



**EPIZONE**

**BOOK of  
ABSTRACTS**

**15<sup>th</sup> EPIZONE  
Annual Meeting  
*New Perspectives  
for the new Era***

**Novi Sad, Serbia  
April 26-28, 2023**

Hosted by Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia



# Welcome



Dear EPIZONE friends,

It is our great pleasure to welcome you to the **15th Annual Meeting of EPIZONE**. The meeting will be held from 26-28 April 2023 in Novi Sad, Serbia, hosted by the Scientific Veterinary Institute "Novi Sad", the Serbian National Research Institute for Animal Health.

The theme of the EPIZONE 15th Annual Meeting will be "**New perspectives for the new era**", referring to the post-COVID time, when everything seems to be different in the world, and the aim of EPIZONE2023 is to point out what are important issues within the field of epizootic diseases research and control today.

The focus is on current research efforts in the field of epizootic animal diseases of livestock, i.e. different animal species, but also wildlife. Respected keynote and invited speakers will be chosen to give abroad perspective, up to date knowledge and the current overview of situation for different epizootic and emerging diseases. Special attention is given to African swine fever and Avian influenza due to their unfavourable epizootic situation and devastating influence on animal health and welfare, public health and economy.

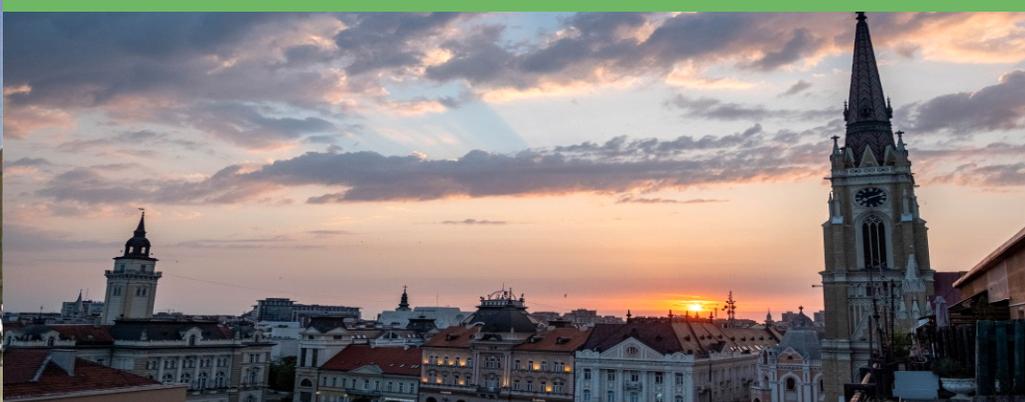
As usual, the meeting shall bring together an outstanding and diverse group of experts from basic to applied science in order to encourage scientific exchange and to establish new contacts and collaborations. Also, the Young EPIZONE group will organize an attractive programme for young scientists in the field of epizootic diseases.

The city of Novi Sad is a multicultural town with rich history and being the European Capital of Culture in 2022 has a lot to offer to our participants.

We are looking forward to hosting you in our beautiful city on the river Danube!

Wim van der Poel  
EPIZONE ERG Coordinator

Tamaš Petrovic  
President of the Organizing Committee of EPIZONE2023



# Organizers

The 15th EPIZONE Annual Meeting  
is organized by:

**The Scientific Veterinary Institute “Novi Sad”  
Novi Sad, Serbia**  
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# Acknowledgement

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- **Biljana Đurđević**, Scientific Veterinary Institute “Novi Sad”, Serbia

## Programme at a Glance

APRIL 26		Wednesday			
Time	Auditorium	Hall 1	Hall 2	Poster Hall	Reception desk
08.00 – 09.00					
09.00 – 11.30					
11.30 – 13.00	Young Epizone meeting		Epizone Coordination Forum and Executive Committee Meeting	Hanging your poster	
13.00 – 14.00	Lunch break in the restaurant for Young Epizone, Coordination Forum and Executive Committee members				Registration
14.00 – 14.30	Opening Session				Speakers check-in
14.30 – 15.00	Keynote lecture 1 Budimir Plavšić, WOAH <b>Challenges to prevent and control emerging diseases</b>			Hanging your poster	All kind of information
15.00 – 15.30	Keynote lecture 2 Claire Guinat, ENVT, INRAE,FR <b>The new era of phylodynamics</b>			Poster viewing available all afternoon	
15.30 – 16.00	Coffee break - poster and stand session				
16.00 – 18.00	Emerging and re-emerging diseases	Pathogen evolution, pathogenesis and immunology			
18.00 – 20.00	Welcome Cocktail – lobby of the Congress Center				

APRIL 27		Thursday		
Time	Auditorium	Hall 1	Poster Hall	Reception desk
09.00 – 09.30	Keynote lecture 3 Bryony Jones, APHA, UK <b>PPR – prospects for eradication</b>			
09.30 – 10.00	Keynote lecture 4 Jasna Prodanov Radulović, NIV-NS, RS <b>African swine fever in Serbia</b>			
10.00 – 10.30	Coffee break - poster and stand session			
10.30 – 12.30	<b>Diagnostic tools and disease surveillance</b>	<b>African swine fever</b>		<b>Registration</b>  Speakers check-in
12.30 – 13.30	Lunch break in the restaurant			All kind of Information
13.30 – 14.00	Keynote lecture 5 Alessio Lorusso, IZSAM, IT <b>Epizootic hemorrhagic disease virus in Europe</b>		<b>Poster viewing available all day long</b>	Issuing a certificate of attendance
14.00 – 14.30	Keynote lecture 6 Rowland Kao, Roslin Institute, Edin., UK <b>Modelling in a Changing World</b>			
14.30 – 15.00	Coffee break - poster and stand session			
15.00 – 17.00	<b>Avian influenza</b>	<b>Epidemiology and risk assessment</b>		
17.00 – 18.30	Social program – more information available soon			
20.00 – 00.30	<b>Gala dinner</b>			



APRIL 28		Friday		
Time	Auditorium	Hall 1	Poster Hall	Reception desk
09.00 – 09.30	Keynote lecture 7 Kerstin Wernike, FLI, Germany <b>Schmallenberg virus vaccine development</b>		Poster viewing	Speakers check-in
09.30 – 10.00	Keynote lecture 8 Tamaš Petrović, NIV-NS, RS <b>Flaviviruses in Serbia</b>			
10.00 – 10.30	Coffee break - poster and stand session			
10.30 – 12.30	<b>Vector-borne diseases</b>	<b>Vaccine development and disease control</b>		Issuing a certificate of attendance
12.30 – 13.00	<b>Closing Ceremony</b>		Poster removal Posters left after lunch will be destroyed	
13.00 – 14.00	Light Lunch in the restaurant			
14.00 – 18.00	Social program – Charm of Srem Region - social & tourist tour			



## 15<sup>th</sup> Annual Meeting of Young EPIZONE Programme at a Glance

**WEDNESDAY 09:00-13:00**

**Hall 1**

- 09:00-09:15h** Welcome  
(dr Tamas Petrovic, dr Biljana Djurdjevic, Vladimir Gajdov)
- 09:15-10:00h** Academic and research career – challenges and opportunities with Q&A session  
(Prof. dr Wim van der Poel, Wageningen University and Research)
- 10:00-10:45h** Digital Epidemiology – Application of Artificial Intelligence in Disease Outbreak Surveillance with Q&A session  
(dr Helmi Zakariah, WHO/ITU Working Group for AI in Health)
- 10:45-11:15h** Break
- 11:15-12:00h** Highly-sensitive diagnostics using type III CRISPR-Cas systems with Q&A session  
(dr Raymond Staals, Wageningen University and Research)
- 12:00-13:00h** Short introduction of Young EPIZONE participants - open discussion and getting to know each other
- 20:00-00:00h** Social event

## Detailed Programme

**Wednesday, April 26, 2023**

08:00 – 18:00	<b>Registration/Speakers check-in/information at Reception desk – Lobby</b>
09:00 – 13:00	<b>Young Epizone – Auditorijum</b> (Restricted meeting)
10:45 – 11:30	<b>Coffee break – lobby and Poster Hall</b>
09:00 – 13:00	<b>Epizone Coordination forum – Hall 2</b> (Restricted meeting)
13:00 – 14:00	<b>Lunch break – Restaurant</b> (Restrictd to Young Epizone, Coordinating forum and Executive Committee members)
14:00 – 14:30	<b>Opening Ceremony</b>
	<b>Welcome addresses:</b> <ul style="list-style-type: none"> <li>- <b>Tamaš Petrović</b> (Local Organizing Committee)</li> <li>- <b>Wim van der Poel</b> (Coordinator of Epizone)</li> <li>- <b>Sava Lazić</b> (Director of Scientific Veterinary Institute Novi Sad)</li> <li>- <b>Miloš Petrović</b> (Chief Veterinary Officer)</li> <li>- <b>Representatives</b> of Local Legal Administration (District and City level)</li> <li>- <b>Representatives</b> of Ministries for Science and for Agriculture</li> <li>- <b>Zoran Milošević</b> (Provincial Secretary for Higher Education and Research)</li> </ul>
14:30 – 15:30	<b>Auditorium</b> <b>Plenary Session 1</b>  <i>Chairs: Wim van der Poel and Tamaš Petrović</i>
14:30 – 15:00	<b>Keynote lecture 1:</b>  <b>Budimir Plavšić (WOAH, Regional representative for Europe)</b> “Challenges to prevent and control emerging and re-emerging animal diseases and zoonoses in Europe: perspective of World Organization for Animal Health”
15:00 – 15:30	<b>Keynote lecture 2:</b>  <b>Claire Guinat (INRAE, France)</b> “The new era of phylodynamics: What pathogen genomes can tell us about epidemic spread?”
15:30 – 16:00	<b>Coffee break - poster and stand session</b>

	Paralel Session – Auditorium	Paralel Session – Hall 1
16:00 – 18:00	<p><b>Emerging and re-emerging diseases</b></p> <p><i>Chairs:</i> Brigitte Cay &amp; Wim van der Poel</p>	<p><b>Pathogen evolution, pathogenesis and immunology</b></p> <p><i>Chairs:</i> Helen Crooke &amp; Lilianne Ganges</p>
16:00 – 16:15	<p><b>Invited Lecture:</b> LSD in Serbia – a successful story of disease control. <b>Vladimir Polaček et al. (Serbia)</b></p>	<p>Bluetongue virus interferes with type I interferon induction by disrupting cGAS pathway. Andrés Louloudes-Lázaro et al. (Spain)</p>
16:15 – 16:30	<p>Engineering recombinant bluetongue viruses expressing reporter genes for in vitro and non-invasive in vivo studies. Javier Ortego et al. (Spain)</p>	<p>Study of goat dendritic cells infection with Peste des petits ruminants virus (PPRV). Pablo Nogales-Altozano et al. (Spain)</p>
16:30 – 16:45	<p>Studies with bat airway organoids of <i>Carollia perspicillata</i> reveal that the respiratory epithelium of bat is not a barrier for interspecies transmission of influenza viruses. Ang Su et al. (Germany)</p>	<p>Characterization of PRCV strains from Europe and the USA. Amalie Ehlers Bedsted et al. (Denmark)</p>
16:45 – 17:00	<p>Cross-Reactivity of Pre-and Post-Pandemic Human, Wild Boar and Farm Animal Sera with Alpha- and Beta-Coronaviruses (CoV) including SARS-CoV-2. Marcel Hulst et al. (The Netherlands)</p>	<p>Interplay between Foot-and-Mouth Disease Virus 3D polymerase and the type I interferon pathway: a contribution to viral persistence? Margan Sarry et al. (France)</p>
17:00 – 17:15	<p>West Nile fever confirmed in a horse in the United Kingdom following recent foreign travel. Nicholas Johnson et al. (United Kingdom)</p>	<p>The porcine low-density lipoprotein receptor (LDLR) plays an important role in Classical swine fever virus (CSFV) infection. Elena Leveringhaus et al. (Germany)</p>
17:15 – 17:30	<p>General discussion</p>	<p>General discussion</p>
17:30 – 18:00	<p><b>Flash presentations (5-6 min each)</b></p> <ul style="list-style-type: none"> <li>- Use of a luminescent reporter virus for rapid assessment of vaccine efficacy against Bluetongue virus. Luis Jiménez-Cabello et al</li> <li>- First report of <i>Porcine respirovirus 1</i> (PRV1) and of <i>Swine orthopneumovirus</i> (SOV) in swine in Italy. Laura Soliani et al.</li> <li>- Bat-borne Issyk-Kul virus: European expansion of a zoonotic agent? Sabrina Canziani et al.</li> <li>- Discussion on presented posters and all other posters in Thematic Session</li> </ul>	<p><b>Flash presentations (5-6 min each)</b></p> <ul style="list-style-type: none"> <li>- Small ruminant lentiviruses: genetic characterization of strains circulating in Italy between 2019 and 2022. Paola Gobbi (<u>Maria Serena Beato</u>) et al.</li> <li>- Milk from rabbit mothers vaccinated against RHD contains a relevant level of specific IgA. Vittoria Di Giovanni et al.</li> <li>- Cytokine secretion in hematopoietic stem cells CD34+ of cattle infected with bovine leukemia virus (BLV). Maria Szczotka et al.</li> <li>- Discussion on presented posters and all other posters in Thematic Session</li> </ul>
18:00–20:00	<p><b>Welcome Cocktail – lobby of the Congress Center</b></p>	

