (World Health Organization, Federal Food Safety and Veterinary Office, European Food Safety Authority, Food and Agriculture Organization, International Atomic Energy Agency) on revising and editing protocols, standard operating procedures, guidelines, and risk assessment plans for emerging diseases complying with the One Health perspective. From 2023 to 2028 I was the Director of the European Society for Vector Ecology (E-SOVE) the most relevant society in Europe for vector ecology. I am now working in the biosafety unit at the Institute of Microbiology of the Department of Environment, Constructions and Design of the University of Applied Science and Arts of Southern Switzerland (SUPSI). Here, I coordinate two projects on emerging vector-borne epidemics (dengue and West Nile virus) funded by the European Commission (HorizonEurope), a project funded by the Federal Food Safety and Veterinary Office on Culicoides vector of bluetonge disease, and a project on the impact of biopesticides on public health and the environment funded by the Swiss National Research Foundation. I authored more than 50 papers and two book chapters. I specialize in working under biosafety containment level 3, providing courses on how to setup arthropod containment laboratories and to work in these facilities including conducting risk assessments for new facilities.

Abstract – How can we control vector-borne disease? Experiences from Switzerland

Vector-borne diseases (VBDs) are among the fastest-growing public and animal health challenges in Europe. Climate change, increasing global connectivity, land-use changes, and the expansion of competent vectors are reshaping the epidemiology of many pathogens, making traditional reactive approaches increasingly insufficient. Effective control requires moving from outbreak response to anticipation through integrated surveillance, early warning systems, and coordinated One Health strategies. Switzerland, and particularly the Canton of Ticino, represents an ideal natural observatory for these challenges. Located at the interface with northern Italy, where arboviruses such as West Nile virus (WNV) and bluetongue virus (BTV) have recently emerged, the region offers a unique opportunity to develop and evaluate surveillance systems capable of detecting pathogens before widespread transmission occurs. Drawing on the experience of the Institute of Microbiology at SUPSI, this keynote will illustrate how integrated surveillance—combining medical and veterinary entomology, molecular diagnostics, epidemiology, and cross-border collaboration—can strengthen preparedness against emerging vector-borne diseases. The lecture will present ongoing activities targeting both vectors and

pathogens, discuss the key components required for effective VBD control, and highlight how surveillance should evolve from a monitoring activity to an operational decision-support system capable of guiding timely public health interventions.

Dr. Martin Eiden

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Lecture: September 4, 09:00 – 09:30, Stadion

Martin Eiden obtained his Diploma in Biology from the University of Würzburg in 1994, followed by a Ph.D. in Biochemistry from the University of Regensburg in 1998. Since 1999 he has been working at the Friedrich-Loeffler-Institut (FLI), Institute of Novel and Emerging Infectious Diseases (INNT), where he serves as Group Leader and Head of the National Reference Laboratories for Rift Valley Fever Virus and Hantaviruses.

Abstract – Emerging and neglected threats: the example of Hantavirus in Europe

Hantaviruses are globally distributed rodent-borne zoonotic viruses, with Puumala virus causing most human infections in Europe, followed by Dobrava-Belgrade virus while Tula and Seoul viruses cause sporadic cases. Persistent infection of specific rodent reservoirs and virus shedding through excreta enable transmission to humans mainly via inhalation of contaminated aerosols. Transmission risk is determined by rodent population dynamics, environmental virus persistence and stable virus circulation in endemic regions. Human infections exhibit marked temporal and geographic variation, with recurrent hotspots in Central Europe. Understanding these drivers of hantavirus circulation is essential for surveillance, outbreak prediction and One Health prevention.

Oral Presentations

Oral Presentations: Best Abstract Submitted

Deciphering early virus-host interactions associated to pathogenic and protective immunity against African swine fever virus

Ezcurra Zubizarreta, Enrique[1,2,3]; Martin, Beatriz[4,5]; Martin-Moraleda, David[1,2,3]; Muñoz, Jordana[1,2,3]; Coatu, Elena[1,2,3]; Tort-Miró, Aida[1,2,3]; Navas, María Jesus[1,2,3]; Muñoz, Marta[1,2,3]; Gonzalez-Oliver, Judith[1,2,3]; Monleon, Paula[1,2,3]; Accensi, Francesc[1,2,3,6]; Vidal, Enric[1,2,3]; Rapullés, Joan[1,2,3]; Cobos, Àlex[1,2,3]; Rodríguez, Fernando[1,2,3]; Esteve-Codina, Anna[4,5]; Montaner-Tarbes, Sergio[1,2,3]; Argilaguet, Jordi[1,2,3]

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3: WOAH Collaborating Centre for the Research and Control of Emerging and Re-Emerging Swine Diseases in Europe (IRTA-CReSA), 08193 Bellaterra, Spain.
4: Centro Nacional de Análisis Genómico (CNAG), Barcelona, Spain.
5: Universitat de Barcelona (UB), Barcelona, Spain.
6: Departament de Sanitat i d'Anatomia Animals. Facultat de Veterinària. Universitat Autònoma de Barcelona (UAB). Bellaterra

African swine fever (ASF) is a major challenge for swine production worldwide, currently uncontrolled, due to the lack of safe and effective vaccines. Live attenuated vaccines (LAVs) represent a valuable experimental tool, inducing protective immune responses without causing the severe disease observed with virulent ASF virus (ASFV) strains. However, the immune mechanisms triggered by LAVs remain poorly characterised. Early immune responses at the site of virus entry are critical in determining infection outcome. Thus, the comparison of virus-host interactions following inoculation with either a virulent ASFV strain or with a LAV may help identify early mechanisms associated with protection or disease. To address this issue, here we leveraged the divergent outcomes induced by LAVs vs virulent ASFV strains to dissect the spatial and temporal immune dynamics associated to protection or pathology. Pigs were intranasally inoculated with the LAV prototype BA71ΔCD2 or its virulent parental strain BA71, and samples from several tissues were collected at early timepoints after infection. Clinical signs, histopathology and viral load quantification confirmed the different outcomes linked to the attenuated and virulent strains. While BA71 caused a systemic infection, BA71ΔCD2 replication was restricted mainly to the tracheobronchial lymph nodes (TB LN). Notably, TB LN from BA71-infected animals displayed marked lymphopenia at later timepoints, consistent with ASF acute pathology, while BA71DCD2-inoculated pigs exhibited an increase of most immune cell subsets analysed. Confocal imaging further revealed that the high viral loads from TB LN of LAV-infected pigs were not associated with tissue damage, in contrast to the severe disruption of lymphoid architecture observed in the virulent infected samples. Bulk RNA-seq analysis of TB LN revealed distinct immune transcriptional profiles. Virulent infection induced a broad and dysregulated activation of innate immune pathways, whereas LAV-infection was associated with a delayed and targeted antiviral and immune-regulatory signatures. Altogether, these findings identify early mucosal immune events present in TB LN as key determinants of protective immunity following intranasal ASFV infection and provide relevant insights for an immunologically driven design of ASF vaccine strategies.

Keywords: Early immune responses, Lymphoid architecture, Differential host responses

Oral Presentations:

Parallel Session 1.1 – One Health and Zoonoses

Date, Time: September 2, 16:00 – 17:30
Location: Stadion

Contents:

Whole-Genome and Antigenic Characterization of Swine Influenza A Viruses in Italy Reveals Extensive Reassortment, Lineage Diversification, and Antigenic Divergence

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Swine influenza A viruses (swIAVs) circulate year-round in the Italian pig population and undergo frequent reassortment involving both swine- and human-origin influenza viruses. Since the late 1970s, the Eurasian avian-like H1N1 virus (H1avN-H1C lineage) has largely replaced the classical 1918-derived H1N1 lineage and continues to diversify. H3N2, historically represented by the Gent/84-like lineage (H3-1970-N2g), declined in prevalence until 2020 but has recently shown substantial evolutionary changes associated with reassortment events involving human seasonal influenza viruses (H3-OtherHuman-N2). H1N2, initially characterized by a human-like H1 (H1B lineage) and then by H1C hemagglutinin, has become the most prevalent and genetically diverse subtype. Since 2009, H1N1pdm09 (H1A lineage) has become established in swine populations, acting as an internal gene donor in multiple reassortant genotypes.
We genetically characterized swIAVs strains detected in Italy from 2023 to 2026 using whole-genome sequencing (n = 432) and assessed their antigenic relationships through hemagglutination inhibition (HI) assays and antigenic cartography on selected strains (n = 139). Among the 432 detected viruses, four subtypes were identified: H1N1, H1N2, H3N1, and H3N2. Whole-genome sequencing revealed extensive genetic diversity, with multiple HA–NA combinations and 29 distinct genotypes generated through reassortment between swine- and human-origin influenza viruses. H1N2 was the most prevalent subtype (49.5%) and showed the highest diversity (14 genotypes). H1N1 accounted for 40.2% of detections and comprised nine genotypes. Although H3N2 was the least frequent subtype (8.8%), several recently identified gene constellations suggest the emergence and potential establishment of novel reassortants carrying a human-origin H3 gene and an N2 gene derived from swine H1N2 viruses. The remaining detections included H3N1 (0.2%) and mixed infections (1.3%).
Antigenic characterization was performed on 139 isolates selected according to genetic features: 101 H1C strains (63 H1C-N2 and 38 H1C-N1), one H1B-N2 strain, 27 H1A strains (22 H1A-N1 and 5 H1A-N2), three H3-1970-N2 strains, and seven H3-OtherHuman-N2 strains. HI assays were performed using a panel of swine antisera targeting representative H1N2, H1N1, and H3N2 genotypes (including H1C-N1, H1A-N1pdm09, H1B-N2, and H3N2 reference sera). The results demonstrated progressive antigenic divergence of circulating strains from reference viruses, characterized by decreasing HI titers and, for some lineages, complete loss of reactivity with available reference antisera. This phenomenon was particularly evident among recent strains belonging to H1C.2.4, H1A.3.3.2, and H3-OtherHuman lineages. These findings highlight substantial antigenic evolution of Italian swIAVs and suggest that increasing genetic and antigenic divergence may compromise antibody cross-reactivity and reduce the effectiveness of current vaccine/reference strain matching.

Keywords: Swine Influenza A viruses, antigenic and genetic characterization, extensive reassortment, Italy

A bovine H5N1 virus efficiently replicates in differentiated human nasal epithelial cells

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Highly pathogenic avian influenza H5N1 viruses of clade 2.3.4.4b have caused unprecedented outbreaks in wild birds and poultry worldwide and have increasingly crossed species barriers to infect a wide range of mammalian hosts, including humans. The recent emergence of H5N1 infections in dairy cattle in the United States has raised concerns about further adaptation of these viruses to mammals and their potential for human-to-human transmission. To evaluate the capacity of these viruses to replicate in the human respiratory tract, we investigated the replication and host interactions of an H5N1 virus isolated from bovine milk in Texas in 2024 (H5N1-Tex/24) using differentiated primary human airway epithelial cultures.

H5N1-Tex/24 replicated in differentiated human nasal and bronchial epithelial cells with kinetics comparable to those of the 2009 pandemic H1N1 virus (H1N1-HH4/09). Notably, H5N1-Tex/24 replicated efficiently at both 37 °C and 33 °C, the latter corresponding to the temperature of the human nasal cavity and representing a key barrier for avian influenza viruses adapting to the upper respiratory tract. In contrast, an earlier clade 2.3.4.4b virus isolated from a pelican in Bern in 2022 (H5N1-BE/22) replicated efficiently at 37 °C but showed markedly reduced replication at 33 °C. The polymerase mutations PB2-M631L and PA-K497R enhanced replication of H5N1-Tex/24 at 33 °C but had little effect at 37 °C. However, introduction of these mutations into H5N1-BE/22 was insufficient to restore efficient replication at 33 °C, indicating that additional viral determinants contribute to cold-temperature adaptation.

Analysis of receptor expression demonstrated that differentiated human nasal epithelial cells express both α2,3-linked and α2,6-linked sialic acid receptors, supporting entry of both avian and human influenza viruses. Consistent with this observation, H5N1-Tex/24 lacked known hemagglutinin mutations associated with a switch to human-type receptor specificity. Furthermore, H5N1-Tex/24 remained highly sensitive to the antiviral activity of interferon-λ (IFN-λ). Nevertheless, infected nasal epithelial cells produced only low levels of endogenous IFN-λ, whereas H1N1-HH4/09 induced substantially stronger IFN-λ responses while exhibiting greater resistance to its antiviral effects.

Together, these findings demonstrate that H5N1-Tex/24 possesses intrinsic properties that support efficient replication throughout the human respiratory tract, particularly in the upper airway, highlighting ongoing adaptation of contemporary clade 2.3.4.4b H5N1 viruses to mammalian hosts and underscoring the importance of continued surveillance and risk assessment.

Keywords: H5N1 avian influenza, Human airway epithelial cells, Clade 2.3.4.4b H5N1, Pandemic risk assessment, Cross-species transmission

Beyond H5N1: Influenza A virus infection in bovine udder organoids

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The host range expansion of highly pathogenic avian influenza virus (HPAIV) H5N1 to dairy cattle sparked veterinary and public health concerns, raising questions about cross-species transmissibility and adaptation beyond the classical avian reservoir. High viral loads in raw milk and mastitis-like disease in naturally infected US cattle highlight the bovine mammary gland as a significant site of viral replication. The non-canonical mammary manifestation of HPAIV challenges current concepts of influenza A virus (IAV) tissue tropism, viral replication capacity, and transmission, with direct impact on dairy production and possibly zoonotic risk. Progress in understanding mammary virus-host interactions has been constrained by the lack of physiologically relevant bovine mammary in vitro models. Against this backdrop, we established a bovine mammary gland organoid (MGO) model derived from primary epithelial cells and maintained in chemically defined media. This scalable, efficient model reconstructs key features of mammary epithelial plasticity and heterogeneity, including luminal–basal polarity, lineage-specific cytokeratin patterns, lactation-related differentiation, and hormone-responsive casein expression. We then used this system to explore IAV infections and their functional effects in the secretory epithelium in vitro across a panel of phylogenetically and phenotypically diverse IAV strains. The model supported productive replication of swine-origin and human pandemic strains, as well as low- and highly pathogenic avian influenza viruses, largely independent of culture conditions. Interestingly, mammalian-origin H1N1 strains exhibited similar infection kinetics to bovine H5N1, while low pathogenic avian H9N2 showed limited replication, indicating subtype-specific differences in mammary tropism. This MGO model enables controlled, tissue-specific in vitro studies of mammary infection biology and influenza pathogenesis aligned with 3R principles and biosecurity requirements. Thus, the organoid platform facilitates detailed molecular research at the wildlife–livestock–human interface, with direct implications for animal health, milk production, and potential zoonotic exposure.

Keywords: Influenza A virus, H5N1, mammary gland, organoids

Tissue Distribution of Coronaviruses in Bats from Great Britain

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Bats are recognised as important reservoir hosts for a wide range of coronaviruses, some of which have demonstrated the ability to cross species barriers and infect humans and other animals. Their ecological diversity and close contact with human environments emphasise the need to better understand how these viruses are maintained and distributed within bat hosts, to inform assessments of zoonotic risk.

This study examines the distribution of coronaviruses across multiple tissues in bat species from Great Britain. Laboratory analyses were conducted at the Animal and Plant Health Agency, Weybridge (UK), where samples were screened using a pan-coronavirus PCR assay, followed by Sanger sequencing for viral characterisation. In total, 382 tissue samples were collected from 68 bat carcasses that had previously tested positive for coronavirus via oral or rectal swabs.

Viral RNA was detected in tissues from ten individual bats. The intestine was the most frequently positive tissue, with viral RNA identified in seven bats: four common pipistrelle bats (Pipistrellus pipistrellus) and three brown long-eared bats (Plecotus auritus). Lower frequency detections were observed in other tissues, including two in the lung, and single detections in the tongue, trachea, heart, stomach, and kidney. Sanger sequencing identified viruses within Alphacoronavirus (including the subgenus Pedacovirus) in 60% (6/10) of positive bats and Betacoronavirus (including Merbecovirus) in 40% (4/10). Both genera were detected across multiple tissue types, with no clear evidence of strict tissue-specific tropism; however, the limited sample size means that definitive conclusions cannot be drawn. Further investigation is ongoing, with additional bat carcasses currently being analysed, and results from this expanded dataset will be presented alongside these preliminary findings.

These results support the existing coronavirus surveillance methods in bat populations, indicating that oral and rectal sampling is adequately sensitive to detect viral presence without routine tissue-specific analyses. While examination of individual tissues can provide additional insight into organ tropism and viral distribution within hosts, it serves primarily as a supplementary approach to established surveillance strategies. This information may enhance understanding of viral persistence and shedding, strengthening zoonotic risk assessment and informing future surveillance strategies.

Keywords: Tissue distribution, Coronaviruses, Bats, Surveillance, Great Britain

High-Resolution Genomic Analysis of SARS-CoV-2 Transmission and Evolution in Danish Mink Farms

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Phylogenetic approaches provide a powerful framework for reconstructing transmission pathways and detecting evolutionary selection using viral genomic data. During the large-scale SARS-CoV-2 outbreaks in Danish mink farms in the fall of 2020, previous investigations demonstrated extensive bidirectional transmission between humans and mink. Using phylogenetic methods, at least 59 independent mink-to-human transmission events and 136 human-to-mink introductions were previously identified (Rasmussen et al., 2024). From a One Health perspective, farmed mink represents a high-risk animal reservoir due to high population density and frequent contact between humans and animals.
The initial dataset consisted of approximately 700 mink-derived SARS-CoV-2 genomes. To improve phylogenetic resolution, an additional 718 samples from 25 farms were selected for sequencing. Of these, 605 yielded complete high-quality genomes suitable for phylogenetic inference, providing an unprecedented within-farm sampling density. All mink sequences were combined with relevant human-derived SARS-CoV-2 sequences to provide epidemiological context and support inference of transmission patterns.
The increased genomic resolution enabled detailed farm-level phylogenetic reconstruction and revealed extensive within-farm viral diversification, transmission-associated mutations, and potential transmission routes between farms. Comparative phylogenetic analyses demonstrated close genetic relationships between mink- and human-derived SARS-CoV-2 genomes, supporting repeated zoonotic and reverse-zoonotic transmission events throughout the outbreak period. Several farm-associated variant clusters and transmission markers were identified, enabling the tracking of viral spread between epidemiologically linked farms. Phylogenetic analyses further revealed clusters of farms infected with closely related variants, suggesting inter-farm transmission facilitated by geographic proximity and indirect transmission routes.
Overall, this study provides a high-resolution view of SARS-CoV-2 evolution and transmission dynamics in Danish mink production systems and associated human populations. The findings highlight the importance of integrated genomic surveillance within a One Health framework for early detection, monitoring and mitigation of zoonotic pathogens capable of establishing secondary animal reservoirs.

Keywords*:* SARS-CoV-2, Mink reservoir, Phylogenetic epidemiology, Zoonotic transmission, One Health

Integrated One Health surveillance reveals West Nile virus expansion beyond endemic areas in France

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The emergence of zoonotic arboviruses in France represents a major public and animal health challenge. West Nile virus (WNV), maintained in an enzootic bird–mosquito cycle, can cause severe neurological disease in humans and horses in 1–10% of infections. Since 2022, WNV circulation has expanded beyond its historical Mediterranean endemic area into regions such as Nouvelle-Aquitaine and Île-de-France.
This study aimed to strengthen WNV surveillance in newly affected regions through a One Health approach and to improve understanding of viral circulation dynamics in animal and environmental compartments.
Regional One Health surveillance networks integrating human and animal health, wildlife, environmental stakeholders, and national authorities were established to support coordinated monitoring and near real-time data sharing. The National Reference Laboratory for WNV conducted operational surveillance activities, including mosquito xenomonitoring through excreta analysis by RT-qPCR, viral genome detection in wild birds admitted to wildlife care centers (n = 744), and equine seroprevalence surveys in Nouvelle-Aquitaine and Île-de-France (n = 484 and 499, respectively).
The findings demonstrated active WNV circulation in Nouvelle-Aquitaine and a more moderate and localized circulation in Île-de-France, with equine seroprevalence rates of 9% and 2.8%, respectively. The detection of WNV-positive mosquito pools and acutely infected wild birds in several departments of both regions further supported these observations. This integrated surveillance framework enabled rapid identification of areas and periods of active viral circulation, facilitated timely alerts to competent authorities, and improved data interpretation through multidisciplinary collaboration.
This study highlights the added value of a One Health approach for emerging arboviruses surveillance. Integrating animal, environmental, and public health surveillance improved understanding of WNV circulation dynamics, strengthened early warning capacities, and supported the development of locally adapted early detection strategies. This framework could serve as a model for other territories and for the surveillance of arboviruses with significant public health impact.

Keywords: West Nile, One Health surveillance

Surveillance of West Nile and Usutu virus in birds in Luxembourg for animal and public health

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While the burden of mosquito-borne diseases has traditionally been high in (sub-)tropical regions, it is expected to also increase in Europe in the future. West Nile (WNV) and Usutu (USUV) viruses are already enzootic in certain European regions, with widening geographic repartition. The two viruses share similar epidemiological characteristics such as bird-to-bird transmission through mosquito (mainly Culex pipiens) bites. Both viruses may also be transmitted to mammals, including humans, leading to a wide range of symptoms. WNV and USUV surveillance in wild birds has previously been shown to be cost-effective and timely to provide early warning signals for increased risk of transmission to human. Passive surveillance was thus initiated in Luxembourg in 2018, when WNV was first detected in neighbouring Germany. Starting at a low pace, 64 dead wild birds were tested by RT-qPCR between 2018 and 2023. The first USUV case was detected in 2020 in a blackbird in Luxembourg capital. Starting in 2024, the surveillance activities were drastically intensified by screening systematically a wider range of bird species, that included birds of prey, Columbiformes and Passeriformes as well as a few waterbirds. A total of 499 dead wild birds were screened in between January 2024 and June 2026. While WNV has not yet been detected in Luxembourg, USUV was detected again in 11 (8.2%) birds (blackbirds, song thrush, common wood pigeon) between July and October 2024. USUV was detected in 22 (8.1%) birds (blackbirds, common wood pigeon, feral pigeon, corvid) between July and September 2025. In 2026, one blackbird was tested positive as early as in April, marking the earliest seasonal detection in the country. For the current season, this signals an earlier and potentially more intense transmission cycle. It also highlights that vector-borne disease surveillance should be carried out over extended periods, if not year-round, to avoid bias and provide early warning. Phylogenetic analyses based on whole genomes revealed the presence of USUV Africa 3 lineage. They also suggested that the 2024-2026 strains do not originate from a new virus introduction, but that USUV was rather maintained in the region since 2020. This finding, together with the overlapping host and vector range of WNV and USUV, suggests that local conditions would likely also allow the maintenance of WNV in the area once introduced, with more severe consequences for public and animal health.

Keywords: Vector-borne diseases, passive surveillance, wild birds, Usutu virus, West Nile virus

Oral Presentations:

Parallel Session 1.2 – Addressing Current Challenges (HPAI, BTV, LSD, FMD, PPR, etc.)

Date, Time: September 2, 16:00 – 17:30
Location: Olympia

Contents:

New FMDV outbreaks in Europe since the beginning of 2026

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Foot-and-Mouth disease (FMD) is a contagious animal disease, caused by the Foot-and-mouth disease virus (FMDV). Some countries are endemic while others are free with or without vaccination. Even if associated, mortality can be estimated low, the morbidity can reach 100% according to species, and the consequences in FMD-free countries can be dramatic, with important economic losses.
The European Reference Laboratory (EURL) for FMD has a role for quick and reliable detection and identification of any FMD case within the EU and of assistance to EU member states to confirm FMD suspicion, by performing viral isolation, molecular and serological analyses.
Between 2011 and 2025, the European Union was free from FMD. After FMD outbreaks in Germany, Hungary, and Slovakia in 2025, causing heavy economic losses including culling of thousands of animals, and finally solved in few months, the disease made two new incursions into Europe in 2026.
In December 2025, the northern part of the island of Cyprus was affected by the disease, which spread rapidly to the southern part of the island (where the first outbreak was confirmed by the Cyprus National Reference Laboratory (NRL) in February 2026). Serotype SAT1/III was identified. This strain is similar to the one that has been circulating in Türkiye for several months (98.48% homology). European vaccination bank was activated and vaccination campaign is still pending.
In March 2026, the Greek NRL confirmed an FMD outbreak on Lesbos Island. Sequencing revealed high similarity to strains in Iran, Azerbaijan, and Türkiye, a finding further confirmed by the EURL. All control measures have been implemented including massive depopulation.
The EURL for FMD has made a significant contribution to characterize the strains and has developed appropriate topotype-specific rtRT-PCR. In parallel Greek and Cyprus NRL still have to analyze many samples every day. To keep up with the pace day after day, Greece is establishing a network of regional laboratories and has asked EURL to evaluate them as part of a proficiency test.
Even if EU was free from FMD for 14 years, four different incursions of FMDV occurred one after another over the course of a year. The outbreaks in Cyprus and Greece are still pending despite the efforts made in each country by stakeholders, veterinary services, governmental measures and laboratory activities, including the NRLs and EURL. These outbreaks remind us that FMD still represent a threat to FMD-free countries and that preparedness is essential.

Keywords: FMDV;outbreak;Europe;EURL;Diagnostic

Lumpy Skin Disease in Italy: From First Introduction in 2025 to Re-emergence in Sardinia in 2026

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Lumpy skin disease (LSD), caused by lumpy skin disease virus, was reported in Italy for the first time in 2025. LSD is a notifiable disease under the Animal Health Law and is classified as a Category A disease, requiring immediate eradication measures upon detection. During 2025 LSD remained largely confined to Sardinia with a total of 79 outbreaks notified, with only one outbreak in Lombardy involving animals imported from Sardinia. Thanks to rapid control and surveillance measures, all outbreaks were eradicated by the end of 2025, and all disease-related restrictions were lifted by mid-February 2026. Following the designation of Sardinia as Vaccination Zone II, island-wide vaccination started on 23 July 2025 and achieved 97.7% establishment coverage and 96.2% cattle coverage by the end of the campaign. Control measures faced some farmer opposition, particularly regarding stamping-out and vaccination. LSD was detected again in Sardinia (Cagliari) on 14 April 2026, affecting mainly unvaccinated 4–6-month-old calves born to vaccinated cows in an area located approximately 100 km from the previous outbreak zones. Genomic sequencing revealed that the LSDV strain identified in Cagliari in 2026 was identical to the strain circulating during the 2025 outbreaks in Sardinia, supporting viral persistence between epidemic waves. The geographic distribution raises questions regarding possible viral persistence, undetected circulation, or new introduction events. Outbreaks in non-vaccinated herds, that occurred during epidemic 2026, may act as reservoirs for the re-emergence and further spread of the disease. The earlier onset of the 2026 outbreak may suggest altered vector dynamics or viral overwintering, although further investigations are ongoing. A new vaccination campaign was launched immediately after the detection of the 2026 outbreaks. At the start of the 2025 vaccination campaign, approximately 330 establishments refused vaccination; following awareness campaigns and veterinary outreach, this number declined to around 100 establishments. Following the first introduction of LSD into Italy in 2025, comprehensive control measures led to the apparent interruption of virus circulation. The subsequent detection of new outbreaks in Sardinia in 2026 prompted further investigation into the epidemiological factors associated with disease re-emergence. Ongoing studies aim to address these uncertainties, while the Sardinian experience reinforces the importance of vigilant surveillance, rapid vaccination responses, and strong collaboration among veterinary authorities and relevant stakeholders to contain emerging transboundary diseases. The recurrence of LSD despite extensive control measures underscores the need for continued vigilance following apparent disease eradication and informs future preparedness and response strategies.

Keywords: Lumpy skin disease, vaccination, transboundary diseases, virus, Animal Health Law.

Immune response to lumpy skin disease virus infection and vaccination measured with in house IgM and IgG ELISAs and IGRA: Insights into isotype switching and cell mediated immunity

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The development of the immune response to lumpy skin disease virus (LSDV) infection and vaccination in cattle is still incompletely understood. Humoral immunity to LSDV is currently assessed with enzyme linked immune assays (ELISA), virus neutralization tests (VNT) and immunoperoxidase assays (IPMA). The commercially available ELISAs employ double antigen for detection of total anti-capripox antibodies, without discrimination between IgM and IgG classes. To investigate antibody development and isotype switching dynamics to LSDV infection and vaccination, we developed indirect anti-LSDV IgM and IgG ELISAs. Together with previously shown development of cell mediated immunity using interferon gamma release assay (IGRA), the results provide a comprehensive overview of the immune response elicited by LSDV infection and immunization in cattle.

Both indirect ELISAs consisted of 96-well plates coated with heat-inactivated whole LSDV antigen intended for use in biosafety level 2 (BSL-2) laboratory settings. The ELISAs were optimized using serum samples from six-month-old bulls (n = 12), experimentally intravenously and intradermally inoculated with a clade 2.5 LSDV (n = 5) or vaccinated with Neethling-based live attenuated vaccines (n = 7). We measured development of immune response over three weeks post inoculation and vaccination.

IgM antibodies were detectable 7-17 days post infection and 10-21 days post vaccination. IgG antibodies were detectable from 10 dpi/dpv maintaining plateau levels throughout the observation period. IgM and IgG ELISAs showed 100% specificity on a panel of negative experimental (n=12) and field (n=23) samples. These preliminary results are a valuable addition to already published IFNg responses measurable and maintained from 7 dpi/dpv.

Both LSDV infection and vaccination stimulate robust cellular and humoral response with substantial levels of interferon gamma and both classes of anti-LSDV antibodies in experimental settings. The combined assessment of IgM and IgG responses provides a tool for identification of recent LSDV infections, and better understanding of immune maturation upon vaccination. Validation using field samples from diverse epidemiological settings is needed to determine the robustness and applicability of these tests for monitoring immune responses under natural conditions.

Keywords: Immune response, anti-LSDV IgM, anti-LSDV IgG, indirect ELISA, seroconversion

Comparative Evaluation of Disease Progression and Host Transcriptomic Responses Following Infection with Romania 2024 and Ivory Coast ’89 PPRV Strains

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Peste des petits ruminants (PPR) is a highly contagious, acute disease affecting sheep and goats. It is caused by the peste des petits ruminants virus (PPRV; family Paramyxoviridae, genus Morbillivirus), which induces transient immunosuppression, high mortality, and robust immune evasion. As disease severity varies considerably among viral strains, characterizing early virus–host interactions is essential to identify the biological correlates of virulence. In this study, we compared the clinical, virological, and transcriptomic dynamics of in vivo infection using two isolates with differing pathogenicity: the emerging Romania 2024 (Rom) strain and the Ivory Coast ’89 (ICV) strain. Under BSL-3 conditions, 25 sheep were assigned to groups and intranasally inoculated with either Rom or ICV, alongside an uninfected control group. Over a 14-day period, rectal temperature and clinical signs were monitored daily. In parallel, swabs and whole blood samples were collected at multiple time points post-infection to quantify viral load (RT-qPCR) and characterize differential gene expression in PBMC (RNA-seq). Sequential necropsies were also conducted to evaluate histopathological changes across organs. Marked differences in disease progression were observed. Infection with the Rom strain resulted in a faster onset of fever, higher peak temperatures, and a more severe clinical course, whereas ICV infection followed a milder trajectory. At the transcriptomic level, key early differences emerged: ICV triggered rapid activation of Toll-like receptor (TLR) signalling pathways at 1 dpi, whereas Rom induced minimal transcriptional changes at this time point, suggesting early evasion of immune detection for this emerging strain. At later stages, both strains elicited a strong interferon-mediated antiviral response and converged toward sustained repression of the host translational machinery. The peak systemic impact was observed at 10 dpi, with more extensive transcriptional dysregulation in Rom-infected animals (4,381 differentially expressed genes) compared to ICV (3,424 genes). These findings suggest that the greater clinical severity associated with the Rom strain may be linked to early suppression and prolonged dysregulation of host immune responses. A deeper understanding of the immunomodulatory mechanisms underlying infection will be critical for improving disease control strategies.

Keywords: PPRV, Transcriptomics, Sheep

Detection of a raccoon dog and fox amdoparvovirus (variant) in domestic dogs in Serbia

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Amdoparvoviruses (family Parvoviridae, genus Amdoparvovirus) infect a wide range of carnivore hosts and are characterized by limited host specificity. Raccoon dog and fox amdoparvovirus (RFAV) has previously been reported in raccoon dogs and foxes, but natural infection of domestic dogs has not been documented. This study describes the detection and genomic characterization of an RFAV in diseased dogs with often fatal outcome in Serbia.
During March–April 2026, samples were collected from Dobermann dogs originating from a kennel in Novi Sad, Serbia, presenting with severe clinical signs and increased mortality among puppies. Following negative results for routinely investigated viral and bacterial pathogens, metagenomic sequencing was performed. Nucleic acids were extracted using the IndiSpin Pathogen Kit (Qiagen), followed by Oxford Nanopore sequencing using the Rapid Sequencing Kit V14 on a MinION platform. Sequencing reads were quality-filtered, host-derived reads were removed using the canine reference genome, and viral reads were identified using a custom DIAMOND viral database. De novo assembly was performed using Flye, followed by polishing with Medaka. Phylogenetic and comparative genomic analyses were conducted using BLAST and reference amdoparvovirus genomes.
A total of 434,220 sequencing reads were generated. Multiple amdoparvovirus-associated reads were detected in affected animals. Assembly and polishing produced a near-complete viral genome of 4,799 bp. BLAST analysis revealed approximately 97% nucleotide identity to previously described raccoon dog and fox amdoparvoviruses. Genome organization was consistent with members of the genus Amdoparvovirus, containing the characteristic non-structural and capsid protein coding regions. Phylogenetic analysis clustered the Serbian strain with RFAV strains previously reported from wildlife hosts. In addition, the molecular (PCR) detection procedure for RFAV canine strain is established.
This study reports the detection of RFAV in Dobermann breed dogs and provides genomic evidence of its close relationship to raccoon dog and fox amdoparvoviruses. The findings expand the known host range of RFAV and suggest that probably cross-species transmission between wildlife and domestic carnivores occurred. The infection is now spreading among dogs (Dobermanns) without connection to wildlife. Further investigations are ongoing to determine the prevalence, pathogenic potential, and epidemiological significance of this virus to canine populations.