Thursday, April 27, 2023

09:00 – 17:00	<b>Registration/Speakers check-in/information at Reception desk – Lobby</b>	
09:00 – 10:00	<b>Auditorium</b> <b>Plenary Session 2</b> <i>Chairs: Nicholas Johnson and Noemi Sevilla</i>	
09:00 – 09:30	<b>Keynote lecture 3:</b> <b>Bryony Jones (APHA, Weybridge, United Kingdom)</b> “Peste des petits ruminants – prospects for eradication”	
09:30 – 10:00	<b>Keynote lecture 4:</b> <b>Jasna Prodanov Radulović (NIV-NS, Novi Sad, Serbia)</b> “African swine fever in Serbia: lessons learned from the domestic pig cycle in smallholder and backyard sector (2019-2022)”	
10:00 – 10:30	<b>Coffee break - poster and stand session</b>	
10:30 – 12:30	<b>Paralel Session – Auditorium</b> <b>Diagnostic tools and disease surveillance</b> <i>Chairs: Miguel Angel Jimenez Clavero &amp; Alessio Lorusso</i>	<b>Paralel Session – Hall 1</b> <b>African swine fever</b> <i>Chairs: Sandra Blome &amp; Jasna Prodanov Radulovic</i>
10:30 – 10:45	Characterization of classical swine fever virus specific epitopes on the D/A domain of glycoprotein E2 Yu-Liang Huang et al. ( <u>Denis Meyer</u> ; Germany)	A multi gene-approach genotyping identifies 24 genetic clusters of European African swine fever genotype II virus isolates circulating from 2007 to 2022. Nadia Casado et al. (Spain)
10:45 – 11:00	Metagenomics-based protocol for rapid detection of outbreak-causing viruses with ONT technology. Irene Aldea et al. (Spain)	Detection of African swine fever virus (ASFV) and blood meals of porcine origin in hemato-phagous insects collected on a Lithuanian pig farm without ASFV-infected pigs. Ann Sofie Olesen et al. (Denmark)
11:00 – 11:15	Improvement of molecular and serological tools for the detection of Venezuelan Encephalitis Virus (VEEV) in equids. Marine Dumarest et al., (France)	An ASFV-host interaction mapping reveals the role of protein CP204L in the regulation of endosomal trafficking. Katarzyna Dolata et al., (Germany)
11:15 – 11:30	Evaluation of a differential detection ELISA test for the presence of antibodies against West Nile virus and Usutu virus in birds. Francisco Llirente et al. (Spain)	Duration of immunity upon infection with African swine fever virus. Virginia Friedrichs et al. (Germany)
11:30 – 11:45	Newcastle Disease virus containing PCR-targets for West Nile virus as a positive control to validate and implement a Real-time PCR. Melle Holwerda et al. (The Netheralands)	Taking a promising vaccine candidate against African swine fever further: Safety and efficacy tests using “ASFV-G-ΔMGF”. Sandra Blome et al. (Germany)

11:45 – 12:00	Novel diagnostic platform based on LAMP assays for the efficient detection of African and classical swine fever viruses using minimal equipment. Jose A. Bohorquez et al. ( <u>Lilianne Ganges</u> , Spain)	General discussion
12:00 – 12:30	<p><b>Flash presentations (5 min each)</b></p> <ul style="list-style-type: none"> <li>- Molecular Pen-Side test for the detection of the Peste des Petits Ruminants Virus. Milovan Milovanović et al</li> <li>- Improvement of point of care diagnosis of African Swine Fever. Cristina Aira et al.</li> <li>- Utilization of ONT NGS in the workflow of veterinary diagnostics. L.L. Cubas-Gaona et al.</li> <li>- Lateral flow device: a promising tool for field diagnosis of Lumpy skin disease. Stefano Baselli et al.</li> <li>- Recombinant Bovine Ephemeral Fever Virus (BEFV) N protein production: evaluation in a competitive ELISA. Roberto Benevenia et al. (<u>Davide Lelli</u>)</li> <li>- Discussion on presented posters and all other posters in Thematic Session</li> </ul>	<p><b>Flash presentations (5-6 min each)</b></p> <ul style="list-style-type: none"> <li>- High-throughput mapping of virus-host interactions to identify new factors of virulence and pathogenicity for ASFV. Juliette Dupre et al. (<u>Marie-Frédérique Le Potier</u>)</li> <li>- Evaluation of African Swine Fever on East Balkan pigs and backyards in Bulgaria: a qualitative assessment. Elena Lazzaro et al.</li> <li>- A deep sequencing strategy for investigation of virus variants within ASFV infected pigs. Camille Melissa Johnston et al.</li> <li>- Discussion on presented posters and all other posters in Thematic Session</li> </ul>
12:30 – 13:30	<b>Lunch break in the restaurant</b>	
13:30 – 14:30	<p><b>Auditorium</b></p> <p><b>Plenary Session 3</b></p> <p><i>Chairs: Ljubo Barbic and Maria Serena Beato</i></p>	
13:30 – 14:00	<p><b>Keynote lecture 5:</b></p> <p><b>Alessio Lorusso (IZSAM, Teramo, Italy)</b></p> <p>“Epizootic hemorrhagic disease virus: a new challenge for Europe?”</p>	
14:00 – 14:30	<p><b>Keynote lecture 6:</b></p> <p><b>Rowland R. Kao (University of Edinburgh, Edinburgh, United Kingdom)</b></p> <p>“Modelling in a Changing World: addressing the challenges of Emergent Infectious Disease problems under Climate Change and Environmental Land Management Decisions”</p>	
14:30 – 15:00	<b>Coffee break - poster and stand session</b>	
15:00 – 17:00	<p><b>Paralel Session – Auditorium</b></p> <p><b>Avian influenza</b></p> <p><i>Chairs: Ana Maria Moreno &amp; Vladimir Polaček</i></p>	<p><b>Paralel Session – Hall 1</b></p> <p><b>Epidemiology and risk assessment</b></p> <p><i>Chairs: Karin Darpel &amp; Ljubo Barbić</i></p>
15:00 – 15:15	Natural selection of H5N1 avian influenza A viruses with increased PA-X and NS1 shutoff activity. Aitor Nogales et al. (Spain)	<b>Invited lecture:</b> Molecular epidemiology as a tool for understanding the viral infections in honeybees. <b>Ivan Toplak et al. (Slovenia)</b>

15:15 – 15:30	Avian influenza surveillance in wild birds in Lombardy and Emilia Romagna regions (Italy) in 2022- Maya Carrera et al. ( <a href="#">Ana Moreno</a> , Italy)	Pigeon paramyxovirus-1 outbreak in captive pigeons in Denmark, 2022. Karen Martiny et al. (Denmark)
15:30 – 15:45	Identification of amino acid residues required for inhibition of host gene expression by influenza virus A/Viet Nam/1203/2004 H5N1 PA-X. Aitor Nogales et al. (Spain)	Novel diagnostic and statistical tool combinations accurately predict time since bluetongue virus infection in ruminants for assessing transmission risk. Kerry Newbrook et al. (United Kingdom)
15:45 – 16:00	Poultry vector vaccines: innovative serological assays for vaccination monitoring and DIVA testing for H5 avian Influenza A. Stéphanie Lesceu et al. ( <a href="#">Adrien Limozin</a> , France)	A Machine Learning Approach for Predicting the Risk of WNV in Fine Spatiotemporal Scale Relying on Earth Observation Data. Dimitrios Sainidis et al. (Greece)
16:00 – 16:15	Development of a 3d Models of Swine Respiratory Cells for the Study of In Vitro Pathogenesis of Influenza A Virus. Aurora De Mattia et al. (Italy)	Wild boar as a reservoir of Aujeszky disease virus, coronaviruses and circoviruses – a study in African swine fever affected population in Poland. Anna Szczotka-Bochniarz et al. (Poland)
16:15 – 16:30	Highly Pathogenic H5n1 Avian Influenza in Free-Living Griffon Vultures. Ursula Höfle et al. (Spain)	African Swine Fever in Italy: effectiveness of eradication measures in newly affected regions and in Sardinia region. Iscaro Carmen et al. (Italy)
16:30 – 17:00	<p><b>Flash presentations (5 min each)</b></p> <ul style="list-style-type: none"> <li>- Pathology associated with highly pathogenic avian influenza H5N1 in naturally infected birds in Serbia in the 2021/2022 epidemiological year. Biljana Djurdjević et al.</li> <li>- Within-host genetic diversity of wild bird-origin LPAIV H7N7 in poultry. Kamila Dziadek et al.</li> <li>- Characterization of High Pathogenicity Avian Influenza Virus H5Nx Detected in Wild Mammals in Denmark, 2021 and 2022. Yuan Liang et al.</li> <li>- Whole genome sequencing of Avian Influenza virus on the Illumina MiniSeq platform. Vladimir Gajdov et al.</li> <li>- Discussion on presented posters and all other posters in Thematic Session</li> </ul>	<p><b>Flash presentations (5-6 min each)</b></p> <ul style="list-style-type: none"> <li>- Risk assessment of foot-and-mouth disease and biosecurity measures on a cattle farm in Serbia. Dejan Bugarski et al (Serbia)</li> <li>- Discussion on presented posters and all other posters in Thematic Session</li> <li>- General discussion for the whole Thematic Session</li> </ul>
17:00 – 18:30	<b>Social program - Discover Novi Sad – Walking tour</b>	
20:00 – 00:00	<b>Gala dinner – Restaurant Alaska Barka</b>	

Friday, April 28, 2023

09:00 – 14:00	<b>Speakers check-in/information/Certificates at Reception desk – Lobby</b>	
09:00–10:00	<b>Auditorium</b> <b>Plenary Session 4</b> <i>Chairs: Ivan Toplak and Piet van Rijn</i>	
09:00 – 09:30	<b>Keynote lecture 5:</b> <b>Kerstin Wernike (FLI, Insel Riems, Germany)</b> “Schmallenberg virus vaccine development”	
09:30 – 10:00	<b>Keynote lecture 6:</b> <b>Tamaš Petrović (NIV-NS, Novi Sad, Serbia)</b> “Flaviviruses in Serbia – One Health Perspectives”	
10:00 – 10:30	<b>Coffee break - poster and stand session</b>	
10:30 – 12:30	<b>Paralel Session – Auditorium</b> <b>Vector-borne disease</b> <i>Chairs: Sara Savić &amp; Tamaš Petrović</i>	<b>Paralel Session – Hall 1</b> <b>Vaccine development and disease control</b> <i>Chairs: Marie-Frédérique Le Potier &amp; 'Thomas Bruun Rasmussen</i>
10:30 – 10:45	<b>Invited lecture:</b> Companion Animals as Sentinels for Viral Zoonoses in Urban Areas. <b>Ljubo Barbić et al. (Croatia)</b>	Adaptation of the ASFV-989 live attenuated virus on continuous cell line, a step forward to become a vaccine candidate. Marie-Frédérique Le Potier et al. (France)
10:45 – 11:00	Automated remote real-time mosquito surveillance by the means of an intelligent sensor trained with machine learning. Maria Gonzales-Perez et al. (Spain)	(Autonomous) Live cell visualization of African Swine Fever Virus DNA replication for the discovery of antiviral compounds. Alicia Mas et al. (Spain)
11:00– 11:15	Transmission of West Nile virus by <i>Culex pipiens</i> Infected with <i>Culex Flavivirus</i> . Albert Burgas Pau et al. (Spain)	Prime-boost strategies using Adeno and MVA vectors for vaccination against PPRV. Ana Carlón et al. (Spain)
11:15 – 11:30	Confirmation of seasonal flavivirus activity at suspect localities through targeted mosquito trapping and testing. Károly Erdélyi et al. (Hungary)	Safety and efficacy upon infection in sheep with Rift Valley fever virus ZH548-rA2, a triple mutant rescued virus. Alejandro Brun et al. (Spain)
11:30 – 11:45	T lymphocytes contribute to protective immunity and pathogenesis in bluetongue virus-infected sheep but not viral replication or transmission. Kerry Newbrook et al. (United Kingdom)	Perspectives of Sterile Insect Technique in control of the Asian Tiger Mosquito: Experiences gained from the first pilot study in Serbia. Aleksandra Ignjatović Ćupina et al. (Serbia)
11:45 – 12:00	General discussion	General discussion

12:00 – 12:30	<p><b>Flash presentations (5 min each)</b></p> <ul style="list-style-type: none"> <li>- Vectors responsible for the transmission of Epizootic Haemorrhagic Disease virus (EHDV) in Italy. Michela Quaglia et al.</li> <li>- Epizootic haemorrhagic disease in Spain, November 2022. Marta Valero-Lorenzo et al.</li> <li>- Screening of Mosquitoes for Usutu Virus in Lombardy region (Northern Italy) 2014–2022. Francesco Defilippo et al. (<a href="#">Davide Lelli</a>).</li> <li>- Seroprevalence of West Nile virus in domestic donkeys in the Special Nature Reserve “Zasavica”, Serbia. Diana Lupulovic et al.</li> <li>- Amplicon-based complete genome sequencing of the West Nile virus. Vladimir Gajdov et al.</li> </ul> <p>- Discussion on presented posters and all other posters in Thematic Session</p>	<p><b>Flash presentations (5 min each)</b></p> <ul style="list-style-type: none"> <li>- MVA vectors carrying chimeric VP2 of African horse sickness virus increased cross-protection against a heterologous serotype. Eva Calvo-Panilla et al.</li> <li>- Functional analysis of the programmed -1 frameshift signal of porcine respiratory coronavirus (PRCV). Tarka Raj Bhatta et al.</li> <li>- Mapping biosecurity measures applied on different animal production systems across Europe – Progress of COST Action BETTER. Jasna Prodanov Radulović et al.</li> <li>- Developing methodologies to profile humoral immune responses against Crimean Congo haemorrhagic fever virus to support effective vaccine design. Sandra Belij-Rammerstorfer et al.</li> </ul> <p>- Discussion on presented posters and all other posters in Thematic Session</p>
12:30 – 13:00	<b>Closing Ceremony</b>	
13:00 – 14:00	<b>Light Lunch in the restaurant</b>	
14:00–18:00	<b><i>Sharm of Srem Region- social &amp; tourist tour</i></b>	

## Keynote lecture

**Budimir Plavšić DVM, MSc, PhD, Research Associate**

**Regional Representative of WOAHA for Europe - [b.plavsic@woah.org](mailto:b.plavsic@woah.org)**

**Auditorium: April 26, 14.30 – 15.00**



Dr Budimir Plavšić is currently the WOAHA Regional Representative for Europe and head of Regional Representation in Moscow. He obtained his Doctor of Veterinary Medicine degree at the Faculty of Veterinary Medicine in Belgrade, where he started his professional career as an assistant professor for Microbiology. He completed a PhD degree, Master of Veterinary Science, and a Specialisation in Veterinary Epidemiology at the same University. He completed many high-level courses, including on exotic animal disease on Plum Island (USA). He also finished the Faculty of International Management. Dr Plavšić was employed by Ministry of Agriculture of Serbia, as a Head of Animal Health in Serbian Veterinary Authority for 15 years, with significant experience in combating of TADs in real time. Before joining the World Organization for Animal Health in November 2018, he was OIE Delegate of Serbia for 4.5 years. His wide experience encompasses areas such as control of animal diseases and zoonoses (namely: Lumpy Skin Disease, Rabies, HPAI, West Nile Fever, Classical and African Swine Fever, FMD, Brucellosis), development of veterinary legislative, contingency planning, pandemic preparedness, and development of complex information systems. Budimir is a permanent member of the Serbian Veterinary Academy. He is also scientist, with a status of Research Associate and published more than 120 articles. Currently, he is committed to the coordination of WOAHA activities in Europe, particularly, with GF-TADs partners as a Secretariat of RSC for Europe, and Quadripartite partners, as active member of Executive Level of the Regional One Health Mechanism for Europe.

## Challenges to prevent and control emerging and re-emerging animal diseases and zoonoses in Europe: perspective of World Organization for Animal Health

Even though there is outstanding progress in medicine, several health threats remain, including emerging infectious diseases, biodiversity loss and climate change with damaging outcomes. Most of these challenges result from complex human, animal and environmental health interactions. During the last 40 years, there have been six pandemics, all of which have originated from the spillover of zoonotic diseases, mainly from wildlife. Risks of emerging pandemic threats underline the validity of the One Health concept in understanding and encountering global health risks. This approach is critical for controlling priority zoonotic diseases such as rabies, HPAI, and many others. Still, its multidisciplinary nature is crucial for preventing animal diseases, such as ASF, FMD, PPR, and other cross-cutting issues, such as antimicrobial resistance, food safety, and healthcare infrastructure. Drivers for increasing health risks, such as climate and land use changes, agricultural practices, globalisation, and wildlife trade, provide multiple opportunities for pathogens to evolve into new forms, making spillover events from animals to humans and vice versa more frequent and intense, and to reach other regions. Therefore, livestock animals and wildlife should be handled with attention and care by Veterinary Services, wildlife authorities and researchers and adequately covered with disease surveillance programmes.

Managing these global health risks requires the cooperation of the animal, human, plant and environmental health sectors. While shaping health policy frameworks, political actors should implement the Whole of Society approach, moving beyond portfolio boundaries of individual health services towards direct collaboration between authorities while engaging intergovernmental organisations, academia, communities, religious institutions, civil society, media, voluntary associations and the private sector.

The World Organisation for Animal Health (WOAHA, founded as OIE) brings its animal health and welfare expertise to the multisectoral partnerships, provides global early warning information, and assists Members in developing national emergency management capacity with cross-border collaborative philosophy. We aim to develop global strategies to tackle major diseases or broader health threats in collaboration with other health organisations and national veterinary authorities. The management of animal health emergencies remains a core function of all governments, particularly with the liberalisation of world trade. Therefore, it is of critical importance to ensure sustainable capacity building of national health services, from the farm level to the final processed product, developing and implementing of animal health policies in line with international standards for disease prevention, preparedness, response and recovery, in close collaboration with national and regional stakeholders.

**Keywords:** multisectoral collaboration, disease prevention, response

## Keynote lecture

**Claire Guinat DVM, MSc, PhD, Research scientist**

**National Research Institute for Agriculture, Food and Environment-INRAE,  
France - [claire.guinat@envt.fr](mailto:claire.guinat@envt.fr)**

**Auditorium: April 26, 15.00 – 15.30**



Dr Claire Guinat obtained her Doctor of Veterinary Medicine degree from the National Veterinary School of Toulouse (ENVT) in 2012. Subsequently, she completed a Master of Science in Animal Health and Epidemiology at the French Agricultural Research Centre for International Development (CIRAD). In 2016, she received her PhD in Veterinary Epidemiology from the Royal Veterinary College (RVC) and The Pirbright Institute. After completing her PhD, Claire completed a four-year postdoctoral fellowship at ENVT. In 2020, she joined ETH Zürich as a Marie-Curie Individual Post-Doctoral Research Fellow. Today, Dr Claire Guinat serves as a Research Scientist at the National Research Institute for Agriculture, Food and Environment (INRAE) in France. She is also member of the scientific expertise committee on Animal Health and Welfare at the French Agency for Food, Environmental and Occupational Health Safety (Anses) and has participated in several working groups on African swine fever for the European Food Safety Authority (EFSA). Currently, she is member of the working group on avian influenza and vaccination. Claire's research interests are centered on investigating the transmission dynamics of zoonotic and animal infectious diseases, with the aim of improving our ability to predict and control future outbreaks. Her work integrates pathogen genome sequences and case surveillance data to reconstruct epidemics and determine the determinants of disease spread. Her research is highly inter-disciplinary, employing a combination of epidemiology, disease modelling, phylodynamics and ecology. Currently, she is involved in projects that focus on avian influenza, African swine fever, peste des petits ruminants, and hepatitis E virus.

## The new era of phylodynamics: What pathogen genomes can tell us about epidemic spread?

Infectious diseases represent a major burden for global economies and public and animal health. Until now, the quantification of the spread of infectious diseases to inform the development of control strategies has traditionally relied on epidemiological data collected during outbreaks. To do this, mathematical models are fitted to epidemiological data (number, date, and location of infected animals or livestock buildings) collected during outbreaks to estimate key transmission parameters, such as the basic reproduction number ( $R_0$ , the expected number of secondary infections generated by an infected epidemiological unit in a completely susceptible population).

Although epidemiological data is of crucial importance for quantifying transmission parameters during outbreaks, pathogen genetic data constitutes another extremely valuable source of information. The increasing accessibility of pathogen genetic data has recently led to extensions in the field of phylogenetics, called phylodynamic approaches, which also allow for this objective to be achieved.

Using genetic sequences from pathogens circulating during an epidemic, phylodynamic approaches allow to infer past transmission events that were not observed using epidemiological data alone. Thus, phylodynamics allow for a greater understanding of the spread of infections, estimation of epidemiological and clinical parameters, and identification of factors influencing the risk of infection. It can provide key information to support policy-making in public and animal health.

We present here examples where phylodynamic approaches have recently been successfully applied to a range of threats to animal and public health. In particular, we provide examples in which these approaches have been relevant to inform on the number of unreported infections, to reconstruct unobserved infections prior to the first officially reported infection, and to distinguish local transmission events vs. importations, which is more difficult with traditional epidemiological methods. Phylodynamics can be used as complement to epidemiological studies.

**Keywords:** phylodynamics, epidemiology, animal infectious diseases, transmission and evolution, animal health

### Keynote lecture

**Bryony Jones BVSc, MSc, PhD, MRCVS**

Lead Analytical Epidemiologist, Animal and Plant Health Agency, Department for Environment, Food and Rural Affairs, UK - [bryony.jones@apha.gov.uk](mailto:bryony.jones@apha.gov.uk)



**Auditorium: April 27, 09.00 – 09.30**

Dr Bryony Jones is currently a Lead Analytical Epidemiologist at the Animal and Plant Health Agency, Department for Environment, Food and Rural Affairs, UK. She obtained her degree in veterinary science at Bristol University, UK, and worked in private veterinary practice for a few years. After completing an MSc in Tropical Veterinary Medicine at Edinburgh University, she worked as a government veterinary officer in the Republic of Kiribati, and then moved to South Sudan where she managed community-based animal health programmes for Unicef and NGOs, and then led the South Sudan rinderpest eradication programme to its successful conclusion. She then spent two years with ILRI (International Livestock Research Institute) in Nairobi, Kenya, building field surveillance capacity for highly-pathogenic avian influenza in East Africa and Egypt. Returning to the UK, she completed an MSc in Vet Epidemiology and joined the Royal Veterinary College Veterinary Epidemiology, Economics and Public Health Group, initially as a Research Assistant while doing a part-time PhD, and then as a post-doctoral researcher and research fellow. Her PhD research was a mixed methods study on small ruminant production, marketing, and health in the Afar Region of Ethiopia, followed by post-doctoral studies focussing on the epidemiology of peste des petits ruminants (PPR) in livestock and wildlife in East Africa. In 2021 she joined the Department of Epidemiological Sciences at the Animal and Plant Health Agency, where she mainly works on the design and analysis of epidemiological studies, and evaluation of disease surveillance. She maintains an interest in PPR and is currently Chair of the PPR Global Research and Expertise Network.

### Peste des petits ruminants – prospects for eradication

Peste des petits ruminants virus (PPRV) causes disease in goats and sheep in Africa, the Middle East and Asia, with a severe impact on livelihoods and livestock trade. It is closely related to rinderpest virus, which was eradicated in 2011, and has been targeted for eradication by the World Organisation for Animal Health and the United Nations Food and Agriculture Organisation by 2030. The characteristics and factors that made rinderpest eradication possible are shared by PPRV, making it feasible to remove one of the major constraints to small ruminant production which would benefit many smallholder and pastoralist livestock keepers. A benefit-cost analysis suggested strong economic returns from a well-coordinated time-bound global eradication programme. The key components for a successful eradication programme include: a good understanding of sheep and goat production systems and prevalent diseases, effective surveillance systems and diagnostics to monitor disease occurrence, effectiveness of control measures, and progress towards freedom from infection, effective vaccination campaigns that take into account population structure and contact networks, and strong coordination and leadership at local, national, regional and global levels. However, success is dependent on the availability of adequate resources for coordinated action. Recent research and experiences related to disease surveillance, vaccination delivery and the role of other domestic hosts and wild species in PPRV epidemiology will be reviewed, and areas for further study will be identified.

**Keywords:** peste des petits ruminants, eradication, surveillance, vaccination, epidemiology

## Keynote lecture

**Jasna Prodanov Radulović DVM, MSc, PhD, Senior Research Associate**

Scientific Veterinary Institute Novi Sad, Serbia - [jasna@niv.ns.ac.rs](mailto:jasna@niv.ns.ac.rs)

**Auditorium: April 27, 09.30 – 10.00**



Jasna PRODANOV-RADULOVIC graduated veterinary medicine at the University of Belgrade, Serbia, and has PhD in veterinary medicine. From the 2000 she works as an epidemiologist specialised for swine diseases at the Scientific Veterinary Institute "Novi Sad", Serbia. Her major research interests are in the field of Epidemiology and Swine Diseases, especially Classical Swine Fever, African swine fever and Pseudorabies. Since 2019 she is Senior Research Associate at the Scientific Veterinary Institute "Novi Sad".

<https://niv.ns.ac.rs/en/team/jasna-prodanov-radulovic/>

## African swine fever in Serbia: lessons learned from the domestic pig cycle in smallholder and backyard sector (2019-2022)

African swine fever (ASF) is currently the most important challenge for domestic pig production worldwide. Among Western Balkan countries, Serbia suffered its first case of ASF in a backyard holding in 2019. Since then, numerous outbreaks in domestic pigs and wild boar have been reported throughout the country. The aim of this research was to analyse specific ASF-risks factors in the pig production system identified in the smallholder and backyards sector. The most important gaps in biosecurity measures related to the human activities recognised as social and cultural identity in Serbia were studied in the period 2019-2022.

Serbia has the highest pig density of the countries in the Balkan region. However, in percentage terms, about 50% of the domestic pig units are located in sector with very low biosecurity measures. Rearing backyard pigs is a very common and traditional practice in country rural areas. The main characteristic is existence of breeding animals and practice of natural mating in the villages. The mixed backyard systems are common, with different livestock and agricultural cropping systems, with a focus on subsistence farming. Another risky activity is related to home-slaughtering practice, which present as a cultural and sociological phenomenon. Backyard pig production is oriented on meat production for home consumption but also for selling, as additional income. The production of meat specialties is recognised tradition in the villages. Finally, outdoor keeping, semi-free range pigs is common practice in some regions. The village inhabitants are frequently hunters and hunting activity may potentially create a direct link between domestic and wild boars. In Serbia, swill-feeding is forbidden by law.

Small-scale farming is common in many countries, especially in rural areas of the Balkan region. Backyards are considered particularly susceptible to ASF introduction and are of interest in programs for disease prevention and control. Farmers, backyard owners still have significant knowledge gaps on ASF and continue to practice various risky behaviours that favour disease spread. Traditional, culture-related aspects were found to be very important obstacles in disease control.