Acknowledgments: This research was supported by the the European Union's Horizon Europe Research and Innovation Project Pipeline for Rapid Diagnostics of Emergency Transboundary Infectious Diseases (PREPARE-TID, grant number 101137132). In addition, this study was also funded by the Provincial Secretariat for Higher Education and Scientific Research Activity of Autonomous Province of Vojvodina, Republic of Serbia (Contract No. 003878144 2025 09418 003 000 001 04 004) and by the Ministry of Science, Technological Development and Innovation of Republic of Serbia, Contract No: 451-03-33/2026-03/200031

Keywords: Raccoon dog and fox amdoparvovirus (RFAV), clinical disease with often fatal outcome, dogs of Dobermann breed, metagenomic sequencing, molecular detection, whole genome sequencing, Serbia

Investigating the virulence change from low to high pathogenicity in avian influenza viruses

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The avian influenza virus (AIV) subtypes H5 and H7 can mutate into highly pathogenic AIV (HPAIV) in farmed poultry. Consequently, both subtypes are notifiable pathogens, and their detection has significant animal welfare, trade and economic consequences. The viral and host factors which influence HPAIV emergence represent a major knowledge gap in understanding AIV pathogenesis.
When novel emergence of HPAIV occurs, it is frequently preceded by circulation of a low pathogenicity (LP)AIV precursor, transmitted from the wild bird reservoir. LPAIVs usually contain a single-basic cleavage site (SBCS) in the haemagglutinin (HA) glycoprotein. HPAIV emergence typically follows mutation to a multi-basic cleavage site (MBCS) in the HA gene, which permits protease cleavage by ubiquitous furin-like host proteases, facilitating systemic infection and increased mortality.
Rare instances of LPAIVs with a di-basic cleavage site (DBCS) have been recorded and may represent an intermediate during LPAIV to HPAIV mutation.
Supporting this, our previous chicken in ovo investigations showed that a reverse engineered H7N7 HPAIV with a DBCS spontaneously mutated, acquiring a MBCS, whereas the SBCS counterpart did not mutate. To continue this work, an H7N7 LPAIV was selected and utilised in conjunction with the H7N7 HPAIV from previous work. Through mutagenesis the native cleavage site (CS) was altered, and DBCS viruses rescued via reverse genetics. Rescued viruses were then characterized in vitro and utilised in the in ovo model.
Emergence of HPAIV in ovo was detected through observations of expanded viral tropism in embryo tissues following immunohistochemical labelling, and CS sequencing confirming MBCS acquisition. In addition to replicating the observation that native HPAIV engineered to possess a DBCS can reacquire a MBCS; we have shown for the first time that native LPAIV harbouring DBCS are also capable of mutating to MBCS, albeit at a lower frequency.
Ongoing work includes investigating this in in ovo models of different bird species (turkey and duck) to explore the role of host factors on AIV pathogenicity switching. Additionally, future work will explore the role of different reassortant viral polymerase permutations on the frequency of MBCS evolution.
Improved understanding of the viral and host factors that contribute to HPAIV evolution will ultimately inform poultry disease policies and interventions, which are critical in managing and controlling AIV outbreaks.

Keywords: Avian Influenza, Pathogenicity, Reverse Genetics, Viral Evolution, Host-Virus Interactions

Evidence for Shedding of Infectious BTV-3 in Semen of Naturally Infected Bulls

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Background:
Bluetongue is an infectious disease of ruminants caused by bluetongue virus (BTV), of the genus Orbivirus and family Sedoreoviridae. Since 2023, BTV serotype 3 (BTV-3) has been circulating in multiple European countries, creating sustained pressure on the livestock industry. BTV is predominantly spread by midges (Culicoides spp.), although other, less common transmission routes occur.
Previous studies have demonstrated shedding of BTV serotype 8 (BTV-8) in semen of naturally infected bulls, with subsequent transmission to heifers via artificial insemination (AI). However, data on this transmission route for other BTV serotypes remain limited. Consequently, current policies governing semen collection and trade are largely based on experiences with BTV-8.

Objectives:
The objectives of this study were to assess whether the strain of BTV-3 circulating in Europe is shed in, and detectable from, semen of naturally infected bulls using standard RT-qPCR techniques. In addition, we investigated the timing of BTV RNA detection in semen in relation to BTV RNA and BTV antibody detection in blood. Finally, the study evaluated the duration of BTV RNA persistence in semen and whether virus present is infectious in cell culture.

Methods:
In February 2025, 163 semen samples were received at The Pirbright Institute, United Kingdom, from fifteen bulls housed at an AI centre in the Netherlands. Samples were collected between July and November 2024 and frozen. All bulls were BTV antibody-negative until July 2024, but subsequently tested positive for BTV RNA and/or antibodies in blood. Semen samples taken from the bulls were later tested at Pirbright, using a BTV segment 10 qRT-PCR assay. Virus isolation was attempted for PCR-positive samples.

Results:
Semen from ten of fifteen bulls were positive for BTV-3 RNA by RT-qPCR in at least a single timepoint, with six bulls positive at multiple timepoints. BTV RNA was detectable in semen for at least seven weeks in three bulls. Infectious BTV was isolated from the semen of one bull at three separate timepoints.

Conclusion:
Shedding of BTV-3 in the semen of naturally infected bulls is conclusively demonstrated using a simple qRT-PCR workflow. The detection of BTV RNA, persistence, and RNA quantities varied considerably between bulls. In addition, infectious virus was successfully isolated from semen in cell culture. This is the first report of BTV-3 in bovine semen, underscoring the potential role of semen in the transmission of BTV-3 and may inform ongoing policies of robust and practical control measures for germinal products.

Keywords: Bluetongue, BTV, semen, transmission

Oral Presentations:

Parallel Session 2.1 – Diagnostic Tools (Focus on Current Challenges with Re-emerging Diseases)

Date, Time: September 3, 11:00 – 12:30
Location: Stadion

Contents:

Nanopore Sequencing in Diagnostic Virology: A Swift Response to Animal Disease Outbreaks in Germany

Calvelage, Sten; Höper, Dirk; Eschbaumer, Michael; Blome, Sandra; Beer, Martin

Friedrich-Loeffler-Institute, Institute of Diagnostic Virology

In Germany, outbreaks of emerging and re-emerging viral diseases affecting wild and domestic animals reached an unprecedented high over the past decade. Among the most significant of these, African swine fever was introduced in several federal states, causing high case numbers, particularly among wild boar, with sporadic outbreaks in domestic pigs. In 2025, a single outbreak of foot-and-mouth disease in a water buffalo herd marked the first occurrence of that disease in Germany since 1988. In all of these cases, collecting genomic data on the causative agent became a fundamental element of epidemiological investigations and the assessment of suitable control measures. While routine diagnostics based on established PCR methods represent the first line of pathogen confirmation due to their rapid and streamlined workflows, these approaches are often limited in their ability to provide detailed genomic characterization of viruses. In contrast, short-read sequencing techniques can generate comprehensive, high-quality sequence datasets but inherently require higher processing efforts and longer turnaround times. As an alternative, nanopore-based long-read sequencing methods promise rapid access to sequence data through fast turn-around times and straightforward library preparation protocols, supporting a timely, data-driven response during an outbreak. Here, we describe our experience implementing a generic nanopore sequencing approach as a complement to standard veterinary diagnostic methods, with a focus on rapid responses to new outbreaks of African swine fever and foot-and-mouth disease in Germany. Nanopore-derived sequencing data were compared with those obtained using the routinely applied short-read sequencing protocols, and the resulting virus sequences were used in molecular epidemiological analyses. Both methods produced highly similar virus sequences for the pathogens investigated, however, the direct comparison also highlighted the advantage of short-read sequencing when sample quantities and qualities were limited. Nonetheless, nanopore sequencing allowed the reliable identification of genetically distinct African swine fever virus variants from geographically separated outbreaks, indicating at least three independent introduction events into German wild boar populations over a five-year period. For the foot-and-mouth disease virus introduction, the accelerated assembly of the viral genome led to a rapid serotype and lineage identification and supported timely phylogenetic analyses. Overall, our results support the use of nanopore sequencing as a flexible and timely complement to established diagnostic and short-read sequencing workflows for genomic surveillance and outbreak management in veterinary virology.

Keywords: Nanopore Sequencing, Veterinary Diagnostic, African Swine Fever Virus, Foot-and-Mouth Disease Virus, Surveillance

Serological approaches to investigate incursions of non-notifiable low pathogenicity avian influenza virus into UK poultry over a 10-year period

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High pathogenicity avian influenza viruses (HPAIVs) have caused significant outbreaks globally in the last 5 years, affecting both avian and mammalian species. Avian influenza viruses (AIVs) are subtyped according to their two envelope glycoproteins, haemagglutinin (HA) and neuraminidase (NA). A total of seventeen HA and nine NA subtypes being detected in avian species globally, with H5 and H7 subtypes being notifiable due to their ability to support a HPAIV phenotype. Spillover of HPAIV into poultry is typically detected and characterised rapidly due to the presence of marked clinical signs. In contrast, incursions of low pathogenic avian influenza viruses (LPAIV) often remain undetected because clinical disease is mild or absent. The UK has a mandatory poultry surveillance program, based on sampling a proportion of apparently healthy UK commercial poultry premises, which are tested serologically for H5/H7-specific antibodies by the established subtype-specific haemagglutination inhibition tests. Such serological surveillance provides an epidemiological snapshot of any significant changes in H5/H7 LPAIV prevalences in different UK poultry sectors and initiate outbreak investigations to exclude active infection. After excluding H5/H7 seropositive flocks, we further investigated sera from the UK poultry survey, collected between 2012-2023 from 204 flocks, to evaluate the presence of antibodies to non-notifiable AIV subtypes. We first tested samples by a generic ELISA to detect viral nucleoprotein antibodies to any AIV subtype, which identified AIV incursion in 45% (n=91/204) of flocks. The highest non-notifiable AIV antibody prevalence occurred in duck flocks (72%; n=62/86), followed by geese (24%: n=12/50), turkey (29%; n=4/14) and chicken (21%; n=10/48) flocks. The 91 AIV seropositive flocks were then tested using a subtype-specific pseudotype virus neutralisation assay (PVNA) to investigate which non-notifiable subtypes may have incurred into UK poultry. PVs were initially generated specifically for the H1, H3, H4, H6, H10 and H11 subtypes for screening the respective HA-specific antibodies by the corresponding PVNA. Among these 91 AIV antibody-positive flocks, 4 (4%), 5 (5%), 6 (7%), 7 (8%), 15 (16%), and 36 (40%) were positive by PVNA for H10, H4, H1, H3, H11, and H6 respectively. The H6 subtype was noted to be particularly prevalent (24/62) among the commercial duck flocks. This subtype-specific PVNA approach is addressing a major knowledge gap concerning the broader epidemiology of non-notifiable AIV subtypes in different UK poultry sectors. The current results have demonstrated that a diverse spectrum of AIV subtypes incursion into UK poultry, with differing seroprevalences between the various poultry species.

Keywords: LPAIV, serosurveillance, PV

Detection of MERS-related coronaviruses in Danish bats using a novel RT-qPCR assay

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Bats are recognized as natural reservoir hosts for numerous viruses and are considered the evolutionary origin of alpha- and betacoronaviruses (CoVs), including SARS-CoV, SARS-CoV-2, and potentially MERS-CoV, a member of the Merbecovirus subgenus. Merbecoviruses have been identified in bat species across Africa, the Americas, Asia, and Europe. Recently, we reported the first occurrence of merbecoviruses in Denmark in Plecotus auritus bats, belonging to a distinct clade. The objective of this study was to develop and optimize an RT-qPCR assay for the detection of merbecoviruses associated with this clade and to apply the assay for surveillance of CoVs in Danish bat populations.
RT-qPCR primers targeting the bat-associated merbecovirus clade were designed both manually and using varVAMP, an automated workflow for designing degenerate qPCR primers. Assays were evaluated and optimized on a viral panel comprising different coronaviruses, including merbecoviruses and alphacoronaviruses. Selected assays were further assessed using a custom in silico primer prediction workflow. Fecal and colon swabs collected from the Danish bat surveillance program in 2025 were screened using the optimized assay. Positive samples were subjected to metagenomic next-generation sequencing, followed by genome assembly and phylogenetic analysis.
Two candidate assays were designed manually, while 10 additional primer sets were generated using an automated workflow. Of these, eight assays were selected for further evaluation. Assays targeting the same ORF1ab region showed the strongest performance and were further optimized into a final RT-qPCR assay. The final assay successfully detected all bat-associated merbecoviruses in the viral panel, including a hedgehog-associated merbecovirus at a high Ct-value, while only limited amplification was observed for a single alphacoronavirus. In silico analysis demonstrated broad inclusivity across known merbecoviruses, incl. MERS-CoV.
Screening of 33 Danish bat samples identified two positive samples. One partial genome was recovered from a Plecotus auritus from Bornholm, consistent with our previous findings, while a full-length genome was obtained from a Pipistrellus nathusii in northern Zealand. Phylogenetic analysis revealed that the P. nathusii virus formed a distinct clade from the previously identified P. auritus-associated lineage, although both belong to a broader vespertilionid-associated merbecovirus lineage. These findings indicate a greater diversity of merbecoviruses circulating in Danish bats than previously recognized.
Our findings expand the known diversity and host range of European bat-associated merbecoviruses and provide a robust molecular tool for their targeted detection. Enhanced surveillance of these viruses is important for understanding coronavirus evolution and assessing future zoonotic risks.

Keywords: coronavirus, RT-qPCR, merbecovirus

Integrated Diagnostic Approaches for Foot-and-Mouth Disease Surveillance, Outbreak Response and Control

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Foot-and-mouth disease (FMD) remains one of the most economically important transboundary animal diseases worldwide, with major consequences for animal health, livestock production, international trade, and food security. Recent outbreaks in previously FMD-free regions, including incursions into countries maintaining freedom through vaccination, have highlighted the importance of rapid, reliable, and fit-for-purpose diagnostic tools for outbreak management, surveillance, and disease control.

Effective FMD control requires diagnostic approaches that address different stages of infection and diverse surveillance objectives. IDvet's diagnostic portfolio includes both direct and indirect methods for the detection of FMDV. For rapid identification of infected animals, the portfolio comprises the ID Gene Lyo™ FMDV Triplex real-time PCR assay, the ID Rapid® FMDV Antigen lateral flow device for field-based testing, and the ID Screen® FMDV Pan-Serotype Antigen Capture ELISA for antigen detection and serotyping. Together, these assays support timely confirmation of infection and characterization of circulating FMDV serotypes.

Complementary serological surveillance is supported by the ID Screen® FMD NSP Competition ELISA, which is suitable for surveillance in free populations, outbreak investigations, and post-outbreak monitoring. Serotype-specific antibody ELISAs provide additional support for vaccination monitoring and, when combined with NSP antibody detection, contribute to differentiation of infected from vaccinated animals (DIVA).

These diagnostic systems are routinely applied across a wide range of epidemiological settings, including endemic regions, countries implementing vaccination programs, and territories seeking to demonstrate freedom from disease. Performance data and field experience from diverse applications illustrate diagnostic challenges and how integrated diagnostic strategies can support risk-based surveillance, early detection, outbreak response, and evidence-based decision-making. Together, these tools provide laboratories and veterinary authorities with flexible solutions to address the evolving challenges of FMD prevention, control, and international trade.

Keywords: FMD, preparedness, diagnostics, PCR, ELISA

Comparison of Mosquito-Magnet® and livestock-aspirated collection of mosquitoes: implications for modelling spread of mosquito-borne animal diseases

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Trap-collected mosquito surveillance data are widely used for mosquito-borne animal disease transmission models. Several input parameters are estimated from such data such as vector-to-host ratios and biting rates. It assumes that traps are adequate surrogate hosts and the numbers and species of mosquitoes it attracts are representative of those attracted by animal hosts. To investigate those assumptions, we evaluated the qualitative and quantitative differences in mosquito collections obtained parallel by aspirating mosquitoes after landing on cows and sheep and by Mosquito-Magnet (MM) traps set on the livestock premises in a controlled field experiment, conducted between May and November at a dairy farm, using a total of 5 different dry cows and 2 sheep in the experiment. Furthermore, 2 MM-traps were installed on the premises of the dairy farm. MM-traps were baited with octanol. For practical reasons, collection of mosquitoes was done separately over 4 periods of the 24-hour diel: a) 2 hours before sunset (SS) up to and including 2 hours after SS; b) 2 hours before sunrise (SR) up to and including 2 hours after SR; c) the hours between the periods indicated under a) and b); d) during daytime. On the livestock hosts, a considerably higher mosquito species richness was observed by aspiration (14 species on the cow and 13 species on the sheep) than by the MM-traps (9 species by the traps). In addition, some mosquito species that attacked and fed on livestock hosts were not captured concurrently by the MM-trap and vice versa. Furthermore, several magnitudes more mosquitoes were captured by aspiration on the livestock hosts compared to the MM traps: 1337, 476 and 171 mosquitoes from the cow, sheep and two MM-traps, respectively, while total collection time per trap was 12 times more compared to aspiration. The proportion blood fed mosquitoes was almost neglectable found in the MM-trap, while approximately 40% and 14% of blood fed mosquitos were collected from the cow and sheep, respectively. This reveals the fallacy of using mosquito surveillance data collected with traps – primarily collected to gain insight into presence or absence of mosquito species and geographical distribution – for mosquito-borne animal disease transmission modelling. Instead, one should invest in field studies with direct collection of mosquitoes from the hosts to estimate vector-to-host ratios and attack and biting rates of mosquito species in representative regions of a country, and use those parameter estimates to feed the transmission models.

Keywords: mosquitoes, species richness, aspiration, Mosquito Magnat trap

Improved whole genome sequencing technique for archived virus samples: sequencing UK's last wild-type rinderpest virus samples

Polo, Noemi; King, Simon; Baron, Michael; Batten, Carrie

The Pirbright Institute, United Kingdom

Rinderpest, also known as cattle plague, caused huge economic losses, threatened food security in developing countries and changed both landscapes and societies impacted for hundreds of years. Although rinderpest has been declared globally eradicated since 2011, the causative virus (RPV) is still held in a small number of laboratories around the world. The existence of wild type virus anywhere poses the risk of re-emergence if reintroduction were to occur through either accidental or deliberate release. The best way to eliminate this risk is to destroy all wild type virus stocks. As a registered Rinderpest Holding Facility, the Pirbright Institute has been contributing to reducing this risk by conducting a series of projects to determine the full genome sequences of, and then destroying, all remaining archived samples of wild type RPV held in the UK. To sequence the last such samples, a probe-enrichment method was developed to improve yields from these low-concentration samples and difficult matrices. Using this technique, sixteen representative samples from eleven distinct isolates of wild type and vaccine strains, some with limited viral nucleic acid, were successfully sequenced. This resulted in complete or near complete genomes of these strains, including one from 1952 (the oldest sample sequenced), prior to sample destruction in March 2026. Through this work, a 6-base insertion was identified in the RPV genome present in two African isolates originating 20 years apart. These are the only wild type RPV isolates sequenced with a genome length different from the 15,882 bases originally identified, the other viruses with additional bases being a subset of the goat-adapted RPV vaccines. It was also discovered that certain strains were detected with very low efficiency using the only published real-time RT-PCR protocol. A modified protocol was developed; including a contingent set of primers and probe, which improves the reliability and sensitivity of this diagnostic test, further contributing to global food, trade, and economic security and disease preparedness. In addition to supporting other countries who might wish to sequence their historic samples of this virus and promoting global biosecurity by destroying the virus once a database of sequences has been produced, this technique may also be adapted to be used for similar viruses for which limited sample material is available.

Keywords: rinderpest virus, genome sequencing, real time PCR, biosecurity, disease preparedness

Oral Presentations:

Parallel Session 2.2 – African Swine Fever

Date, Time: September 3, 11:00 – 12:30
Location: Olympia

Contents:

Genomic and biological characterization of a genetically distinct African swine fever virus genotype II variant detected in Spain

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African swine fever (ASF) remains one of the most important transboundary animal diseases affecting domestic pigs and wild boar worldwide. Since the introduction of ASF virus (ASFV) genotype II into Georgia in 2007, the disease has spread across Europe and Asia, causing substantial economic and ecological impacts. In November 2025, ASF was detected in wild boar in northeastern Spain after more than 30 years. Initial molecular investigations identified a genetically distinct ASFV genotype II variant characterized by a unique multigene profile and a large deletion within the left variable region (LVR), raising questions regarding its origin, evolution, and biological properties.

To investigate this variant, epidemiologic, serologic, genomic, and experimental studies were performed. Whole-genome sequencing of eight representative ASFV-positive wild boar cases detected between November 2025 and February 2026 revealed a stable approximately 9.8-kb deletion affecting multiple MGF110 and MGF360 genes. Outside the deleted region, the Spanish viruses remained highly similar (>99.9% nucleotide identity) to the Georgia 2007/1 lineage despite 30 nucleotide differences and assignment to a novel genetic group (group 29). Analysis of six additional genomes confirmed the temporal stability of the deletion and high genomic conservation throughout the outbreak, with only a single synonymous SNP detected in one virus. Notably, the probable index case already carried the same deletion, suggesting that this genomic feature was present before introduction into Spain.

Serologic analyses performed during outbreak follow-up identified ASFV-specific antibodies in 44 of 137 PCR-positive wild boar (32.1%), confirming the initial findings in the index animals and suggesting a more prolonged course of infection than typically observed with highly virulent genotype II ASFV strains. To further investigate the biological behaviour of the Spanish isolate, experimental infection studies were conducted in domestic pigs. Although the virus remained highly pathogenic and lethal, infected animals developed detectable antibody responses and showed a longer incubation period than generally reported for hypervirulent genotype II isolates.

These findings demonstrate the emergence of a genetically distinct ASFV genotype II variant in Spain and highlight the importance of continued genomic and epidemiologic follow-up of ASF outbreaks to better understand the evolution, circulation, and biological significance of emerging ASFV variants.

Keywords: African swine fever; Genotype 2; LVR; pathogen evolution.

Passive surveillance efficiency for African Swine Fever detection in wild boar in Italy.

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African swine fever (ASF) poses a critical threat to the global pig industry. In Europe, wild boar represents the epidemiological reservoir for the genotype II virus. Management strategies target wild boar populations and rely heavily on early virus detection, through passive rather than active surveillance. Although passive surveillance is widely regarded as the most effective tool for ASF detection, its efficacy over active surveillance is occasionally questioned, due to the high effort and long-term commitment required for its implementation. This study aims to compare the outcomes of passive and active surveillance activities within the framework of the Italian surveillance and eradication plan to assess their relative sensitivity in detecting infected wild boars. In Italy, ASF is endemic in wild boars within a large north-western cluster encompassing five regions. Passive surveillance relies on testing reported found-dead carcasses. Enhanced passive surveillance is also implemented through targeted active carcass searches. This activity involves the inspection, via foot patrols and/or teams comprising a handler and a trained dog, of small areas of territory selected based on the epidemiological situation. Data from both active and passive surveillance activities are recorded in the Italian veterinary information system www.vetinfo.it. Since early 2022, a total of 84.226 wild boars were tested for ASF virus: 73.394 through active surveillance and 10.832 through passive surveillance. The prevalence of ASF-positive carcasses was significantly higher in passive surveillance (27.41%) than in active surveillance (1.04%) (p-value < 0.001). Within the enhanced passive surveillance activities, the probability of detecting at least one carcass within a 1 km2 grid cell was significantly greater when detection dogs were employed (13.23%) compared with searches conducted without them (0.94%) (p-value < 0.001). Among successful searches, the probability of finding multiple carcasses was more than twice as high when using detection (30.71%) than during foot patrols alone (12.95%) (p-value < 0.001). Our findings demonstrate that testing found-dead wild boars is far more sensitive than testing hunted or killed animals for ASF detection and monitoring. Furthermore, the use of trained dogs significantly improves the effectiveness of passive surveillance, and it is therefore hoped that the number of trained dog-handler teams will be increased soon. Therefore, passive surveillance represents a cornerstone of ASF surveillance programs, tracking epidemiological evolution in endemic zones, while supporting carcass removal activities, reducing environmental viral contamination, and contributing to the assessment of conditions required for the recovery of ASF-free status.

Keywords: African Swine Fever, Passive surveillance, Eradication

African swine fever early detection kinetics in boar semen following oronasal exposure to high and low virulent ASFV strains

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African swine fever (ASF) was previously detected in semen from 4 boars inoculated intramuscularly with ASFV Estonia 2014 strain; virus detected as early as 1 day post inoculation (dpi) in blood and 2 dpi in semen. The virus was transmitted to naïve gilts followed by abortions and viral replication in fetal tissues. The purpose of the present study was to compare a more natural oronasal infection to determine the shedding patterns of ASFV in semen and various sample types as well as to establish a timeline for the onset of clinical signs, after infection with highly virulent ASFV Georgia 2007/1 and attenuated ASFV-GUS-Vietnam strains. In experiment one, nine boars (n=9) and in experiment two, eight boars were inoculated oronasally with ASFV Georgia 2007/1 and ASFV-GUS-Vietnam respectively with a dose of 2 x 105 TCID50 in 4mL per boar (2mL oral and 1mL/ nostril). Boars were evaluated and semen collection was attempted daily together with blood, blood swab, serum, oral swab and oral fluid collection. The samples were tested by four different real time PCR assays. A clinical scoring system was used to evaluate the clinical condition, and rectal temperatures were collected daily. All boars infected with ASFV Georgia 2007/1 developed clinical signs of acute ASF starting 3 dpi. Rectal temperatures increased starting 3 dpi, peaked at 5 dpi and remained high, with most boars 41°C or higher. Viral genome was detected as early as 1 dpi in oral fluids, 2 dpi in blood and 3 dpi in semen. In general, virus was detected in semen one day after it was detected in blood. Hind limb ataxia, reduced appetite, and fever were the most notable early clinical signs. All boars reached humane endpoint by 7 dpi. All boars infected with ASFV-GUS-Vietnam also developed clinical signs and fever although relatively delayed. Viral genome was detected as early as 2 dpi in oral fluids, 3 dpi in blood and 4 dpi in semen. Overall, viral genome was detected in semen 1-4 days after detection in blood indicating blood as a comparatively better sample type for early detection of ASF in boars. Boars continued to mount the dummy and collect despite high fever with clinical signs. Using effective sampling strategies while monitoring for early clinical signs such as fever, reduced appetite, and hind limb ataxia are critical to prevent distribution of infectious semen to protect significant numbers of sow farms from ASF downstream.

Keywords: African swine fever, boars, semen, shedding, early detection

African swine fever virus lacking C717R is highly attenuated in pigs and fails to protect against homologous challenge infection.

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African swine fever (ASF) is a severe and economically important hemorrhagic disease of domestic pigs and wild boar caused by ASF virus (ASFV). No vaccines against ASF are available yet in most parts of the world except Vietnam and China. This is due essentially to safety concerns since only live attenuated ASF vaccines have proven sufficient efficacy so far. The ASF virion has a complex icosahedral structure with a double-stranded DNA genome encoding more than 160 genes, most of which remain poorly characterized. Understanding the ASFV gene functions and their interactions with the swine immune system is crucial for vaccine development. In this study, we analyzed a mutant of the genotype II Georgia 2007 (GRG) strain from which we deleted the previously uncharacterized C717R gene encoding a putative serine/threonine kinase. The mutant virus – GRG-ΔC717R-eGFP – showed severely impaired replication in both primary monocyte-derived macrophages (MDMs) and immortalized porcine kidney macrophages (IPKM) when compared with the parental GRG isolate. A GRG virus reconstructed form the GRG-ΔC717R-eGFP backbone – GRG-C717R-Rev – and a GRG-ΔC717R-eGFP-derived virus in which we replaced the non-essential EP402R gene with C717R recovered replication of the parental GRG in cell culture. In vivo, experimental infection of domestic pigs produced neither viremia nor clinical signs, underscoring the essential role of C717R in ASFV virulence. Nevertheless, all pigs infected with GRG-ΔC717R-eGFP seroconverted after two virus injections, as detected by anti-p72 ELISA, yet none were protected against disease when challenged with parental GRG. In conclusion, our data demonstrate that C717R contributes substantially to efficient ASFV replication in vitro and in vivo, and that vaccination with GRG-ΔC717R-eGFP does not protect pigs against ASFV challenge infection.

A minimal infectious dose of African swine fever virus induces lethal disease with implications for environmental persistence and molecular surveillance

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IRTA, Spain

African swine fever (ASF) remains one of the most important transboundary diseases affecting domestic pigs and wild boar populations worldwide. The continued circulation of African swine fever virus (ASFV) genotype II across several European countries, particularly within wild boar reservoirs, represents a major threat to regions with high pig density. The recent detection of ASFV-positive wild boar in the Barcelona area further emphasizes the increasing risk of environmental spread and local transmission in densely populated swine-producing regions.

Despite extensive control efforts, several aspects of ASF epidemiology remain incompletely understood, particularly those associated with environmental transmission dynamics and viral persistence in carcasses and decomposing tissues. In this context, low-dose exposure scenarios and the molecular stability of ASFV in degraded biological matrices represent critical factors requiring further investigation under field-relevant conditions.

In the present study, we evaluated whether an extremely low ASFV dose could establish infection following intranasal exposure. Twenty animals were inoculated with 10 HAD of the highly virulent ASFV Georgia genotype II strain, representative of the lineage currently circulating in Europe, including its emerging field variants. Only one of the inoculated animals became infected; however, this animal developed severe ASF-compatible clinical disease comparable to that observed following high-dose exposure. These findings suggest that exposure to very low amounts of ASFV may still represent a biologically relevant transmission scenario under natural environmental conditions.

Additionally, we investigated the persistence of ASFV DNA in decomposing tissues subjected to thermal stress conditions. Bone marrow, tongue, ear tip, and muscle samples were longitudinally analyzed by qPCR and LAMP assays. Among the evaluated matrices, bone marrow showed particularly robust molecular detectability, maintaining detectable ASFV DNA levels even during advanced stages of tissue degradation for up to 160 days.

Overall, these findings contribute to a better understanding of the mechanisms that may support ASFV environmental persistence and carcass-associated transmission. Furthermore, they highlight the value of decomposing tissues, particularly bone marrow, as reliable matrices for carcass-based molecular surveillance and risk assessment in endemic or affected areas where infected animals are found dead under field conditions.

Keywords: African swine fever virus (ASFV); minimal infectious dose; decomposing tissues, bone marrow, environmental transmission

African swine fever virus hijacks the host cell cytoskeleton to induce tunneling nanotube-like projections: a comparative study of primary target cells and ASFV isolates

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African swine fever (ASF) is one of the most devastating viral diseases affecting domestic pigs and wild suids, causing mortality rates of up to 100% and substantial economic losses worldwide. The causative agent, African swine fever virus (ASFV), primarily infects macrophages, yet the cellular mechanisms exploited by the virus to disseminate remain poorly understood. Improved knowledge of ASFV–host cell interactions is essential for the development of effective control strategies and antiviral interventions.
Here, we investigated ASFV-induced cytoskeletal remodelling in primary porcine macrophages using conventional and confocal immunofluorescence microscopy combined with transmission electron microscopy. Infection of monocyte-derived macrophages (MDMs) with the ASFV Belgium 2018/01 isolate resulted in the formation of long membranous projections exhibiting morphological characteristics of tunneling nanotubes (TNTs). Strikingly, 57.6% of infected cells established connections with distant neighbouring cells through these structures, suggesting a potential mechanism for direct intercellular communication and viral dissemination.
To evaluate the biological relevance of this phenomenon, we compared multiple ASFV target cell populations, including pulmonary alveolar macrophages (PAMs), splenic macrophages, progressively matured monocytes, and both pro- and anti-inflammatory polarized MDMs. TNT-like projections were absent in luminal monocyte/macrophage populations such as PAMs and short-term cultured monocytes, whereas their induction was unaffected by macrophage polarization status, indicating that cellular differentiation rather than activation state governs susceptibility to TNT formation.
Furthermore, a comparative analysis of 19 ASFV field isolates spanning diverse genotypes and virulence profiles, together with the Vero cell-adapted Ba71V strain, revealed that TNT induction is a conserved hallmark of ASFV infection. Although quantitative differences were observed among isolates, attenuated genotype I strains induced significantly more projections than virulent genotype I strains. The detection of TNT-like structures in infected Vero cells further points to conserved host pathways involved in their formation and highlights the utility of this model for mechanistic studies.
Collectively, our findings uncover a previously unrecognized cytopathic effect of ASFV infection and identify TNT-like structures as a conserved feature across viral strains and susceptible cell types. These results provide a strong foundation for future studies aimed at deciphering the molecular mechanisms underlying TNT biogenesis and determining their contribution to ASFV transmission and pathogenesis.

Keywords: Macrophages, Tunneling nanotubes, African swine fever virus

Oral Presentations:

Parallel Session 3.1 – Vaccine Development

Date, Time: September 3, 15:45 – 17:00
Location: Stadion

Contents:

Persistence of maternal vaccine-induced immunity under field conditions against BTV-3 and exploratory evidence of heterologous immune transfer through bovine colostrum

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Following the emergence of bluetongue virus serotype 3 (BTV-3) in Belgium and the ongoing circulation of BTV-8 in neighboring regions, mandatory vaccination against both serotypes was implemented in 2025. Although transfer of BTV-specific maternal antibodies from vaccinated ewes to their offspring has previously been demonstrated, the persistence of maternally derived antibodies following BTV-3 vaccination under field conditions remains poorly characterized. Determining how long these antibodies persist is important to optimize vaccination timing in young lambs, and comparison with BTV-8 may provide insight into serotype-specific differences. In addition, little is known about the use of bovine colostrum for heterologous transfer of BTV-specific antibodies to lambs.