**Keywords:** African swine fever, domestic pig cycle, backyards, Serbia

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## Keynote lecture

**Alessio Lorusso DVM, PhD, Virologist**

Veterinary Medical Officer at IZS-Teramo, Italy - [a.lorusso@izs.it](mailto:a.lorusso@izs.it)



**Auditorium: April 27, 13.30 – 14.00**

Dr Alessio Lorusso is currently employed at the IZS-Teramo as permanent Veterinary Medical Officer. He obtained his Doctor of Veterinary Medicine degree at the Faculty of Veterinary Medicine of the University of Bari; next he obtained his PhD degree on the reverse genetics of canine coronavirus after a two-years training period at the Virology Unit of the Faculty of Veterinary Medicine of Utrecht, The Netherlands. He moved to the NADC-USDA of Ames-Iowa (USA) as a Post Doc (Research Microbiologist) on the epidemiology and pathogenesis of swine influenza viruses including the pandemic 2009 H1N1. He also obtained from the University of Teramo a post-graduate school of specialisation degree in Animal Health. Currently, he is attending the Executive Master in Management of Health and Social Care Organizations (Emmas-Milan). His research activities are not restricted to bluetongue and related orbiviruses but they also include morbilliviruses, flaviviruses, and coronaviruses. Deputy Director of the FAO Reference Laboratory for zoonotic coronaviruses. Areas of interest: virus discovery and characterization; virus evolution, next generation sequencing; genome editing via reverse genetics of RNA viruses and pathogenesis studies in animal models of orbiviruses, flaviviruses, coronaviruses, morbilliviruses; zoonoses, public health; diagnosis of infectious diseases via innovative molecular methods; one health, virus emergence.

## Epizootic hemorrhagic disease virus: a new challenge for Europe?

The World Organisation for Animal Health (WOAH) lists epizootic hemorrhagic disease (EHD) as a disease of wild and domestic ruminants caused by EHD virus (EHDV). EHDV is related to bluetongue virus (BTV), the etiologic agent of the bluetongue disease of ruminants. Both viruses belong to the genus *Orbivirus* and circulate in multiple serotypes. Their viral genomes consist of 10 segments (S1–S10) of double-strand RNA; the structural outer capsid protein (coded by S2) determines serotype specificity. Both viruses cause similar clinical signs in cattle and are transmitted by *Culicoides* spp. biting midges. BT primarily affects sheep and in recent decades has been described multiple times in the European Union (EU), causing devastating repercussions on animal trade. Most European BT outbreaks had a direct origin in North Africa because of wind-driven dissemination of BTV-infected midges from this region. At the end of October and beginning of November 2022, respiratory distress, fever, erosions of the muzzle and oral mucosa, and drooling were reported in cattle farms in the south-western part of Sardinia and western Sicily. All animals were positive for EHDV RNA. The outbreak was reported to Italy's Ministry of Health, which in turn notified WOAH and the European Commission, which imposed animal movement restrictions within a 150-km radius of the outbreak sites. On November 18, 2022, EHD was also reported in Andalusia regions of Spain, in the cities of Cadiz and Seville. WGS demonstrated that the Sardinian and Sicilian EHDV-8 strains shares high nucleotide sequence identity (>99.9%) with multiple EHDV-8 TUN 2021 strains sequenced so far. Predicting future scenarios for the EU cattle production system is difficult, but EHD will probably pose new challenges to EU veterinary authorities. The lessons learned with BT should be a reference for choosing proper control and prevention strategies for EHD. Overall, these events further emphasize the importance for countries of Europe of having robust collaborations with authorities in North Africa on public and animal health. The prompt detection of EHDV-8 in Sardinia and Sicily is the most recent example of the benefits that such relationships could yield. This collaboration proved crucial; it led to development of a specific and accurate molecular test for detecting EHDV-8, given that knowledge of the genome constellation and the genomic relatedness of EHDV-8 with extant EHDV serotypes had already been achieved. Vaccine development needs to be boosted because vaccination is the only strategy to reduce virus circulation and prevent direct and indirect economic losses. In the keynote lecture, the most recent updates upon all aspects of EHDV-8 pathobiology are discussed.

**Keywords:** EHDV-8, cattle, Europe, restrictions

### Keynote lecture

**Rowland R. Kao, PhD**

Sir Timothy O'Shea Professor of Veterinary Epidemiology and Data Science, Roslin Institute & School of Physics and Astronomy, University of Edinburgh, UK - rowland.kao@ed.ac.uk



**Auditorium: April 27, 14.00 – 14.30**

Rowland Kao is a mathematical biologist who studies infectious disease dynamics, mainly with respect to the role of demography in the spread and persistence of livestock diseases, such as foot-and-mouth disease, bovine tuberculosis, scrapie, BSE and avian influenza in poultry. This work includes the development of theoretical models of disease transmission on social networks and applications to the transmission of livestock diseases using simple differential equation models, analysis of social networks, statistics and simulations. Increasingly, it involves the integrated analysis of genetic and epidemiological data to determine the characteristics of disease outbreaks, with bovine Tuberculosis being a lead example. As such it integrates a wide variety of topics, most importantly the analysis of networks, but also elements of human behaviour and the management of land resources (why do farmers move livestock the way they do, and what would happen if the conditions under which they moved livestock, changed), risk-based surveillance (can we use livestock movements and other forms of contact to identify individual farms most at risk of disease, and/or of transmitting it) and parameter inference (from observed disease data, can we estimate the relative and absolute importance of different routes of contact). From 2020, he has been part of the UK's COVID-19 response, as a member of SPI-M (the Scientific Pandemic Influenza Modelling Group, providing operational and more recently, strategic advice to the UK government on COVID-19 preparedness). He chairs the UK Government Dept. of the Environment, Food and Rural Affairs (Defra) Exotic and Emergent Diseases Committee, a subcommittee of Defra's Science Advisory Council, of which he is also a member.

### **Modelling in a Changing World: addressing the challenges of Emergent Infectious Disease problems under Climate Change and Environmental Land Management Decisions**

The world is facing simultaneous challenges to address climate change, the reversal of biodiversity loss and maintain food security. This will require a conscious, sustained effort to balance land use across these requirements. Many of the strategies to address these issues rely on increased connectivity, however such increased connectivity also provides opportunities for new pathogens to emerge and for old ones to threaten previously low risk populations. Here, we shall outline key aspects of the problem, using models that capture disease transmission risks via livestock movement networks, to explore how the intersections of human decision-making, land use change and climate change have the potential to exacerbate emergent infectious disease challenges.

We use combinations of simulation and analytical models in a network analysis framing, to illustrate how disease risk interacts with changes in patterns of connectivity. (i) In the first example, we use a simple agent-based network model of simulated farms trading livestock with each other. In this model we explore the disease outcomes associated with a system where there is a higher value associated with maintaining a healthy herd, but higher costs arise when livestock purchased from healthier herds carry a premium. (ii) in the second example, we use a combination of detailed information on farm and wildlife locations, livestock movements, and bacterial whole genome sequence data, and analyse these via machine learning analysis and phylodynamic models. Using these models, we explore the drivers of an emergent bovine Tuberculosis (TB) problem in Northern England, in an area previously considered at low risk of sustained TB circulation. From example (i) we show that despite having no inherent behavioural differences in simulated individuals, divergent buying patterns can emerge solely due to stochastic differences in disease experience. From example (ii) we show that isolates of bacteria from cattle and badgers that are more genetically similar and therefore more closely related to each other, are not only be more closely linked in time and space, but also linked via 'network' measures associated with farm management, and with cattle playing a prominent role in circulation.

Here we have provided evidence that changes in land management practices can play a role in the emergence of infectious disease which in turn may impact on our ability to reach environmental, biodiversity and food sustainability targets. As infectious diseases risks are likely to become increasingly unpredictable, incorporating this risk in assessments into land management decision-making is urgently required.

**Keywords:** land use, climate, biodiversity, emergent infections

**Funding source:** The results presented here have been funded by The Wellcome Trust and the BBSRC

## Keynote lecture

**Kerstin Wernike, DVM, PhD**

**Head of WOAH Reference Laboratory for Bovine Viral Diarrhea and German National Reference Laboratory for Bovine Viral Diarrhea and Schmallenberg virus, Friedrich-Loeffler-Institut, Germany - kerstin.wernike@fli.de**



**Auditorium: April 28, 09.00 – 09.30**

PD Dr. Kerstin Wernike studied veterinary medicine in Berlin, Germany, has a doctoral degree in veterinary medicine (LMU Munich, Germany) and is a board-certified expert in veterinary virology. She gained her habilitation (*venia legendi*) in animal health and animal welfare at the University of Rostock. Since 2016, Kerstin Wernike is senior scientist at the Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, Germany. She is head of a WOAH reference laboratory for bovine viral diarrhoea and of the German national reference laboratories (NRLs) for bovine viral diarrhoea/mucosal disease and Schmallenberg virus. In addition, she is deputy head of the German NRL for SARS-CoV-2 infections in animals since 2020. Kerstin Wernike has 13 years' expertise in working with different animal viruses under high containment conditions. Her main research interests include emerging animal viruses, host-virus-interaction, immunoprophylaxis and the development of diagnostic test systems.

## Schmallenberg virus vaccine development

The orthobunyavirus Schmallenberg virus (SBV) was initially detected in 2011 near the German/Dutch border region. Thereafter, it spread rapidly throughout the European continent, thereby causing a large-scale epizootic of fetal malformation in the ruminant population. By now, SBV established an enzootic status in Central Europe with regular wave-like re-emergence, which has prompted intensive research efforts in order to elucidate the pathogenesis and to develop countermeasures. SBV is transmitted by insect vectors (*Culicoides* biting midges) and, therefore, vaccination is one of the most important tools of disease control, especially since experience with viruses related to SBV, like Akabane virus and Aino virus in Japan, has demonstrated that vaccination is a very useful and adequate instrument for disease control. Here, we present which vaccine formulations have been tested to prevent SBV infections in the major target species, namely cattle, goats and/or sheep.

In a first step, inactivated SBV candidate vaccines were developed, and it could be shown that they efficiently protect against experimental SBV infection, in contrast to a heterologous multivalent Akabane/Aino vaccine that was tested at the same time and that was not able to provide protection from SBV infection. However, the inactivated SBV vaccines did not enable the differentiation of infected from vaccinated animals (=DIVA capability). In order to combat this obstacle, a series of further approaches ranging from modified live, live-vectored, subunit and DNA-mediated vaccine delivery to multimeric antigen-presentation on scaffold particles was developed and evaluated. In short, it was repeatedly demonstrated that the N-terminal half of the glycoprotein Gc, composed of the Gc head and the head-stalk, is highly immunogenic, with a superior immunogenicity of the complete head-stalk domain compared to the Gc head only. Furthermore, in all Gc protein-based vaccine candidates, immunized animals can be readily discriminated from animals infected with the field virus by the absence of antibodies against the viral N-protein.

The unexpected emergence of SBV has demonstrated the constant threat that yet unknown insect-transmitted viruses could suddenly appear in previously unaffected regions. During the decade since SBV was discovered, the virus has become an important model virus to study orthobunyaviruses in general and for the development of vaccines, especially as different orthobunyaviruses share a very similar structural organization. It could be shown that holistic approaches combining immunization-challenge infection studies with structural analyses provide essential knowledge required for an improved vaccine design.

**Keywords:** Schmallenberg virus, Bunyavirus, arbovirus, vaccine, immunoprophylaxis

## Keynote lecture

**Tamaš Petrović DVM, MSc, PhD, Principal Research Fellow**

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**Auditorium: April 28, 09.30 – 10.00**

Tamaš PETROVIĆ, D.V.M., M.Sc, PhD, Principal Research Fellow, Scientific field: Microbiology – virology. Graduated at the Faculty of Veterinary Medicine, University of Belgrade, 1997. Master thesis and doctoral dissertation on the subject of bovine viral diarrhoea virus in the field of contagious diseases and zoonosis defended on the Faculty of Veterinary Medicine, University of Belgrade in 2002 and 2006. Scientific titles: 1998 Research Trainee; 2002 Research Assistant; 2007 Research Associate; 2011 Senior Research Associate; 2016 Principal Research Fellow. Basic Research interest: Veterinary virology, food and environmental virology, vector-borne infections, prevention, diagnostic, control and eradication of contagious diseases and zoonosis. Main working and research activities: detection of animal viral diseases by conventional laboratory methods (virus isolation, VNT, ELISA, IH, AGID), and detection and characterization of viruses by molecular diagnostic methods. Participating and or coordinating 20 national, 5 bilateral and 9 international research projects. Published more than 300 research or professional papers. Employed in Scientific Veterinary Institute Novi Sad on Department of virology from 1997. Current position: Head of the Research Division, President of the Scientific Council and Head of Virology Department. <https://niv.ns.ac.rs/en/team/tamas-petrovic/>

## Flaviviruses in Serbia – One Health Perspectives

Today we are witnessing an increasing number of zoonoses, especially vector borne ones, that are emerging and spreading throughout the world because of climate change and the increasingly intense movement of people and goods. Only the multidisciplinary approach that is the basis of "One Health" provides a comprehensive and precise collection of data that is necessary for quick and effective action for timely discovery and for the establishment of a system for monitoring and control of the existing and new vector borne zoonoses. The aim of this work is to summarize the data about the presence/prevalence of some zoonotic flaviviruses that circulate in Serbia in the last decade, and to point the necessity of One Health approach in their surveillance and control.

After some historical data, the first data on the presence of West Nile virus (WNV) in Serbia was obtained by serological testing of horses in the northern part of the country, i.e. in 12% (46/349), 28.6% (72/252), 49.2% (64/130) and in 46.9% (45/96) blood sera of horses sampled during 2009/2010, 2011, 2012 and 2013. WNV circulation was also confirmed by 5/92 (5.4%) WNV antibody positive blood sera and 8/82 (9.8%) WNV RNA positive tissues from 134 wild birds collected during 2011/2012. In 2010 and 2013, 3/50 (6%) and 28/306 (9.15%) sampled mosquito pools tested positive for WNV RNA. Also, 5.04% (17/337) human blood sera tested WNV antibody positive in 2010. Human WNV clinical outbreaks were recorded each year, starting from 2012, when the first human epidemic was recorded. Until now, the biggest human epidemics were detected in 2013 (302 cases / 35 deaths), in 2018 (415 cases / 36 deaths) and in 2022 (226 cases / 12 deaths). As a response to this epidemiological situation, the integrated national WNV surveillance programme, conducted and funded by the state veterinary system, has been established since 2014 and has been successful in detection of the WNV presence in sentinel animals, wild birds, and mosquitos before human outbreaks in each season. Despite the fact that tick-borne encephalitis (TBE) has been a notifiable disease in Serbia since 2004, there is no active TBE surveillance program of serologic or molecular screening of TBE virus (TBEV) infections in humans in the country. In the last years, TBEV presence and circulation is continuously confirmed by seropositive findings in sporadic testing of humans, often without clinical manifestation of the disease, like findings of 0.37% (1/267) seropositive human sera collected between 2014 and 2015, and 13.27% (15/113) seropositive humans among those exposed to tick bite in 2020. Sporadic clinical human cases of TBE in Serbia are periodically detected. Several decades after the first published data, the presence of TBEV was demonstrated by real-time RT-PCR and virus sequencing from *Ixodes ricinus* tick samples collected during 2014 and 2015 from the Fruška Gora Mountain and the territory around Belgrade (prevalence 2% (1/50) and 6.6% (30/450) at 2 out of 17 tested localities). One TBEV isolate from *I. ricinus* tick was sequenced and typed as the Western European subtype. In 2017, the clinical outcome of TBE was detected in one horse in the central part of the country. However, TBEV was not detected in *Ixodes ricinus* ticks collected from 2018 to 2022.

For Usutu virus (USUV) presence in Serbia, only recent data are available. USUV RNA was confirmed in 0.4%, 0.93% and 2.75% of tested *Culex pipiens* mosquito pools in 2014, 2015, and in 2017. One isolate from 2014 was genotyped as USUV Europe 1 lineage, and two isolates from 2017 as USUV Europe 2 lineage. The obtained results confirmed the first findings of specific antibodies in human's blood sera from 2015, horses from 2010, and wild boars from 2011/2012. Although the circulation of USUV in wild birds has been confirmed by serological test in 2012, the presence of USUV in wild birds has not been detected until now.

In Serbia, the integrated monitoring system for arbovirus infections is only partially present. The first steps have been established, but it is necessary to regulate this system in a number of aspects that include: 1. Strengthen the concept of "One Health", integrating surveillance systems in the veterinary and public health sectors, based on legislation; 2. Establishment of a common database of zoonotic events and zoonotic agents related to public health and veterinary sectors; 3. Development of a national plan for zoonotic epidemics within the One Health concept; 4. Strengthen the links between the surveillance systems in public health and veterinary sectors by providing systematic exchange of high quality data, during routine surveillance and crisis events; 5. Development of a national plan for vector control and 6. Strengthening the laboratory capacities in the field of zoonotic diseases.

**Key words:** Flaviviruses in Serbia, One Health concept, integrated surveillance system

**Acknowledgments:** This work was funded by the Ministry of Science, Technological development and Innovation of Republic of Serbia by the Contract No: 451-03-47/2023-01/200031.

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*First Parallel Session*

**Emerging and Re-Emerging Diseases**

*Oral Presentations*

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## Invited lecture

### LSD in Serbia – a successful story of disease control

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Lumpy Skin Disease (LSD) was last diagnosed in Serbia in 2016, when it spread to many Balkan and Eastern European countries. In 2016, 225 outbreaks of this disease were registered in Serbia. The disease was not detected in the north of the country (north of the Danube River). During the disease suppression, a number of measures including stamping out on the affected farms and vaccination were used. The vaccination was carried out using homologous attenuated vaccine (*Neethling strain*) on the territory of the whole country, continuously from 2016 and during the following 5 years (until 30<sup>th</sup> June 2021). From 2017, both active and passive surveillance, including serological and virology monitoring was carried out on the territory of the whole country. In addition, the country was divided into zones where the disease occurred and where the risk of spreading from neighboring countries was higher. The control and surveillance measures differed according to the established zones. These were more complex in the southern parts of the country, where the disease was last detected. This surveillance was carried out until 2022. To date, no new cases of this disease have been registered. The following factors were found to have contributed most to the success in LSD suppression in Serbia:

the capacity of the veterinary service that managed to vaccinate a high percentage of cattle in a short period of time; formation of National Expert Group on LSD National Task Force on prevention and control of LSD (led by Prime minister's office); the willingness of the state to provide financial resources for emergency vaccination of the entire population of cattle in Serbia; a joint coordinated vaccination campaign on the territory of the entire region; a quick compensation policy; the motivation of the veterinary service on the field; trained veterinary specialist staff on the field; laboratory diagnostic capacities with introduced molecular methods for distinguishing the vaccine from the field strain; good coordination between crisis centers; trained teams and equipment for rapid control of infection on the field etc.

With all the measures implemented by the veterinary service of Serbia, especially in 2016, including all the efforts and with the financial support of the EU, the spread of this infectious disease to other territories of the countries of the European continent was successfully prevented.

**Key words:** Lumpy Skin disease, cattle, Serbia, Neethling vaccine

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## Engineering recombinant bluetongue viruses expressing reporter genes for in vitro and non-invasive in vivo studies

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Bluetongue virus (BTV) is the causative agent of the important livestock disease bluetongue (BT), which is transmitted via *Culicoides* bites. BT causes severe economic losses associated with its considerable impact on health and trade of animals. By reverse genetics, we have designed and rescued reporter-expressing recombinant (r)BTV expressing Nanoluc luciferase (Nluc) or the Venus fluorescent protein.

To generate these viruses, we custom synthesized a modified viral segment 5 encoding NS1 protein with the reporter genes located downstream and linked by the Porcine teschovirus-1 (PTV-1) 2A autoproteolytic cleavage site. Therefore, fluorescent signal or luciferase activity are only detected after virus replication and expression of non-structural proteins.

Fluorescence or luminescence signals were detected in cells infected with rBTV-Venus or rBTV-Nluc, respectively. Moreover, marking of NS2 protein confirmed that these reporter genes were only expressed in BTV-infected cells. Growth kinetics of rBTV-Nluc and rBTV-Venus in Vero cells showed a replication rate comparable to that of rBTV. Regarding the stability of these viruses, rBTV-Venus were stable for at least three passages and rBTV-Nluc was stable up to passage six in Vero cells. Infectivity studies of these recombinant viruses in IFNAR (-/-) mice showed a higher lethal dose for rBTV-Nluc and rBTV-Venus than for rBTV indicating that viruses expressing the reporter genes are attenuated in vivo. Interestingly, luciferase activity was detected in the plasma of viraemic mice infected with rBTV-Nluc. Furthermore, luciferase activity correlated with viremia levels of infected mice throughout the infection. In addition, IFNAR-/- mice were subcutaneously infected with rBTV-1-Nluc and in vivo imaging of Nluc expression was detected in the target tissues for BTV replication in IFNAR-/- mice and ruminants.

We have for the first time investigated the in vivo replication and dissemination of BTV in IFNAR (-/-) mice using BTV-Nluc and non-invasive in vivo imaging systems (IVIS). rBTV expressing reporter genes provide an ideal biotechnological asset in the ongoing fight to better characterize BTV and identify new therapies for BT disease.

**Keywords:** bluetongue, fluorescent, luciferase, reporter, infection

**Funding source or Acknowledgement:** PID2020-112992RR-I00, PRE2021-097320.

## Studies with bat airway organoids of *Carollia perspicillata* reveal that the respiratory epithelium of bat is not a barrier for interspecies transmission of influenza viruses

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Bats are a natural reservoir for many viruses and therefore important in the interspecies transmission of viral pathogens. To find out whether bat airway cells can be infected by viruses of other mammalian species we developed an organoid culture model derived from the respiratory tract of *Carollia perspicillata*. The cell composition of organoids resembled that of bat trachea and lungs as determined by immunofluorescent staining. Infection studies revealed that bat airway organoids (AOs) from either the trachea or lung, respectively, are susceptible to infection by two different swine influenza A viruses. The bat AOs were also used to develop an air-liquid-interface (ALI) culture system of filter-grown epithelial cells. Infection of these cells showed the same characteristics including a lower virulence and enhanced replication and release of the H1N1/2006 virus compared to infection with H3N2/2007. These observations were in agreement with the results obtained by the infection of porcine ALI cultures with these two virus strains. Interestingly, bat airway cells were found to contain only a low amount of alpha 2,6-linked sialic acids, the preferred receptor determinant for mammalian influenza A viruses. By contrast, alpha 2,3-linked sialic acid, the receptor determinant for avian influenza viruses, is abundantly present on bat cells. Therefore, bat airway cells are expected to be highly susceptible not only to mammalian but also to avian influenza viruses. Our culture models can be extended to other parts of the airways and to other species and thus provide a promising tool to analyse the transmission of viruses both from bats to other species and from other species to bats.

**Keywords:** bat, airway organoid, air-liquid interface, influenza virus

## Cross-Reactivity of Pre-and Post-Pandemic Human, Wild Boar and Farm Animal Sera with Alpha- and Beta-Coronaviruses (CoV) including SARS-CoV-2

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Pre- and post-pandemic farm animal, wild boar and human sera, including sera collected from humans with known SARS-CoV-2 virus neutralizing titers, were tested for cross-reactive antibodies towards different beta-coronaviruses (beta-CoV's), including Bovine coronavirus (BCV), Porcine hemagglutinating encephalomyelitis virus (PHEV) and SARS-CoV-2, and the alpha-CoV Transmissible gastroenteritis virus (TGEV).

Fixed dilutions of sera (1:50) were tested in neutralization assays with ascending concentrations of BCV and TGEV (50 - 5000 and 100 -10.000 TCID<sub>50</sub> units per well BCV and TGEV, respectively). Sera from animals experimentally infected with BCV, TGEV, PHEV and Porcine epidemic diarrhea virus (an alpha-CoV) showed that cross-neutralization of BCV was restricted to animals infected with beta-CoV's, and for TGEV to animals infected with alpha-CoV's.

Testing of panels of sera revealed high percentages of BCV-neutralizing sera (pre- and post-pandemic sera together) for humans (59%), pigs (39%), cows (100%), wild boars (29%), sheep (36%) and rabbits (60%). TGEV-infection was only neutralized by pig sera (68%) and a few wild boar sera (4.6%), whereas all sera tested from humans, cows, sheep, and rabbits did not neutralize TGEV. Part of these BCV-neutralizing sera also stained PHEV infected cells and reacted with immobilized recombinant spike (S1-S2) and NP proteins of SARS-CoV-2 in ELISA's. However, none of these BCV-neutralizing pre-pandemic human sera and pre- and post-pandemic wild boar and farm animal sera were able to neutralize SARS-CoV-2 infection, emphasizing the unique surface structure evolved in SARS-CoV-2. The percentage of human sera able to neutralize BCV was significantly higher for post-pandemic SARS-CoV-2 convalescent sera (78%) compared to pre-pandemic human sera (39%). Moreover, significantly more of these SARS-CoV-2 convalescent human sera were able to neutralize a high concentration of BCV (5000 TCID<sub>50</sub>). Interestingly, for post-pandemic sera collected from wild boars living near densely populated areas in the Netherlands also a higher percentage and stronger BCV-neutralization was observed in comparison to pre-pandemic sera from the same area.

We conclude that SARS-CoV-2 infection in humans may have activated a "memory" antibody response directed against structurally related or common epitopes exposed on the surface of a broad range of  $\beta$ -CoV's. Characterization of these epitopes may contribute to the development of vaccines that confer a broad protection against future emerging, disease threatening beta-CoV's and SARS-CoV-2 VOC's. Further research is needed to elucidate if a symptomless infection and/or environmental exposure to SARS-CoV-2 or another beta-CoV triggers the assumed "memory" antibody response in wild boars.

**Keywords:** SARS-CoV-2, beta-Coronaviruses, pre- and post-pandemic, human sera, farm animal sera, wild boar sera, cross-neutralization

## West Nile fever confirmed in a horse in the United Kingdom following recent foreign travel

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West Nile virus (WNV), a zoonotic mosquito-borne flavivirus, is increasing its geographic range in Europe, although infection has not been detected in the United Kingdom (UK) through surveillance. Equines are susceptible to WNV infection, causing poliomyelitis in a small proportion of cases that can lead to severe disease and death. In November 2022, a 7-year-old mare returned to the UK from Andalucía in Spain. Upon arrival in the UK, clinical signs of ataxia, hyperesthesia and rootedness were observed, and the mare was transferred to an equine hospital. Further signs, including marked muscle fasciculations and tremors were noted, most prominently in the face and over the shoulders, and bilateral hind limb weakness. A nasopharyngeal swab sample was negative for both Equine herpesvirus 1 and 4. Due to seasonal presence of WNV in Spain, combined with the clinical presentation, serum was drawn and submitted for WNV antibody testing. WNV IgM antibodies were detected, indicative of recent infection or vaccination. However, the mare had no record of WNV vaccination. A WNV specific RT-qPCR failed to detect virus in serum and cerebrospinal fluid samples. The mare was treated with anti-inflammatories and its health improved, allowing discharge from hospital. However, the mare's condition subsequently deteriorated and after re-admission it required euthanasia on welfare grounds. Following necropsy, WNV RNA was detected in brain tissue confirming the diagnosis of West Nile fever. Sequence analysis confirmed the virus as belonging to WNV lineage 1. The region of Andalucía, where the horse had travelled to, experienced a WNV lineage 1 outbreak among equines in 2020 with subsequent cases reported in 2021 and 2022. It is likely that WNV was transmitted to the mare while in Spain. This case is a timely reminder that vaccination against WNV is highly recommended in equines that are travelling to areas where WNV is seasonally present or endemic, and particularly during the vector active season.