To address this, fifteen ewes, originating from three Belgian sheep flocks and vaccinated against BTV-3 and BTV-8 during the 2025 Belgian vaccination campaign, together with their lambs (n = 21), were followed longitudinally. Serum samples were collected in March, April and May 2026 and analyzed using a competitive ELISA. Samples collected in March were also tested in virus neutralization tests (VNT) against BTV-3 and BTV-8, while additional VNT analyses and follow-up sampling are ongoing. Furthermore, two BTV-3-vaccinated cows, their calves, and two lambs that received bovine colostrum from these cows were followed serologically.

All ewes (15/15) were ELISA-positive throughout the study period (March, April and May) with no apparent decline in ELISA S/N values. Neutralizing antibodies against BTV-3 and BTV-8 were detected in all ewes in March. All lambs (21/21) were ELISA-positive in March, and neutralizing antibodies against BTV-3 and BTV- 8 were detected in 20 and 19 lambs, respectively. In April, 20 of the 21 lambs remained ELISA-positive, whereas by May this number had declined to 16. Ongoing VNT analyses will determine whether and to what extent neutralizing antibodies decline after birth. In a separate study, BTV-3 neutralizing antibodies persisted for at least five months in calves born to BTV-3-vaccinated cows. In two lambs receiving bovine colostrum, BTV-3 neutralizing antibodies remained detectable for at least three months and titers were within the range observed in lambs receiving ovine colostrum.

These preliminary longitudinal field data demonstrate efficient transfer and progressive decline of maternal antibodies in lambs. Initial VNT results confirmed transfer of BTV-3 and BTV-8 neutralizing antibodies, while ELISA indicated persistence in most lambs up to approximately 3 months of age, although ongoing analyses will further refine their duration. Bovine colostrum may represent an alternative source of BTV-specific passive immunity in lambs when ovine colostrum is unavailable.

Keywords: Bluetongue virus, Maternal antibodies, Lambs, Vaccination, Colostrum

Production and characterization of Lv17/WB/Rie1-ΔEP153R/ΔEP402R vaccine candidates with serially deleted MGF 300 and MGF 360 genes

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Currently, live-virus vaccines are the only effective means of preventing ASF disease. Vaccine candidates can be generated by removing multigene family (MGF) genes responsible for virulence and immunosuppression. However, classical CRISPR/Cas-mediated gene knockout in ASFV remains time-consuming, hindering the systematic functional analysis of MGF gene deletions.
To accelerate this process, we engineered a novel Cas-based mutagenesis workflow that reduces recombinant virus production time by several weeks. This method enables the rapid generation of serial deletion mutants. Over a 10-week period, we constructed four recombinant viruses (LV17d1–4) derived from the parental Lv17/WB/Rie1-ΔEP153R/ΔEP402R strain. These variants carry semi-nested deletions spanning the 16251–26107 genomic region, targeting 1, 2, 5, and 7 MGF genes (comprising four MGF 360 and three MGF 300 genes).
Ex vivo replication kinetics of the mutants were evaluated in PAMs at a low MOI infection. At 96 hours post-infection (hpi), only the maximum-deletion mutant (LV17d4) exhibited viral yields exceeding those of the parental strain. The specific infectivity of the virions strongly correlated with viral titers at 108 hpi. Notably, the replication of the LV17d3 mutant was significantly impaired compared to all other strains.
In vivo safety and efficacy were assessed in domestic pig trials. Vaccination with LV17d1 and LV17d2 (10 000 FFU) induced clinical symptoms and viremia, with anti-ASFV antibodies detectable from day 9. In contrast, LV17d3 and LV17d4 administered at low (100 FFU) and high (10 000 FFU) doses elicited no or mild clinical signs, respectively. Due to low seroconversion by day 21, a secondary booster was administered, triggering robust antibody production across all cohorts. Animals were challenged intramuscularly with 100 TCID50 of the virulent Armenia/07 strain.
Groups vaccinated with LV17d1, LV17d2, low-dose LV17d3, and high-dose LV17d3 demonstrated protection rates of 80%, 100%, 40%, and 60%, respectively. Conversely, the LV17d4 cohorts exhibited no protection.
In conclusion, deleting multiple MGF 300 and 360 genes does not exert a calculable additive effect on ex vivo viral replication, but vaccine candidates with beneficial impact on phenotypic attenuation and vaccine safety can be selected in in vivo experiments.

Keywords: ASFV, vaccine, rational design

Construction of gene-deleted African swine fever viruses based on C717R-mediated positive selection: a novel approach to generate attenuated viruses

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African swine fever ASF is a lethal haemorrhagic disease in domestic pigs and wild boar caused by ASF virus (ASFV), a large, complex DNA virus that encodes more than 160 proteins, depending on the genotype. A large proportion of ASFV-encoded proteins are not essential for the completion of the virus life cycle but modulate virus host interactions and immune responses. Exploring the functions of non-essential ASFV genes through genome editing is crucial for understanding ASF pathogenesis and for the development of gene-deleted live attenuated vaccines. Homologous recombination with gene transfer plasmids remains the standard method for ASFV genome editing. However, this method requires labour-intensive and time-consuming purification of mutant from parent virus. Here we report on the development of a novel positive-selection strategy associated with homologous recombination for accelerating the generation of ASFV gene-deleted mutants. The principle relies on a replication-impaired *C717R* gene-deleted ASFV backbone in which the *C717R* gene is reinserted in place of a non-essential gene of interest. Due to the enhanced replication characteristics of viruses that have reintegrated *C717R*, the recombinant viruses can easily be purified and further used to investigate the function of the deleted genes of interest *in vitro*. Surprisingly, gene-deleted viruses that were generated with *C717R*-based selection from the *C717R* gene-deleted backbone were attenuated *in vivo* when compared with viruses from which the corresponding gene was deleted from the parental GRG backbone. Thus, we propose this strategy as a novel approach for the construction of live attenuated ASFV vaccines.

Novel African swine fever virus live attenuated vaccines based on relocation of C717R associated with deletion of non-essential genes

Nolwen Husson[1,2,3], Obdulio García-Nicolás[1,2], Maksym Samoilenko[1,2,3], Francisco Brito[1,2], Matthias Liniger[1,2], Noelle Donze[1,2], Caroline Lehmann[1,2], Sylvie Python[1,2], Markus Gerber[1,2], Liudmyla Dovhanych[1,4], Kateryna Sytnyk[1,4], Valentyna Fomina[1,4], Raffael Fricker[1,4], Charaf Benarafa[1,2], Nicolas Ruggli[1,2], Artur Summerfield[1,2], Kemal Mehinagic[1,2]

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African swine fever (ASF) is a lethal viral disease caused by African swine fever virus (ASFV), a large and highly complex DNA virus infecting both domestic and wild pigs. Infection with highly virulent ASFV strains results typically in acute hemorrhagic fever, severe immune dysregulation and mortality rates approaching 100%, leading to devastating impacts on pig populations and agricultural economies. Despite extensive research efforts, no ASF vaccine has yet been authorized for use in most parts of the world. Among the various vaccine platforms investigated, live attenuated vaccines (LAV) have shown the greatest potential in terms of efficacy. The present study employs a novel strategy developed recently which enables rapid generation of ASFV recombinants by dramatically enhancing selection of targeted deletion mutants through positive selection. The system provides additional attenuation by reintroduction of the *C717R* gene at the location of the depleted genes (relocC717R). Using this approach, we developed six LAV candidates lacking either *B125R* or *B318L (*ΔB125R-relocC717R and ΔB318L-relocC717R, respectively), with or without additional expression of Chemerin or CXCL12. These chemoattractants were selected for their ability to recruit plasmacytoid dendritic cells (pDCs) and other immune cell populations. *In vitro* characterization demonstrated that the LAV candidates exhibited attenuated growth compared to wild-type ASFV. Chemotactic activity towards pDCs and other immune cell populations was observed following macrophage infection with the LAVs expressing a chemoattractant. Evaluation in pigs showed that all vaccines were highly attenuated and provided full clinical and virological protection against highly virulent genotype II challenge. Vaccination induced robust virus-specific antibody and T-cell responses. Taken together, these results demonstrate the successful development of safe and highly efficacious novel LAV candidates, using a novel efficient genetic recombination platform for the construction of next-generation ASF vaccines.

Three of the most commonly used sheep pox vaccine strains offer sufficient protection against a challenge with a highly virulent sheeppox virus strain

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Exotic and vector-borne diseases, Sciensano, Belgium

Vaccination is an important tool in the control of sheeppox. A wide variety of vaccines is available, and they are often derived from local circulating strains. Although there is some data available on their effectiveness in the field, data from trials in a controlled environment is often missing. Therefore, we evaluated the safety and efficacy of three commonly used vaccine strains (Romanian, RM65 and Bakirköy) in controlled experimental trials.
For each strain, 28 to 36 sheep were included in the vaccine evaluation. Because of the high number of animals involved, the vaccines were tested in five different trials, each with a similar set-up. Animals were vaccinated according to the manufacturer's instructions and were followed for 21 days to assess the safety of the vaccine. After this period, a part of the vaccinated animals was challenged with a virulent sheeppox strain. In addition, naïve animals were also challenged in each trial to serve as infection controls. After challenge, all animals were again monitored for 21 days. A daily clinical examination was performed and swabs, blood and biopsies were collected regularly.
In the period after vaccination, strong local reactions at the vaccination site were observed in animals vaccinated with the Romanian (60% of animals) and RM65 (50%) strain. In addition, a high fever (>41°C) was also detected in the majority of the animals vaccinated with these strains (Romania: 82% and RM65: 72%). For the Bakirköy strain, this strong local reaction was not observed and high fever was also limited (4%).
In the period after challenge, there was almost no local reaction at the challenge site, nor any other clinical signs in the animals vaccinated with the Romanian and RM65 strains. In some animals vaccinated with the Bakirköy strain, a strong local reaction (14%), fever (7%) and small nodules (7%) were observed. Viremia and nasal excretions were also more often detected in these animals. The difference between the Bakirköy strain and unvaccinated animals after challenge was however still very clear. All of the unvaccinated animals had a strong local reaction, fever and nodules, resulting in p-values below 0,01 for all these parameters compared with the Bakirköy strain.
Despite the differences between the vaccine strains, all of them were considered safe and effective. The side effects remained limited to a local reaction and fever and the different vaccine strains offered a 100% protection against mortality and a 79-100% protection against morbidity.

Keywords: Sheeppox, Vaccination, Safety, Efficacy

Oral Presentations:

Parallel Session 3.2 – Disease Surveillance, Epidemiology, Risk Analysis

Date, Time: September 3, 15:45 – 17:00
Location: Olympia

Contents:

Cryptic invasion and evolutionary dynamics of peste des petits ruminants virus across Europe

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The disease called peste des petits ruminants (PPR) is spreading in Europe since July 2024, raising growing concern, with hundreds of thousands of sheep and goats culled due to the ongoing outbreaks. Our objectives were to identify the likely geographical origins of viral introductions into the affected countries, quantify viral spread between countries and assess the role of live small ruminant trade in shaping spatial transmission dynamics. We generated full genome sequence and performed phylodynamic analyses of PPR viruses circulating in small ruminants in Europe from 2024 to early 2026. We leveraged this genetic analysis with the results of epidemiological investigations carried out by Veterinary authorities. We infer that eastern Romania acted as the primary source of regional viral dissemination, with repeated introductions to Greece, sporadic spread to Bulgaria and Hungary, movements from eastern to western Romania, and further dissemination to the South Eastern Europe, including Albania, Kosovo, and more recently Croatia. Cross-border transmission was strongly associated with official records of live animal trade, and outbreak investigations indicate additional roles for illegal trade and indirect transmission. PPR is likely circulating undetected in multiple areas in South Eastern Europe, notably due to the high variability of clinical signs in infected animals. Awareness of the difficulty in detecting the disease based on mild or atypical clinical signs should be disseminated widely across the veterinary sector. Coordinated cross-border surveillance, timely data sharing, and integrated control measures are needed to stop the spread of PPR into Europe

Keywords: small ruminant; phylodynamics; live animal trade; disease emergence; morbillivirus

Serological Screening for Avian Influenza in Cattle in the Netherlands

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Introduction
Avian influenza (AI) primarily affects birds but has expanded its host range in recent years. In 2024 the US reported the first large-scale outbreak of Highly Pathogenic Avian Influenza (HPAI) in cattle. The H5N1 clade 2.3.4.4b virus replicated strongly in the mammary glands and was shed in the milk leading to cow-to-cow transmission. No infections in European cattle have been reported.
In January 2026, HPAI H5N1 virus was detected in a cat on a dairy farm in the Netherlands. Subsequent sampling of cattle on the farm revealed no viral RNA, but H5-specific antibodies in both dairy cattle and youngstock. A nationwide serological screening was therefore initiated to investigate the situation in cattle in the Netherlands.

Methods
At the affected farm, 70 individual milk samples, two bulk tank milk samples, and serum samples from 72 lactating cows and 38 young stock animals were collected. Samples were analyzed using RT-PCR, NP-ELISA, H5-specific ELISA, virus neutralization test (VNT), and a multiplex Luminex assay. In addition, bulk milk samples of the affected farm that had been stored at Royal GD were tested in an NP blocking ELISA.
For the nationwide follow-up screening, 3,019 serum samples from 427 cattle farms collected between November 2025 and January 2026 were tested for antibodies against AI virus. This included 1,444 samples from 113 farms in the region surrounding the affected farm and 1,575 samples from the rest of the Netherlands. All samples were screened using NP-ELISA, with positive samples confirmed by H5-specific ELISA and VNT.

Results
No viral RNA was detected at the affected farm, indicating absence of active virus replication. However, H5N1-specific antibodies were identified. Antibody prevalence in lactating cows based on NP-ELISA and VNT was 20.0% in milk, 47% in serum samples, and 63% in young stock. Stored bulk milk samples of the affected herd from November and December were negative, and bulk milk samples from January and February were tested positive in NP ELISA.
In the nationwide screening, 30 samples (~1%) were positive in the initial NP-ELISA screening. However, all samples tested negative in confirmatory testing (H5-specific ELISA and VNT), indicating no additional H5-specific AI antibody detections.

Conclusion
These results suggest that AI infection in cattle in the Netherlands is rare and that the case was likely isolated, despite widespread circulation of AI virus in wild birds. However, the possibility of other isolated cases cannot be excluded.

Keywords: HPAI, Influenza, dairy cow, cattle, screening

Bluetongue Virus Serotype 3 in Switzerland: Producer Perspectives, Epidemiological Dynamics and Disease Impact

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Bluetongue disease is a vector-borne viral disease affecting ruminants. Since the first de-tection of serotype 3 (BTV-3) in Switzerland in August 2024, the disease has spread rapidly, resulting in almost 6,000 laboratory-confirmed infected farms nationwide. However, com-prehensive data on animal health, economic, and epidemiological impacts remain limited.
To address these knowledge gaps, a study comprising three complementary components was conducted. First, a nationwide online survey was distributed to approximately 9,000 cattle and sheep farms, both with and without confirmed BTV-3 infection, collecting infor-mation on clinical signs, reproductive disorders, vaccination strategies, preventive measures, economic impacts, and communication with stakeholders. Second, pre-vaccination antibody prevalence was assessed in 554 animals from eight dairy herds sam-pled in spring 2025. Third, infection dynamics were investigated longitudinally in an addi-tional dairy cattle herd (48 animals) and sheep herd (57 animals), with repeated serological testing performed during the month following the first confirmed BTV-3 case.
A total of 964 complete survey responses were received (response rate: 10%). Among farms without laboratory-confirmed BTV-3 infection, nearly half considered their herds likely affected based on observed clinical signs. Willingness to vaccinate was high, with most farms having administered at least a primary vaccination course since 2024. Nevertheless, about one-third had not vaccinated animals and did not intend to do so. Respondents re-ported a median perceived annual economic impact associated with BTV-3 of approximate-ly CHF 8,500 per farm. Communication by authorities, veterinarians, media outlets, and agricultural organizations was generally rated as satisfactory. Serological investigations re-vealed substantial virus circulation before vaccination. Among the eight dairy herds, 64% of tested animals had antibodies against BTV, with within-herd apparent prevalence ranging from 20% to 94%. In the longitudinal examination of the additional dairy cattle and sheep herd, antibody prevalence increased rapidly following detection of the first case. One week after case detection, antibody prevalence was 75% in the dairy herd and 42% in the sheep herd, increasing to 92% and 67%, respectively, after four weeks.
The results provide important insights into the epidemiology, animal health consequences, and economic burden of BTV-3. Rapid within-herd transmission and widespread exposure among Swiss livestock populations were observed, highlighting the high relevance for farm-ers, as reflected by the willingness of a substantial number of survey participants to share their private data for further in-depth analyses on disease impact.

Keywords: Disease Transmission, Economic Impact, Seroprevalence, Dairy cattle, Sheep

Genomic Surveillance and Epidemiology of West Nile and Usutu Viruses in France

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West Nile virus (WNV) and Usutu virus (USUV) are neurotropic zoonotic arboviruses belonging to the genus Orthoflavivirus (family Flaviviridae). Both are maintained in enzootic transmission cycles involving ornithophilic Culex mosquitoes and avian reservoir hosts, whereas humans and horses are considered incidental dead-end hosts. WNV is widely distributed and comprises nine recognized lineages (WNV-1 to WNV-9), with lineages 1 and 2 responsible for most cases of viral encephalitis in humans and horses. In Europe, WNV infection is a notifiable disease in humans, equids and birds. USUV emerged more recently in Europe and currently includes eight recognized lineages, three African (Africa 1–3) and five European (Europe 1–5). Except for Africa 1, all lineages have been detected in Europe and have caused major epizootics in wild and captive birds. Although human infections remain rare, increasing numbers of cases highlight the importance of USUV surveillance within a One Health framework.

In France, WNV and USUV are considered emerging pathogens due to their expanding geographic distribution and increasing outbreak frequency. The European and French National Reference Laboratory (EURL/French NRL) contributes to a multidisciplinary One Health surveillance network involving medical, veterinary and entomological partners. Alongside routine diagnostic investigations of clinically suspected cases in horses and captive birds using serological and molecular assays, whole-genome sequencing of circulating strains has been integrated into the surveillance workflow. We performed whole-genome sequencing of all WNV- and USUV-positive samples available at the EURL/French NRL and collected over the past decade, using Oxford Nanopore and Illumina sequencing technologies.

In addition to sequence database enrichment, WNV and USUV viral genomes were used for genomic epidemiology analyses. We will present a One Health genomic investigation of WNV strains circulating in France in recent years, with particular emphasis on the introduction and circulation of WNV in the Paris region in 2025. Finally, we will provide an overview of the epidemiology and spatiotemporal dynamics of USUV in wild birds based on samples collected over the past decades by the EURL/French NRL.

The increasing circulation of WNV and USUV, together with the emergence of WNV into previously unaffected areas of France, highlights the need to strengthen and expand surveillance across the national territory. Early detection through integrated One Health surveillance and genomic epidemiology is essential to support risk assessment, guide control measures and mitigate potential public health impacts.

Keywords: West Nile virus, Usutu virus, One Health, genomic surveillance, molecular epidemiology.

Spatiotemporal and phylogenetic dynamics of Newcastle disease virus in wild birds in Catalonia, Spain: implications for reservoir dynamics and risk assessment

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Newcastle disease virus (NDV) remains enzootic in poultry and wild birds, with high genetic diversity driven by wildlife reservoirs and transmission dynamics at the wildlife-livestock interface. Despite vaccination in some regions, the epidemiological situation in Europe has worsened since late 2025, with increasing poultry outbreaks and the emergence of virulent genotype VII strains in countries like Spain. These trends highlight the need to understand NDV dynamics in wild bird populations to improve surveillance and risk assessment. We aimed to characterize spatiotemporal patterns of NDV in wild birds and identify host species involved in virus maintenance in Catalonia (Spain).

A retrospective epidemiological and phylogenetic analysis was conducted on avian samples collected through passive surveillance in Catalonia (2018-2025), tested using molecular methods and analyzed by year, location, and species. Phylogenetic analyses were based on NDV sequences from Catalan wild birds, complemented with Spanish wild bird and poultry outbreak sequences (2025-2026) for contextual comparison.

In Catalonia, NDV was detected in 189/3,209 samples (5.9%), confirming sustained circulation. Prevalence varied annually (4.3-18.6%), with the highest value in 2019 likely due to limited sampling, and remained relatively stable (4.3-7.5%) despite increased sampling from 2022 onwards. Spatial distribution was heterogeneous, with higher prevalence in Barcelona and Girona, potentially reflecting urbanization and higher densities of synanthropic Columbiformes compared to more rural Lleida and Tarragona provinces. Streptopelia decaocto was the main host associated with NDV detection (49.1%), followed by Columba livia (11.9%). From 2023 onwards, sporadic detection in mallards, passerines, and gulls suggested incidental spillovers and broader circulation.

Phylogenetic analysis revealed co-circulation of multiple genotypes (I.2, VI.2.1.1.2.1, VII.1.1, XXI.2) in Spanish wild birds. Genotype I showed high diversity across host species, consistent with repeated introductions; genotype VI formed a pigeon-associated lineage suggestive of local evolution; and genotype XXI comprised co-circulating pigeon-associated clades linked to other European countries. In contrast, genotype VII viruses linked to recent poultry outbreaks formed a homogeneous cluster related to poultry strains from the eastern part of the European region. Further investigations are needed to determine whether this reflects a recent introduction or local emergence.

These findings highlight sustained NDV circulation, with Columbiformes as key reservoirs and wildlife acting both as a source of viral diversity and a potential interface for spillover into poultry. Integrating epidemiological and evolutionary data is essential to improve risk assessment and guide targeted surveillance and control strategies within a One Health framework.

Keywords: Newcastle disease virus (NDV), Epidemiology, Wild birds, Columbiformes, Phylogenetics

Mapping transmission: PRRSV phylodynamics and swine movement networks in Austria

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Porcine Reproductive and Respiratory Syndrome (PRRS) remains an economically devastating viral disease impacting the swine industry. In Austria, PRRSV is endemic, posing a continuous threat to herd health. Austria serves as a critical transit hub for swine trade, presenting ongoing challenges for disease control. In response, national initiatives like the PRRS Stabilization program aim to limit field strain spread. Despite these structured efforts, long-term evolutionary dynamics and explicit spatial transmission pathways of PRRSV in Austria remain uncharacterized. While diagnostic data have been collected since 2012, a comprehensive longitudinal analysis has not yet been reported.
Our objectives are twofold: first, to characterize regional genetic diversity and infer localized sequence clusters within the broader European landscape using Upper Austrian PRRSV sequences (2012–2025); second, to couple viral evolutionary data with animal movement networks to infer farm-to-farm transmission pathways and identify structural risk factors.
A total of 457 PRRSV ORF5 sequences (606 bp) collected from swine farms in Upper Austria were aligned using MAFFT (globalpair). A maximum-likelihood phylogeny was reconstructed using RAxML-NG with a GTR+I+G nucleotide substitution model and 1,000 bootstrap replicates. Genetic clustering was determined using ClusterPicker with a bootstrap support threshold $>90\%$ and a maximum pairwise genetic distance of 14.2%. Phylogenetic temporal signals of prioritized clusters were verified using TempEst. Time-scaled phylogenies were constructed in BEAST using a 10-million MCMC chain length, GTR+I+G model, uncorrelated relaxed clock, and Bayesian skyride model, with the first 10% discarded as burn-in. Maximum clade credibility (MCC) trees were utilized in TransPhylo to infer transmission networks under incomplete sampling, which were subsequently overlaid with swine movement data.
The analysis revealed a highly diverse genetic landscape with a mean pairwise distance of 14.2%, yielding 32 distinct clusters. Cluster 7 was the largest ($n = 71$; 15.5%), and Cluster 23 exhibited the highest homogeneity (n = 30). The absence of a single common ancestor demonstrates that PRRSV endemicity in Upper Austria is sustained by multiple independent introduction events. However, the dominance of Cluster 7 indicates a variant that primarily driving regional transmission. Overlapping genomic data with trade networks demonstrates that livestock movement maintains this endemic strai

Keywords: phylodynamics, viral transmission, molecular surveillance, swine movement networks, PRRSV

Oral Presentations:

Parallel Session 4.1 – Vector-borne Diseases

Date, Time: September 4, 10:30 – 12:00
Location: Stadion

Contents:

Understanding the pathogenesis of the transboundary bluetongue virus serotype 3 (BTV-3) in Europe through experimental sheep infection

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Bluetongue (BT) is a debilitating notifiable disease that affects wild and domestic ruminants, which typically affects sheep more severely. The disease is produced by the infection with bluetongue virus (BTV), an orbivirus transmitted by the bite of Culicoides spp. This virus possesses multiple serotypes, and little cross-protection exists between these. In 2023, BTV serotype 3 emerged in northern Europe and rapidly spread across the continent, reaching Scandinavia, Greece and the Iberian peninsula by 2024. This unprecedent spread was accompanied by unusually severe disease in sheep, yet the biological basis underlying the virulence and rapid transmission of this remains largely unknown. Here, we present the first comprehensive experimental characterization of BTV-3 infection in an European sheep breed, integrating clinical, virological, immunological and histopathological assesments. All infected sheep developed an acute disease with moderate clinical signs. Viremia was already detectable by day 2 post-infection (dpi), peaked at 7dpi, and infectious virus could be recovered until 10dpi, indicating a faster replication kinetics faster than previously reported for other BTV serotypes. The infection induced a marked leukopenia with severe depletion of circulating monocytes between 2 to 5 dpi, demonstrating early innate immune suppression not described for other serotypes. Histopathology analyses revealed novel features of BTV-3 pathognecity, including early lymphoid activation followed by marked macrophage expansion in the splenic red pulp and haemophagocytosis in lymph nodes at later stages of infection. Pulmonary lesions evolved from congestion and oedema to a mild mononuclear interstitial pneumonia, while hyperplasia of the bronchiolar associated lymphoid tissue was a common feature. Several animals developed corneal opacity associated with peripheral keratouveitis, broadening the recognized clinical spectrum of BTV-3 infection. Adaptive immunity developed in a pattern consistent with BTV infection. However, neutralizing antibodies to BTV-3 showed little cross-reaction with other circulating European serotypes, indicating that prior exposure to endemic BTVserotypes is unlikely to confer protection against the emergent BTV-3. Overall, our findings demonstrate that BTV-3 possesses unique immunomodulatory and pathogenic properties, characterized by rapid replication and early disruption of innate immunity, which likely contribute to its enhanced virulence and transboundary spread. This work provided novel mechanistic insight into BTV-3 pathogenesis and underscore the vulnerability of regions with historical BTV circulating to emerging orbivirus incursion.

Keywords: Bluetongue virus serotype 3, sheep, viremia, pathogenesis, immune response

Development of vaccines based on recombinant VP2 protein of Bluetongue virus and evaluation in an IFNAR-/- murine model

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ANSES/INRAe/ENVA, UMR 1161 Virology

Bluetongue Fever (BT) is an infectious disease affecting domestic and wild ruminants, transmitted by blood-feeding midges of the Culicoides genus. This virus, considered exotic until the 2000s, emerged in continental Europe in 2006. Since then, numerous epizootics involving different serotypes/strains of this virus have been observed in Europe. Thus, in France, since 2018, three serotypes are considered endemic (BT-3, 4, and 8). To control this disease, vaccines are available consisting of inactivated wild-type BT strains, but they do not allow differentiation from infected animals nor do they confer cross-protection between serotypes. The VP2 structural protein of the BT virus induces the production of neutralizing antibodies in infected or vaccinated hosts. Our approach is based on the expression of recombinant VP2s of the BT virus in a eukaryotic system in order to evaluate their immunogenic properties in the IFNAR -/- murine model.
The coding sequence of VP2s of serotypes 3 (BTV3 France 2024), 4 (BTV4 Corsica 2016), or 8 (BTV8 France 2006 and BTV8 France 2023) was inserted into a pVote plasmid by Invitrogen Gateway recombination. The recombinant Modified Vaccine Ankara (MVA) viruses generated allow the expression of these proteins in fusion with a FLAG tag in BSR cells, under IPTG induction.
Following transient expression, VP2 proteins from serotypes 3 and 4 demonstrated solubility. Within the BTV-8 strains, the recombinant VP2 from France 2023 was soluble, whereas the France 2006 variant was not. Soluble VP2 proteins were immunoprecipitated and formulated with Montanide ISA71 (SEPPIC) adjuvant to vaccinate IFNAR-/- mice subcutaneously. Following a day 21 booster, sera were collected on day 42 to assess neutralizing antibody (NAb) responses, and a homologous wild-type challenge was administered on day 49. Strikingly, despite identical production and purification methods, these recombinant VP2 proteins elicited varying degrees of protection (from partial to complete) across the 10, 4, and 1 µg dose groups.

Keywords: Bluetongue Virus, Recombinant Protein, VP2

Serological and molecular evidences of Crimean-Congo Hemorrhagic Fever virus (CCHFV) among animals and ticks in Israel

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Background
CCHFV, an RNA virus (family Nairoviridae), is the etiological agent of CCHF, a tick-borne disease, endemic in many parts of Asia, Europe and Africa. Ticks, mainly of the genus Hyalomma, are considered the main vector as well as reservoir of the virus. Generally, animals are asymptomatic to the disease with transient short viremia. In contrary, CCHFV infections in humans may develop into a severe hemorrhagic fever that could be lethal. Most human infections result from tick bites, although direct contact with infected blood or tissues of animals or humans is considered as an alternate way of infection.

Methods
Twenty beef cattle herds sampled during 2024-2025 and thirty-three camel herds sampled during 2013-2025 for sera and ticks. Presence of antibodies in the sera samples was studied using ELISA test. Additionally, the presence of CCHFV in the ticks was tested using RT-qPCR.

Results
In seventeen of the beef cattle herds, located in the north and central Israel, we have found seropositivity ranging from 3-100%. Moreover, we have identified prior exposure of camels in thirty of the herds collected during 2013 to 2025 in ranging prevalence of 5-30%. Lastly, we detected the presence of CCHFV in several species of ticks collected from the cattle herds and from wild animals. Phylogenetic analysis of a partial sequence of the S segment, have clustered to the Asia-1 genotype, suggesting the origin of the virus relates to the Middle-east linage.

Conclusions
These results demonstrate the presence of CCHFV in Israel. The high prevalence of prior exposure to CCHFV in cattle herds in the north and center part of Israel and in camels in the south part of the country, back traced as early as 2013. In addition, we have identified the presence of the virus in ticks, demonstrating the spread of CCHFV to new locations across the Mediterranean basin. Although no human cases were reported, so far, the prevalence, distribution and history of the animals' exposure suggest the virus is circulating in the country for at least 12 years. This One Health approach emphasizes the risk for populations such as animal breeders, veterinarians and healthcare workers.

Keywords: CCHFV, Ticks-borne, emergence, zoonotic, hemorrhagic disease

Wesselsbron virus infection in neonatal lambs causes severe viscerotropic hepatitis associated with neutrophil infiltration

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Wesselsbron virus (WSLV), a zoonotic mosquito-borne orthoflavivirus in the YFV serogroup, primarily infects ruminants and causes abortions and high mortality in young animals. WSLV-infected sheep develop hepatitis, and milk-borne transmission is described. Here, we established a mucosal WSLV infection model in lambs to better define immune mechanisms associated with severe outcomes. Lambs were inoculated orally or nasally with doses equivalent to virus levels in milk and nasal secretions of infected ewes and were monitored for clinical signs, virology, serum biochemistry, cytokines, and chemokines. Blood and liver transcriptomes were analyzed using blood transcriptional modules (BTM), and liver pathology and neutrophil infiltration were characterized by histology, myeloperoxidase (MPO) immunostaining, and digital image analysis.
After mucosal inoculation, WSLV efficiently infected lambs, developing viremia and fever, followed by jaundice and reduced body weight gain. One third of infected animals developed severe disease, reaching humane endpoints with a mortality rate equivalent to that of natural WSLV outbreaks. The virus showed strong viscerotropism, with the liver presenting the highest viral loads, correlating with histopathological and biochemical markers of liver injury and a MELD XI like severity score. Severely ill lambs presented with multiorgan failure (liver and kidney), resembling viscerotropic YF in humans.
Systemically, we detected a transient IFN α response, with elevated IL 6 and IL 10, and a chemokine burst of CCL3/CCL4 (decreasing over several days), and sustained CXCL10 production. Severe cases showed very low IFN α with higher IL 6 and CXCL10. In convalescent animals, BTMs revealed early antiviral/myeloid activation followed by strong B and T cell and cell cycle modules, coinciding with high neutralizing antibody titers and viremia clearance. In severe cases, BTMs revealed a persistent neutrophil related signature, weak NK cell and adaptive responses, with poor neutralizing antibody production.
In the liver, severe cases showed transcriptomic profiles characterized by myeloid and neutrophil activation and altered metabolic and extracellular matrix pathways, whereas convalescent livers showed strong activation of NK cell, T cell, and proliferation modules. Furthermore, neutrophil associated genes and MPO staining were markedly increased in the liver of severe cases, which was strongly associated with viral load and lesion severity; also showing the highest levels of soluble MPO. The present study established a model of natural mucosal WSLV infection in lambs that resembles many characteristics of the YFV-induced viscerotropic hepatitis and identifies a neutrophil-driven, NK-deficient inflammatory axis linked to fulminant disease; providing a model to study pathogenesis and interventions for orthoflavivirus-induced hepatitis.

Keywords: Wesselsbron virus, lamb, viscerotropic hepatitis, System immunology, neutrophils

Characterization of Japanese Encephalitis Virus Replication Kinetics and Target Cells in Porcine PBMCs

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Japanese encephalitis virus (JEV) is a zoonotic mosquito-borne flavivirus endemic in Asia which poses a threat to Europe. Pigs serve as important amplifying hosts and are thus of high epidemiological importance. While JEV interaction with immune cells is important for virus spread and immune modulation in humans and mice, such information is largely lacking in pigs. This study therefore aimed to characterize JEV replication kinetics in porcine PBMCs and identify target cells.
Frozen PBMCs from 3 pigs were infected with JEV at MOI 0.1, 1 and 10 and studied at 8, 24 and 48 hours post-infection (hpi) by qRT-PCR, virus isolation, fluorescence immunocytochemistry and flow cytometry, in triplicate per pig.
qRT-PCR showed that porcine PBMCs supported viral replication, with Ct values decreasing over time in an MOI-dependent manner, consistent with virus titration results. Infectious virus was detected in supernatants at 8, 24 and 48 hpi for MOI 10, 1 and 0.1, respectively, with titers reaching up to 10^8.55 $TCID_{50}/10^6$ PBMCs at 48 hpi.
Flow cytometry identified CD172a+ myeloid antigen-presenting cells (mAPCs) as the only susceptible immune subset, based on NS3 detection. Infection increased with MOI and time; at 48 hpi and MOI 10, a mean of 12.64% of mAPCs were NS3+, with inter-individual variation (19.81% vs. 9.05%). Monocytes (CD172a+CD14+) accounted for 87.07% of infected mAPCs, with 25.16% of monocytes being infected. Among monocyte subsets defined by CD14 and CD163, immature ($CD14^{high}CD163^{-}$) and intermediate-like ($CD14^{high}CD163+$) monocytes were the predominant infected populations (26.51% and 29.46% of JEV+ cells; 25.5% and 39.7% infected within each subset, respectively). No JEV infection was detected in lymphocytes.
These results indicate that JEV mainly targets porcine monocytes, particularly intermediate-like and immature subsets, which support viral replication to high titers. These cells may play a key role in viral dissemination and tissue invasion, consistent with observations in humans and other flavivirus models, and support the usefulness of this model to investigate JEV pathogenesis and immune evasion.

Keywords: Japanese encephalitis virus, porcine PBMCs, cell tropism, viral replication kinetics

Ticks and tick-borne human and animal pathogens in high-footfall areas in Luxembourg

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Tick-borne diseases of humans and animals are on the rise due to the geographical expansion of their tick vectors and higher incidence of infected ticks. The distribution and density of ticks, as well as the prevalence of tick-borne pathogens are currently under-characterized in Luxembourg. Therefore, nationwide surveillance activities were implemented in the country in 2025.
From April to November, questing ticks were collected monthly via blanket dragging at 41 high–footfall sites across Luxembourg, with 300 m² surveyed per month at each site. The sites were categorized as urban, suburban, or rural and contained two to seven transects representing different microhabitats. Nucleic acids were extracted from all collected ticks and screened for tick-borne pathogens, including tick-borne encephalitis virus (TBEV), Borrelia spp., Anaplasma phagocytophilum, Neoehrlichia mikurensis, Babesia spp., Spiroplasma ixodetis, Bartonella spp., Rickettsia spp., Coxiella burnetii and Francisella tularensis.
A total of 4,486 ticks were collected and analyses indicated that tick populations were heterogeneously distributed not only between but also within most collection sites. In addition, there was a strong influence of location and microhabitat type on tick abundance. In none of the ticks, TBEV was detected, but about 17% were positive for Borrelia spp. Characterization of Borrelia genospecies and screening for other tick-borne pathogens are ongoing, and first results will be shared at the conference.
The nationwide tick and tick-borne pathogen surveillance will provide critical insights into our understanding of the driving factors behind tick abundance and tick-borne disease risk in Luxembourg, particularly in green spaces with high public usage. The data will contribute to European efforts to monitor and mitigate the risk of tick-borne diseases of public and veterinary importance.

Disclaimer:
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Keywords: tick-borne pathogens, nationwide surveillance, tick distribution and density, Borrelia genospecies

Oral Presentations:

Parallel Session 4.2 – Pathogenesis and Virology

Date, Time: September 4, 10:30 – 12:00
Location: Olympia

Contents:

Chicken Lung Organoids as a Physiologically Relevant Model to Study Avian Influenza Infection

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Avian influenza poses a major global threat to animal health, especially due to the continuous emergence of novel viral strains that challenge surveillance, preparedness, and control. Robust in vitro models that accurately reflect avian physiology are essential for advancing host–pathogen research and enabling rapid assessment of emerging viruses during outbreak situations while reducing the need of animal experimentation.
We investigated whether chicken lung organoids recapitulate key features of the avian respiratory mucosa and provide a suitable platform for avian influenza infection studies. Chicken lung organoids were generated from three individuals and maintained in proliferation media before inducing differentiation in organoid-derived monolayers. Comprehensive characterization was performed using quantitative PCR and immunofluorescence to assess expression of respiratory epithelial markers and identify major epithelial cell types.
Both PCR and immunofluorescence analyses confirmed the presence of key cell populations found in the respiratory epithelium, including goblet cells, ciliated epithelial cells, and pneumocytes. Expression of cell-type-specific markers increased during differentiation, including those associated with goblet (MUC2), ciliated (IFT88, TUBB4B), and pneumocyte (CAV1, SFTPA2) populations, progressively approaching levels observed in parental lung tissue. Donor-specific variation in marker expression and differentiation responses reflected natural biological diversity among individual animals.
To assess susceptibility to infection, differentiated monolayers infected with an H7N1 high pathogenicity avian influenza virus were monitored for 48 hours using an IncuCyte SX5. Cultures supported productive infection and showed clear cytopathic effects. Infection at a MOI of 0.1 led to a marked cellular apoptosis 16 hours post-inoculation, as detected by a fluorescence-based caspase assay in which green fluorescence is emitted upon caspase activation. These results demonstrate that organoid derived monolayers are permissive to viral replication and suitable for comparative infection studies.
Together, these findings establish chicken lung organoids as a physiologically relevant in vitro system for studying influenza virus infection of the respiratory tract. This platform offers a controlled model to investigate host–virus interactions and supports rapid assessment of emerging avian influenza strains, complementing in vivo approaches.

Keywords: avian influenza virus, chicken lung organoids, host-pathogen interactions, avian respiratory epithelium, in vitro infection model

Homologous and heterologous influenza A virus infections of bovine, porcine and human well-differentiated airway epithelial cells: virus loads and host responses

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Background
Recent introductions of highly pathogenic avian influenza (HPAI) virus into dairy cattle illustrate the propensity of Influenza A viruses to cross species barriers and adapt to novel hosts. Ongoing transmission between cattle in the USA has resulted in zoonotic transmission to humans and allows viral adaptation to replication in mammals. We aimed to study infection and host response of well-differentiated airway epithelial cells (WD-AEC) of human, bovine and porcine origin after inoculation with homologous and heterologous influenza A viruses (IAV).

Methods
We prepared WD-AEC cultures grown at air-liquid interface (ALI) using airway organoids derived from the lower respiratory tract of cattle, pigs and humans (3 donors per species). These cultures were inoculated with 10^4 TCID50 of IAV strains derived from humans (pH1N1), pigs (H1N1, H3N2), or four HPAI-H5N1 strains derived from chickens, fox or cattle. We measured virus loads in apical washes 1- and 2-days post inoculation and host responses in cells harvested 20 hours post inoculation by RNAseq.

Results
Clade 2.3.4.4b HPAI H5N1 viruses derived from USA cattle or from a Dutch fox (with either PB2-627E or -627K) efficiently infected WD-AEC of all three host species, with highest virus loads and decreases in trans-epithelial electrical resistance in cattle and humans. Interestingly, the HPAI H5N1 virus isolated from chickens replicated poorly in all three host species. Swine influenza viruses and pH1N1 replicated best in WD-AEC of humans and pigs. RNAseq analysis revealed differentially expressed genes (DEGs) involved in immune processes, like interferon and cytokine signaling. Notably, highest numbers of DEGs were detected in human WD-AEC. Although the pH1N1 and swine H1N1 viruses efficiently replicated on human WD-AEC the number of DEGs was limited compared to the other viruses. The highest number of DEGs were observed for the fox PB2-627K virus in both human and bovine WD-AECs

Discussion
Here we show that inoculation of bovine, porcine or human WD-AEC with different IAV strains resulted in different levels of virus replication, tissue damage and host responses. Our results illustrate that organoid-derived WD-AEC of different host species can be used to assess viral traits and zoonotic potential of IAV strains.

Keywords: HPAI H5N1, virus evolution, host range, organoids, RNAseq

Host Immune Signatures Distinguish Clinically Silent Persistence from Severe Disease during Classical Swine Fever Virus Infection

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Persistent classical swine fever virus (CSFV) infection represents a major challenge for disease control because infected animals may maintain prolonged high viraemia and viral shedding in the absence of detectable humoral responses or overt clinical signs. However, the host immune mechanisms determining whether infection remains clinically silent or progresses toward severe disease remain poorly understood.

In this study, we investigated host transcriptional programs associated with divergent clinical outcomes following experimental infection of neonatal piglets with the moderately virulent CSFV strain Catalunya01. Peripheral blood mononuclear cells (PBMCs) collected during early infection (7 dpi) and later disease stages (22 dpi) were analyzed by RNA sequencing and targeted TaqMan RT-qPCR validation.

Despite similarly elevated viral loads across infected animals, transcriptomic analyses revealed markedly distinct immune-associated signatures. Early infection was characterized by activation of interferon-associated antiviral pathways, including increased expression of OAS1, IRF7, and ISG12, consistent with a strong innate antiviral state. In contrast, animals developing mild/moderate persistent disease showed attenuation of interferon and inflammatory programs together with increased expression of checkpoint-associated regulatory genes, including TIGIT, ZBTB32 and CTLA4. Severe disease progression was associated with increased inflammatory activation, including IL1B, TLR2, and CCL2, together with strong exhaustion-associated immune signatures.

RT-qPCR validation confirmed OAS1 as a marker of early antiviral activation, whereas TIGIT expression progressively increased according to disease severity, suggesting transition toward exhaustion-associated immune dysfunction during persistent infection.

Collectively, these findings support the existence of a coordinated IFN–Inflammasome–Exhaustion axis associated with divergent clinical trajectories during persistent CSFV infection and suggest that disease outcome is determined not only by viral burden, but also by the host immune state established during infection

Keywords: Classical swine fever virus (CSFV), Persistent infection, Type I interferon, Inflammasome, Immune exhaustion, Transcriptomics

The outcome of Orbivirus co-infections is influenced by the viral strains involved, cell line and timings of infection

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The genus Orbivirus (family Sedoreoviridae) is a group of arthropod-borne, non-enveloped viruses with genomes composed of 10 segments of linear dsRNA. The orbiviruses bluetongue virus (BTV), epizootic haemorrhagic disease virus (EHDV) and African horse sickness virus (AHSV) cause disease of high consequence for livestock and equids. The rapid evolution of such segmented viruses, and consequent emergence of new strains, can be facilitated through genome-segment reassortment following co-infection with different viral strains. Indeed, genome reassortment between circulating BTV strains occurs extensively in the field. The current co-circulation of multiple BTV strains in Europe raises the possibility of reassortment between strains, with unpredictable outcomes of disease severity and vector transmission phenotypes. Reassortment, however, is constrained by superinfection exclusion (SIE), whereby an initial and established viral infection blocks infection of the same cell by a second, superinfecting virus. SIE has been observed in BTV, however, the mechanisms of SIE, the extent SIE constrains reassortment in BTV, and whether SIE is a shared phenotypic trait across orbiviruses, remain poorly understood.

In this study, we investigated the interactions during co-infection of bovine or Culicoides-cells between homologous and heterologous Orbivirus species of relevance to the current European ecosystem, including BTV-3 and EHDV-8. Using a combination of strain- and segment-specific qRT-PCR, and fluorescent in situ hybridisation (FISH) assays, we showed that synchronous co-infection between homologous or heterologous orbiviruses results in neutral co-existence, whereas asynchronous co-infection leads to total or partial exclusion of the superinfecting virus. The varying degree on SIE was found to depend largely on the strain of the initial and superinfecting virus. Furthermore, our findings indicate that in either synchronous or asynchronous BTV co-infections, reassortment was frequent providing there was no total SIE. The constellation and frequencies of BTV genotypes that arose in bovine-host cells, however, differed from those arising in Culicoides-vector cells, suggesting potential biases. Although partial SIE did not prevent reassortment, it did reduce the viral diversity resulting from reassortment. In conclusion, Orbivirus co-infection dynamics are complex and may impact viral diversity, evolution and the emergence of novel viral strains.

Keywords: Orbivirus, bluetongue virus, Co-infection, Superinfection Exclusion, Reassortment

Functional analysis of neurotropic porcine bastrovirus by reverse genetics

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Bastroviruses (BastV) are non-enveloped, single-stranded, positive-sense RNA viruses that have recently been discovered in the feces of various animals and humans. Their genomes are approximately 5–7 kb in length and are organized into at least two open reading frames (ORFs). ORF1 encodes non-structural proteins with sequence similarity to hepeviruses, whereas ORF2 encodes structural proteins and shows sequence similarities to astroviruses.
Recently, we identified a porcine bastrovirus strain (PoBastV CHE/2022) in the brain tissues of domestic pigs presenting with fatal neurological disease. The full-length genome of PoBastV CHE/2022 was obtained by high-throughput sequencing. In addition to ORF1 and ORF2, we identified a third ORF (ORF3A) at the 3' terminus of the genome, which contains an internal ORF3B. Another ORF, designated ORF4, overlaps with the 5' end of ORF2. These ORFs were found to be conserved in PoBastV detected in pig feces. Whether viral proteins are translated from these ORFs and what function they serve in viral replication remains unknown.
To enable further functional studies, we generated a molecular cDNA clone of PoBastV CHE/2022 and rescued infectious virus using reverse genetics in intestinal porcine enterocytes (IPEC-J2). This reverse genetics system allowed us to investigate viral protein expression and its potential role in virus replication, using antisera-based immunofluorescence detection and targeted virus mutagenesis. Initial immunofluorescence data demonstrated the expression of proteins encoded by ORF1, ORF2, ORF3A, and ORF4, but not by ORF3B, in infected IPEC-J2 cells. To understand the function of ORF3A, we generated an ORF3A-knockout cDNA clone and successfully rescued the infectious virus in IPEC-J2 cells. The viral load of the ORF3A-mutated virus in these cells was significantly lower than that of the wild-type virus. These data demonstrate that the protein encoded by PoBastV ORF3A is dispensable for viral replication; however, it appears to increase replication efficiency.
Taken together, we have established a reverse-genetics platform for PoBastV, laying the groundwork for further studies into the molecular biology and pathogenesis of emerging bastrovirus infections.

Keywords: Bastroviruses, reverse genetics, virus mutagenesis, immunofluorescence, pathogenesis

Pathobiology and Protective Immune Transfer Against Wesselsbron Virus in Ifnar1-/- Mice

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Wesselsbron virus (WSLV) is a neglected mosquito-borne flavivirus endemic to sub-Saharan Africa. Its transmission routes remain poorly defined, and several domestic and wild animals—sheep, goats, cattle, horses, and rodents—have been proposed as reservoirs. Infection in adult ruminants is typically asymptomatic, but gestational infection causes abortion and congenital malformations. Newborn animals may develop high fever and lethargy, sometimes leading to death. WSLV also infects humans, with documented cases of acute febrile illness. We recently demonstrated vector-independent transmission in sheep, showing that infected ewes can transmit WSLV to their lambs via milk. Although current prevalence is unknown, recent surveys in livestock and mosquitoes indicate a rising emergence potential of clade I strains. This study addresses the lack of a small-animal model suitable for investigating protective immunity and supporting antiviral and vaccine development.
WSLV strains SAH177 (clade II) or recombinant SA999 (rSA999, clade I) were administered subcutaneously into the footpad of wild-type (WT, C57BL/6J) or Ifnar1-/- mice. WT mice were inoculated with 10^4 TCID50 of rSA999 to generate immune sera, collected four weeks later. Passive immunization of Ifnar1-/- mice was performed by intraperitoneal injection of immune or control serum before challenge with the same strain. Clinical signs and body weight were monitored daily. At defined time points, mice were euthanized and blood, spleen, liver, and brain were collected. Viral loads were quantified by RT-qPCR targeting the conserved 3'UTR, and neutralizing antibodies were assessed by VSNT.
Adult and young WT mice remained asymptomatic after inoculation with up to 10^6 TCID50 of either strain, showing absent or minimal viral replication. Sera from infected WT mice displayed strong cross-neutralization between clade I and II strains. In contrast, Ifnar1-/- mice inoculated with 10^4 TCID50 developed clinical disease with significant weight loss. rSA999 caused transient paw swelling beginning at 5 dpi, followed by full recovery, whereas SAH177 infection resulted in death or humane euthanasia by 5–6 dpi. At a lower inoculum 10^2 TCID50, both strains produced high viral loads in blood and organs at 2 and 4 dpi, with SAH177 replicating to higher levels. Passive immunization with WT immune serum protected Ifnar1-/- mice from footpad swelling and reduced viral RNA in serum and tissues by up to three logarithmic scales.
Here, we demonstrate that the Ifnar1-/- mouse model offers a valuable tool for investigating the mechanisms of protective immunity and advancing vaccine and antiviral development against WSLV.

Keywords: Wesselsbron virus, passive immunization, mouse model

Poster Presentations

Participants of the Poster Slam

Location: Stadion

Poster Slam – Round 1

Participant Name (poster no.)	Abstract Title
Yamazaki, Wataru (1.14)	Detection of African swine fever virus DNA in pig farm wastewater and Amblyomma javanense ticks in Vietnam
Coatu, Elena (2.06)	Intradermal and intranasal vaccination with microparticulated ASFV proteins induces distinct immune profiles in pigs
Soliani, Laura (1.28)	Genetic diversity of Influenza D viruses circulating in cattle in Northern Italy between 2020 and mid-2026
Tomás, Gisela (2.05)	Protein-only microparticles as a vaccine platform against infectious bursal disease and high pathogenicity avian influenza
Johnston, Camille Melissa (1.31)	Coronavirus diversity in wildlife in Denmark: Insights from bats and mustelids
Gelskov, Laura Vebæk (1.16)	Recurrent detection of Usutu virus in blackbirds in Denmark, 2025
Sopbue, Ines (3.11)	Mapping Rift Valley fever emergence risk in Cameroonian livestock
Holopainen, Riikka (1.15)	Monitoring ruminant antibodies may enhance identification of new TBE risk areas for humans
Domb, Ana (1.27)	Assessing the epidemiological impact of EHDV-8 emergence in France: seroprevalence, herd-level mortality, and individual reproductive performance in cattle

Poster Slam – Round 2

Participant Name (poster no.)	Abstract Title
Meyer, Denise (1.19)	Alternative sample matrices for the detection of antibodies against classical swine fever virus
Lazov, Christina M. (1.21)	Swab type and storage conditions have limited impact on PCR detection of Aujeszky's disease virus
Trogu, Tiziana (1.22)	Development of a Sandwich ELISA and LFD for Rapid Detection of Seneca Valley Virus
Kang, Hyeonjeong (1.30)	Comparative genomic characterization of Cattle- and Elephant- derived FMDV O/ME-SA/Ind-2001e reveals cross-species transmission in Cambodia
Al-Rashed, Ali M. (1.32)	Evolutionary Emergence of the Foot-and-Mouth Disease Virus A/ASIA/G-VII Lineage in Western Asia
Abicht, Helge (1.24)	New IBR serum double-antigen sandwich ELISA
Singh, Balwant (1.17)	Development of a Recombinant, Non-Infectious Competitive ELISA Platform to Support Post-Eradication Preparedness for Rinderpest virus
Zeiske, Sophie (3.12)	Virus detection in Culicoides biting midges during the BTV-3 outbreak in north-west Germany 2024
Obishakin, Emmanuel (1.20)	Molecular detection, serotyping and genomic characterisation of multiple Bluetongue virus and Epizootic haemorrhagic disease virus circulating in ruminants in Nigeria

Sewgobind, Sanam (1.23)	Evaluation of West Nile virus Single-Round Infectious particle as a Surrogate virus in a neutralisation test compared with plaque reduction neutralisation test
Lelli, Davide (3.13)	New Insights into the Ecology and Clinical Relevance of Hypsugopoxvirus in European Bats
Byaruhanga, Tim (1.25)	Platinum IV Chloride Viability PCR assay for the assessment of virus disinfectants

Poster Slam – Round 3

Participant Name (poster no.)	**Abstract Title**
von Wyttenbach, Stefano (4.16)	Unbiased N-Terminomic approaches reveal new proteolytic targets of the African swine fever virus protease pS273R in primary porcine macrophages
Poljakovic, Robin (4.12)	Vinculin: an unexplored player in classical swine fever virus infection
Puente Marin, Sara (4.13)	Persistent classical swine fever virus infection is associated with early cytotoxic immune dysregulation despite absence of clinical disease
Grau Roma, Llorenç (4.15)	Unraveling Infectious Disease Pathogenesis through Digital Pathology: The Case of Wesselsbron-Induced Hepatitis
Lambert, Fanny (4.14)	The 3d polymerase of foot-and-mouth disease virus interacts with the card domain of porcine mavs and inhibits type i interferon signaling
Newbrook, Kerry (1.26)	Longitudinal BTVPUR®4-8 vaccination trial highlights appropriate BTV vaccination status in cattle
Sajewicz-Krukowska, Joanna (4.17)	Antibiotic administration reduces early infectious bronchitis virus shedding and modulates antiviral responses in chickens

Poster Presentations:

Poster Session 1 – Diagnostics, Epidemiology

Location: Fair Play

Contents:

Poster 1.01

Differentiation of Vaccine and Circulating Lumpy Skin Disease Virus in an Evolving Epidemiological Landscape

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Lumpy skin disease (LSD) is a transboundary cattle disease for which vaccination remains a central control strategy. PCR-based diagnostics are essential for outbreak confirmation, surveillance, animal movement certification, and demonstration of freedom from infection as outlined in the WOAH Terrestrial Manual. Historically, molecular assays capable of differentiating vaccine and classical field strains have supported disease control programs and facilitated the investigation of post-vaccination reactions associated with live attenuated Neethling-based vaccines.
In recent years, this diagnostic paradigm has become increasingly challenged. Alongside classical field and vaccine strains, recombinant LSDV variants have been reported in multiple regions. These viruses contain genomic signatures associated with both vaccine and field strains and may circulate in affected populations. Consequently, the distinction between vaccine-associated detections and infection with circulating virus has become increasingly difficult, reducing the discriminatory capacity of several established PCR-based differentiation assays.
To address this challenge, the optimized ID Gene Lyo™ LSD DIVA Triplex 2.0 qPCR kit was developed for the simultaneous differentiation of vaccine and circulating LSDV, including recombinant variants, within a single diagnostic workflow. The assay incorporates an endogenous internal control to ensure reliable interpretation of results.
Performance was evaluated using diagnostically relevant sample types, including whole blood, oral and nasal swabs, and skin lesion material. The assay reliably differentiated vaccine-derived detections from circulating virus and provided valid results across all sample matrices tested.
The ability to distinguish vaccine-associated findings from infection with circulating LSDV is increasingly important for clinical investigations, surveillance, outbreak response and disease eradication programs. Robust molecular tools that accommodate the evolving genetic diversity of LSDV can support accurate diagnosis and strengthen evidence-based control strategies in vaccinated populations.

Keywords: Lumpy skin disease, Capripox, diagnostics, DIVA

Poster 1.02

Structure and dynamics of reported livestock trade networks in Africa

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Livestock trade is a major driver of live animal movements worldwide and plays a key role in the transmission of transboundary animal diseases such as foot-and-mouth disease (FMD). However, the structural organization and temporal dynamics of these networks remain insufficiently characterized, particularly in Africa.
In this study, trade networks were reconstructed using UN Comtrade data from 37 out of 54 African countries over 2015–2025, covering four FMD-susceptible species (cattle, pigs, goats, and sheep). The contribution of each country (node) was categorized using weighted PageRank centrality and the most influential ones classified as hubs. Trade corridors (edges) were defined as persistent when present >70% of the study period and dominant when accounting for ≥5% of annual trade volume.
At the multi-species level, the network comprised 40 actors, both within and outside Africa, with four major hubs: South Africa, Kenya, Morocco, and Palestine. North African countries, particularly Morocco and Egypt, structured several corridors toward Europe, with Egypt also connected to Brazil. South Africa emerged as a key exporter linking African markets to the United Arab Emirates (UAE) and island territories such as Mauritius.
Species-specific analyses revealed marked heterogeneity. The cattle network closely reflected the multi-species structure, with continent-wide participation and strong connectivity. Small ruminant networks were more regionally constrained, involving 22 and 16 actors for sheep and goats, respectively, mainly in Southern Africa. Identified hubs included South Africa (both species), Lesotho, Ivory Coast, and Kenya (sheep). Sheep trade exhibited extended export corridors (South Africa–Senegal, South Africa–UAE), whereas goat trade appeared more fragmented but remained regionally diverse.
In contrast, the pig trade network was smaller and more centralized, comprising 10 actors and 9 corridors. Tanzania emerged as the sole hub, with the Zambia–Tanzania corridor representing the main trade pathway, while South Africa acted mainly as a regional exporter without central hub status.
Across species, a limited number of hubs (notably South Africa, Egypt, Morocco, Tanzania, Kenya, and Uganda) were consistently connected through persistent, high-volume corridors, concentrating both trade intensity and connectivity. These node - corridor configurations represent key pathways for disease dissemination. Targeting these strategic nodes and corridors provides a robust approach for risk-based surveillance and control, with vaccination prioritization in high connectivity areas likely to enhance the effectiveness of FMD control strategies across the continent.

Keywords: FMDV, trade, connectivity, transboundary, network, Africa

Poster 1.03

Comparison of Buffers to Protect the Integrity of Viral RNA in Foot-and-Mouth Disease Samples

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The accurate and rapid diagnosis of foot-and-mouth disease (FMD) is critical for effective implementation of control strategies requiring that samples from livestock with suspected vesicular disease must first be transported to centralised laboratories for testing. Established methods rely upon the isolation of viruses in cell culture, necessitating that the virus remain infectious. In endemic regions, the combination of distance and unreliable infrastructure can result in delays of several days for samples to arrive at the laboratory. Such delays, especially in the absence of a cold chain, negatively impact the quality of samples, decreasing the reliability of virus isolation. In contrast, real-time RT-PCR is widely used for laboratory diagnosis of FMD and does not rely upon infectious virus. Therefore, the aim of this study was to optimise transport methods that prioritise RNA integrity.

A variety of commercial buffers designed to preserve nucleic acid are available. In this study we tested and compared four such buffers (DNA/RNA Shield, Zymo Research, Primestore MTM, Longhorn Diagnostics, RNAlater, Invitrogen and an in-house formulation of the RNAlater buffer) for their ability to preserve FMDV RNA. A pool of swab eluate was generated by washing swabs in nuclease free water, before aliquoting the solution into tubes containing the selected buffers. These tubes were then held at -4°C, 37°C, or at room temperature for selected time points until 56 days. RNA was extracted and tested by the 3D real-time RT-PCR according to the protocols of the WOAH Terrestrial Manual.

At 4°C and at room temperature, samples stored in either DNA/RNA Shield or Primestore MTM showed no significant increases in CT value, while for RNAlater and the in-house equivalent minor increases in CT value were observed after 14 days. At 37°C CT values increased gradually for samples stored in DNA/RNA Shield and Primestore MTM and the in-house buffer, but for RNAlater no increase in CT was observed for the duration of the experiment. In contrast, CT values increased immediately and dramatically at every temperature when using nuclease-free water as a control.

The extracted RNA was also demonstrated to be of sufficient quality to transfect into LFBK cells and consequently recover live virus. This demonstrates that, despite the buffers being too toxic to directly use with cell culture, the use of an RNA-stabilising buffer does not exclude the possibility of performing virus isolation.

This work demonstrates that RNA stabilising buffers can be used to improve sample integrity for FMDV diagnostics.

Keywords: Foot and mouth disease virus, transport buffer, diagnostics

Poster 1.04

ASFV MONODOSE-LAMP® for rapid on-field detection of African swine fever virus in whole blood

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African swine fever virus (ASFV) is a highly contagious and lethal pathogen affecting domestic pigs and wild boar, requiring rapid and reliable diagnostic tools to support disease control, particularly under field conditions. Loop-mediated isothermal amplification (LAMP) offers a fast and simple alternative to conventional molecular methods, enabling on-site detection without the need for complex laboratory infrastructure.
The aim of this study was to evaluate a fluorimetric LAMP assay for the direct detection of ASFV in whole blood samples from wild boars, analyzed in the Barcelona area, and domestic pigs, infected with the Georgia strain. The assay was performed using specific primers targeting ASFV in a ready-to-use MONODOSE-LAMP® format, allowing direct testing without prior nucleic acid extraction. Reactions were carried out using the portable Genie® II device, enabling in situ analysis under field conditions. LAMP reactions were completed within 30 minutes, and ASFV was successfully detected directly from blood samples. The performance of the assay was validated against the WOAH-recommended real-time PCR, showing high concordance between both methods. The ability to detect ASFV without extraction significantly reduced processing time and simplified the workflow.
In conclusion, the fluorimetric LAMP assay, combined with the portable Genie® II platform represent a rapid, robust, and field-deployable approach for ASFV detection. By enabling reliable on-site diagnostics and reducing dependence on centralized laboratories, highly trained personnel, and Biosafety Level 3 (BSL-3) facilities, this method supports early disease detection and the timely implementation of outbreak response measures.

Keywords: LAMP, fluorimetric, ASFV, monodose, on-site

Poster 1.05

Development of a Duplex Real-Time PCR Assay for Rapid Identification of a Distinct African Swine Fever Virus Variant Detected in Spain

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African swine fever (ASF) remains one of the most important transboundary animal diseases affecting domestic pigs and wild boar worldwide. Following more than three decades without ASF, the disease was detected in wild boar in northeastern Spain in November 2025. Molecular investigations identified an unusual African ASFV genotype II variant characterized by a large deletion in the left variable region (LVR) of the genome. Rapid identification of this variant became particularly relevant for source-tracing investigations and for screening large numbers of samples generated during epidemiological and laboratory investigations associated with the outbreak.

To support these activities, a duplex real-time PCR assay was developed combining a universal ASFV detection target based on the p72 gene with a second target designed to identify the characteristic LVR deletion associated with the Spanish variant. This approach enables simultaneous ASFV detection and rapid discrimination of the Spanish variant from conventional genotype II ASFV strains.

The assay was evaluated using a comprehensive panel of more than 550 samples. This panel included over 300 experimental samples obtained from domestic pigs infected with different ASFV genotype II isolates and collected at multiple time points post-infection to assess assay performance throughout the course of infection. Different sample matrices were represented, including whole blood, serum and tissues collected during experimental studies. In addition, more than 250 field samples from domestic pigs and wild boar originating from ASF-affected European countries were included, covering a broad range of epidemiological situations and viral loads. Samples associated with the Spanish variant, ASFV challenge-virus stocks and material from animal experiments were also analysed.

The duplex PCR demonstrated a diagnostic specificity of 100% and a diagnostic sensitivity of 98.8%. All samples associated with the Spanish variant were correctly identified, while no false-positive variant detections were observed among conventional genotype II isolates. The assay showed robust performance across sample matrices and infection stages, supporting its application under both experimental and field conditions.

The assay provides a rapid and reliable alternative to whole-genome sequencing for screening large numbers of samples and supports molecular tracing of the Spanish ASFV variant. Following validation, the method was successfully transferred to the Spanish National Reference Laboratory and incorporated into investigations related to the outbreak. This study demonstrates how genomic signatures identified during outbreak investigations can be rapidly translated into practical diagnostic tools supporting ASF surveillance, source tracing and disease control.

Keywords: African swine fever virus (ASFV); duplex real-time PCR; genotype II; molecular tracing; variant-specific detection.

Poster 1.06

Efficacy of the Curing Process in Inactivating African Swine Fever Virus in Italian Salami

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The Italian swine industry has faced major challenges since 2019 due to the spread of African swine fever (ASF) across Europe and North America and its incursion into Italy in 2022. Despite the current situation, Italy remains a leading producer of Protected Designation of Origin (PDO) pork products, accounting for more than 50% of EU-27 PDO production. ASF is a highly contagious viral disease of suids transmitted through direct and indirect contact, including contaminated pork products, food waste and fomites. Current knowledge on ASF virus (ASFV) survival in cured pork products is largely based on studies conducted in the late 1980s with less sensitive methodologies and focusing on genotype I strains, no longer circulating outside Africa. To fill these knowledge gaps, the ASFree M.e.a.t. project aims to investigate ASFV persistence in cured pork products and provide updated scientific evidence to support international trade. A consortium of four Italian Experimental Zooprophylactic Institutes, coordinated by the National Reference Centre for Swine Fevers (CEREP), produced and experimentally contaminated five representative types of Italian dry-cured salami: Milano, Spianata, Napoli, Felino, and Cacciatore. The study evaluated the efficacy of curing process and High-Pressure Processing (HPP) in inactivating ASFV to produce safe commodities. Artificial contamination trials were conducted according to UNI EN ISO 20976-2:2022 and FSIS guidelines using the ASFV strain BA71/V, under BSL-3 conditions. Salami were inoculated with 1% (w/v) virus suspension and subjected to controlled curing. Throughout the process, weight loss, water activity, pH, and mesophilic lactic acid bacteria counts were monitored. Samples collected during curing and after HPP treatment were tested by Real-Time PCR and virus isolation on VERO cells to assess viral persistence and infectivity. Preliminary results show a progressive reduction in ASFV infectivity during curing in all salami types. Virus isolation was positive in the initial raw meat mixture but negative in samples collected at the end of curing and after HPP treatment. In Milano salami, infectivity was detected up to the drying phase, while analyses of the remaining salami are still ongoing. These data suggest traditional curing processes, particularly when combined with HPP, can substantially contribute to ASFV inactivation in short-cured salami. Future work will include viral DNA quantification by digital PCR and in vivo confirmation studies through feeding trials using negative products by virus isolation. These results may provide important scientific evidence to support risk-based trade policies and help maintain international market access for Italian cured meat products.