**Keywords:** West Nile virus, importation, phylogeny

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*First Parallel Session*

**Emerging and Re-Emerging Diseases**

*Posters with Flash Presentation*

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## P01. Use of a luminescent reporter virus for rapid assessment of vaccine efficacy against Bluetongue virus

Luis Jiménez-Cabello<sup>1,\*</sup>, Sergio Utrilla-Trigo<sup>1</sup>, Alejandro Marín-López<sup>2</sup>, Miguel Illescas-Amo<sup>1</sup>, Eva Calvo-Pinilla<sup>1</sup>, Piet Van Rijn<sup>3,4</sup>, Aitor Nogales<sup>1</sup> y Javier Ortego<sup>1</sup>

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Generation of reporter viruses by reverse genetics (RG) is a useful molecular tool to explore and characterize viral diseases. Replication-competent reporter viruses expressing luminescent tags have been key for studying different viral aspects, and very handy for high-throughput screening assays. For Bluetongue virus (BTV), the etiological agent of Bluetongue (BT), just a modified replication-competent BTV-1 that expresses the fluorescent mCherry fused to the NS3 protein has been constructed so far. Therefore, designing of recombinant reporter viruses would enhance knowledge on BTV as well as provide a tool that will upgrade evaluation of novel vaccine candidates against this virus. By reverse genetics, we have previously designed and rescued a recombinant BTV-8 backbone-based virus expressing NanoLuciferase (rBTV-8/NLuc). To generate this virus, we synthesized a modified genome segment 5 encoding NS1 protein with the NLuc reporter gene located downstream and separated by the Porcine teschovirus-1 (PTV-1) 2A autoproteolytic cleavage site. In this work, we evaluated the potential of this newly generated recombinant BTV expressing the NanoLuciferase reporter gene to facilitate and accelerate vaccine efficacy studies against this relevant viral disease.

A group of IFNAR(-/-) mice (n=10) was immunized with the commercial inactivated vaccine VETIA's BLUEVAC®BTV(8) against BTV-8 following a homologous prime-boost regime administered four weeks apart. A group of IFNAR(-/-) mice (n=10) was left untreated. Four weeks after the boost dose, both groups of animals were challenged with a lethal dose of rBTV8/NLuc (1000 PFU) and mice were monitored daily for signs of disease and survival. After viral challenge, viral replication and NLuc activity were determined in blood and plasma samples, respectively.

Immunization with the commercial vaccine was fully protective, preventing illness or death in vaccinated mice, while most of mock-vaccinated mice succumbed to the challenge and only two out of ten mice survived. Consistent with these observations, viral replication and NLuc measures were higher in mock-vaccinated mice at all the time points evaluated. As the reporter gene was fused to the non-structural protein NS1, luciferase activity is only detected after virus replication. In this sense, the bioluminescent signal kinetics in plasma samples were consistent and correlated with virus titer kinetics.

All these data demonstrates that, at least in mice, NLuc-expressing rBTVs are a suitable biotechnological instrument for the rapid and easy preclinical assessment of novel vaccines against BTV.

**Keywords:** Bluetongue virus (BTV), reverse genetics, reporter gene, luciferase, vaccine

**Funding source or Acknowledgement:** PID2020-112992RR-I00

### P03. First report of *Porcine respirovirus 1 (PRV1)* and of *Swine orthopneumovirus (SOV)* in swine in Italy

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*Porcine Respirovirus 1 (PRV1)*, which was previously known as *Porcine Parainfluenza virus 1*, and *Swine Orthopneumovirus (SOV)* are both single-stranded, non-segmented, negative sense RNA viruses. Both PRV1 and SOV are emerging porcine respiratory viruses. As of now, PRV1 and SOV infections in pigs, their pathogenicity and their effect on swine health are still unknown. While there have been reports of the detection of these viruses in some European countries, no data are available regarding their circulation in Italy.

From September 2022 to February 2023, 343 samples of swine origin, collected for diagnostic purposes, were screened to detect PRV1 and SOV. 59% of the samples were collected from pigs in Northern Italy, as part of passive surveillance, in which clinical specimens are routinely tested for swine influenza and for porcine reproductive and respiratory syndrome (PRRS). 41% of the samples were collected from wild boars during a regional monitoring plan and were routinely tested for influenza A virus. After nucleic acids extraction, *real-time* RT-PCR was performed with primers and probes specific for the hemagglutinin-neuraminidase gene of PRV1 and for the nucleocapsid gene of SOV.

Out of 343 samples, 16 and 9 samples tested positive for PRV1 and for SOV respectively. Mean Cq values of positive samples were of 32.35 for PRV1 and of 32.15 for SOV. Interestingly, three samples collected from pigs tested positive for both viruses, while only one wild boar sample tested positive for PRV1 and one for SOV. Moreover, six pig samples tested positive for both PRV1 and PRRS. Taken together, these preliminary results highlighted a relatively low prevalence for both viruses in pigs (7.3%-PRV1, 3.9%-SOV). Although, it is worth noticing that the analysed samples came from passive surveillance, and because of that, could not be representative of the real prevalence.

To the best of our knowledge, this is the first report regarding PRV1 and SOV circulation in the *Suidae* population in Italy. Further investigations are needed to better understand PRV1 and SOV molecular epidemiology and transmission routes. Given that, additional activities will focus on trying viral isolation and to perform WGS, in order to investigate the genetic diversity of both viruses, and to better understand if they may play a role in the Porcine Respiratory Disease Complex (PRDC).

**Keywords:** Swine, respiratory viruses, *porcine respirovirus 1*, *swine orthopneumovirus*

**Funding source:** Study partially funded by Italian Ministry of Health, PRC2020005.

**Acknowledgement:** We would like to thank Professor Timm Harder and Doctor Annika Graaf from Friedrich-Loeffler-Institut for kindly providing RNA positive controls for both PRV1 and SOV

## P04. Bat-borne Issyk-Kul virus: European expansion of a zoonotic agent?

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*Issyk-Kul virus* (ISKV), *Nairoviridae* family, is a zoonotic virus firstly detected in 70'- 80' in Central Asia from both *Nyctalus noctula* bats and bat ticks. Recently, ISKV genome has been detected in bats in Germany (*Eptesicus nilssonii*) and Sweden (*Myotis brandtii*) suggesting a possible geographical expansion to Europe. In this study, following the previous isolation and genome characterization of ISKV from an *Hypsugo savii* sampled in Northern Italy in 2021, we performed a specific ISKV surveillance in bat populations in order to acquire epidemiological information on its viral ecology and diffusion.

Bats were collected from wildlife rehabilitation centers (WRC) in Lombardy and Emilia-Romagna regions (Northern Italy) from 2017 to 2022. The presence of lyssavirus was excluded by PCR on brain samples before performing any other analysis. DNA barcoding was used for bat species identification. Tissue samples from different organs (lung, heart, spleen, gut and brain) were collected and analyzed by a specific ISKV End-point PCR targeting L gene specifically developed for the purpose.

A total of 387 bats belonging to 13 different species were collected. *Hypsugo savii* and *Pipistrellus khulii* were the most representative species, 48.38% and 40.86% respectively. Other than the first ISKV strain (IT-297348-34/2022) isolated from an adult female of *Hypsugo savii* in Bergamo province in 2022, we found five more ISKV positive samples, all from *Hypsugo savii* bats recovered in WRCs in 2017, 2020 and 2021. Molecular and phylogenetic analyses are in progress in order to characterise the new sequences and compare them to the first ISKV Italian isolate, which in turn showed high nucleotide similarity for each gene segment (L, M, S) to ISKV strains detected in bats in Germany (strain PbGER) and Central Asia (strain LEIV-315K).

Italian ISKV appears to be associated to *Hypsugo savii*, a synanthropic and sedentary bat species which roost in buildings, thus suggesting even a possible public health implication. Retrospective analysis trace back the presence of ISKV in Northern Italy to 2017 suggesting that it has been probably present in this area for several years.

**Keywords:** ISKV, Bat, Italy

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*First Parallel Session*

**Emerging and Re-Emerging Diseases**

*Poster Presentations*

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## P02. Establishment of an ovine cell line derived from peripheral blood monocytes and its potential use as in vitro model to study livestock viral infections

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Virus isolation is still used as the most common assay to identify the viral agent for diagnosis on infections in veterinarian laboratories when the results of molecular testing or serology are unavailable or equivocal. Primary cells of bovine, ovine or porcine origin or immortalized cell lines are used as a tool to isolate viruses from biological samples or to grow virus in large amounts that can be used for the production of inactivated vaccines or viral antigen in diagnosis techniques. The main disadvantage is that these cell lines are less susceptible to be infected and could lead to a higher number of false negatives. Mesenchymal stromal cells (MSC) are a population of undifferentiated stem cells isolated from several tissues defined by their ability to adhere to plastic surfaces, express a certain panel of phenotypic markers and can differentiate into different lineages when cultured in specific inducing media. Therefore, the main aim of this work was the characterization, culture and differentiation of a cell line generated from mesenchymal stromal cells isolated of ovine peripheral blood.

The cell line was characterized by flow cytometry (FACS) using a panel of monoclonal antibodies and were analysed by real time quantitative polymerase chain reaction (RT qPCR) using specific gene primer pairs sequences from surface markers of hematopoietic cells or mesenchymal cells. Furthermore, the differentiation potential to osteoblasts was evaluated using specific differentiation medium. Finally, the susceptibility of the cell line to be infected with different viruses causing disease in ruminants was also studied.

Experimental results obtained by RT qPCR and FACS showed that cells expressed different mesenchymal markers and were negative to hematopoietic markers. MSCs were efficiently differentiated to osteoblasts, this was confirmed by specific staining with Alizarin red. In addition, the cell line was susceptible to infection to all several zoonotic and/or ruminant viruses tested (RVFV, AKV, Toscana virus, BTV, AHSV, FMDV, SBV, PPRV, EHDV, Influenza and WNV).

We describe a newly developed ovine cell line generated from mesenchymal stem cells isolated from ovine peripheral blood. These cells express mesenchymal markers and are able to differentiate into osteoblasts. This cell line was productively infected with all viruses tested and may represent a useful tool in veterinarian laboratories and to carry out studies of virus-cell interactions.

**Keywords:** ovine cells, mesenchymal cells, real time PCR, flow cytometry, differentiation, infection.

**Funding source or Acknowledgement** [This work was supported in part by grant PID2021-122567OB-I00 funded by MCIN/AEI/10.13039/501100011033/ FEDER, UE]:

## P05. Mixed human migrations and transboundary animal diseases spread: the Western Balkan route case

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Transboundary animal diseases (TADs) impact animal health and countries' economies. This work aimed to assess if the Land Western Balkan route for mixed human migration could play a role in spreading TADs with a focus on African Swine Fever (ASF) and Lumpy Skin Disease (LSD).

Between 2019 and 2022, six field missions were performed in Romania, Serbia, Greece, Albania, Bulgaria and Lebanon for data collection using participatory methodological tools and field inspection of strategic points. Overall, 110 people among veterinarians, farmers, hunters, academics, and humanitarian sector representatives were involved.

The Western Balkan Route is one of the main migratory pathways into Europe. After the peak of the migration crisis in 2015, mixed migration flows decreased to a few thousand people annually until last year, when a new rise has registered. Also, the pace of movement has changed over the years: in 2015, migrants moved fast from their countries of origin, mainly Syria and Afghanistan. Since 2016, migrants have spent long periods in Turkey or been stuck at the borders between Balkan and European countries. Most migrants must use a mix of types of transport to move along the migration route: on foot far from main roads, by small boats, taxis, and buses. In all these cases, the presence of live animals is not reported except for the rare exception of a few migrants who arrived in the Balkans with pets (Syrian families with dogs). Products of animal origins are not reported as usual. Livestock presence is not allowed in the camps in the EU or Balkan countries.

Moreover, most migrants are Muslim, and the food ratio in all countries excludes pork. Hunting of hunted small animals, e.g. rabbits and birds, has been mentioned, while wild boar is not considered an option. Migrants spend significant time in forest areas, setting up jungle camps with poor hygienic conditions. That could become points of aggregation of wild boar that could represent a risk in ASF-affected areas.

Moreover, walking in ASF-infected forests has been reported as a risk for ASF spread. It was finally reported that during the religious festivity, migrants purchased high numbers of small ruminants (no cattle or pork was mentioned).

Data collected showed the low impact of long-distance human mixed migrations through the Western Balkan route towards Europe on the spread of TADs. Further studies should be done to explore the more recent events linked to the Ukraine crisis.

**Keywords:** Mixed human migrations, African Swine Fever, Lumpy Skin Disease, Transboundary Animal Diseases

**Acknowledgement:** DEFEND project, funded from the European Union's Horizon 2020 RIA programme under the grant agreement N°773701

## P06. Visualizing bioluminescent bluetongue virus infection in living IFNAR(-/-) mice

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Optical imaging of reporter gene labelled viruses in small animal models can allow us to perform real-time analysis of the infection process without sacrificing the host. Bluetongue virus (BTV) affects ruminant livestock such as cattle, sheep, goats and wild ruminants such as deer and camelids. In our laboratory we characterized adult mice with gene knockouts of the interferon  $\alpha/\beta$  receptor (IFNAR<sup>-/-</sup>) as a model of lethal BTV infection. By reverse genetics, we have designed and rescued a recombinant bluetongue virus serotype 1 expressing Nanoluciferase (rBTV-1-Nluc). The objective of this work is monitoring BTV infection using a reporter-expressing virus in the IFNAR<sup>-/-</sup> mice model.

To generate these viruses, we custom synthesized a modified genome segment 5 encoding NS1 protein with the reporter gene located downstream and separated by the Porcine teschovirus-1 (PTV-1) 2A autoproteolytic cleavage site. A group of IFNAR<sup>-/-</sup> mice (n = 4, 2 males and 2 females) were subcutaneously infected with 10<sup>3</sup> PFU/mice of rBTV-1-Nluc and bioluminescence was monitored every 2 days until day 6.

The Nluc expression was readily detected in infected mice at 3 d.p.i. In vivo imaging of Nluc expression was initially detected in the skin at the site of infection and the lymph nodes and later, when systemic spread occurred, in spleen, lung, thymus and ovaries, target tissues for BTV replication in IFNAR<sup>-/-</sup> mice and ruminants. Maximum levels of Nluc expression were detected at days 5–6 post-infection and could not be monitored at later times since all of them succumbed to viral infection. At day 6 p.i, immediately after imaging, mice were euthanized and lungs, liver, brain, spleen, kidney, testis and ovaries were collected to quantitate Nluc expression using ex vivo imaging. Similar to our previous results, Nluc expression was detected in lung, spleen, ovaries and also in liver.

The new rBTV-NLuc is not only a powerful tool for studying different aspects of bluetongue pathogenesis in a non-invasive manner, but can also be used for in vitro and in vivo antiviral and vaccine candidate screenings.

**Keywords:** Bluetongue, Reverse genetic, Reporter gene, Nanoluciferase, Bioluminescence

**Funding source or Acknowledgement:** PID2020-112992RR-I00, PRE2021-097320.

## P07. Molecular biology diagnostic tool and methodology of Foot and Mouth Disease (FMD) in Georgia

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Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals. FMD is caused by an aphthovirus, an RNA virus with a positive-sense single-stranded genome, in the family of Picornaviridae. There are seven serotypes of FMD virus, namely O, A, C, ASIA 1, SAT 1 (South African Territories), SAT 2, and SAT 3. Susceptible animal species are domestic cattle, pigs, sheep, goats, buffalo and all species of wild ruminant and pigs. FMD outbreaks in developed countries and their significant economic impact have increased concern worldwide. FMD is probably the most important constraint to trade in live animals and their products. The goal is to have correct molecular biology tool for accurate RT-PCR diagnostics.

SLA utilizes PCR technique using Real-Time PCR assay for the detection of viral RNA based on the Roche Light Cycler for FMD. The test specifically detects 3D (Callahan et al., 2002) region of the gene. The viral RNA was extracted by using RNA extraction kits: Qiagen catalog #52906 and FMD Real-Time PCR assay catalog #TC-9081-064 provided by Tetracore. RT-PCR cycle conditions: 48°C 15 min, 95°C 2 min, 95°C 10 sec, 60°C 40 sec, (45 cycle).

for this research used field samples n=31 and samples n=10 (Proficiency test panel samples). In total, 41 samples have been tested at SLA (2021-2022). Field samples were received from different regions of Georgia that were negative and 7 samples out of 10 (proficiency testing samples) were positive. SLA regularly participates in European Union Reference Laboratories (EU-RL) Proficiency Testings, receives reports indicating that all criteria are met, It ensures reliability of diagnostic tools and the results.

Testing results showed that techniques play an increasingly valuable and unique role in laboratory diagnostics that enables early detection of the disease in case of occurrence, carries out complex measures for controlling FMD. If necessary, SLA will conduct similar studies in the future in Georgia.

**Keywords:** Molecular biology, Virus, FMD, Diagnostics, Laboratory Technique

## P08. Equine brain organoids: tools to better understand West Nile virus infection of the equine central nervous system and to identify antiviral molecules

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West Nile virus (WNV) is a mosquito-borne virus, member of the *Flaviviridae* family that can cause severe, sometimes fatal, neurological disorders to horses and humans. With its constant re-emergences in Europe, WNV is a pathogen that threatens the health of these two species, for which there is currently no treatment. Interactions of WNV with its hosts have mostly been studied in mice and cell lines. Although the use of these models have led to major discoveries, they poorly predict the interactions happening between the virus and the human or equine central nervous system (CNS). Recently, three-dimensional (3D) cellular models called human brain organoids (hBOs) have been developed with the aim to better represent the tissue organisation of the human CNS. They are a new alternative to model neurotropic viral infections. However, to our knowledge, equine brain organoids (eBOs) are not yet available. The goals of this study were to develop eBOs and to use them as a pathological model of WNV infection in horses and as a high value support for antivirals identification.

Equine BOs were generated from equine induced pluripotent stem cells and were differentiated for 70 days. Their size and morphology were evaluated by microscopy and cortical markers expression was assessed by RT-qPCR. Equine BOs were infected with 50, 500 or 5000 pfu of WNV-NY99 for 24, 48, 72, 96 hours and 7 days. Three molecules were tested for their anti-WNV activity and increasing doses were added in eBOs culture media 2 hours before and 72 hours post infection. Viral replication in eBOs and antiviral activity of molecules were assessed through quantification of viral RNA (RT-qPCR) and/or viral particles (TCID50) in supernatants.

We showed that eBOs are spheroids which grow overtime and express early and late cortical markers. WNV inoculation led to a progressive increase of WNV RNA in the supernatant, showing that the virus replicated efficiently in eBOs. One molecule, out of the three tested, caused a dose-dependent decrease of infectious viral particles in eBOs supernatants.

Our results thus demonstrate that eBOs are 3D structures made of neural cells that are permissive to WNV and that they can be used for the identification of antiviral molecules. They represent a novel and unique, physiologically relevant, 3D *in vitro* model of WNV infection of the equine brain that could accelerate the development of antiviral treatments for horses.

**Keywords:** West Nile virus, brain organoids, 3D-model, antiviral molecules

**Funding source:** INRAE, ENVA

## P09. Preparedness - Aligning Japanese encephalitis virus diagnostics in Europe

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The geographical range of mosquito-borne Japanese encephalitis virus (JEV) has expanded from its endemic region, Asia, to Australia. JEV emergence is usually detected when there is an increase in stillborn and weak piglets with neurological signs. Outbreaks can have severe health and economic impacts and cause ~68,000 human cases in Asia each year. Given that suitable vertebrate host and mosquito vector species appear to be present in Europe, JEV introduction could lead to disease outbreaks among humans and horses, and establishment of the pathogen in livestock or wildlife. Introductions could occur through movements of infected pigs and mosquitoes, but also migratory birds that act as reservoirs. Introduction and establishment of the virus in these populations may not be apparent at first, providing time for the virus to spread before spill over. Further complicating JEV detection is the extensive cross-reactivity in many serological assays with other flaviviruses circulating in Europe (i.e., tick-borne encephalitis virus, West Nile virus, Usutu virus). In addition, the viremia in clinical cases may be short, making it difficult to detect the virus. Thus, in order to be prepared for future potential emergence of JEV in Europe, it is necessary to establish sensitive and specific diagnostic tools. To enable European preparedness for detection, surveillance and monitoring of JEV introduction and spread, five international veterinary laboratories collaborated (CoVetLab), with the aim to align JEV diagnostics. The objectives are to 1) standardize serological assays for antibody detection in swine, 2) establish a pipeline for molecular virus detection and genotyping of JEV.

The partner institutions conducted an inventory of their current JEV detection assays, samples and diagnostic interests. Protocols were shared and reviewed; for serological and PCR assay development institutes are building on these shared protocols. All institutes participate in the EURL for equine diseases flavivirus serological and molecular proficiency tests to assess established and newly developed assays for the detection of JEV infections. Lastly, a collaborative approach will be used to develop amplicon-based sequencing protocols and to test bioinformatics pipelines.

Overall, this project provides a framework for communication and collaboration between arboviral researchers at five international veterinary institutes, enhancing ease of collaboration and alignment of diagnostic techniques. In addition, the sharing of knowledge and expertise has enabled the identification of JEV diagnostic capability gaps at each participating institution and will enable enhancement of existing diagnostics pipelines.

**Keywords:** Arbovirus, diagnostics, emerging pathogens, flavivirus, zoonosis

## P10. Investigating the emergence of a zoonotic virus: phylogenetic analysis of European Lyssavirus Bat 1 in the UK

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The European bat lyssavirus 1 (EBLV-1, *Lyssavirus hamburg*) is predominantly found in serotine bats (*Eptesicus serotinus*) and is responsible for the majority of bat rabies cases in mainland Europe. A passive bat rabies surveillance scheme at the Animal and Plant Health Agency (UK) detected the virus in the UK for the first time in October 2018 and as of February 2023, a total of twenty cases have been reported. Analysis in this study focused on the nine isolates received between October 2018 and December 2021.

We investigated the emergence of EBLV-1 by undertaking comprehensive whole genome sequence analysis and Bayesian phylogenetics. The dataset included the nine UK sequences and 106 sequences covering six countries in mainland Europe (France, Spain, Germany, Netherlands, Denmark, and Russia) from between 1968 to 2019, including 18 French EBLV-1 positive RNA samples sequenced for this study.

Sequence analysis revealed extreme similarity between UK EBLV-1 sequences (99.9-100%), implying a single source of introduction rather than multiple events from a number of sources. Bayesian analysis revealed the UK EBLV-1 sequences shared their most recent common ancestor with an EBLV-1 sequence from a serotine bat detected in Brittany, France in 2001, with an estimated date of divergence of 1997. Within the UK sequences, the earliest divergence was estimated to occur in 2009.

This study provides valuable insights into the dynamics and epidemiology of an emerging zoonotic pathogen, crucial to better understanding the risk it poses to public and animal health.

**Keywords:** European Bat 1 lyssavirus, EBLV-1, *Eptesicus serotinus*, serotine, next generation sequencing, Bayesian analysis, emerging infectious diseases, rabies

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*Second Parallel Session*

**Pathogen evolution, Pathogenesis  
and Immunology**

*Oral Presentations*

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## Bluetongue virus interferes with type I interferon induction by disrupting cGAS pathway

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Bluetongue virus (BTV) is a double-strand RNA virus of the *Orbivirus* genus and the *Reoviridae* family. It is an arthropod borne disease that affects ruminants. Although BTV has been described to antagonize IFN signalling, the mechanism by which the virus interferes with IFN-I induction has not been well described. Cyclic GMP-AMP synthase (cGAS) is a DNA sensing cellular receptor that was canonically understood to respond to pathogen and host derived DNA. However, recent studies have shown that some RNA viruses are capable of activating this pathway, inducing IFN-I production. The aim of this research is to study potential interactions between BTV and cGAS pathway as a possible mechanism of inhibition of IFN-I induction.

Interferon reporter assays were carried out using HEK-293T cells expressing firefly luciferase under the control of an IFN- $\beta$  promoter (293T-IFN $\beta$ ) (Rodríguez-Madoz JR, *et al.* 2010). IFN- $\beta$  promoter induction of cells transfected with a plasmid containing the sequence of BTV-NS3 protein was measured using the neolite luminescence reporter gene assay system (PerkinElmer). To study potential cGAS or STING degradation, 293T cells (ATCC CRL-1573) were transfected with pCMV-Lenti-cGAS-HA, pcDNA3.1-STING and pIRES-BTV-NS3 or pIRES-BTV-NS4 or infected with BTV-8 (NET2006/04). Cell lysates were analyzed via SDS-Page and immunoblotting. To evaluate interferon production, immortalized sheep thymus cells were infected with BTV and stimulated with *E. coli* DNA or MVA. 16 hours post-stimulation, cell lysates were collected for nucleic acid extraction using RNeasy Micro Kit (Qiagen), which were then analyzed by qPCR using specific primers for sheep IFN $\alpha$  and ISG15 genes.

BTV infection inhibits DNA-induced type-I IFN transcription in sheep thymus cells. This inhibition correlates with cGAS degradation during infection. BTV-NS3 protein has been identified as the protein responsible for cGAS degradation, which occurs via an autophagy-dependent mechanism in which ubiquitinated NS3 binds to cGAS.

In this study we describe a new mechanism by which bluetongue antagonizes the interferon response. During BTV infection, IFN-I induction is hindered due to cGAS degradation mediated by NS3 protein through an autophagy-dependent mechanism.

**Keywords:** Bluetongue, interferon, cGAS-STING.

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## Study of goat dendritic cells infection with Peste des petits ruminants virus (PPRV)

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Peste des petits ruminants (PPR) is a World Organisation for Animal Health notifiable disease affecting sheep and goats. It is caused by Peste des petits ruminants virus (PPRV), a *morbillivirus* of the *Paramyxoviridae* family. PPRV infection induces immunosuppression, which can cause animal death due to opportunistic infections. In general, the disease is more severe in goats than in sheep, but the mechanisms that explain this phenomenon are still unknown. Dendritic cells (DC), which are central to mounting adequate immune responses to pathogens, can be targeted by PPRV in sheep. In the present work, we assessed PPRV effects on goat DC.

Monocytes were obtained from caprine peripheral blood mononuclear cells (PBMCs) using density gradient centrifugation and antibody magnetic sorting. Monocytes were then differentiated into immature monocyte-derived DC (iMoDC) with growth factors for 72h and matured with the TLR7/8 agonist R848 for 24h. To study the effect of the virus, iMoDCs were infected after 48h differentiation and surface marker expression, phagocytosis and antigen presentation assessed.

In this study, we were able to establish a protocol that differentiated goat MoDC expressing the classical MoDC markers (MHCI, MHCII, CD1, CD1w2, CD80, CD86, CD40, CD209, CD11b, CD11c and CD172a) and typical morphological characteristics. PPRV was able to infect caprine MoDC and this led to the upregulation of maturation markers CD40, CD80 and CD86. Moreover, the phagocytic capacity of infected MoDC was decreased when compared to mock counterparts, which further indicated that the infection produced MoDC maturation. Mixed lymphocyte reaction assays however showed that caprine PPRV-infected MoDC capacity to stimulate CD4<sup>+</sup> and CD8<sup>+</sup> T cell proliferation was reduced. PPRV can therefore target goat DC to disrupt their ability to present antigen.

A protocol for the obtention of functional goat MoDC has been established. Besides, we found that PPRV infection affected the characteristics of these caprine DCs, producing a partial maturation status that is inefficient at antigen presentation.

**Keywords:** PPRV, *morbillivirus*, goats, immunosuppression, DC

**Funding source:** The work was funded by grant RTI2018-094616-B-100, and PID2021-124872OB-I00 from Ministerio de Ciencia e Investigación (Spain).