Keywords: AFRICAN SWINE FEVER VIRUS, ASFREE MEAT, SAFE COMMODITIES

Poster 1.07

Whole gag gene sequencing of Italian Small Ruminant Lentiviruses

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Introduction: Small ruminant lentiviruses (SRLVs) affect sheep and goats worldwide, causing a variety of clinical diseases. SRLVs are currently classified, based on gag-pol and pol sequencing, into five genotypes (A-E) and many sub-genotypes. The presence of asymptomatic animals, high genetic variability, and low proviral load may hamper an efficient diagnosis. Moreover, the use of several sequencing protocols and diverse genetic fragments leads to ambiguities in the genomic classification. Employing longer sequences (i.e. full gag gene) facilitates the generation of more robust and accurate genetic data. The aim of our work was to develop a primer walking technique to sequence the full gag gene of SRLV-positive samples already gag-pol sequenced between 2019 and 2026 at the Italian Reference Laboratory for Ruminant Retroviruses (CEREL).
Material & Methods: The method was developed using primers retrieved from the bibliography or newly designed based on the alignment of 945 SRLV sequences retrieved from Genbank, obtaining three overlapping fragments that cover the entire gag gene. To validate the methodology, a panel of 26 genotype A gag-pol sequenced samples, collected by CEREL through passive surveillance, was tested, including two German strains used as references. Nucleic acids were extracted, reverse transcribed, and amplified through RT-PCR. After Sanger sequencing, phylogenetic analyses were done using different software (MEGA X, Geneious and NGPhylogeny), and results were compared.
Results & Discussion: Overlapping fragments were obtained for twelve out of the 26 selected samples (46.15%). The alignment of sequences was made with 42 complete A genomes retrieved from Genbank, along with B and C genotypes as outliers. With our newly developed protocols, six samples clustered with the genomes of the same sub-genotypes identified with the gag-pol protocol. Five samples clustered together, likely due to the lack of complete reference genomes in Genbank. The only exception is one sample that was previously classified as A9 (for which reference sequences are present in the alignment) and now cluster with the A19 sub-genotype, probably because it was previously misclassified. German samples, belonging to the A21 sub-genotype, which is not present in Italy, clustered separately from the Italian ones. Moreover, the phylogenetic trees gave concordant results and high bootstrap values, supporting the classification. These PCR protocols facilitate complete gag phylogenetics, therefore contributing to the generation of robust and accurate genetic data useful for adequate genetic SRLV classification.

Keywords: Lentivirus, SRLV, full gag, genetic characterization, Italy

Poster 1.08

Validation of Duplex Real-Time RT-PCR Assays for Simultaneous Detection and Differentiation of Bluetongue Virus and Epizootic Hemorrhagic Disease Virus and Serotyping of Key European Bluetongue Virus Serotypes

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Bluetongue virus (BTV) and Epizootic Hemorrhagic Disease virus (EHDV) represent two major pathogens of ruminants remaining high impacts in livestock animal health. In Europe a complex epidemiological situation of emergence, repeated incursions and co-circulation of both diseases, also with multiple BTV serotypes, pose significant diagnostic and surveillance challenges. Here we describe the optimization and validation of the use as multiplex of some real time RT-PCR assays described in the bibliography and widely employed in official diagnostic laboratories throughout Europe. Firstly, pan-BTV/EHDV real-time RT-PCR assays for simultaneous detection and differentiation of both orbiviruses, including an endogenous beta-actin internal control to monitor RNA integrity and minimize false negatives. Secondly, real-time RT-PCR assays designed for rapid serotyping of epidemiologically relevant European BTV serotypes: BTV-3/BTV-8, predominant throughout the European continent; and BTV-1/BTV-4, with a greater presence in Mediterranean regions. The assays exhibited high analytical and diagnostic performance, validated according to WOAH standards using circulating viruses, reference strains, and extensive field samples from blood and viscera of domestic and wild ruminants. The multiplex formats substantially decrease time and costs of the analysis, becoming appropriate for implementation in official diagnostic laboratories. These methods constitute a useful approach for addressing emerging threats in areas with intricate co-circulation dynamics and complex Orbivirus situations.

Keywords*:* Bluetongue virus, Epizootic Hemorrhagic Disease Virus, molecular diagnosis, real time RT-PCR, validation

Poster 1.09

Rapid nanopore-based identification of avian H5 virus strains

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Avian influenza virus (AIV) surveillance requires rapid and reliable genotyping methods to support surveillance and outbreak control. The H5 subtype is of particular concern due to its association with highly pathogenic avian influenza (HPAI) strains circulating in wild and domestic bird populations. The Finnish Food Authority is the EU National Reference Laboratory (NRL) for Avian Influenza and Newcastle Disease in Finland and responsible for avian influenza surveillance.
Recent advances in sequencing technologies, particularly nanopore-based platforms, offer significant potential to accelerate virus identification and characterization. In this study, we evaluated a rapid nanopore sequencing approach for the identification of avian H5 virus strains using the Oxford Nanopore MinION platform. The workflow focuses on amplicon-based sequencing coupled with a ligation-based library preparation protocol to enable flexible and scalable genotyping.
Amplicon samples targeting the eight different genomic segments (PB1, PB2, PA, HA, NP, NA, MP, NS) of the H5-virus were prepared and sequenced using the MinION system. Sequencing performance was assessed in terms of detection capability, reliability, and suitability for routine surveillance applications. Preliminary results demonstrate that the ligation-based nanopore sequencing protocol enables reliable detection of avian influenza strains from amplicon-derived material: the MinION sequencer was able to produce identical consensus sequences to those obtained from a previous Illumina-based sequencing run. The method provides notable advantages in turnaround time compared to conventional sequencing approaches, while also offering flexibility in sequencing depth and the number of samples multiplexed per run.
These findings highlight the potential of nanopore sequencing as a rapid and adaptable tool for AIV genotyping in a surveillance setting. However, further optimization is required to define the optimal sequencing depth and sample multiplexing strategies needed to ensure robust and reproducible genotype identification across diverse sample types and viral loads.
In conclusion, nanopore-based sequencing represents a promising approach for enhancing avian influenza surveillance, supporting faster decision-making and improved outbreak management. Ongoing work will focus on method validation and standardization for routine diagnostic implementation.

Keywords: avian influenza, genotyping, nanopore

Poster 1.10

Enhancing serological surveillance of swine Influenza A Virus using an Hemagglutinin H1B COBRA-derived indirect ELISA

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Swine influenza A virus (IAV-S) is highly transmissible and poses economic and public health concerns. Hemagglutinin and neuraminidase undergo antigenic drift and shift, driving the frequent emergence of new strains. H1 viruses are divided into lineages 1A, 1B and 1C. Increasing demands for virological and serological surveillance require updated diagnostics. The hemagglutination inhibition (HI) assay represents the serological reference assay but its diagnostic value relies on the choice of the correct antigens and can yield variable results. To broaden antibody detection capabilities, we developed an indirect ELISA (iELISA) to detect H1B-specific antibodies in swine. Using the Computationally Optimized Broadly Reactive Antigen (COBRA) approach, we designed an H1B consensus-derived antigen, which was expressed as full-length (H1B-FL) and globular domain (H1B-GD) recombinant proteins.
The H1B COBRA sequence was codon-optimized. H1B-FL (with trimerization domain) and H1B-GD were cloned into a modified pCDNA3.4 vector, expressed in mammalian cells and purified. Antigens were titrated with hyperimmune serum from an H1B-vaccinated pig. For iELISA, plates were coated with 3µg/mL of H1B-FL or H1B-GD (one column per antigen), sera were applied as two-fold serial dilutions (1/50–1/3200) to antigen-coated and blank columns (no antigen) and detection was performed with anti-swine IgG-HRP antibody. Endpoint antibody titers were determined from the dilution curves. The panel included 31 IAV-negative samples, used to establish the assay cut-off set at an OD of 0.2 at a 1/50 dilution, and 44 sera (33 piglets, 11 sows) from an Italian farm with circulation of H1B, H1A and H1C viruses. All farm sera were previously screened by a commercial NP ELISA and NP-positive samples were further tested by HI against representative strains of H1A, H1B, H1C and H3 lineages.
HI titers were generally higher against the circulating H1B strain than against the other strains. Concordance with HI was 79.5% for H1B-FL iELISA and 72.7% for H1B-GD iELISA. Although some discordant results were observed between assays, H1B-FL showed higher detection rates and consistently higher antibody titers than H1B-GD. These findings indicate that the H1B-FL antigen performs better than the H1B-GD in detecting H1B-reactive sera. The H1B-FL antigen represents a promising tool for serological surveillance in swine. Future work will extend the COBRA approach to additional circulating swine influenza virus lineages to improve serological characterization and lineage-specific antibody detection.

Keywords: Influenza A Virus, Serology, Hemagglutinin, ELISA, Swine

Poster 1.11

Establishment and Application of the FMD Serum Bank at the National Reference Laboratory in Korea for Diagnostic Standardization

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The Animal and Plant Quarantine Agency (APQA) of Korea, serving as the National Reference Laboratory for foot-and-mouth disease (FMD) diagnosis, operates the FMD Serum Bank to support standardized FMD diagnostics and research. Since 2017, the APQA FMD Diagnostic Division has systematically collected and managed sera from diverse sources, including naturally infected animals, vaccinated animals, and sera obtained from FMD-free countries and regions. This effort aims to maintain and expand standardized serum resources to enhance the reliability and consistency of FMD diagnostic methods. Following collection, sera are categorized by animal species, collection year, infection status, vaccination status, SP serotype (O, A, and Asia1), and NSP antibody reactivity, and then evaluated using established serological assays, including SP/NSP ELISA, virus neutralization tests (VNT), and enzyme-linked immunoelectrotransfer blotting (EITB). Following evaluation, qualified sera are registered in the Serum Bank. Currently, approximately 3,247 FMD-related sera are preserved, of which 964 sera have been selected to establish standardized serum panels. These panels are utilized for the standardization of antibody diagnostic methods, validation of antibody diagnostic kits, proficiency testing of regional animal health laboratories, evaluation of SP and NSP surveillance assays, and validation of O-type SP antibody titration assays. Candidate reference sera were aliquoted at 800 µL into lyophilization vials according to standardized in-house procedures established by the APQA FMD Diagnostic Laboratory and subsequently freeze-dried under validated conditions. The resulting lyophilized sera underwent systematic quality assessment, including homogeneity and stability testing, and only batches meeting predefined acceptance criteria were incorporated into the standardized reference serum panels. Building upon this established foundation, the APQA FMD Diagnostic Division will continue to expand and refine national reference serum panels to further strengthen the accuracy, comparability, and quality assurance of FMD diagnostic activities. Future efforts will focus on broadening the diversity of available serum resources, including the establishment of panels representing additional FMD virus serotypes such as SAT1 and SAT2, which are important for preparedness against potential transboundary incursions. In addition, the APQA FMD Diagnostic Division will explore opportunities for collaboration with international reference laboratories to secure and characterize serum resources with performance characteristics comparable to internationally recognized reference standards. These continued initiatives are expected to enhance the capacity of the national FMD diagnostic system, further support APQA's role as a National Reference Laboratory, and contribute to global efforts for improved FMD surveillance, preparedness, and disease control.

Keywords: Foot and Mouth Disease, Serum bank, Vaccination, Infection, Reference serum

Poster 1.12

Seroprevalence of Peste des Petits Ruminants (PPR) and Foot-and-Mouth Disease (FMD) in Small Ruminants in Nowshera District, Khyber Pakhtunkhwa, Pakistan

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Peste des Petits Ruminants (PPR) and Foot-and-Mouth Disease (FMD) are among the most important transboundary animal diseases affecting small-ruminant production worldwide. These diseases cause substantial economic losses through reduced productivity, increased mortality, and trade restrictions. The present study was conducted to determine the seroprevalence of PPR and FMD in sheep and goats in Nowshera District, Khyber Pakhtunkhwa, Pakistan.
A total of 125 farms were visited during the study. The surveyed population consisted of 1,560 goats and 914 sheep. For serological investigation, 600 samples were collected for PPR testing and 500 samples for FMD testing. Samples were analyzed using standard diagnostic procedures to assess the occurrence of both diseases.

The results showed that 190 of 600 samples were positive for PPR, indicating a seroprevalence of 31.7%. Similarly, 164 of 500 samples tested positive for FMD, corresponding to a seroprevalence of 32.8%. The findings demonstrate that both diseases remain prevalent in small-ruminant populations in the study area and may adversely affect livestock productivity and farmers' livelihoods.

The study highlights the need for strengthened disease surveillance, effective vaccination programs, and improved biosecurity measures to reduce the burden of PPR and FMD. These interventions are essential for improving animal health, enhancing farm productivity, and supporting resilient livestock systems in Pakistan.

Keywords: PPR, FMD, seroprevalence, sheep, goats, livestock health, Pakistan.

Poster 1.13

Genomics for Animal and Plant Disease Consortium (GAP-DC): Pathogen Surveillance in Great Britain via an Integrated Initiative.

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The impact of next generation sequencing technologies on pathogen surveillance, detection and characterisation is significant. As the cost of sequencing declines and its accessibility improves, the potential for genomic surveillance to mitigate threats posed by endemic and (re)-emerging diseases increases. A One Health framework that leverages genomic surveillance for animal and plant pathogens can therefore address critical challenges for biosecurity and animal and public health, particularly in an era characterized by climate change and globalization.
GAP-DC (GAPDC | Genomic Surveillance) was launched in July 2023, a partnership between key British animal and plant health agencies, namely APHA, Royal Veterinary College, CEFAS, The Pirbright Institute, Fera Science and Forest Research. GAP-DC takes a cross-sectoral approach across animal, plant and aquatic health domains to address technological and policy challenges shared among multiple diseases and sectors. Through this broader scope, GAP-DC aims to promote unified responses among agencies and improve public communication during disease incursions.
GAP-DC's scope is defined by six interconnected work packages (WPs). The first WP focuses on enhancing frontline pathogen detection at high-risk locations, such as border control posts. The second WP targets the spillover of pathogens between wild and farmed populations. The third WP seeks to advance the identification and understanding of the disease agents contributing to syndromic or complex diseases, including both notifiable agents and those not currently recognized by legislation or identified as disease-causing agents. The fourth WP develops frameworks for detecting and managing outbreaks of new and re-emerging diseases. The fifth WP explores innovative strategies for mitigating endemic diseases and the sixth WP focuses on enhancing coordination among key stakeholders, including potential end users.
From an animal health perspective, the project has detected a novel virus in wildlife populations, improved genetic characterisation for an endemic production disease, developed probe panels to generate focussed sequencing datasets and investigated potential viral incursions via illegal entry of products of animal origin. As GAP-DC is in its final year, data mentioned above from the WPs will be shared and the positives and pitfalls of the multi-agency multi sector approach will be discussed.

Keywords: Preparedness; Genomics; Surveillance

Poster 1.14

Detection of African swine fever virus DNA in pig farm wastewater and Amblyomma javanense ticks in Vietnam

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African swine fever virus (ASFV) is a major threat to pig production, but the environmental distribution of viral DNA in Southeast Asian pig-farming systems remains poorly characterized. We investigated ASFV DNA in pig farm wastewater and Amblyomma javanense ticks collected from rescued Sunda pangolins in Vietnam, and evaluated a polyethylene glycol (PEG) precipitation-based concentration method for wastewater testing. Ninety-three wastewater samples from 16 commercial pig farms and 40 tick samples were tested by real-time PCR. ASFV DNA was detected in 7 wastewater samples (7.5%) using the PEG-based method, compared with 3 (3.2%) by the reference extraction method, and in 3 tick samples (7.5%). The PEG-based method improved analytical sensitivity by approximately 100-fold, lowered Ct values by 3.05 to 7.09 cycles in concordant positive wastewater samples, and showed 100.0% diagnostic sensitivity and 95.6% diagnostic specificity relative to the reference method. Infectious virus was not recovered from ASFV-positive wastewater samples, and sequencing of tick amplicons was unsuccessful, probably because of low viral loads. These findings show that ASFV DNA can be detected in farm wastewater and wildlife-associated ticks, supporting the value of environmental and vector-oriented surveillance for ASFV monitoring in endemic regions.

Keywords: African swine fever virus; wastewater; Amblyomma javanense; real-time PCR; Vietnam

Poster 1.15

Monitoring ruminant antibodies may enhance identification of new TBE risk areas for humans

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In Finland, tick-borne encephalitis (TBE) is focally endemic, particularly in the Åland Islands, coastal regions, and southeast of the country, where vaccinations have been implemented since 2006. During the 2020s, sporadic human cases have emerged in new areas, with the northernmost focus located in the municipality of Simo in Lapland. To assess whether monitoring ruminants could help detect emerging TBE risk areas for humans, TBE virus (TBEV) antibodies in ruminants were studied in 2024–2025 as part of the EU co-funded OH4Surveillance project.
Serum samples were collected at slaughter from 1,695 cows from 330 suckler herds, 1,034 sheep from 99 herds, and 10 goats from 1 herd. The samples were analyzed using an ELISA test for TBEV antibodies and due to its limited sensitivity, borderline results were examined using a virus neutralization test. Human surveillance data from the Finnish Infectious Diseases Register was used to indicate regional TBE incidence.
TBEV antibodies were detected in both cows and sheep, with a higher prevalence in sheep (4.0% of animals and 13.1% of herds) than in cows (2.6% of animals and 7.3% of herds). No antibodies were detected in goats. Geographically, most seropositive herds were located in known TBE regions. Antibodies were also detected in herds located in municipalities without reported human cases, suggesting possible emerging TBE foci. No seropositive herds were found in southeastern Finland despite relatively high human incidence in that area.
The study demonstrates that TBEV circulates in Finnish ruminants, particularly in sheep, and that livestock surveillance can complement human disease mitigation. Detecting antibodies in areas without reported human cases indicates potential new risk areas that warrant further investigation. These insights may help guide targeted human vaccination strategies and support timely identification of human infections. Physicians should be aware of these findings.

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Keywords: TBE, ruminant TBEV antibody surveillance, new TBE risk areas for humans, OH4Surveillance

Poster 1.16

Recurrent detection of Usutu virus in blackbirds in Denmark, 2025

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The Orthoflavivirus Usutu virus (USUV) was detected for the first time in Denmark in 2024, primarily affecting Eurasian blackbirds (Turdus merula) resulting in a noticeable population decline (Gelskov et al. 2026). A total of 85 blackbirds were submitted for examination, of which 56 tested positive for USUV RNA by RT-qPCR. Infected birds were identified across most parts of the country, and three distinct lineages were detected (Europe 2, Europe 3 and Africa 3).
In 2025, USUV was once again detected in wild birds in Denmark, although fewer birds were submitted for examination. Among 28 blackbirds examined at the Veterinary Pathology Section at the University of Copenhagen, six tested positive for USUV RNA by RT-qPCR with high viral loads in the brain material (Ct 11-22). Pathological findings were similar to those observed in 2024, with splenomegaly as the most common finding, and many of these birds were emaciated.
Whole genome sequencing using a targeted NGS approach revealed two distinct USUV lineages, Europe 2 and Africa 3, which were also the most frequently detected lineages in Denmark the previous year. In selected positive birds, additional RT-qPCR analyses of multiple organs (heart, lung, liver, spleen, kidney, and intestine) and cloacal and oropharyngeal swabs all yielded positive results, demonstrating widespread viral distribution within the birds. Furthermore, infectious virus was isolated from liver, kidney, and spleen, as well as from all swab samples from these birds.
The recurrent detection of USUV in 2025, together with the identification of multiple lineages, suggests either repeated introductions in 2025 or sustained local circulation within Denmark. These findings including the continued detection of the Europe 2 lineage, which has been associated with the highest number of reported human USUV infections in Europe, highlights the importance of continued surveillance for both animal and public health.

Reference: Gelskov, L.V., Johnston, C.M., Hammer, A.S.V. et al. First detection of Usutu virus in wild birds in Denmark, 2024. Sci Rep 16, 5156 (2026). https://doi.org/10.1038/s41598-026-35874-y

Keywords: Usutu virus, Blackbird, Emerging infectious disease, Vector-borne infections, Zoonosis

Poster 1.17

Development of a Recombinant, Non-Infectious Competitive ELISA Platform to Support Post-Eradication Preparedness for Rinderpest virus

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Following the global eradication of Rinderpest virus (RPV), sustained diagnostic capability remains essential to support surveillance, contingency preparedness, and compliance with WOAH–FAO post-eradication frameworks, while reducing reliance on Rinderpest Virus-Containing Material (RVCM). Our project has focused on the development of a fully recombinant, non-infectious competitive ELISA (cELISA) platform to replace legacy virus-based assays without compromising analytical performance. The haemagglutinin (H) protein of the RBOK vaccine strain was selected as the diagnostic antigen based on its immunological relevance and established recognition by monoclonal antibody (mAb C1) used in the WOAH-approved cELISA. A truncated ectodomain construct (aa 149–609) of H was codon-optimised, expressed in Expi293 cells, and purified via Ni-NTA affinity chromatography. Protein integrity and antigenicity were confirmed by SDS-PAGE and Western blot using anti-His and mAb C1. In parallel, recombinant mAb C1 was generated from sequenced hybridoma variable regions and expressed in mammalian cells, establishing a sustainable recombinant antibody supply chain. Functional ELISA assays demonstrated strong, specific, and reproducible binding between recombinant RBOK-H and mAb C1. Site-directed mutagenesis of RPV H at two previously characterised residues (H309S, G311S) abolished binding, validating the predicted location of the epitope and generating a robust negative control antigen. Analytical specificity testing against a panel of non-RPV morbillivirus haemagglutinins additionally confirmed no cross-reactivity with mAb C1, demonstrating high diagnostic specificity. Stability and blocking studies further confirmed reagent robustness and operational flexibility. Systematic checkerboard optimisation of RBOK-H concentration and mAb C1 dilutions established reproducible assay conditions, achieving a linear dynamic range (OD 0.6–1.0) suitable for competitive inhibition analysis. Screening of archived sera from RBOK vaccinated (n=93) and RPV convalescent animals (n=71) demonstrated clear serological discrimination consistent with expected antibody kinetics, supporting preliminary sensitivity and specificity assessments. Collectively, these results establish validated recombinant reagents and demonstrate the feasibility of a scalable, non-infectious cELISA framework aligned with WOAH–FAO post-eradication priorities. Following further standardisation and inter-laboratory validation across Rinderpest holding facilities (RHFs), this platform has the potential to reduce dependence on high-containment infrastructure while maintaining diagnostic robustness, thereby strengthening global RPV biosecurity resilience and preparedness in the post-eradication era.

Keywords: Rinderpest virus, post-eradication preparedness, competitive ELISA (cELISA), Recombinant diagnostics, Haemagglutinin, Serological assay, Diagnostic validation, Biosecurity

Poster 1.18

Ensuring Biosafety and Nucleic Acid Integrity: PrimeStore® MTM Effects on African Swine Fever Virus

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African swine fever (ASF) is a devastating transboundary disease of domestic and wild suids characterized by high mortality rates, that poses a threat to the global swine industry. Controlling ASF in wildlife is challenging and depends largely on passive surveillance and active carcass retrieval. Collecting clinical samples from degraded carcasses is a major bottleneck for conclusive diagnosis, due to biosafety risks and to the need to preserve viral nucleic acids required for downstream molecular testing. In this context, PrimeStore® molecular transport medium (PS-MTM) has been already investigated as a potential solution capable of simultaneously inactivating ASF virus (ASFV) and stabilizing viral DNA, with successful outcomes at +4°C and room temperature for 21 days. In the present study, we assessed PS-MTM ability to inactivate ASFV and to preserve ASFV DNA for 15 days by stressing environmental conditions with higher temperatures, thus simulating samples' suboptimal transport and storage settings. We employed the genotype 1 ASFV BA71V virus as a reference strain, lab-adapted to grow in VERO continuous cell line. Viral suspensions were mixed with PS-MTM at a 1:3 ratio and incubated for 30 minutes, 7 days, and 15 days at temperatures of +4°C, +22°C, and +37°C. Viral infectivity was subsequently assessed through cell culture assays, while nucleic acid stability was evaluated by extraction and analysis using Real-Time PCR. The results demonstrated that PS-MTM completely inactivated ASFV after only 30 minutes of exposure, regardless of the incubation temperature, with no evidence of viral replication or cytopathic effects (CPE) observed in cell cultures. Furthermore, the medium effectively preserved ASFV DNA throughout the study period, even at +37°C. These findings confirm that PS-MTM may enhance biosafety, facilitating the transport of diagnostic specimens, and ensuring the reliability of molecular diagnostics under challenging environmental conditions. Its use may significantly reduce the risks and costs associated with the movement of potentially infectious biological materials between laboratories and across geographical regions, while maintaining the integrity of viral nucleic acids required for accurate diagnostic testing.

Keywords: African swine fever, ASFV, MTM, inactivation, nucleic acid stability

Poster 1.19

Alternative sample matrices for the detection of antibodies against classical swine fever virus

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Introduction and Objectives
Classical swine fever (CSF) is a severe systemic disease of domestic pigs and wild boar worldwide. Affected wild boar populations have often maintained the infection over months or even years and threatened domestic pig holdings. Therefore, passive surveillance of fallen wild boar plays an important role in early detection of disease introduction. As CSF shows comparable pathological findings to African swine fever (ASF), which nowadays affects many countries worldwide, differential diagnosis is performed. Especially, if no serum sample can be collected from wild suids, blood swabs represent an alternative matrix for ASF diagnosis. Based on this, the present study focused on the evaluation of blood swabs for the detection of antibodies against CSF virus (CSFV).
Materials and Methods
Quick-drying swabs were soaked with EDTA blood, which was collected from pigs experimentally infected with CSFV, as well as from non-infected animals. Pieces of the swab were taken up in sample dilution buffer or cell culture medium and were analyzed in CSF antibody ELISAs or virus neutralization assay (VNT). To assess potential species effects, antibody negative EDTA blood samples from wild suids (wild boar, warthog, red river hog) were spiked with CSFV antibody positive serum.
Results
By comparing serum and blood swabs in CSF antibody ELISAs and VNT, a moderate loss of sensitivity was observed, but this had no impact on sample classification when the material was collected ≥ 21 days post infection. For samples collected during early seroconversion, which usually have only low antibody levels, sample classification can be affected. Moreover, no species effects were observed using blood swabs of various wild suids.
Conclusion
Taken together, blood swabs of wild suids represent an alternative sample matrix for antibody detection when the general preferred sample matrix serum is not available even though there are minor limitations for samples collected at an early time point after infection. In addition, this sample matrix is also applicable for CSFV genome detection and can be combined with ASF diagnosis.

Keywords: Classical swine fever, blood swabs, serology, differential diagnosis, wild suids

Poster 1.20

Molecular detection, serotyping and genomic characterisation of multiple Bluetongue virus and Epizootic haemorrhagic disease virus circulating in ruminants in Nigeria

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Background:
Bluetongue virus (BTV) and Epizootic haemorrhagic disease virus (EHDV) are members of the genus Orbivirus, family Sedoreoviridae, primarily transmitted by the vector Culicoides biting midges. Both viruses affect ruminants, with significant implications for animal health and trade. Despite their relevance, they remain underreported in Africa, and molecular data from Nigeria are particularly limited.
Objectives: To investigate the prevalence and molecular diversity of BTV and EHDV in ruminants across selected regions of Nigeria.

Methods: A total of 550 blood samples were collected from cattle and sheep in Plateau (North-Central), Sokoto (North-West), and Oyo (South-West) States of Nigeria. Samples were analysed at the Pirbright Institute, United Kingdom using real-time RT-PCR assays targeting BTV genome segment 10 and EHDV genome segment 9. Both BTV and EHDV-positive samples were serotyped using segment 2-based real-time RT-PCR. Eight BTV and two EHDV positive samples were also subjected to whole genome sequencing (WGS) using the Illumina Miseq platform.
Results: Seventy-one samples (12.9%) tested positive for BTV. Of these, twenty-one samples were successfully serotyped using segment 2-based real-time RT-PCR and WGS, revealing eleven classical serotypes: BTV-1, -3, -4, -7, -8, -9, -10, -11, -13, -22, and -24. The serotype of the remaining fifty samples could not be detected using real-time RT-PCR targeting BTV genome segment 2. Ten samples (1.8%) were positive for EHDV. Both real-time RT-PCR and WGS assays identified four serotypes in six positive samples: EHDV-1, -7, -8, and -9. One sample showed evidence of co-infection with both BTV (serotype 10) and EHDV (serotype 1). Segment 2-based real-time RT-PCR serotyping did not reveal any serotypes in four EHDV positive samples.
Conclusion: This study provides the first comprehensive molecular evidence of diverse BTV and EHDV serotypes concurrently circulating in Nigeria. The high proportion of non-serotyped BTV and EHDV positive samples suggests the possible presence of novel or divergent strains, warranting further genomic investigation which is ongoing. Overall, these results highlight the need for enhanced surveillance and further genomic analyses, to better understand the epidemiology in the region. These findings provide significant and important baseline data for Nigeria and West Africa, supporting improved surveillance and informing regional control strategies for Orbivirus diseases.

Keywords: Bluetongue virus, Epizootic haemorrhagic disease virus, whole genome sequencing, Nigeria, Culicoides, RT-PCR, Serotyping

Poster 1.21

Swab type and storage conditions have limited impact on PCR detection of Aujeszky's disease virus

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Aujesky's disease (AD) is classified as category C+D+E in the Animal Health Law and is therefore a notifiable disease in the EU. The causative alphaherpesvirus is present in various wild boar populations in Europe, and some countries experience outbreaks of AD in domestic pigs. Nevertheless, compared to years ago, when the virus was widely present in the EU, there is limited research on this topic. Additionally, there is no European Reference Laboratory (EURL), limiting the contact and exchange of knowledge between different National Reference Laboratories (NRLs).
Sampling, sample type and sample transport are critical components in case of a suspicion and during an outbreak response. This CoVetLab project (AUJESAMPLE) aimed to optimise and harmonise these components by investigating the limitations of each sample type and the different storage protocols used.
Three national reference laboratories (SSI, APHA and WBVR) performed in vitro studies, while ANSES, the WOAH reference laboratory for Aujeszky's disease, performed an in vivo animal infection study and shared selected ADV strains and samples among the project partners.
In the in vitro study, three different ADV isolates, a local ADV and two reference isolates (Bartha and NIA3), were used to spike two swab types (cotton and flocked). Replicate swabs were then stored either dry or in various transport media under different temperatures for up to 96 hours. Following storage, the presence of ADV was assessed by qPCR using the standard laboratory protocols of each NRL. Overall, neither the swab types nor storage media influenced the sensitivity of ADV detection by qPCR when samples were stored for up to 96 hours at room temperature, 4°C, or 37 °C.
Data from the in vivo experiment showed that all sample types (oropharyngeal and nasal swabs, nasal wipes, oral fluid, tonsillar brushes) were PCR positive from day 3 to day 10 post infection, and most remained positive until day 21, the end of the experiment. However, tonsillar brushing appeared to be more sensitive, i.e. lower Cq values, for detecting the virus by PCR. Similarly, ADV detection was consistently more sensitive using flocked swabs in PBS than with dry cotton swabs. Concordant results were reported from all the NRL when testing shared samples from the in vivo experiment.
The results of this project will support updates of guidelines for the optimal sampling of animals suspected of AD and for the proper storage and transport of the samples before testing at the NRL.

Keywords: **Sample transport model, swab comparison, transport medium comparison, experimental infection**

Poster 1.22

Development of a Sandwich ELISA and LFD for Rapid Detection of Seneca Valley Virus

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Seneca Valley virus (SVV) belongs to the Picornaviridae family (genus Senecavirus) and primarily affects pigs, although its host range may be broader. The virus was first isolated from pigs in 1988 in the United States, after spreading to countries in South America and Asia; in 2022, five clinical outbreaks occurred in England, UK, marking its first documented entry into Europe. The characteristic clinical signs represented by vesicular lesions on the snout and feet, are clinically indistinguishable from those of foot-and-mouth disease (FMDV). From a preparedness perspective, it is of paramount importance to undertake a rapid and reliable differential diagnostic process. For this reason, it is essential to have reliable diagnostic tools that enable the accurate diagnosis of this disease, thereby resolving any diagnostic uncertainty.
The aim of this project was to develop diagnostic tools for SVV detection, using the hybridoma technology for the production of monoclonal antibodies. Four separate immunizations of mice with an inactivated strain of SVV (sw/USA/09-34037) yielded 74 hybridomas, which were subsequently characterized, titred and selected using virus neutralization assays, direct ELISA, and trapping ELISA. A total of 7 monoclonal antibodies were selected as candidate capture antibodies and 5 were selected for use as detection antibodies (HRP-conjugated) based on their higher affinity for binding the SVV antigen and testing 35 combinations against 21 different SVV isolates. The profiles obtained from these tests identified a capture antibody and a pool of two detection antibodies which were taken forward to develop a simple-to-use ELISA kit. Subsequent testing involved 144 samples derived from experimental infections and clinical infections, including vesicular epithelium and other types of biological matrices.
Analytical validation demonstrated a reliable analytical sensitivity (aSe) until 1:125 dilution, with good correlation to real-time RT-PCR and the absence of cross-reactivity to other picornaviruses (SVDV, FMDV types O, A, and Asia1). Diagnostic validation using field samples revealed that vesicular epithelium was the most suitable matrix for virus detection using the sandwich ELISA, achieving a diagnostic sensitivity (dSe) of 73.1% and diagnostic specificity (dSp) of 100% in non-infected tissues. Currently we are developing a rapid LFD test using the same monoclonal antibodies, in order to make the tool faster, more reliable, and field-friendly, facilitating the diagnosis and the management of potential outbreaks, where initial results show good agreement with the ELISA results.

Keywords: Monoclonal antibodies, hybridoma, preparedness

This study is co-funded by the Italian Ministry of Health (PRC 2023004) and by the European Union's Horizon Europe Project 101136346 EUPAHW

Poster 1.23

Evaluation of West Nile virus Single-Round Infectious particle as a Surrogate virus in a neutralisation test compared with plaque reduction neutralisation test

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West Nile virus (WNV) is an emerging mosquito-borne flavivirus of public and animal health interest. The measure of virus-neutralising antibodies is essential for serological surveillance and immunological studies. The plaque reduction neutralisation test (PRNT) is widely regarded as the 'gold-standard assay' for the detection of WNV-specific antibody neutralisation; however, it is labour-intensive, time-consuming, and requires the handling of infectious virus. Single-round infectious particles (SRIPs) offer a safer and potentially higher-throughput alternative. Here, SRIPs encode a pre-membrane and envelope proteins of wild-type WNV, enabling cellular entry while remaining replication-incompetent due to a defective genome. WNV SRIPs were successfully generated, applied in a neutralisation assay and results were compared with those obtained using PRNT.

WNV SRIPs were generated by transient co-transfection of HEK293T producer cells with plasmids encoding a YFV sub-genomic replicon, YFV Capsid protein and WNV structural proteins. Supernatants were harvested that contained the SRIPs and titrated onto Vero cells prior to use in neutralisation assays. Production yielded SRIP titres of up to 3.5 x 105 TCID50/ml, demonstrating efficient particle generation suitable for downstream applications e.g. in neutralisation assays. A panel of sera collected from WNV-challenged ponies was analysed using both the SRIP-based neutralisation assay and a conventional PRNT. Neutralising antibody titres were determined using half-maximal inhibitory concentration (IC50) values for the SRIP assay and percentage plaque reduction for PRNT.

The assay enabled successful quantification of WNV-neutralising antibodies across the serum panel, with preliminary comparison with PRNT indicating agreement between the two methods, supporting the potential option of the SRIP assay as a surrogate virus for live-virus neutralisation assays. The greater percentage of plaque reduction calculated from PRNT, the greater the IC50 value obtained from SRIP neutralisation test. Further analysis is ongoing to characterise assay correlation and performance metrics. These findings demonstrate the feasibility of using WNV SRIPs as a safer and potentially more scalable alternative to conventional PRNT for WNV serology, diagnostic capabilities and as a flavivirus research tool.

Keywords: Neutralisation, Single-round infectious particle, PRNT, Flavivirus

Poster 1.24

New IBR serum double-antigen sandwich ELISA

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While IBR eradication is progressing in Europe, the incidence remains a concern in some areas (Valas, 2026). To address the challenges of IBR eradication in the final phase of programs, the sensitivity and specificity of diagnostic tools must be maximized. With this in mind, a new ELISA has been developed: the IBR Serum Ab test. This test uses a new single-step double antigen sandwich ELISA and employs a recombinant protein as the antigen.
The IBR Serum Ab test allows the testing of individual serum, plasma and meat juice samples, as well as pools of up to 10 serum or plasma samples, with the same kit.
Sensitivity, specificity, and reproducibility parameters were evaluated with a view to submitting a validation dossier to the French and German regulatory authorities, including the use of reference samples from these countries.
The results showed improved analytical sensitivity for approximately a third of the samples analyzed (a gain of up to two 2-fold dilution steps) compared to other tests available on the market, as well as significantly improved specificity, particularly for challenging samples from animals positive for antibodies against BoHV2.
A sensitivity of 100% (95% CI: 99.1% - 100%) and a specificity of 99.9% (95% CI: 99.2%-100%) were obtained with a panel of positive (n=522) and negative (n=847) field samples, respectively. Finally, field trials involving laboratories in France (n=2), Germany (n=2) Switzerland (n=1) and Austria (n=1) demonstrated the good reproducibility and repeatability of the test (CV < 15%).
Thanks to its superior specificity and ease of use, the IBR Serum Ab ELISA should contribute to strengthening the effectiveness of IBR control programs in the field.

Keywords: IBR, BoHV1, BoHV2, ELISA, recombinant protein

Poster 1.25

Platinum IV Chloride Viability PCR assay for the assessment of virus disinfectants

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Biosecurity in laboratories relies on effective disinfection. Validation of disinfects against viruses by cell culture can be lengthy and technically challenging, especially for fastidious or highly pathogenic viruses. Viability PCRs use reagents that ensure only nucleic acids from inside intact viral virions is amplified by PCR. We assessed Platinum IV Chloride PCR as a method to detect inactivation of both enveloped and non-enveloped viruses.
VirkonS or a 70% ethanol/5% Isopropanol mixture were added to 10^5 viral copies of the non-enveloped Murine Norovirus (MNV) and Porcine Circovirus-2 (PCV-2), as well as the enveloped Porcine Reproductive and Respiratory Syndrome virus (PRRSV). After 10 minutes (VirkonS) or 1 minute (alcohol mix) incubation at room temp, 10mM PtCl4 was added (10 minutes at 5˚C) and viral nucleic acid extracted with viral RNA mini kit before MNV, PCV-2 and PRRSV quantified by virus-specific qPCRs.
No viral nucleic acid was detected using the PtCl4 method for all three viruses after treatment with 0.86 % (final concentration) of Virkon S indicating loss of virus viability. Virkon S is a disinfectant with several mechanisms that cooperate. It disrupts viral envelopes and protein capsids, which will allow PtCl4 to penetrate into disrupted virions and covalently bond to free nucleic acids thereby interfering with downstream PCRs. In contrast similar or only slightly lower viral nucleic quantities than non PtCl4 treated controls were observed for the alcohol treatment for all three viruses. Alcohols act by denaturing proteins and disrupting lipid envelops and are highly effective against enveloped viruses. The apparent lack of reduction using the PlCl4 method may indicate that alcohols rather trap the nucleic acid inside a shell of virion proteins that are partially denatured, but not sufficiently disintegrated.
This work provides evidence for the application of PtCl4 as a marker for viable/ inactivated virus and is applicable to highly pathogenic and fastidious viruses that may be incompatible to traditional virus isolation methods. However, the method is only applicable to methods that result in a sufficient destruction of the virus core to allow PtCl4 access.
Methods like these are important to allow for the verification of inactivation under various circumstances and for different purposes. We propose to foster discussions within the EPIZONE network to capture virus inactivation processes, manage knowledge and avoid duplication of efforts.

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Keywords: Virus viability assay, virus inactivation, disinfection

Poster 1.26

Longitudinal BTVPUR®4-8 vaccination trial highlights appropriate BTV vaccination status in cattle

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Bluetongue virus (BTV) is an arbovirus transmitted by Culicoides biting midges. It causes an infectious haemorrhagic disease, bluetongue, in domestic and wild ruminants. There has been a widespread shift in BTV distribution in recent decades and strains of several BTV serotypes (including BTV-3, BTV-4 and BTV-8) are now co-circulating across Northern Europe. Inactivated monovalent vaccines were used previously to control BTV-8 outbreaks and bivalent vaccines are available in areas with BTV co-circulation. Vaccination remains the most effective control measure, but the neutralizing antibodies generated only protect ruminants from re-infection with strains of a homologous serotype. With 28+ BTV serotypes, this poses a significant challenge in a rapidly evolving orbivirus landscape. To develop effective cross-protective vaccines, a greater understanding of the ruminant immune response to BTV vaccination is required. Field reports suggest that not all cattle have detectable anti-BTV antibodies following vaccination, requiring the need to determine “appropriate vaccination status” for effective disease control and safeguarding trade.
Under controlled field conditions, sixty cattle were vaccinated with two doses of inactivated bivalent BTVPUR®4-8 vaccine (Boehringer Ingelheim) and followed longitudinally for one year to characterize humoral immune responses. Serum was screened for antibodies against BTV VP7 by competitive ELISA and dynamics of IgM- and IgG-specific subclasses further characterized. Serum neutralization tests were used to quantify neutralizing antibodies. Sera was also collected from 406 UK-imported cattle with known vaccination history and humoral immune responses characterized for comparison.
All 60 cattle seroconverted one month after completing their vaccine course, confirming by competitive ELISA that all cattle developed an antibody response to BTVPUR®4-8 vaccination. However, these IgG-specific anti-VP7 antibody responses were short-lived. Whilst only 19.2% and 38.2% cattle had neutralizing antibody titres to BTV-4 and BTV-8 respectively one month after prime vaccination, nearly all cattle (92-97%) had neutralizing antibodies to at least one BTV serotype one-month post-booster vaccination. However, for some cattle this response was short-lived, and others had no detectable BTV-4 neutralizing antibodies. Just 40-58% cattle had neutralizing antibodies up to 12 months. Neutralizing antibodies, however, were the only immune parameter to be consistently detected up to 12 months post-vaccination in a high proportion of cattle, highlighting its use as an indicator of appropriate vaccination status. Serological responses in field-vaccinated cattle were far more inconsistent and variable, highlighting potential differences between monovalent and bivalent formulations. This study highlights that current diagnostic tools can be used to identify appropriate vaccination status in BTVPUR®4-8 vaccinated cattle.

Keywords: Bluetongue virus, Orbivirus, BTVPUR4-8, vaccination

Poster 1.27

Assessing the epidemiological impact of EHDV-8 emergence in France: seroprevalence, herd-level mortality, and individual reproductive performance in cattle

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Epizootic Hemorrhagic Disease (EHD) is an emerging vector-borne viral disease that recently reached Europe, with the first French cases detected in September 2023. As bluetongue virus (BTV), EHDV belongs to the Orbivirus genus and is transmitted by Culicoides biting midges, posing a threat to cattle health. In France, the introduction of EHDV serotype 8 led to the declaration of 3,636 clinical outbreaks by the end of 2023, followed by 2,716 additional outbreaks in 2024. Despite its rapid spread, the large-scale epidemiological impact of EHDV-8 on cattle productivity in mainland France remains poorly quantified. This study aimed to characterize the impact of EHDV emergence by combining individual-level data from the French National Cattle Register (BDNI) with serological surveys. We analyzed nine administrative departments along the southwest-to-northeast expansion front. Within-herd, between-herd, and animal-level true seroprevalence values were estimated using a Bayesian framework to account for diagnostic uncertainty and clustering. To assess the impact on productivity, we analyzed mortality rates and reproductive performance (calving intervals) in the surveyed herds. Generalized linear mixed models (GLMMs) with negative binomial and gamma distributions were used to evaluate the association between EHDV seroprevalence and these performance indicators, while adjusting for age, production type, BTV seroprevalence, and temporal trends. Results showed that true seroprevalence increased sharply between the 2023-2024 and 2024-2025 seasons. Between-herd prevalence reached 100% in the southwestern introduction areas during the first year. By 2024-2025, infection had become widespread across all studied southwestern departments, with a between-herd prevalence of 100%. At the same time, within-herd and animal-level seroprevalence increased substantially in areas along the expansion front. Statistical models revealed no significant impact of EHDV on herd-level mortality rates. However, a significant increase in individual calving intervals was observed in the sampled herds. These results suggest that EHDV-8 induced a subclinical economic burden rather than a major lethal crisis in the French cattle population. By filling these knowledge gaps, this study provides essential data to characterize the first emergence of EHDV-8 in France. Our results provide a baseline from which the force of infection across the emergence front could be reconstructed, and could inform mechanistic SEI models integrating vector transmission and animal movements. This will allow an evidence-based evaluation of current control measures, ultimately improving preparedness for future re-emergence or the introduction of new Orbivirus strains in Europe.

Keywords: EHDV-8, Seroprevalence, Herd-mortality, Reproductive performance, France

Poster 1.28

Genetic diversity of Influenza D viruses circulating in cattle in Northern Italy between 2020 and mid-2026

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Influenza D virus (IDV) is an emerging orthomyxovirus first identified in 2011 in the US in swine presenting with respiratory disease. Since then, IDV has been detected worldwide in multiple animal species, with cattle recognized as the primary reservoir. Molecular epidemiological studies have identified two major circulating clades, D/OK and D/660, while additional divergent clades have been reported in geographically restricted regions, such as Japan (D/Japan). In Europe, IDV was first reported in France in 2012 and subsequently detected in Italy, Luxembourg, Ireland, the UK, Switzerland, and Denmark. The aim of this study was to assess the prevalence of IDV on cattle farms in Northern Italy and to characterize the genetic diversity of circulating strains.
Between January 2020 and mid-May 2026, a total of 4,615 respiratory specimens (lungs, nasal swabs, bronchoalveolar lavages, and others) were collected by field veterinarians for diagnostic purposes during outbreaks of Bovine Respiratory Disease Complex (BRDC) in 1,498 cattle farms in Northern Italy. Samples were tested for IDV by real-time RT-PCR. Virus isolation was attempted on RT-PCR-positive samples. A subset of viral isolates and/or PCR-positive clinical samples was sequenced with Illumina technology, followed by segment-specific phylogenetic analysis. Hemagglutinin-esterase-fusion (HEF) gene clades were assigned using the IDV Typer. Sequence alignments were performed with MAFFT, manually edited in Aliview, and maximum-likelihood phylogenies were constructed using iqtree2.
Overall, 187 cattle farms tested positive for IDV, with 293 RT-PCR-positive samples identified during the study period, with positivity rates ranging from 3,9% to 10,2% (2020-7,1%; 2021-4,8%; 2022-6,5%; 2023-8,2%; 2024-4,2%; 2025-3,9%; 2026-10,2% based on partial data). The highest proportion of IDV-positive samples was detected among nasal swabs (269/293; 91,81%), followed by lungs (13/293; 4,44%), BALs (8/293; 2,73%), and tracheal swabs (3/293; 1,02%). Among these, 131 samples were successfully isolated, and WGS was performed on a subset. Based on the HEF gene analysis, the D/OK lineage accounted for 59,68% of sequenced isolates, while D/660 accounted for 40,32%, and reassortment among internal genes was detected. Notably, the D/660 clade was first identified in Italy in 2019, became predominant during 2020-2022, whereas D/OK increased in prevalence from 2023 onward.
These results provide evidence of active IDV circulation in cattle populations in Northern Italy, and demonstrate the co-circulation of D/OK and D/660 lineages, with evidence of reassortment. This genetic diversity likely reflects ongoing viral evolution within cattle populations and highlights the importance of genomic surveillance for monitoring IDV spread, lineage dynamics, and reassortment events.

Keywords: Influenza D virus, cattle, molecular surveillance, whole-genome sequencing, phylogenetic analysis

Poster 1.29

Reconstructing the molecular epidemiology of rabies in Switzerland from outbreak to elimination

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Rabies virus (RABV) remains one of the deadliest zoonotic pathogens, with the vast majority of human deaths occurring in Asia and Africa despite decades of vaccination efforts. While elimination has been achieved in selected regions, the evolutionary dynamics of RABV during sustained epidemic transmission and eradication campaigns remain incompletely understood.
This research investigates the long-term sylvatic rabies epidemic in Switzerland (1967-1997), the first country to eradicate wildlife rabies through oral vaccination, providing a well-characterized epidemic setting to examine viral evolution under defined ecological and intervention pressures. Over 99% of the whole-genome sequences obtained from 88 RABV isolates derived from brain samples representing 13 host species, including humans, across 17 cantons over three decades enabled high-resolution phylogenetic, molecular clock, and SNP-based analyses. Three temporally structured clades were identified: 1967–1976 (red), 1976–1988 (blue), and 1984–1996(green). These clades suggest multiple independent introductions of RABV into Switzerland, with ancestral links to strains from Poland and Germany (red), France (blue), and a combination of France and Germany (green). Molecular clock inference dated the epidemic origin to the late 1950s to mid-1960s, aligning with historical rabies expansion in Central Europe. The predominance of synonymous substitutions indicates strong purifying selection throughout the epidemic, with no evidence of major genomic changes associated with the hosts. A small subset of SNPs was temporally associated with epidemic peak spanning years, including a single nonsynonymous mutation in the glycoprotein (G) that, based on a deep mutational scanning study, has been shown to enhance viral entry. Overall genomic stability suggests that epidemic persistence was driven primarily by ecological and demographic processes, including fox population dynamics and dispersal, rather than by rapid viral adaptation. These findings indicate that long-term viral epidemics do not always require major genetic adaptation but may instead be driven mainly by ecological factors such as host density and spatial structure.

Keywords: Molecular epidemiology, virus evolution, virus emerging, zoonosis

Poster 1.30

Comparative genomic characterization of Cattle- and Elephant- derived FMDV O/ME-SA/Ind-2001e reveals cross-species transmission in Cambodia

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Foot-and-mouth disease virus (FMDV) is a highly contagious transboundary animal disease that primarily affects domestic and wild cloven-hoofed animals. Although infection is generally restricted to artiodactyl hosts, sporadic infections in non-cloven-hoofed species, including elephants, have occasionally been reported. However, most previous elephant-associated cases relied on serological evidence or partial genomic characterization, and comprehensive genome-based analyses of spillover events remain limited. In this study, we investigated FMDV infection in an Asian elephant (Elephas maximus) during the 2024 FMD outbreak in Cambodia. We compared the elephant-derived virus with contemporaneously circulating cattle-derived strains.

A male Asian elephant at the Phnom Tamao Wildlife Rescue Center exhibited vesicular and erosive lesions on the trunk, feet, and oral cavity. Clinical samples were subjected to real-time RT-PCR, virus isolation, serological analysis, whole-genome sequencing, and phylogenetic analysis. Serum samples collected from the two elephants showing clinical signs tested positive for antibodies against both FMDV structural (SP) and non-structural (NSP) proteins, and for neutralizing antibodies against FMDV serotype O.

Phylogenetic analysis based on VP1 and whole-genome sequences classified the elephant-derived virus within the O/ME-SA/Ind-2001e lineage. VP1 nucleotide sequence identity ranged from 95.73% to 99.68% compared with O/ME-SA/Ind-2001e strains circulating in livestock in Cambodia in 2024. A whole-genome comparison revealed a 99.8% nucleotide sequence identity between the elephant-derived virus and the cattle-derived strain circulating in Cambodia. The elephant isolates differed from cattle-derived viruses from Kampot and Prey Veng provinces by only a single amino acid substitution in the 2C and 3Cpro regions, respectively. No amino acid substitutions were detected in capsid proteins, including the VP1 GH loop containing the conserved RGD receptor-binding motif, or in the host-range–associated 3A region. Additional substitutions were limited to scattered changes in non-structural proteins (Lpro, 2C, 3Cpro, and 3Dpol), while a synonymous substitution was observed in 2B. Comparative in vitro analyses demonstrated that elephant- and cattle-derived viruses exhibited similar replication kinetics across multiple cell lines, including BHK-21, IBRS-2, LFBK, LFBK-αvβ6, ZZR, and MDBK.

In conclusion, these findings provide strong genomic, virological, serological, and functional evidence that the elephant infection represented a recent livestock-to-wildlife spillover event rather than sustained wildlife transmission or host-adapted viral evolution.

Keywords: FMDV, Asian elephant, Spillover, Whole-genome sequencing

Poster 1.31

Coronavirus diversity in wildlife in Denmark: Insights from bats and mustelids

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Coronaviruses (CoVs) comprise a diverse group of RNA viruses that infect a broad range of hosts and are of major importance to human and animal health. Bats are established reservoirs of mammalian CoVs and have been implicated in the emergence of multiple zoonotic viruses. Mustelids represents key hosts at the human–animal interface, as highlighted by SARS-CoV-2 outbreaks in farmed mink. In this study, we applied targeted metagenomics to recover and characterize full-length CoV genomes from Danish wildlife.

Fecal and colon swabs were collected from the Danish bat surveillance program between 2021 and 2024, while mustelid fecal and throat swabs were collected as part of a project assessing selected pathogens in native Danish mustelids. Samples were screened by RT-qPCR using pan-CoV ORF1b assays and a SARS-CoV-2 E-gene assay. Positive samples were subjected to metagenomic next-generation sequencing (mNGS) on Illumina MiSeq and NextSeq platforms. Full-length genomes were assembled using our metagenomic workflow. Global phylogenetic analysis based on total average nucleotide identity was used to determine placement within the subfamily Orthocoronavirinae, followed by local maximum-likelihood phylogenetic analyses at the subgenus level.

Following RT-qPCR screening and confirmation by Sanger sequencing and/or mNGS, alpha-CoVs were detected in 15 of 222 bat samples and in one of 203 mustelid samples. Among bats, positives were identified in Myotis dasycneme (n=1), M. daubentonii (n=12), and Pipistrellus pygmaeus (n=2). Full-length genomes were recovered from one M. dasycneme, six M. daubentonii, and one P. pygmaeus sample, with partial genomes obtained from two additional M. daubentonii samples. In mustelids, a full-length genome was recovered from the alpha-CoV–positive Mustela putorius (European polecat) sample.

Phylogenetic analysis showed that M. daubentonii viruses clustered within a single Pedacovirus clade associated with Danish hibernacula, suggesting host-associated circulation. In contrast, the M. dasycneme virus belonged to a more heterogeneous Pedacovirus lineage spanning multiple bat genera and geographic regions. The Nyctacovirus genome from P. pygmaeus clustered closely with Danish and Swedish sequences (96.4–98% identity), indicating long-term regional circulation.

The M. putorius virus represents the first detection of an alpha-CoV in a wild mustelid in Denmark. It clusters within Minacovirus alongside European polecat and ferret viruses and is distinct from farmed mink-associated lineages, with no evidence of spillover from farmed mink.

None of the bat samples or mustelid samples tested positive for SARS-CoV-2.

Keywords: Coronavirus, metagenomics, wildlife surveillance, bats, mustelids

Poster 1.32

Evolutionary Emergence of the Foot-and-Mouth Disease Virus A/ASIA/G-VII Lineage in Western Asia

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Trans-pool movements of foot-and-mouth disease viruses (FMDV) can substantially impact a region's risk profile. The A/ASIA/G-VII lineage has been historically circulating FMD endemic countries in Southern Asia (primarily in India). However, exotic outbreaks due to this lineage were reported in Western Asia in 2015-2018. This study aimed to investigate the molecular evolution, geographic spread, and selective pressures driving the emergence and spread of the A/G-VII lineage. We analysed 350 FMDV sequences encoding for the VP1 region and a further 48 complete genomes, retrieved from FMDbase (www.fmdbase.org). Bayesian inference using VP1 data estimated the ancestral origin of the A/G-VII lineage during 1987 and its evolutionary rate of 7.90 × 10^-3 substitutions/site/year. Phylogeographic analysis reconstructed the westward spread, from India into Western Asia, of viruses characterised by a deletion at residue VP3-59, beginning in 2014-2015. Spread of this lineage involved repeated geographic virus transitions from India into Saudi Arabia, and from India to neighbouring FMD Endemic Pool 2 countries. Branch-specific analysis revealed evidence of positive selection at residues flanking the VP3-59 deletion for viruses introduced into both Saudi Arabia and Pool 2. Additional positively selected sites were identified within the VP1-coding region. Signals of within-lineage recombination were detected in 5 out of the 48 complete genomes analysed. In summary, the A/G-VII lineage expanded geographically with evidence of associated selective pressures. These findings underscore the need for a continued FMD genomic surveillance to better inform risk-based regional control strategies.

Keywords: Foot-and-Mouth Disease Virus, Molecular Epidemiology, Molecular Evolution, Outbreak

Poster Presentations:

Poster Session 2 – Vaccines

Location: Dialog

Contents:

Poster 2.01

Up to one year duration of immunity in cattle for a vaccine against Bluetongue virus serotype 3 (BTV 3)

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Background and Objectives:
The Blue Tongue Virus (BTV) is the causative agent of a severe seasonal transboundary disease affecting ruminants. Since its emergence in the Netherlands in 2023, Bluetongue virus serotype 3 (BTV3) has continued to spread widely across Europe, with significant impacts on animal health and causing severe losses for farmers. Mass vaccination of herds is the most efficient tool against the disease and establishing the duration of immunity (DoI) provided by BTV3 vaccine (Bultavo 3) in cattle is therefore essential to enable long-term control strategies. This study aimed to evaluate the efficacy of the vaccine six months and one year after administration.

Material and Methods:
BTV3 inactivated vaccine at a minimum dose was administered to 16 calves (21 - 36 days old) with 2-shots, 21 days apart, and 16 calves were left unvaccinated. Half the animals in each group were challenged at 6 and 12 months after vaccination respectively, with a virulent BTV3 heterologous strain, isolated from the 2023 outbreak in The Netherlands. Clinical signs, rectal temperatures and general condition were recorded daily for 21 days in all animals and viral RNA was measured by a BTV3 specific qRT-PCR.

Results:
The vaccine showed a good safety profile, with no local or systemic reactions following either administration. Following challenge, clinical signs like conjunctivitis and/or nasal discharge and hyperthermia were observed in all control cattle. All vaccinated animals were protected from development of clinical signs at both 6 months and 1 year after vaccination. Presence of BTV3 RNA was detected in blood from all control animals. Following challenge 6 months after vaccination, viraemia was absent in all vaccinated calves at all sampling times. Following challenge 1 year after vaccination, viral RNA was undetectable in 7 out of 8 vaccinated animals, and only transient, low level viraemia occurred in one calf. Vaccination reduced viral load 100% and 96.3% after 6 months and 1 year, respectively.

Conclusion:
In conclusion, a two dose regimen of the inactivated Bultavo 3 vaccine conferred robust one year protection in cattle, fully preventing clinical disease. The vaccine provided complete prevention of viraemia after 6 months and significantly reduced viraemia after one year. These results demonstrate that vaccination not only protects individuals but also effectively blocks onward transmission, supporting its use in BTV 3 control strategies.

Keywords: BTV-3, Bultavo 3, vaccine, cattle, duration of immunity

Poster 2.02

One year duration of immunity in sheep for a vaccine against Bluetongue virus serotype 3 (BTV 3)

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Background and Objectives:
The Blue Tongue Virus (BTV) is the causative agent of a severe seasonal transboundary disease affecting ruminants. Since its emergence in the Netherlands in 2023, Bluetongue virus serotype 3 (BTV3) has continued to spread widely across Europe, with significant impacts on animal health and causing severe losses for farmers. Mass vaccination of herds is the most efficient tool against the disease and establishing the duration of immunity (DoI) provided by BTV3 vaccine (Bultavo 3) in sheep is therefore essential to enable long-term control strategies. This study aimed to evaluate the efficacy of the vaccine one year after administration.

Material and Methods:
Eight animals (32 - 36 days old) were vaccinated with one-shot of BTV3 inactivated vaccine at a minimum dose and challenged one year later. One group of 8 non-vaccinated animals served as control. One year after primary vaccination, all animals were challenged with a virulent BTV3 heterologous strain, isolated from the 2023 outbreak in The Netherlands. Animals were observed for clinical signs and rectal temperatures for 21 days post-challenge and presence of viral RNA in serum were measured using a BTV3 specific qRT-PCR at regulator intervals.

Results:
The vaccine demonstrated a good safety profile, with no local or systemic reactions following administration. Following challenge, clinical signs like oedema, locomotion troubles, anorexia, apathy, conjunctivitis and/or nasal discharge, and hyperthermia were observed in all control sheep. In contrast, none of the vaccinated animals developed clinical signs. Presence of BTV3 RNA was detected in blood samples from all control animals from day-3 post challenge and persisted until study termination. No viral RNA was detected in any of the vaccinated animals at any sampling time following challenge one year after vaccination, corresponding to a 100 % reduction in viral load compared with controls.

Conclusion:
In conclusion, in this study, a single dose regimen of the inactivated Bultavo 3 vaccine conferred robust one year protection in sheep, preventing clinical disease and viraemia after heterologous challenge. These results demonstrate that vaccination not only protects individuals but also effectively blocks onward transmission, supporting its use as a key component of BTV 3 control strategies in an increasingly enzootic European context.

Keywords: BTV-3, Bultavo 3, vaccine, sheep, duration of immunity

Poster 2.03

Immune performance of concomitant administration of two inactivated vaccines against multiple epizootic serotypes of bluetongue virus in cattle

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Background and Objectives:
Multiple serotypes of bluetongue virus (BTV) circulate across Europe and pose significant health and management challenges for cattle breeders. Vaccination protocols must ensure strong and protective immunity while avoiding repeated handling of animals that may increase operational constraints and animal stress. Concomitant administration of inactivated vaccines targeting different BTV serotypes could offer a practical solution to facilitate implementation of vaccine programs and streamline field procedures. The objective of this study was to assess whether the simultaneous administration of two inactivated bluetongue vaccines induced an antibody response not lower than that obtained when each vaccine is administered separately. Tolerance of the procedure was also evaluated.

Methods:
Approximately 70 healthy cattle of various breeds, aged 7-17 months, with confirmed pan-BTV and EHDV anti-VP7 seronegative status and blood qPCR negative at inclusion, were enrolled across five farms in France. Animals were randomized into four groups: BTV-3 inactivated vaccine (BulTaVo®3), BTV 4 and 8 inactivated vaccine (BTVPUR®4-8), both vaccines on the same day, and unvaccinated controls. The injections were performed 21 days apart. Rectal temperature was recorded at each vaccination and for the following two days. Serum samples were collected on Days 0, 21 and 42 upon vaccination. Antibody responses to VP7 were analysed using a commercial competition ELISA (Innovative Diagnostics, Grabels, France). Neutralizing antibody titres targeting VP2 were determined by seroneutralisation tests (SNT) for BTV 3 and BTV 8. Results were compared between groups of concomitant or single vaccine immunization using Student's and Chi-squared tests.

Results:
Clinical assessment showed stable rectal temperatures following vaccination, and no adverse events were reported.
The analyses demonstrated that concomitant administration did not affect either the magnitude or the kinetics of vaccine-induced antibody responses against BTV3 and BTV-8. All vaccinated groups exhibited 100% positive virus-neutralising antibody titres higher than 3.2 log2 against both viruses following the recommended vaccination regimen, with no statistically significant differences observed between singly vaccinated and with the concomitant vaccination group.

Conclusion:
This study provides field evidence supporting the compatibility and immunological performance of concomitant administration of two inactivated bluetongue vaccines in cattle. Findings are expected to support practical vaccination strategies that reduce animal handling requirements while maintaining adequate protection against multiple circulating bluetongue virus strains for improvement of vaccination acceptability.

Keywords: BTV-3, BTV-4, BTV-8, vaccination, cattle

Poster 2.04

Establishing a streamlined foot-and mouth disease virus neutralisation test for vaccine matching

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INTRODUCTION

Selection of appropriate vaccine strains is critical for effective control of foot-and-mouth disease (FMD), requiring assessment of antigenic match between vaccines and circulating strains. The virus neutralisation test (VNT) is the preferred in vitro method for vaccine matching; however, it is laborious, low throughput and can exhibit high variability. The current method uses five virus doses, linear regression analysis to calculate neutralisation titres and relationship coefficient (r_1 value) between vaccine and field isolates measure antigenic similarity . This study aims to improve VNT efficiency by reducing virus input dose and optimise assay conditions while maintaining reliability in vaccine matching.

METHODS

Archived vaccine matching datasets from 2017 and 2023 for SAT2 vaccine strains SAT2 Eriteria and SAT2 ZIM 83 were assessed against twenty-nine field isolates. Comparative statistical analyses were conducted using linear regression data from five, three and one virus doses. Neutralisation titres were evaluated using boxplots to assess distribution and variability, while agreement in r_1 values was determined using Cohen's kappa (κ) statistic. In addition, a linear regression model using ten virus doses was applied to examine the relationship between virus dose levels and neutralisation responses for SAT2, O and A serotypes across both vaccine strains and field isolates.

RESULTS

Descriptive analysis showed no major difference on neutralisation titres for five vs three virus doses in comparison to five vs one virus dose. Overall neutralisation responses remain comparable irrespective of the number of doses used. Relationship coefficient (r_1 value) for SAT2 Eriteria showed perfect agreement according to Cohen's kappa (κ) statistic for both comparisons (five vs three and five vs one virus doses). In contrast, SAT2 ZIM 83 showed substantial to moderate agreement for five vs three and five vs one respectively. Linear regression analysis for SAT2, O and A vaccine and field viruses showed linear relationships are maintained at certain virus doses on either side of 2 log10 (100TCID50) virus dose, thus maintain assay reliability.

DISCUSSION

This preliminary data shows potential to reduce virus doses in vaccine matching VNT assay, improving efficiency, throughput and reliability while maintaining acceptable accuracy when using three virus doses instead of five. This analysis could be further extended to other serotype vaccines dataset. Overall, the increasing demand from vaccine manufacturers to include their vaccines in routine vaccine-matching testing could be met by enhancing testing capacity, enabling a greater number of vaccine strains to be evaluated against more field isolates.

Keywords: Vaccine matching, virus neutralisation test, antigenic match, vaccine strains, field isolates, neutralising antibody, Foot and mouth disease virus

Poster 2.05

Protein-only microparticles as a vaccine platform against infectious bursal disease and high pathogenicity avian influenza

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Highly contagious avian diseases including infectious bursal disease virus (IBDV) and high pathogenicity avian influenza viruses (HPAIVs), remain major threats to poultry health, food security and outbreak preparedness. Although vaccination is essential for disease control, current strategies may be limited by vaccine-associated immunosuppression and incomplete prevention of viral shedding. This highlights the need for safe, non-replicative and adaptable vaccine platforms.
Immunostimulatory protein-only microparticles (ISPOMs) offer a promising platform adaptable to different viral antigens. They enable the gradual release of antigens in nanoparticle form, induce antigen-specific antibody and T-helper responses, and promote cytotoxic T-cell activation through cross-priming. Since no vaccine virus replication occurs, this platform may preserve immunocompetence while improving vaccine safety.
In this study, we evaluated ISPOMs using two antigenic models based on proteins from IBDV (VP2) and HPAIV (hemagglutinin, HA). VP2-ISPOMs were assessed in SPF chickens and commercial broilers following a subcutaneous prime-boost vaccination strategy, while HA-ISPOMs were evaluated in SPF chickens to characterize dose- and route-dependent immune responses. Humoral immunity was measured by commercial and antigen-specific ELISAs and, for HPAIV, by hemagglutination inhibition assay. Cellular responses were evaluated by IFN-γ ELISpot and CFSE-based T-cell proliferation assays.
In the IBDV model, responses differed between SPF and commercial chickens. In SPF birds, VP2-ISPOMs induced antibody responses and significantly improved survival (72%) compared to controls (6%) following challenge. Notably, protection in some seronegative birds suggested a contribution of cellular immunity. In contrast, commercial broilers showed no clear increase in VP2-specific antibodies, likely due to maternal antibody inference, and developed severe bursal lesions in most cases. Overall, VP2-ISPOMs showed potential as an IBDV vaccine, although their effectiveness was limited in the presence of maternal antibodies. In the HPAIV model, conventional humoral assays failed to detect H5-specific antibodies, whereas an in-house HA-ISPOM ELISA identified antigen-specific antibodies, particularly following in ovo and subcutaneous vaccination. Cellular assays revealed heterogeneous but detectable IFN-γ production and CD4+ T-cell responses, more pronounced after subcutaneous vaccination. Protective efficacy against HPAIV challenge remains to be determined, but these findings support the potential of HA-ISPOMs and highlight the need for further optimization.
Taken together, ISPOMs show promise as a non-replicative vaccine platform for avian viral diseases. Further optimization of formulation and dosing, together with challenge studies, will be essential to determine whether this approach can provide robust protection, particularly against HPAIV.

Keywords: Protein-only microparticles, infectious bursal disease virus, high pathogenicity avian influenza, chickens, cellular immunity, vaccine platform

Poster 2.06

Intradermal and intranasal vaccination with microparticulated ASFV proteins induces distinct immune profiles in pigs

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African Swine Fever (ASF) is a major threat to the swine industry due to its severe socioeconomic impact and the lack of safe and effective vaccines. Although live attenuated vaccines (LAVs) can induce robust immunity, biosafety concerns restrict their use, making subunit vaccines the preferred alternative. In this context, understanding how vaccination routes modulate immunity is critical. A previous study using a viral vector suggested potential for combined intranasal (IN) and parenteral administrations. However, the individual contribution of mucosal versus systemic compartments remains unknown.
This study evaluated how independent and combined vaccination routes influence the immune responses induced by an ASFV protein cocktail consisting of zinc microparticulated p30, p54, p72 and p72c, alongside soluble CD2v. Following a prime-boost regime, pigs vaccinated via intradermal (ID) and IN+ID routes seroconverted and developed strong ASFV-specific IgG responses, mainly directed against p54, p72c and p30. Additionally, unaccompanied ID inoculation induced p54-specific IFN-g-producing cells, whereas IN vaccination alone failed to elicit significant pre-challenge humoral or cellular responses. Following challenge with the virulent Georgia2007/1 strain, no protection was achieved in any of the immunized groups, reaching humane endpoints within a week. Interestingly, IN-immunized pigs exhibited an accelerated post-challenge antibody boost compared to controls, demonstrating an undetected mucosal priming effect. Overall, while the novel microparticle platform successfully induced specific responses, these were insufficient to control a lethal ASFV infection, likely due to the weak cellular responses induced.

Keywords: intradermal, intranasal, ASFV, subunit vaccine, pig, immune response

Poster Presentations:

Poster Session 3 – Emerging Viruses and Vector-borne Diseases

Location: Spirit

Contents:

Poster 3.01

Occurrence of zoonotic and livestock pathogens in European wild boar in Austria

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Wild boar can be carriers of various pathogens with zoonotic potential or danger to domestic pigs. With increasing wild boar populations in Europe over the last decades, the risk of disease transmission has also increased. This study investigated the prevalence of selected pathogens of relevance in Austrian wild boar: hepatitis E virus (HEV), suid alphaherpesvirus 1 (SuAHV-1), influenza A virus (IAV), Brucella spp. and Leptospira spp.. Tissue samples of 257 wild boar (116 spleens and 141 organ pools, comprising tonsil, lymph nodes, spleen, liver, lung and kidney) collected in 2022 were analysed by PCR. HEV was found in five organ pools (3.5 %) and three spleen samples (2.6 %). Subsequent sequencing allowed the identification of subtypes 3a, 3i and one unassigned subtype. SuAHV-1 was detected in none of the organ pools but in two spleen samples (1.7 %). IAV was detected in four organ pools (2.8 %). Brucella were found in two organ pools (1.4 %) and seven spleens (6 %). While intermediate Leptospira could be detected in two organ pools (1.4 %) and two spleens (1.7 %), all samples were negative for pathogenic Leptospira. Although overall prevalences seem low, we cannot exclude the possibility of former infections since we could not test for the presence of antibodies due to the lack of blood samples. Our findings highlight the relevance of wild boar in the epidemiology of multiple infectious agents and the need for continuous surveillance to better assess the potential risks associated with infectious diseases in wild boar populations.

Keywords: wild boar, hepatitis E virus, suid alphaherpesvirus 1, swine influenza A virus, PCR

Poster 3.02

Expert Knowledge Elicitation: Development of guidelines for assessing data gaps in risk assessment

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Lack of data in veterinary risk assessment can be addressed through expert knowledge elicitation (EKE). EFSA provides a formal 'standard' EKE approach (EFSA, 2014). However, this approach is resource-intensive, time-consuming and requires specific training, making it less suitable when results of a risk assessment are needed at short notice or when limited budget is available. The need for a 'lighter' approach was addressed within a European collaboration of veterinary research institutes. The aim was to develop fit-for-purpose guidelines for performing an EKE that address data gaps in veterinary risk assessment within available resources and time, without compromising output quality and robustness.
A literature review was carried out to get an overview of EKE methods used in veterinary risk assessment and related fields, such as food safety. Additionally, an inventory was compiled of the EKE methods that project team members had carried out in the past. Finally, a case study was conducted to assess whether this 'lighter', yet structured, EKE approach would lead to useful results. The knowledge and experience obtained from the literature review, the inventory of collective expertise, and the case study were used as a basis for drafting guidelines, including a decision tree. These guidelines outline different approaches for performing a 'light' EKE, tailored to various practical scenarios, such as short deadlines, a small budget or insufficient expert availability.
This compilation of approaches to a 'light' EKE is designed to guide and support users in selecting and implementing a simplified EKE in a way that remains transparent, structured and robust.

Reference: EFSA, 2014. Guidance on expert knowledge elicitation in food and feed safety risk assessment. EFSA Journal 2014; 12(6):3734

Keywords: Risk assessment, expert knowledge elicitation

Poster 3.03

The return of the Berne virus: Equine torovirus discovered in Algeria 50 years after its first and only isolation in Switzerland

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It was in 1972 that Bernese veterinary virologists isolated a new virus from a rectal swab sample of a horse with diarrhea, designating it as “Berne virus” (BEV). This virus was later found to represent a new type of enveloped, positive-sense, single-stranded RNA virus. While it shares molecular similarities with coronaviruses, its unique doughnut-shaped morphology led to the name equine torovirus (EToV). Although serological investigations suggested that BEV is geographically widespread and can infect different host species, it has never been isolated or directly detected since. Yet, BEV became the prototype of toroviruses because it grew readily in equine dermis cell cultures and served as a model for basic molecular biology research in the following decades.
In the framework of a virome study on fecal samples from Algerian horses using viral metagenomics, we detected BEV-specific sequences in a healthy adult horse. By combining Illumina short-read sequencing, Oxford Nanopore long-read sequencing, Sanger sequencing, and RACE RT-PCR, we determined the near-complete genome sequence of 27,981 nt, which showed the characteristic features of toroviruses. We designated this new virus strain as EToV Algeria. Open reading frame (ORF) sequences encoding the pp1a, pp1b, and N proteins were 98.8% to 99.4% identical to those of BEV. However, ORFs encoding the S and HE proteins were only about 75% identical to BEV, but around 95% identical to Bangali torovirus. Bangali torovirus was recently identified in camel-derived ticks in East Africa. These results raise the question of whether EToV Algeria derived from genomic recombination between BEV and Bangali torovirus.
Our findings provide the first genetic evidence of a BEV-like virus in decades and highlight the potential role of genetic recombination in the evolution and host-switching capabilities of equine toroviruses.

Keywords: Berne virus, torovirus, algeria, recombination

Poster 3.04

First Small Ruminant Lentivirus detection in roe deer in Italy

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Introduction: Small ruminant lentiviruses (SRLVs) are worldwide distributed, affecting sheep and goats and representing one of the most insidious viral infections impacting these species. In the last decades, SRLVs antibodies were detected in several wild ruminants in the United States and Europe, although molecular detection has been reported only in French ibexes and in red deer in Poland. These findings may suggest a potential role of wild ruminants in the epidemiology of SRLVs. The aim of our work was to investigate the susceptibility of roe deer to SRLVs via a passive surveillance study conducted from 2023 to 2025 in Central Italy.
Material and Methods: The monitoring program was based on the analysis of carcasses originated from road-killed animals and individuals that died in wildlife rescue centers in two central Italian regions, Umbria and Marche. Organs were collected and analyzed at National Reference Laboratory for SRLV (CEREL). All samples were tested by a nested RT-PCR targeting the gag-pol region, positive samples were subsequently Sanger sequenced and phylogenetic analysis were performed using IQ-TREE and ClusterPicker software.
Results & Discussion: 179 roe deer were sampled between January 1, 2023, and December 31, 2025. We obtained 245 samples, comprising 132 spleens (53.87%), 56 lungs (22.85%), 43 pooled organs (17.55%), and 14 blood clots (5.71%). One spleen from a road-killed female collected in the Marche region tested positive for SRLV by nested end-point PCR, first time in Europe. The sample belongs to sub-genotype A23, which is infrequent in Italy, according to phylogenetic analyses. The sequence clustered with strains previously identified in domestic small ruminants in southern Italy. Our data showed that cervids may be susceptible to SRLV and can act as reservoirs. Limited data on SRLV in wild ruminants hinders origin identification, therefore monitoring and systematic screening is essential to clarify wildlife epidemiological role in virus maintenance and spillover transmission.

Keywords: Lentivirus, SRLV, Roe deer, wild ruminants, Central Italy

Poster 3.05

First detection of Usutu virus in mosquitoes from Denmark, 2024

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In 2024, Usutu virus (USUV) was reported in birds in Denmark for the first time. The virus appeared to primarily affect the Eurasian blackbird (Turdus merula) population. Overall, USUV was detected in two thirds of blackbirds submitted for analysis (56/85) in the autumn 2024, and follow-up sequencing revealed that the viruses belonged to three distinct lineages (Europe 2, Europe 3 and Africa 3) (Gelskov et al., 2026). Subsequently, USUV was also detected, for the first time, in mosquitoes collected during the same year.
The mosquitoes were collected within the framework of the three-year EU4Health project, entitled 'OH4Surveillance - Setting up a coordinated surveillance under the One Health approach'. As part of this project, targeted mosquito surveillance in Denmark have been expanded. Within OH4Surveillance, the primary focus is on analyzing the collected mosquitoes for the presence of project priority pathogens, e.g. West Nile virus (WNV). Further, collected mosquitoes are also made available for surveillance activities for additional mosquito borne pathogens, including USUV.
None of the collected mosquitoes tested positive for WNV. However, using USUV RT-qPCR, viral RNA was detected in pools of Culex mosquitoes (pipiens/torrentium) collected in late summer 2024 from the Southern parts of Denmark. Sequencing revealed that the viruses belonged to the Europe 2 and Africa 3 lineages, which were also the dominant lineages detected in the blackbird population that year.
The detection of USUV in Culex mosquitoes collected in the Southern parts of Denmark indicates that the virus could potentially become established in the local mosquito population within that area. Furthermore, these findings provide a proof-of-concept for the mosquito sampling strategy established and implemented within OH4Surveillance (e.g., strict implementation of freeze-chain following mosquito collection), demonstrating that this setup provides high-quality samples suitable for laboratory detection of mosquito-borne pathogens.

The OH4Surveillance project is co-funded by the European Union under Grant Agreement No 1011324. Disclaimer: Views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or HaDEA. Neither the European Union nor the granting authority can be held responsible for them.

Reference: Gelskov LV, Johnston CM, Hammer ASV, Jensen TK, Lohse L, Belsham GJ, Rasmussen TB, Olesen AS. First detection of Usutu virus in wild birds in Denmark, 2024. Sci Rep (2026). https://doi.org/10.1038/s41598-026-35874-y

Keywords: USUV, Scandinavia, surveillance, vectorborne diseases

Poster 3.06

Entomological monitoring for Lumpy Skin Disease in Sardinia (Italy) during 2025-26 outbreaks

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Lumpy Skin Disease (LSD) is a viral disease that affects cattle, caused by Capripoxvirus (LSDV). Vectors, such as hematophagous arthropods, are likely to transmit the virus mechanically. Following the disease outbreaks in Sardinia (Italy), mapping the composition, abundance, and viral prevalence of local insect vectors has become a priority to better understand the epidemiology of the disease. The present study analyses the arthropod populations sampled within Sardinian outbreak farms

Monitoring was conducted at affected cattle farms in Sardinia using direct collection on animals (cattle), environmental/stable sampling, and deploying light traps for hematophagous insects. The specimens were identified to the species, genus or family level using morphological characteristics. The detection of LSDV DNA was conducted by Real-Time PCR.

Ticks were found to be present in high numbers on livestock and in the environment, with Rhipicephalus bursa (172 specimens) and Hyalomma marginatum (101) being the most prevalent species. Concurrently, a strong pressure by both hematophagous and opportunistic flies was identified directly on the cattle, including blood-feeding species such as Stomoxys calcitrans (35), Haematobia irritans (28), and Hippobosca equina (22), and opportunistic dipterans, such as Musca domestica (27) and Sarcophaga carnaria (5). Biting midges (Culicoides spp.) constituted the numerically dominant dipteran group, with a total of 601 individuals intercepted. A total of 32 mosquitoes were sampled from cattle and light traps, including 16 Culex pipiens and 11 Aedes detritus. A very high density of hematophagous Psychodidae (374) was registered using light traps. In addition, beetles and minor dipterans of secondary veterinary importance were sampled.

Ticks accounted for the most substantial number of PCR positive samples, with 18 R. bursa and 13 H. marginatum testing positive. The virological screening also revealed LSDV DNA in 13 H. equina and one S. calcitrans, while among synanthropic and opportunistic flies, the virus was detected in Musca domestica (10 specimens) and Sarcophaga carnaria (2). Finally, molecular screening confirmed one positivity each for Ae. detritus and Culicoides jumineri.

The monitoring results outline a clear high-intensity multi-vector scenario within the Sardinian outbreaks. The high number of positive samples found in ticks (Rhipicephalus and Hyalomma) and in both hematophagous (Hippobosca, Stomoxys, Culicoides, Aedes) and closely associated or opportunistic dipterans (Musca, Sarcophaga) demonstrates that the mechanical transmission of LSDV in Sardinia could benefit from an ecological synergy between flying vectors and ectoparasites. These data provide crucial scientific evidence for implementing integrated vector management plans and monitoring epidemiological risks across the regional territory

Keywords: Lumpy Skin Disease, Arthopods, Vectors, Ticks, Sardinia

Poster 3.07

Identification of Rickettsia raoultii-infected foci in the expanding Dermacentor reticulatus tick population in Luxembourg

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Dermacentor reticulatus (Fabricius, 1794) has recently become established in Luxembourg. This tick species is known to transmit several human and/or animal pathogens, including Babesia canis, Rickettsia spp., and tick-borne encephalitis virus (TBEV). However, there is currently limited data on the distribution of D. reticulatus ticks in Luxembourg and no information on the pathogens they harbour.
Ticks were collected by flagging and direct inspection of the vegetation. In 2024, collections were conducted along two rivers and at 33 sites throughout Luxembourg. Based on the findings from 2024, two sites were resampled in 2025. Nucleic acids extracted from ticks were screened using qPCR assays that target a range of tick-borne pathogens. Dermacentor reticulatus distribution was modelled based on tick occurrence, land-cover composition, climate, environmental, and human population data.
A total of 510 ticks from 2024 and 46 ticks from 2025 were analysed. Rickettsia raoultii was the predominant pathogen detected in both 2024 and 2025, with prevalences of 11.4% and 17.4%, respectively. Francisella tularensis was detected in two ticks (0.4%), and Borrelia spp., Borrelia miyamotoi, Neoehrlichia mikurensis, and Spiroplasma ixodetis were each detected in one tick (0.2%) in 2024 but not in 2025. No ticks tested positive for TBEV, Anaplasma spp., or Babesia spp. Spatial heterogeneity in R. raoultii prevalence was observed and areas with high prevalence identified in 2024 were confirmed in 2025. Species distribution modelling identified southern Luxembourg as highly suitable habitat for D. reticulatus and indicated a potential for further range expansion.
The D. reticulatus population in Luxembourg harbours several pathogens of veterinary and public health importance, with R. raoultii identified as the predominant species. The detection of these pathogens and the habitat suitability of southern Luxembourg highlight the need for continued surveillance of tick populations and associated pathogens to better assess and manage emerging health risks.

Disclaimer:
Part of this study was supported by co-funding from the European Union's EU4Health programme under Grant Agreement Nr 101132473 OH4Surveillance.Views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or HaDEA. Neither the European Union nor the granting authority can be held responsible for them.

Keywords: Dermacentor reticulatus, Rickettsia raoultii, tick-borne pathogens, habitat suitability modelling

Poster 3.08

Serological surveillance for five arboviruses in sentinel cattle in South Korea from 2009 to 2025

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Arthropod-borne viruses (arboviruses) are transmitted by haematophagous arthropod vectors, such as mosquitoes and Culicoides biting midges. Arboviruses cause abortion, stillbirth, and congenital malformations, resulting in significant economic losses to the livestock industry. This study aimed to investigate the long-term serological trends of arbovirus infections in sentinel cattle in South Korea using virus neutralization test (VNT). From 2009 to 2025, blood samples were collected from 500 unvaccinated sentinel calves under one year of age across all provinces of South Korea twice annually, in May and October, for serological surveillance of five arboviruses. VNT were performed to detect neutralizing antibodies against Akabane virus (AKAV), Aino virus (AINOV), Bovine ephemeral fever virus (BEFV), Chuzan virus (CHUV), and Ibaraki virus (IBAV). The mean seropositive rates determined by VNT were 6.5% for AKAV, 6.7% for AINOV, 3.0% for CHUV, 4.5% for BEFV, and 3.1% for IBAV, respectively. A substantial increase in the nationwide mean seropositive rates for AKAV (40.2%), AINOV (33.2%), and CHUV (29.1%) was observed in 2010, after which the seropositive rates remained at approximately 5%. In 2024, a temporary increase in the seropositive rates for AKAV (7.3%), AINOV (20.3%), BEFV (28.6%) was observed. However, in 2025, the seropositive rates for all five viruses declined to below 5%. This study demonstrates the prevalence of arbovirus infection in Korea over a 17-year period. Climate change caused by global warming is expected to influence the survival and distribution of vector insects, thereby facilitating the spread of viral infections in the livestock industry. Therefore, regular vaccination programs should be further strengthened along with continuous monitoring.

Keywords: Akabane virus, Aino virus, Bovine ephemeral fever virus, Chuzan virus, Ibaraki virus

Poster 3.09

West Nile virus integrated monitoring program in Vojvodina Province of Serbia – data obtained in 2021, 2023 and 2025

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The concept and results of the national integrated West Nile virus (WNV) monitoring program implemented in 2021, 2023 and 2025 in the territory of the Vojvodina Province of Serbia are presented. The WNV monitoring program, funded by the Veterinary Directorate was implemented by the veterinary service in cooperation with entomologists and ornithologists. The goal of the program was the early detection of WNV circulation in the environment and timely reporting to public health institutions and local governments in order to increase preparedness, initiate prevention of infection in humans and initiate targeted mosquito control actions. The program was based on the detection of the presence of WNV 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 (from 2023) for the presence of WNV-specific IgM and IgG antibodies.
WNV circulation was detected in all districts of Vojvodina Province from beginning of August until end of October in 2021, then from end of June to September in 2023, and from July to September in 2025. The presence of WNV IgM antibodies in the blood serum of horses was detected in 1.08% (6/555), 1.81% (10/554) and 0.34% (2/567) of the tested animals in 2021, 2023 and 2025 consequently. The presence of WNV IgG antibodies in young cattle was detected in 14.20% (161/1134) and in 8.68% (98/1134) of tested animals in 2023 and 2025. The virus (WNV) presence was detected in 11.43% (12/105) of wild bird samples in 2021, then in 7.42% (2/27), 5.56% (2/36) and 0% (0/4), as well as in 6.94% (5/72), 0% (0/29) and 0% (0/10) samples of dead, live captured and shot wild birds in 2023 and in 2025 consequently. Also, WNV was detected in 10.74% (26/244), 5.71 % (18/315) and 4.2% (12/285) of tested pooled samples of Culex pipiens mosquitoes.
The integrated WNV surveillance program in 2025 detected a very low intensity of WNV circulation, much lower comparing to 2021, and even lover of the level of virus circulation in 2023. Despite the low intensity of virus circulation, especially in 2025, the WNV monitoring program in all three years showed satisfactory results in terms of sensitivity and capacity to indicate the spatial distribution of the risk for humans.

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Keywords: WNV national surveillance program in 2021, 2023 and 2025, surveillance in horses, calves, wild birds and mosquitos, Vojvodina Province of Serbia

Poster 3.10

Sentinel Value of Companion Animals for West Nile Virus Surveillance in Croatia

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West Nile virus (WNV) is an emerging mosquito-borne zoonosis endemic in continental Croatia, causing recurrent outbreaks in humans and animals. Human cases are increasingly reported in populated areas, highlighting the need for effective surveillance to detect early virus circulation. Horses are widely recognised as sentinel species for WNV surveillance, while companion animals may provide additional epidemiological information due to their close association with humans and their frequent presence in urban environments. Therefore, this study aimed to assess WNV seroprevalence in horses, dogs and cats in Croatia and evaluate the potential role of companion animals as sentinels of WNV circulation.
Serum samples from horses, dogs and cats were collected across Croatia in 2025 as part of the national WNV surveillance programme and the CROOH (Croatia One Health) zoonotic disease surveillance project. Sera were screened for WNV antibodies using a commercial ELISA (INgezim® West Nile Compac, Ingenasa, Madrid, Spain). All ELISA-positive and equivocal samples were further tested by virus neutralisation test (VNT) against WNV, tick-borne encephalitis virus (TBEV) and Usutu virus (USUV). A fourfold difference in neutralising antibody titre was used as the cut-off for determining seropositivity for virus species. Samples that did not exhibit this difference were classified as flavivirus reactive. Comparisons between species and regions used appropriate statistical tests with significance set at $p<0.05$.
In total, 1,439 equine, 144 canine, and 83 feline samples were tested. ELISA reactivity was observed in 171/1,439 (11.9%) horses, 17/144 (11.8%) dogs and 11/83 (13.3%) cats (no significant difference between species, $p=0.88$). WNV-neutralising antibodies were detected in 8.3% of horses, 4.2% of dogs and 1.2% of cats, with a significant difference among species ($p=0.017$). Flavivirus-reactive samples occurred in 4.9% of horses, 4.9% of dogs and 3.6% of cats. In Zagreb, ELISA seroreactivity was similar in horses and dogs (18.6% and 17.6%, respectively); no seropositive cats were detected.
Horses remain the primary sentinel species for WNV surveillance in Croatia. Comparable seropositivity in dogs, including in urban settings such as Zagreb, and the spatial co-occurrence of WNV-seropositive dogs, cats and horses at the county level support the value of companion animals as supplementary sentinels. The low seroprevalence in cats suggests a more limited sentinel role. These findings support integrated One Health surveillance that incorporates companion animals to improve early detection of WNV circulation.

Keywords: West Nile Virus, sentinel surveillance, companion animals, One Health, Croatia

Poster 3.11

Mapping Rift Valley fever emergence risk in Cameroonian livestock

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Background: Rift Valley fever virus (RVFV) is a major emerging zoonotic pathogen, with high epidemic potential. In Cameroon, ecological heterogeneity—from forests to arid savannas—creates diverse conditions for RVFV emergence, yet no clinical cases have been officially reported despite IgG and occasional IgM detections. This study aims to identify high-risk areas for RVF emergence in livestock at a spatial resolution of 1 km² and temporal coverage from 2010 to 2025. It proposes division-specific prevention strategies based on dominant risk factors.
Methodology/Principal Findings: Using a GIS-MCDA (Geographic Information Systems - Multi-criteria Decision Analysis) framework, we combined insights from a multidisciplinary, multisectoral experts panel, with literature evidence via a Weighted Linear Combination (WLC) approach. Expert and literature-derived weights were equally integrated across twelve standardized environmental, entomological, zootechnical and human variables for dry and wet seasons. High-risk areas were concentrated in the North-West, West, Adamawa and Far North Regions. In the dry season, small ruminant density was the dominant risk factor across most divisions in the North-West, West and Far North, whereas cattle density predominated in the Adamawa Region. Proximity to protected areas was also the key risk factor in the North- West and Far North Regions. In the wet season, Aedes mosquito density emerged as the primary driver.
Conclusions/Significance: The maps guide prioritization of high-risk divisions for targeted serological surveys in humans, livestock and wildlife, vector control in favourable habitats, and sentinel herd programs. Optimal implementation relies on coordinated actions among animal health, wildlife, human health, and environmental sectors, supported by participatory surveillance and strengthened laboratory capacity ensuring timely detection and response.

Keywords: Rift Valley fever; Risk mapping; Knowledge integration; Livestock; Cameroon

Poster 3.12

Virus detection in Culicoides biting midges during the BTV-3 outbreak in north-west Germany 2024

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Bluetongue virus (BTV), Schmallenberg virus (SBV) and epizootic haemorrhagic disease virus (EHDV) are arthropod-borne viruses transmitted by biting midges of the genus Culicoides. In Central Europe, the importance of biting midges as vectors increased after the emergence of BTV-8 in 2006 and SBV in 2011. In the following years, SBV reached an enzootic status and several BTV serotypes were repeatedly introduced and caused outbreaks all over Europe. Furthermore, in 2022, EHDV was detected for the first time in southern European regions.
SBV, BTV and EHDV affect domestic and wild ruminants. The resulting diseases may cause animal suffering and economic losses due to management and control measures such as vaccination costs or trade restrictions. In 2023, BTV serotype 3 was detected for the first time in the Netherlands and caused a severe outbreak in Germany in 2024. The objective of this study was to monitor the viruses present in the biting midge population and to compare the prevalence of the viruses in the vectors.
For the monitoring, biting midges were caught from April to September 2024 on 14 farms in north-west Germany by traps equipped with UV-light. Of the trapped individuals, 337,359 females were selected and sorted into 7,549 pools, with up to 50 individuals per pool, based on subgenus, trapping site and trapping date. The pools were tested by PCRs for the genomes of SBV, BTV and EHDV. BTV-RNA was detected in a total of 1,359 pools (18.0%) and SBV-RNA in 402 pools (5.3%). None of the tested pools was positive for EHDV.
In this study, we confirmed the ongoing circulation of SBV, as well as an extensive BTV circulation in the Culicoides fauna in north-west Germany in 2024. Both viruses were first detected in the biting midges in early summer and reached their highest levels in August. Though not all factors influencing the transmission of viruses by biting midges are fully understood, they seem to affect the transmission of viruses of different orders and characteristics, such as BTV and SBV, in the same way.

Keywords: bluetongue virus, Schmallenberg virus, Culicoides, biting midges, monitoring study

Poster 3.13

New Insights into the Ecology and Clinical Relevance of Hypsugopoxvirus in European Bats

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Bats are increasingly recognized as major reservoirs of emerging zoonotic viruses, with important implications for animal health, wildlife conservation, and public health. Hypsugopoxvirus (HYPV), first identified in Hypsugo savii bats in Italy in 2017, remains the only bat-associated poxvirus described in Europe. However, knowledge of its ecology, epidemiology, tissue tropism and pathogenic potential is still limited. This study aimed to expand current knowledge on HYPV circulation and its possible clinical relevance in European bat populations.
Within ongoing passive virological surveillance activities on bats in Northern Italy, based on specimens collected through wildlife rescue centers (CRAS), selected samples were additionally screened for HYPV using a pan-poxvirus end-point PCR integrated into the routine diagnostic workflow of IZSLER.
The diagnostic protocol included organ homogenate preparation, virus isolation in cell culture, electron microscopy (EM) and molecular investigations mainly targeting lyssaviruses and coronaviruses followed by Sanger and/or NGS sequencing. Bat species identification was performed using both morphological criteria and COI gene sequencing.
A total of 382 bats collected between 2019 and 2026 were screened for HYPV. Eight tested positive for HYPV, corresponding to a prevalence of 2.1%. All positive cases involved Hypsugo savii, supporting a strong host association. Infectious virus was successfully isolated in 6 of 8 positive cases, supporting active viral circulation in Northern Italian bat populations.
Notably, two viral isolates (286977-1/2024 and 286977-3/2024) were obtained directly from patagial cutaneous lesions in infected Hypsugo savii bats, suggesting a possible association between HYPV infection and skin alterations.
Electron microscopy performed directly on lesion-derived material revealed abundant brick-shaped poxvirus-like particles morphologically consistent with Chordopoxvirinae, further supporting active viral replication within affected tissues. These two isolates yielded complete, high-quality genome sequences suitable for detailed genomic characterization. Comparative genomic analyses demonstrated >99% nucleotide identity with the previously described HYPV strain 251170-23/2017 (GenBank accession no. MK860688), indicating the circulation of highly conserved HYPV strains several years after the original identification of the virus. Partial genomic sequences were obtained from all additional positive samples, while full-genome sequencing of the remaining isolates is ongoing.
Overall, these findings demonstrate the continued circulation of HYPV in bat populations and reinforce its association with the bat species Hypsugo savii. Moreover, the combined virological, electron microscopy and genomic findings provides preliminary support for a possible association between HYPV infection and cutaneous lesions, warranting further investigations into the epidemiology, host adaptation and pathogenic potential of this novel European bat poxvirus.

Keywords: Bats, Poxvirus, Surveillance

Poster Presentations:

Poster Session 4 – Pathogenesis

Location: Time-out

Contents:

Poster 4.01

Nonlinear and lagged associations between ambient temperature and pre-weaning piglet mortality in Cuba

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Climate change is increasing the frequency of hot conditions, raising concern for thermally sensitive stages in swine production, yet evidence from humid tropical systems remains limited. We quantified the immediate and delayed association between daily mean ambient temperature and pre-weaning piglet mortality using daily records from 14 commercial farms located in 12 municipalities across Cuba during 2022–2024 (17,599 deaths). Farrowing accommodation consisted mainly of open or semi-open naturally ventilated maternity buildings with standardized farrowing crates/cubicles, implying limited active microclimate control and closer coupling between outdoor weather and the maternity-room microclimate. Daily mean temperature and relative humidity were derived from ERA5-Land reanalysis and linked to farm-day death counts at the municipality level. We applied a space–time stratified case-crossover design implemented with conditional quasi-Poisson regression, controlling for long-term trends, seasonality, and day-of-week. Temperature effects were modeled with a distributed lag non-linear model (DLNM) over lags 0–7 days while adjusting for relative humidity. The cumulative temperature–mortality relationship was U-shaped and strongly heat-dominated, with a minimum-mortality temperature (MMT) of 23.7 °C; above the MMT, cumulative risk increased steadily and accelerated toward the upper tail. Temperature-related effects were predominantly acute, with increased lag-0 risks at both temperature tails: RR = 1.49 (95% CI: 1.06–2.09) at the maximum observed mean temperature of 31.2 °C and RR = 2.26 (95% CI: 1.27–4.02) at the minimum observed mean temperature of 14.3 °C. However, cold-related effects attenuated rapidly and contributed little to the overall attributable burden. Non-optimal temperature accounted for 16.5% of deaths, almost entirely attributable to heat (15.4%), indicating that heat already represents the dominant temperature-attributable burden for Cuban piglets. Importantly, most heat-attributable deaths occurred on non-extreme warm days above the MMT, while the hottest 1% of days (≥99th percentile) contributed a smaller but measurable fraction (0.7%). These findings suggest that reducing pre-weaning losses in tropical swine systems requires routine heat-risk management in farrowing and nursery environments, integrating ventilation, air movement, water access, and feasible cooling strategies into daily practice, alongside preparedness for episodic hot extremes to support climate-resilient production.

Keywords: Pre-weaning piglets, heat, cold, case-crossover design, distributed lag non-linear model

Poster 4.02

Maternal transfer and decay of Crimean-Congo haemorrhagic fever virus antibodies in sheep: establishment of a longitudinal ewe-lamb cohort in Bulgaria

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Background: Crimean-Congo haemorrhagic fever virus (CCHFV) is a tick-borne zoonotic pathogen that can cause severe disease in humans but circulates asymptomatically in livestock. While livestock studies have traditionally focused on seroprevalence, the immune responses that allow natural hosts to control infection without disease remain poorly understood. Understanding these responses could provide important One Health insights relevant to animal and human health. Maternal antibodies represent a unique opportunity to study early-life immunity in naturally exposed animals, yet little is known about the antigen-specific antibody profiles transferred to lambs, how these profiles change over time or how they relate to subsequent infection under field conditions.
Methods: A longitudinal ewe-lamb cohort was established in Burgas Province, Bulgaria, a CCHFV-endemic region. Adult ewes from commercial sheep farms were screened for pre-lambing antibodies using the ID Screen® CCHF Double Antigen Multi-species ELISA (IDvet). Seropositive animals were enrolled into prospective follow-up. Paired ewe serum, colostrum and lamb serum samples were collected shortly after birth, and longitudinal sampling of lambs is ongoing (every 3-4 weeks until end of October 2026). Farm management, housing conditions (temperature and humidity), tick-control measures and animal characteristics are recorded throughout the study.
Results: A total of 104 adult ewes from eight commercial sheep farms were screened during the pre-lambing period (January 2026). Farms were purposively selected from areas previously identified as CCHFV hotspots. Overall, 99/104 (95.2%) animals were seropositive and 5/104 (4.8%) were seronegative, consistent with intense, long-standing CCHFV circulation on these farms. Following screening, a subset of 27 ewes was selected for longitudinal follow-up, ensuring representation across farms. Seronegative animals were retained as controls where available. These animals produced 32 lambs, including five twin pairs, forming the ewe-lamb cohort. Paired maternal and neonatal samples have been collected and longitudinal follow-up is underway through both the indoor barn phase, when tick exposure is minimal, and the subsequent pasture phase, when tick activity and CCHFV transmission is expected to increase.
Conclusions: This cohort provides platform for studying maternal immunity and early-life infection dynamics under natural CCHFV exposure conditions. Samples will be used to quantify maternal transfer and decay of antibodies to CCHFV Gc, NP and GP38 antigens and to investigate associations between residual maternal immunity and subsequent evidence of infection. The findings will improve interpretation of livestock serosurveillance data and support preparedness activities, diagnostic development and future vaccine strategies within a One Health framework.

Keywords: Crimean-Congo haemorrhagic fever virus, antibody response, sheep, vaccine

Poster 4.03

Dynamics of splenic monocytic cells during acute African swine fever

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To elucidate the underlying dynamics of macrophages infection, depletion and replacement, we sequenced immune cell populations at a single-cell resolution (scRNA), with a focus on macrophages, monocytes, and dendritic cells from the spleen of nine infected pigs, over three time points - from day 0, until pig death at day 5.
To observe the progression of infection over time, we focussed on the on the trajectory and profiles of macrophage subsets. However, viral gene expression at day 5 amounted to upwards of 92% of total reads per cell, making their origin indistinguishable and leading to the clustering of all infected cells into one group. In order to identify the origin of infected macrophages in this cluster, we used linear regression approaches (elastic net) to define their unique transcriptomic signature before infection and allowing us to characterize specific infected sub-populations and their likely origin. Tissue-resident macrophages were shown to be strongly depleted over time, and monocyte-derived macrophages showed a cycle of recruitment, differentiation into macrophages, infection and depletion. In addition, we show the progression of the innate immune response in infected and bystander cells and their cell specific signatures, elucidating additional potential pathogenic mechanisms.

Keywords: African swine fever, macrophages, inflammation

Poster 4.04

Madin-Darby canine kidney cells express multiple transmembrane serine proteases supporting trypsin-independent replication of various influenza viruses

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A key determinant of influenza A virus (IAV) virulence is the envelope glycoprotein hemagglutinin (HA), which mediates host cell binding and membrane fusion. Fusion competence requires proteolytic cleavage of HA into HA1 and HA2. Most IAV viruses possess a monobasic cleavage site recognized by trypsin-like proteases expressed only in specific cell types in vivo and in vitro.
This study aimed to compare IAV replication in established cell lines with and without trypsin, identify the subcellular site and endogenous protease(s) responsible for HA activation, and investigate additional molecular determinants of trypsin-independent viral replication.
Screening of different cell lines showed that the human lung adenocarcinoma-derived cell lines A549 and Calu-3 did not support efficient virus replication of H1N1(PR8) in the absence of exogenous trypsin. In contrast, Madin-Darby canine kidney (MDCK) cells supported trypsin-independent replication, although virus replication was delayed and reached lower titers than in trypsin-supplemented cultures. Three MDCK sublines (MDCK-I, MDCK-II, and MDCK-NBL2) all supported trypsin-independent virus replication. In particular, the MDCK-NBL2 cell line allowed trypsin-independent replication of multiple IAV strains, including human H1N1, porcine H3N2, and low-pathogenic avian H7N7, H9N2 and H5Nx viruses. Analysis of low-pathogenic H5Nx viruses revealed marked differences in the proteolytic activation despite identical HA cleavage motifs.
Treatment with the serine protease inhibitors camostat mesylate, nafamostat mesylate, and pefabloc (AEBSF), inhibited trypsin-independent H1N1(PR8) replication in MDCK-NBL2 cells, indicating that endogenous serine proteases mediate HA cleavage. RT-PCR analysis detected expression of multiple transmembrane serine proteases in MDCK-NBL2 and MDCK-II cells, including TMPRSS1, TMPRSS2, TMPRSS4 and TMPRSS11D (human airway trypsin-like protease, HAT). In addition, serum antibodies directed against individual canine TMPRSS proteases partially inhibited H1N1(PR8) replication in MDCK-NBL2 cells, suggesting that HA activation is mediated by multiple serine protease rather than a single enzyme.
In summary, these findings support the use of MDCK cell lines, particularly MDCK-NBL2, for influenza virus isolation from clinical specimens and for screening protease inhibitors as antiviral agents, while reducing the need for animal-derived trypsin in influenza vaccine production.

Keywords: MDCK, avian influenza, HA cleavage, serine protease, TMPRSS

Poster 4.05

Modelling Zoonotic Flavivirus Infection using Brain Organoids

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Zoonotic orthoflaviviruses, including the arthropod-borne Zika (ZIKV), West Nile (WNV) and dengue (DENV) viruses, as well as the tick-borne encephalitis virus (TBEV), are increasing in incidence and geographic spread, posing a growing threat to global health. These viruses can aAect the human nervous system across life stages, from congenital neurodevelopmental disruption to encephalitis later in life. Human-relevant models are necessary to better define disease mechanisms and test therapeutics. We employ human neural organoids (hNOs) as a versatile platform to address both needs. Early-stage hNOs, modelling fetal brain development, were used to systematically compare the neuroteratogenic potential of ZIKV, WNV and DENV. All three viruses established sustained infection with distinct tropism and severity: ZIKV preferentially targeted neural progenitor cells, driving apoptosis and severe growth restriction; WNV predominantly infected neurons, causing partial depletion of the neuronal compartment; and DENV had minimal impact on growth or transcriptional profiles. Bulk and spatial RNA sequencing further resolved antiviral, stress and apoptotic signatures across cell types as well as disruption of neurodevelopmental pathways. To extend the platform toward therapeutic applications, we employ more mature hNOs to model TBEV infection of diAerentiated neural tissue. Using this system, we performed an antiviral compound screen, demonstrating the platform's capacity to support drug discovery against neurotropic viral infections. Together, these applications establish hNOs as a physiologically relevant platform to study neurotropic flavivirus infection, spanning disease mechanisms to antiviral screening. This approach supports assessment of neurodevelopmental risks and the identification of countermeasures against zoonotic flaviviruses.

Keywords: flavivirus, zoonoses, organoids, neurotropic, west nile virus

Poster 4.06

Pathogenesis of Rift Valley fever virus in an intranasally challenged ferret model, indicating multiple routes of neuroinvasion with consequential encephalitis and ophthalmitis

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Rift Valley Fever virus (RVFV) is a zoonotic mosquito-borne virus. Human infection can be acquired through mosquito bite or mucosal infection via exposure to infected animal materials. Infection in humans is often asymptomatic or mild, although severe disease can manifest as haemorrhagic fever, ophthalmitis, and in some cases fatal encephalitis. In this study, the ferret intranasal infection model was used to simulate mucosal RVFV infection through occupational exposure, investigating the subsequent central nervous system (CNS) invasion and ophthalmic pathogenesis. Female ferrets (n=15) were inoculated intranasally with 1e7 plaque forming units (pfu) of RVFV strain ZH501. Clinical signs, temperature and bodyweight were monitored daily. Three infected ferrets were euthanised at 3 days post-infection (DPI), reflecting an early pre-clinical timepoint; remaining ferrets were euthanised when reaching predetermined clinical end points. Control ferrets (n=3) were mock-inoculated and assessed in parallel. RVFV infection was confirmed through RT-PCR of tissue samples and swabs, and immunohistochemistry of fixed tissues. Sera were investigated for viremia and neutralising antibodies, while selected CNS tissues were assessed for modulation of RNA transcripts for innate immune markers associated with neuropathogenesis. At the pre-clinical time-point of 3 DPI, mild multifocal suppurative rhinitis and multifocal interstitial pneumonia were detected in the nasal cavity and lungs, respectively, supported by detection of viral antigen and RNA. From 6 DPI, infected ferrets exhibited neurological signs, and by 6–8 DPI, a mild-to-severe non-suppurative meningoencephalitis and myelitis was observed, progressing rostrally to caudally, with ophthalmitis observed from 7 DPI and 100% mortality by 8 DPI. Mild-to-moderate hyperaemia of the meninges was observed macroscopically in all infected ferrets. These findings demonstrate progressive RVFV invasion of the CNS over time post-infection, accompanied by virus dissemination to the eye and lung, but with minimal evidence of hepatic involvement. Viraemia was detectable at clinical endpoint (6–8 DPI). The mounting of a neutralising antibody response failed to prevent neuropathogenesis and clinical disease progression. The predominance of neurological disease in RVFV-infected ferrets was associated with statistically significant transcriptional up-regulation for IL-6, TNF-α and CCL5 in the cerebellum at clinical endpoint (6–8 DPI). Although these data suggest primary neuroinvasion via the olfactory route through the nasal turbinates to cranial nerves, concurrent virus replication in the lungs and presence of viremia implies that haematogenous dissemination into the CNS as a secondary route is possible. This relevant experimental ferret model may further contribute to understanding the pathogenesis of human RVFV infection.

Keywords: Rift Valley fever virus, ferret, intranasal, encephalitis, ophthalmitis

Poster 4.07

Recovery of infectious FMDV from RNA using an optimised chemical transfection approach

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Foot-and-mouth disease virus (FMDV) is a highly contagious livestock disease with significant economic impact, necessitating accurate and complete diagnosis for effective control and vaccine selection. However, the FMDV capsid is highly labile and disassembles as a result of heating or imperfect pH, rendering the virus non-infectious. Field samples often arrive at international reference laboratories after having been transported in suboptimal conditions which, among other reasons, limits conventional virus isolation and scope for downstream characterisation. Since 2007, 10,669 samples have been tested at the World Reference Laboratory for FMD (WRLFMD), of which 18% were rRT-PCR positive but failed to yield virus in cell culture, in turn constraining serotyping by ELISA, sequencing and vaccine-matching work. To address this challenge, we optimised a chemical transfection protocol for the recovery of infectious FMDV directly from extracted viral RNA.

Experiments were initially conducted to assess different transfection kits, cell preparation, and reagent/RNA input requirements using WRL-LFBK-αvβ6 cells. Initial screening of TransIT-mRNA, Lipofectamine MessengerMAX, and Lipofectamine 3000 demonstrated that FMDV could be rescued using all three reagents, although performance varied depending on transfection setup and RNA handling. Subsequent optimisation identified cell confluence at the point of transfection as an important determinant of reproducibility, with approximately 70–80% confluence identified as optimal. RNA dilution and volume-response studies further showed that rescue efficiency declined with lower RNA input, and increased with higher reagent:RNA ratios up to 3× the recommended volume, plateauing at higher amounts. MessengerMAX provided the greatest analytical sensitivity and the final protocol was established using these data.

The optimised method was applied to a panel of field samples and archival materials that had failed to yield live virus by standard isolation methods. Using chemical transfection of extracted RNA, infectious virus was successfully recovered and serotyped from samples originating in multiple countries across different FMDV endemic pools, including serotype SAT1, SAT2, SAT3 and O viruses. These findings demonstrate that RNA-based recovery can generate infectious virus from samples that would otherwise return limited information.

This protocol provides a practical route to recover infectious FMDV from PCR-positive, cell culture-negative samples, improving access to isolates for serotyping, sequencing, and vaccine-strain matching. Its implementation will substantially increase the value of field submissions and strengthen regional and global FMDV surveillance and vaccine selection efforts.

Keywords: FMDV, RNA Transfection, Virus Recovery, Diagnostics

Poster 4.08

Tropism of an avian H5N1 virus for bovine cells is determined by the HA2 subunit regulating membrane fusion

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In 2024, H5N1 highly pathogenic avian influenza (HPAI) viruses of clade 2.3.4.4b were reported for the first time in dairy cows in Texas, USA. Unlike typical influenza infections, the virus replicated primarily in mammary gland tissue rather than the respiratory tract, causing a mastitis-like disease and resulting in high titers of infectious virus in milk.
To investigate viral determinants of bovine cell tropism, we compared the replication of three H5N1 viruses in bovine macrophage (BoMac) and bovine kidney (MDBK) cells. For comparison, replication was also assessed in canine kidney (MDCK) and human lung epithelial (Calu-3) cells. The viruses included the bovine isolate H5N1Tex/24, the highly pathogenic avian isolate H5N1Yam/04, and the low-pathogenic avian isolate H5N1Hok/04. To permit work under biosafety level 2 conditions, the polybasic HA cleavage sites of H5N1Tex/24 and H5N1Yam/04 were modified to monobasic motifs. Reverse genetics was used to generate reassortant viruses and identify genomic determinants of cell tropism.
In BoMac cells, H5N1Tex/24 and H5N1Yam/04 replicated efficiently, whereas H5N1Hok/04 showed poor replication. Both H5N1Tex/24 and H5N1Hok/04 replicated poorly in MDBK cells, while all viruses reached high titers in MDCK and Calu-3 cells. Reassortment experiments revealed that exchanging polymerase (PB2, PB1, PA), NP, or NS segments had little effect on replication in bovine cells. In contrast, replacement of the HA segment of H5N1Hok/04 with that of H5N1Tex/24 or H5N1Yam/04 enabled efficient replication in BoMac and MDBK cells. Conversely, introducing the H5N1Hok/04 HA into H5N1Tex/24 markedly reduced replication, identifying HA as a key determinant of bovine cell tropism.
To further investigate this restriction, H5N1Hok/04 was serially passaged in BoMac cells. After 6–7 passages, the virus acquired enhanced replication capacity. Genome sequencing identified mutations within the HA2 subunit, and transfer of the adapted HA segment to H5N1Hok/04 conferred efficient replication of the reassortant virus in bovine cells. Polykaryon assays demonstrated increased fusion activity of the adapted HA compared with the parental virus.
These findings indicate that influenza virus cell tropism is influenced not only by HA receptor-binding properties but also by HA-mediated membrane fusion activity.

Keywords: H5N1, Hemagglutinin, bovine cells, reassortant viruses, fusion activity

Poster 4.09

Astrovirus associated fatal encephalitis in a European Shorthair cat, Switzerland 2026

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Astroviruses are single-stranded, positive-sense RNA viruses with high genetic diversity found in a wide spectrum of animal species. In most mammalian species, astroviruses primarily replicate in the gastrointestinal tract, and infections remain subclinical. However, in humans, infections can develop into severe disease, especially in children and immunocompromised patients. In recent years, neuroinvasive astrovirus infections have been reported in humans, as well as in minks, pigs, and ruminants.
Here, we report a fatal case of astrovirus infection in an 11-year-old European Shorthair cat without evidence of immunosuppression. The animal presented with acute, and progressive neurological signs, including tetraparesis, and was euthanized due to a poor prognosis. A neuropathological investigation revealed disseminated, severe poliomeningoencephalitis with neuronal necrosis and neuronophagia, indicating a viral etiology. Using viral metagenomics, we identified the complete genome sequence (6,657 nt) of a novel feline astrovirus, which we designated feline astrovirus CH16. Phylogenetically, this virus clusters with other neuroinvasive astroviruses found in humans and animals. Furthermore, RNA in situ hybridization on formalin-fixed, paraffin-embedded brain tissue sections showed strong viral RNA labeling in association with the histopathological lesions, supporting a causal link between the astrovirus infection and the disease.
To our best knowledge, this is the first reported case of astrovirus-associated encephalitis in companion animals. It highlights the expanding host range of neuroinvasive astroviruses and underscores the need to include astroviruses encephalitis in the differential diagnosis of unexplained neurological diseases in cats.

Keywords: encephalitis, neurology, cat, astrovrius

Poster 4.10

Clinical, immunological and transcriptomic determinants of age-dependent susceptibility to classical swine fever virus

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Classical swine fever virus (CSFV) remains one of the most important transboundary viral pathogens affecting domestic and wild pigs worldwide. While disease outcome is known to depend on viral virulence, host-related factors, particularly age, are also believed to play a critical role in determining susceptibility and clinical progression. However, the molecular mechanisms underlying age-associated differences in disease severity remain poorly understood.
The objective of this study was to investigate the clinical, virological, immunological, and transcriptomic factors associated with age-dependent susceptibility to infection with a highly virulent CSFV strain. Three age groups of domestic pigs were experimentally infected with 10,000 viral particles of the highly virulent Margarita strain. The study included ten neonatal piglets (3 days old), six pigs aged 11 weeks, and six pigs aged 28 weeks. Animals were monitored throughout infection for clinical evolution, survival, viral replication kinetics, and humoral immune responses.
Marked differences in disease outcome were observed among age groups. Infection resulted in severe clinical disease and mortality in neonatal piglets and 11-week-old pigs, whereas animals infected at 28 weeks of age remained clinically healthy despite exposure to the same infectious dose and viral strain. These findings support a strong influence of host age on susceptibility to CSFV infection and disease progression.
To identify molecular mechanisms potentially associated with these contrasting outcomes, transcriptomic analyses based on RNA sequencing (RNA-seq) are currently being performed. Differential gene expression, pathway enrichment analyses, and age-specific immune signatures will be investigated and integrated with clinical, virological, and immunological findings.
The poster will present the comparative clinical outcomes, viral replication dynamics, antibody responses, and the first transcriptomic data obtained from the different age groups.
This study provides a unique experimental model to investigate age-related determinants of susceptibility and resistance to CSFV infection and may contribute to the identification of host pathways and biomarkers relevant for disease control, surveillance, and vaccine development.

Keywords: African swine fever virus (ASFV); minimal infectious dose; decomposing tissues, bone marrow, environmental transmission

Poster 4.11

Efficient CRISPR-Cas9 gene targeting of primary porcine macrophages for virological and immunological studies

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The understanding of pathobiological mechanisms of porcine viral diseases and associated immune responses is limited by the scarce availability of broadly applicable genetic tools to test molecular mechanisms essential for virus entry and replication cycle and the function of immune response mechanisms. The challenge is even stronger when studying myeloid cells, which are notoriously difficult to handle using classical transfection and transduction systems, which tend to activate immune cells and modify their responses.
To address this gap, we optimized conditions for electroporation of freshly isolated porcine blood monocytes with CRISPR/Cas9 ribonucleoprotein (RNP) complexes to efficiently target porcine genes. Transfected cells were then differentiated in vitro into macrophages and validated in studies of inflammatory cytokine production, integrin-mediated cell-cell interactions, and entry of macrophage-tropic viruses such as porcine reproductive and respiratory syndrome virus (PRRSV) and African swine fever virus (ASFV).
We show that parameters previously optimized for RNP nucleofection of human and mouse monocytes can be further improved for enhanced gene targeting in porcine monocytes, where >80% of the resulting differentiated macrophages do not express the target protein detected by flow cytometry. We found that targeting Tnf efficiently blocks the release of this cytokine in targeted differentiated macrophages stimulated with E. coli lipopolysaccharide (LPS). We showed that targeted deletion of the PRRSV receptor CD163 was associated with reduced infection. Furthermore, we confirmed that deletion of the integrin CD11c in macrophages is essential for modulation of type I interferon responses in co-cultured porcine plasmacytoid dendritic cells. We efficiently targeted the expression of major histocompatibility complex II molecules by targeting the class II transactivator (CIITA). However, deletion of CIITA did not block ASFV entry in macrophages. Finally, we also identified that the gene targeting efficiency can differ depending on the supplier of recombinant Cas9 and that some Cas9 production batches contain unidentified contaminants that can trigger inflammatory cytokine secretion and a subsequent tolerance state that inhibit further stimulation of macrophages by LPS.
Overall, we have optimized and efficient CRISPR/Cas9 approach in porcine blood monocyte-derived macrophages, which can be used for a range of immunological and virological studies focusing on porcine epizootic viruses.

Keywords: CRISPR/CAs9, macrophage, African swine fever, cytokines

Poster 4.12

Vinculin: an unexplored player in classical swine fever virus infection

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Background and Objectives
Vinculin (VCL) is a cell junction and adhesion protein with multiple functions. Little is known about its role in viral infections, despite evidence suggesting its relevance for important human pathogens, including dengue virus (DENV) and human immunodeficiency virus (HIV). For classical swine fever virus (CSFV), an economically highly relevant animal pathogen, a possible role of VCL in cell entry was suggested based on a single knockdown screen in epithelial cells. In this study, we investigated the impact of VCL on cell permissivity to CSFV.

Material and Methods
VCL knockout cells were engineered by two different CRISPR/Cas9 gene editing strategies applied on two porcine epithelial cell lines. Corresponding rescue cells were generated by lentiviral trans-complementation. Modified cells were genetically and phenotypically characterized for expression of VCL and the tight junction protein Zonula occludens-1 (ZO-1). Subsequently, genetically modified cells were used in CSFV infection experiments. The proportion of infected cells was determined, and viral load was quantified.

Results
Successful VCL knockout and VCL rescue was confirmed by genetic characterization as well as on protein level. Viability was demonstrated to be similar when compared to the parental cells. The absence of VCL led to a distorted ZO-1 expression pattern which was largely restored by VCL rescue. Knockout in both cell lines resulted in an enhanced infection with CSFV that could be partly reversed by VCL rescue, depending on the virus strain, cell line and experimental conditions. Effects varied across viral strains, with the most pronounced effects showing an increase of infected cells (about 20-fold) and an increase in viral titres (about 40-fold) at 72 hours post infection.

Conclusion
The presented findings suggest that the absence of VCL increases permissivity of different porcine epithelial cells to CSFV, although its knockdown was reported to reduce permissivity. In line with our findings, knockdown of VCL and its interaction partner Talin were previously shown to result in an enhanced infection with HIV pseudotypes whereas overexpression had opposite effects. Our findings and existing literature suggest a complex and dynamic role of VCL in viral infection, potentially dependent on the presence but also on the amount of VCL. Future studies will focus on elucidating the underlying mechanism and investigate whether observed effects are result of altered cell barrier integrity or other functions relevant for viral replication.

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Keywords: classical swine fever virus, virus–host interaction, viral infection

Poster 4.13

Persistent classical swine fever virus infection is associated with early cytotoxic immune dysregulation despite absence of clinical disease

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Classical swine fever virus (CSFV) persistent infection represents a major obstacle for disease control because persistently infected animals remain clinically healthy while maintaining high viral loads and continuous viral shedding, acting as silent reservoirs for viral dissemination. However, the early immunological mechanisms underlying the establishment of persistent infection remain poorly understood.
In this study, two litters of neonatal piglets (15 and 16 animals) were intranasally inoculated at 6 days of age with the moderately virulent CSFV Cat01 strain, previously characterized by its ability to induce postnatal persistent infection. In parallel, a third litter of 15 piglets was vaccinated with the live attenuated C-strain vaccine. Clinical, virological, immunological, and transcriptomic analyses were performed at 7 days post infection or vaccination, before the appearance of clinical signs or detectable antibodies.
Despite the absence of clinical manifestations, Cat01-infected piglets showed markedly elevated CSFV RNA loads in serum and secretions together with significantly increased serum IFN-α levels compared with vaccinated animals. RNA-seq analysis of PBMCs revealed clearly distinct immune signatures between groups. Persistently infected piglets exhibited significant upregulation of cytotoxic effector genes, including gnly, prf1, gzmb, faslg, tbx21 and runx3, together with enrichment of natural killer cell-mediated cytotoxicity, cytokine signaling, and JAK–STAT pathways. In contrast, vaccinated animals showed higher expression of genes associated with antigen presentation and adaptive immune priming, consistent with a more coordinated protective immune response. Increased expression of the immunoregulatory genes lag3 and ido1 was also detected in persistently infected animals, suggesting simultaneous activation of inhibitory pathways associated with dysfunctional antiviral immunity. Importantly, RT-qPCR validation confirmed marked upregulation of gnly expression specifically in persistently infected animals but not in vaccinated piglets.
These findings demonstrate that early persistent CSFV infection is characterized by a dysregulated cytotoxic immune state associated with impaired adaptive immune programming, providing new insights into the mechanisms underlying viral persistence and identifying candidate biomarkers for early discrimination between persistent infection and vaccination.

Keywords: CSFV, persistent infection, transcriptomic, cytotoxicity, IFNalpha

Poster 4.14

THE 3D POLYMERASE OF FOOT-AND-MOUTH DISEASE VIRUS INTERACTS WITH THE CARD DOMAIN OF PORCINE MAVS AND INHIBITS TYPE I INTERFERON SIGNALING

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Foot-and-mouth disease (FMD) is a highly contagious and devastating viral disease affecting domestic and wild artiodactyls. The causative agent, Foot-and-Mouth Disease Virus (FMDV), is a picornavirus of the genus Aphthovirus comprising seven immunologically distinct serotypes (O, A, C, Asia1, SAT1-3) and many subtypes. Although Europe had remained FMD-free since 2011, outbreaks re-emerged in 2025 and 2026. Viral persistence in more than 50% of ruminants following clinical recovery, regardless of vaccination status, remains a major challenge for FMD control. Underlying mechanisms are poorly understood, but previous work suggested that a long-lasting but inefficient innate antiviral response may contributes to virus/host equilibrium. We previously identified interactions between FMDV and the type I interferon (IFN-I) pathway in four susceptible species, including an interaction observed exclusively in pigs, where persistence does not occur, between the viral 3D polymerase (3Dpol) and MAVS, a central adaptor in the IFN-I pathway, linking MDA5 sensing to TBK1 and IRF3 activation. The FMD-Scape project aims to characterize FMDV/host interactions during both acute and persistent phases of infection.
Within this project, our objective was to map the interaction domains between 3Dpol and porcine MAVS using truncated MAVS and GST pull-down affinity chromatography method. In parallel, we extended our previous findings showing that 3Dpol from serotypes O and SAT1 partially inhibits IFN-I induction, reducing pathway activation by 40%, by using luciferase reporter assays to investigate its effects at different stages of the IFN-I induction signalling cascade.
Our results demonstrate that 3Dpol interacts with porcine MAVS through its CARD domain, required for downstream IFN-I signalling. In addition, luciferase reporter assays revealed that 3Dpol from serotypes O and SAT1-3 partially and differentially inhibits IFN-I signalling depending on the stimulated effector. When the pathway was activated through MAVS overexpression, 3Dpol serotype O inhibited signalling by 60%, whereas 3Dpol serotypes SAT1-3 reduced activation by 30%.
We thus identified the interaction domain between 3Dpol and porcine MAVS and showed that 3Dpol exerts inhibit IFN-I signalling upon MAVS overexpression. Taken together, these results suggest that 3Dpol may play an unsuspected role in FMDV evasion of the host IFN pathway and potentially persistance. Further studies will investigate the impact of the 3Dpol/MAVS interaction on innate antiviral responses and viral replication. This work is part of a broader “interferactomics” analysis of FMDV/IFN-I pathway across species including African buffalo, the natural reservoir of SAT viruses, aiming to improve understanding of immune evasion and support development of control strategies for FMD.

Keywords: Foot-And-Mouth Disease Virus (FMDV); 3D Polymerase; MAVS interaction; Interferon Pathway; Virus–Host Interactions; Interactions Domains

Poster 4.15

Unraveling Infectious Disease Pathogenesis through Digital Pathology: The Case of Wesselsbron-Induced Hepatitis

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The digital transformation is revolutionizing the field of pathology. By converting histological features into quantitative data, AI-assisted digital pathology offers new opportunities to characterize disease processes and to detect biological differences that may be difficult to appreciate by conventional microscopic assessment.
In our previous work, we demonstrated that Wesselsbron virus (WSLV), a zoonotic mosquito-borne orthoflavivirus infecting ruminants, can spread from sheep to lambs through milk consumption and observed distinct tissue tropisms for different viral clades. In addition, clinical and laboratory data indicated that the clade I rSA999 strain induced more pronounced hepatic damage than the clade II SAH177 strain. However, the study was based on a relatively low number of animals, and the hepatic lesions were generally mild. Consequently, traditional manual semiquantitative scoring assessment of formalin-fixed paraffin-embedded (FFPE) liver sections stained with hematoxylin and eosin was unable to clearly demonstrate differences between WSLV strains.
To address this limitation, we applied computational pathology techniques in immunohistochemical stains from these liver samples, aiming to further characterize the WSLV-induced hepatic lesions and to investigate histopathological differences between both strains. Immunohistochemical stains targeting viral antigen, T lymphocytes, B lymphocytes, histiocytes, and hepatocyte proliferation were used in FFPE liver samples from WSLV-infected ewes and lambs, including mock-inoculated controls. The stains were analyzed using whole-slide imaging and deep learning-assisted computational analyses with commercial software.
Computational quantification revealed predominantly T-cell and histiocytic hepatitis accompanied by increased hepatocyte proliferation. Moreover, the analysis identified a stronger inflammatory response in animals infected with the more virulent rSA999 strain, providing quantitative evidence for biological differences that had remained difficult to appreciate by conventional histopathological evaluation. Digitally derived parameters also correlated with hepatic WSLV RNA loads and with biochemical markers of hepatic injury (aspartate transferase [AST], bilirubin), and inflammation (adenosine deaminase [ADA]), indicating that digital histopathology reliably detects liver damage and disease severity.
By leveraging whole-slide imaging and deep learning, we present an objective, reproducible approach to characterizing virus-induced hepatitis in FFPE tissues, potentially applicable to other infectious diseases.

Keywords: flavivirus; hepatitis; immunohistochemistry; machine learning; zoonosis.

Poster 4.16

Unbiased N-Terminomic approaches reveal new proteolytic targets of the African swine fever virus protease pS273R in primary porcine macrophages

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African swine fever virus (ASFV) is a large double-stranded DNA virus causing African swine fever (ASF), a lethal disease of domestic and wild pigs with a mortality of up to 100%. The current ASF outbreaks in Europe and Asia have severe animal welfare and socioeconomic consequences due to challenges in wild boar control, biosecurity, and the lack of an effective vaccine. Understanding the >150 ASFV genes is crucial for deciphering virulence and guiding vaccine design. Among the 68 structural proteins, pS273R is the only known viral protease, essential for processing the polyproteins pp220 and pp62 during virion assembly. Although pS273R is known to interact with host proteins, its broader functional roles remain unclear.
To investigate its proteolytic activity, we produced recombinant pS273R and a catalytically inactive mutant. Porcine monocyte-derived macrophages (MDM) were treated with recombinant pS273R or infected with the genotype II ASFV Georgia 2007 strain in independent experiments. Lysates collected at 5 and 30 hours post-infection were analysed using 16-plex tandem mass tag (TMT) labelling and liquid chromatography-mass spectrometry (LC-MS/MS). Cleaved N-termini were identified with the TermineR pipeline and validated using recombinant proteins, infected cells, and synthetic peptide substrates.
Recombinant pS273R efficiently processed pp62 and cleaved SUMO1 and SUMO2/3 substrates in a dose- and time-dependent manner, with preference for SUMO1. LC-MS/MS revealed GlyGly/Xaa as the favored cleavage motif of pS273R. Analysis of ASFV-infected MDM at 30 hpi showed new host-derived neo-N-termini, including six peptides with GlyGly/Xaa sequences. Proteolytic processing of tubulin alpha 1A and SUMO-specific protease 3 (SENP3) was verified in vitro, and SENP3 degradation confirmed in infected cells. Expected cleavage of pp220 and pp62 and identification of G1340L as a novel ASFV protein target highlight pS273R's broader substrate range.
We show that pS273R cleaves host proteins during ASFV infection unveiling new mechanisms contributing to ASFV life cycle and pathobiology. Importantly, we discovered that G1340Las is a novel viral protein target of pS273R suggesting a new mechanism regulating the viral transcription machinery.

Keywords: ASFV, Proteomics, Protease

Poster 4.17

Antibiotic administration reduces early infectious bronchitis virus shedding and modulates antiviral responses in chickens

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Infectious bronchitis virus (IBV) remains one of the most important viral pathogens affecting poultry worldwide, causing substantial economic losses despite widespread vaccination programs. Increasing evidence indicates that the intestinal microbiota plays an important role in shaping host immunity and influencing the outcome of viral infections. However, its involvement in the pathogenesis of IBV infection remains poorly understood.

The aim of this study was to investigate whether antibiotic administration affects the course of IBV infection and host immune responses in chickens. Specific pathogen-free (SPF) chickens were treated with a broad-spectrum antibiotic cocktail prior to infection with two genetically distinct IBV genotypes, QX and 793B. Viral shedding, tissue viral loads, and expression of selected immune-related genes were assessed using quantitative RT-PCR.

At 3 days post-infection, antibiotic-treated chickens exhibited markedly reduced viral shedding from both the respiratory and intestinal tracts compared with untreated birds. The most pronounced effect was observed in cloacal swabs from chickens infected with the 793B strain. In contrast, no significant differences in viral loads were detected in the examined tissues, including the trachea, lungs, intestine, kidneys, and spleen.

Antibiotic administration was also associated with alterations in host immune responses. Although the expression of type I interferons was reduced following infection, antibiotic-treated birds showed increased expression of interferon-stimulated genes, including IFIT5 and OASL, together with enhanced expression of selected pro-inflammatory cytokines. These findings suggest activation of alternative antiviral pathways that may contribute to limiting viral dissemination and shedding during the early stage of infection.

In conclusion, although the use of antibiotics as an antiviral intervention cannot be justified, this study revealed unexpected relationships between antibiotic administration, viral shedding, and host immune responses. The findings suggest that antibiotic-induced changes within the intestinal environment may influence the early stages of IBV infection. Further studies, including microbiome profiling, are required to elucidate the mechanisms underlying these observations and to better understand the role of microbiota-host-virus interactions in IBV pathogenesis.

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Keywords: Infectious bronchitis virus (IBV), host-pathogen interactions, gut microbiota, innate immunity, viral shedding