## Characterization of PRCV strains from Europe and the USA

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Coronaviruses infect a broad spectrum of avian and mammalian hosts and may cause a wide array of symptoms. As seen with SARS-CoV-2, coronaviruses may also change host- or tissue tropism over time. Porcine respiratory coronavirus is another example of such a virus, being a deletion variant of transmissible gastroenteritis virus (TGEV). A prerequisite for a change in coronavirus host- or tissue tropism is the adaptability of the viral structural proteins (spike (S), envelope (E), membrane (M), and nucleocapsid (N)) in order for the virus to enter and replicate in this new environment. In the present study, we aimed to investigate sequence variations in the genes encoding the structural proteins between various TGEV and PRCV sequences, and to express and characterize the structural proteins of a European strain.

An Italian PRCV strain (PRCV 15087/12 III NPTV Parma) was sequenced, and was, along with an unpublished sequence of a Belgian strain (PRCV 91V44), included in a sequence and phylogenetic analysis of about forty TGEV and nine PRCV sequences from Europe and the USA. The structural proteins of the Italian strain were transiently expressed in BHK-21 cells, and the cell lysates were analyzed in ELISA assays and Western blots. In the sequence analysis, we found variations among the genes encoding the four structural proteins for the PRCV strains from Europe and from the USA. The deleted region in the S gene was not the same size for all strains, which could indicate that multiple events have occurred and altered the sequence although the host- and tissue tropism of the viruses appears unchanged. Phylogenetic analyses showed that PRCV strains from Europe clustered separately from the PRCV strains from the USA. We transiently expressed the four structural proteins of the Italian PRCV strain and demonstrated this by the specific recognition of the proteins in serological and blotting analyses.

We found variations among the European and American PRCV strains, including variations in the deletion in the S gene of PRCV compared to TGEV. Furthermore, we demonstrated the transient expression of the structural proteins of an Italian PRCV strain by ELISA assays and Western blots.

**Keywords:** Porcine respiratory coronavirus (PRCV), sequence analysis, structural proteins, serological response

## Interplay between Foot-and-Mouth Disease Virus 3D polymerase and the type I interferon pathway: a contribution to viral persistence?

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One important issue associated with Foot-and-Mouth disease (FMD) is the ability of FMD virus to persist in ruminants for several months. Despite considerable work done, the underlying mechanisms remain unknown. However, we have shown previously that persistent infection in bovine primary cells is associated with a durable but ineffective innate antiviral response that may rely on protein-protein interactions (PPI) between FMDV and its host. While FMDV persistence has been reported in ruminants, it has not been reported in pigs. This differential persistence provides an opportunity to compare virus-host interactions between species, determine their host specificity and identify potential links between PPI and viral persistence.

We focused on the interplay between FMDV proteins and 16 cellular proteins belonging to the type-I interferon (IFN I) pathway, described as involved in more than 75% of virus-host PPI. Plasmids expressing these 16 proteins from cattle, pig, sheep and goat, as well as expression vectors for the 15 serotype-O FMDV proteins were built by Gateway method. PPI were identified by NanoLuc-2-Hybrid screening and confirmed using affinity chromatography GST-Pull-Down. The phenotypic impact of some FMDV proteins on the IFN pathway has also been investigated via luciferase reporter assays.

The NanoLuc-2-Hybrid approach revealed several PPI between the IFN pathway proteins, and the viral proteins tested. The most promising results concerned the 3D<sup>pol</sup> for which no interaction with the IFN pathway has been described. This protein was used to screen cattle, sheep, goat and pig NanoLuc plasmid libraries. When comparing the screening results, it appears that most of the interactors are shared with relative strength across species. The FMDV-host PPI have been confirmed by GST-Pull-Down and functional validation is ongoing. Furthermore, we have demonstrated by luciferase reporter assays the inhibitory effect of 3D<sup>pol</sup> on the IFN pathway induction phase.

We have demonstrated novel interactions between FMDV 3D<sup>pol</sup> and cellular proteins involved in the IFN pathway from four virus-susceptible species. A direct effect of 3D<sup>pol</sup> on the IFN pathway induction phase was also revealed. Combined, these results strongly suggest that 3D<sup>pol</sup> could play an unsuspected role in FMDV escape from the host IFN pathway. A FMDV modulation of the antiviral response, through interactions with its host, is one hypothesis explored to understand FMDV persistence. Thus, it is conceivable that the 3D<sup>pol</sup> protein, by interacting with certain IFN pathway proteins, might contribute to the establishment and/or maintenance of a FMDV-host equilibrium.

**Keywords:** protein–protein interactions; virus–host interactions; foot-and-mouth disease virus (FMDV); 3D polymerase; interferon pathway

## The porcine low-density lipoprotein receptor (LDLR) plays an important role in Classical swine fever virus (CSFV) infection

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Classical swine fever virus (CSFV), a pestivirus within the family *Flaviviridae*, is the causative agent of classical swine fever, an economically important epizootic disease of pigs. Although several host cell factors required for replication of CSFV have been identified, its entry mechanism is still poorly understood. The virus-host cell interaction of other, distantly related porcine pestiviruses, such as Bungowannah pestivirus (BuPV), is little studied. The low-density lipoprotein receptor (LDLR) is involved in cell entry and post-entry processes of various viruses including other members of the *Flaviviridae*, e.g. hepatitis C virus (HCV). In order to better understand the entry mechanism of CSFV and other porcine pestiviruses, the relevance of LDLR in infection with CSFV and BuPV was investigated.

The role of LDLR in infection with three different CSFV strains and BuPV was initially studied by antibody-mediated blocking of LDLR on the surface of permissive cells. For confirmation, the permissivity of porcine cell lines genetically engineered by CRISPR/Cas9 to express either low or high levels of LDLR was evaluated at 20 and 72 hours post infection (hpi).

Infections with genetically distinct CSFV strains were strongly reduced at 20 hpi after blocking with an LDLR-specific antibody, e.g. for the highly pathogenic strain Kozlov resulting in 55-fold reduction of infectious titers. BuPV showed a less pronounced dependency on LDLR with about 10-fold reduced titers. Blocking had no impact on uptake of low-density lipoprotein (LDL), suggesting a role of LDLR that is independent of the availability of LDL. The altered LDLR expression of the engineered cell lines had no clear effect on BuPV infection. In contrast, the reduced and enhanced LDLR expression levels had a significant impact on permissivity to CSFV that became apparent already at 20 hpi and was still prominent at 72 hpi, providing further evidence for a role of LDLR in entry and possibly also in post-entry processes of CSFV.

LDLR appears to play an important role in the replication cycle of CSFV, while it seems to be less relevant in BuPV infection, indicating differences in the virus-host cell interactome of diverse porcine pestiviruses. The newly identified dependence of CSFV on LDLR reveals parallels to other members of the family *Flaviviridae*, which might allow the transfer of findings between these viruses. Similar to HCV, LDLR probably represents one of several cellular factors mediating the initial interaction between CSFV and its host cell.

**Keywords:** Classical swine fever virus, Bungowannah pestivirus, Low density lipoprotein receptor, viral entry, host factor

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*Second Parallel Session*

**Pathogen evolution, Pathogenesis  
and Immunology**

*Posters with Flash Presentation*

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## P11. Small ruminant lentiviruses: genetic characterization of strains circulating in Italy between 2019 and 2022

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Small Ruminant Lentiviruses (SRLVs) are retroviruses that include VISNA-MAEDI and CAEV, which cause disease of different severity in sheep and goats. Since control and eradication plans are not mandatory in several countries, including Italy, SRLVs are distributed worldwide. SRLVs are characterized by a high genetic variability, with five genotypes (A-E) and several sub-genotypes (A1-24; B1-5; E1-2). In Italy three genotypes and fourteen sub-genotypes were identified so far, two of which have been recently described, through a retrospective study conducted on positive cases diagnosed from 1998 to September 2019. This highlights that molecular characterization of positive samples is pivotal to understand and the SRLV evolution in Italy. The present study phylogenetically characterized SRLV diagnosed during the syndromic surveillance conducted from October 2019 to May 2022 in Italy. During this period, 182 sheep and goat farms distributed in fifteen Italian regions and 31 provinces were subjected to SRLV diagnosis, with 630 samples tested. Samples were analyzed by nested PCR targeting the gag-pol conserved region, generating an amplicon of about 800 bp. Extraction of proviral DNA or RNA was performed from different matrices (somatic cells, buffy coat, joint fluids, organ homogenates) Positive samples were Sanger sequenced and sequences generated aligned with reference strains for genotyping. Maximum Likelihood (ML) phylogenetic analysis was performed with 10,000 replicates and bootstrap values >70%. During the study period, 59 farms out of 182 tested (30.84%) were positive, distributed in ten regions and nineteen Italian provinces. In 2019, 27/74 farms resulted positive, in 2020 16/51, in 2021 8/42 and in 2022 8/22. Eighty-nine out of 630 samples analysed resulted positive (14.12%), of which 40 were successfully sequenced. Three genotypes (A, B, E) and seven sub-genotypes (A9, A19, A24, B1, B2, B3 and E2) distributed in nine regions were identified. The presents study identified the circulation of A9 sub-genotype in Northern Italy (Veneto) in 2021 for the first time, previously reported solely in central (2010-2018) and south (2017-2019) Italy. In addition, the phylogenetic analysis conducted revealed the circulation of the A24 sub-genotype in South Italy for the first time. As regards the B genotype (1-3), this is widely distributed across the in peninsular Italy, with the B1 being the most widespread. The genetic characterization of SRLVs is an essential tool for mapping the circulation and distribution of the sub-genotypes in sheep and goat farms in Italy, the effectiveness of which would be enhanced by continuous and constant monitoring of the infection in the field.

**Keywords:** small ruminant lentiviruses, genotyping

## P12. Milk from rabbit mothers vaccinated against RHD contains a relevant level of specific IgA

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Members of the class Mammalia use milk to passively protect infants from infectious diseases, thus giving the developing immune system time to mature and become autonomous. Rabbit mothers actively provide IgG from the serum through the placenta to the foetus, that is after birth protected at the systemic immune level. Therefore, at first colostrum and then milk contain immunoglobulins, mainly IgA, to passively protect the young rabbit's mucous membrane during the lactation period. However, very few studies have been conducted to understand the origin of specific IgA, particularly in relation to the vaccination protocol. Here, we analysed the quantity and specificity of IgA present in the milk of farmed rabbits, vaccinated against RHDV and RHDV2, two lagoviruses that cause rabbit haemorrhagic disease (RHD), a lethal hepatitis with a high mortality rate.

We collected blood and milk samples of 20 does vaccinated with two monovalent vaccines respectively for RHDV and RHDV2. Samples were taken at different intervals after birth and sera were tested with specific cELISAs (total Ig) for both viruses. Furthermore, to detect total and specific IgA against RHDV/RHDV2, we performed direct and reverse ELISA using specific Mab anti IgA and/or anti-viruses. A sandwich ELISA based on MAb anti IgA was used for quantifying IgA.

cELISAs showed that vaccinated mothers were seropositive with a good titer for both RHDV (~1/320) and RHDV2 (~1/160). Interestingly, titres obtained with cELISAs by testing both milk and sera of the same rabbits were comparable. The amount of IgA was  $350 \pm 100$  ug/ml, with around 20% of sIgA in the sera and 5-10 mg/ml of sIgA in the milk. Both direct IgA ELISA and the reverse ELISA resulted positive with comparable titre, even if direct ELISA showed a better sensibility, limiting the risk of false negative results.

Although preliminary, our results on milk collected from vaccinated rabbits for RHD, clearly showed the presence of specific IgA at relevant titres, similarly in cELISA to those found in the serum. Considering the relevance of humoral immunity in protecting rabbits from RHD, our results indicate that young rabbits born from vaccinated mothers benefit from a dual passive protection, both at systemic (serum IgG), as already previously shown, and mucosal (milk IgA) level. However, given the novelty of the data, further studies are needed to confirm this conclusion.

**Keywords:** rabbit, IgA, milk, vaccination, RHD

**Funding source:** This research was supported by Italian Ministry of Health, project PRC2019/007 to IZSLER

## P13. Cytokine secretion in hematopoietic stem cells CD34+ of cattle infected with bovine leukemia virus (BLV)

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Bovine leukaemia virus (BLV) is a B-lymphotropic oncogenic member of *Retroviridae* family and is an etiologic agent of enzootic bovine leukosis, the most common neoplastic disease of cattle. BLV infection is characterized by a long viral latency and by the absence of viremia. The mechanisms of viral persistence and disease progression remain poorly understood at present. Bone marrow is populated by hematopoietic stem cells (HSCs) and by a cell population not directly involved in haematopoiesis - mesenchymal stem cells (MSCs). These stem cells reside in the bone marrow and are capable of homing to the BM cavity and regenerating a full array of hematopoietic cell lineages. The HSCs are rare and represent only 0,005% to 0,01% of all nucleated BM cells and they express surface antigens CD34 and CD35, produce and release a number of cytokines involved in the maintenance of haematopoiesis. The aim of the study was determination the profile of cytokine production by stem cells CD34+ of cattle naturally infected with BLV.

The CD34+ cells were generated from the blood and lymphoid organs of infected with BLV and control healthy cows with the use of immunomagnetic method and monoclonal antibodies anti CD34+ (Miltenyi Biotec). Isolated CD34+ cells were cultivated for 2 weeks *in vitro*. The level of cytokines: IL-6, IL-10, IL-12p40, IL-12p70, IFN and TNF was determined in culture fluid in flow cytometer with the use of monoclonal antibodies for cytokines.

The levels of cytokines produced by blood HSCs: IL-6, IL-12p70 and TNF- $\alpha$  were higher in infected than in control cows. In bone marrow HSCs of leukemic cows, the greater concentrations of: both IL-12, TNF- $\alpha$  and IFN- $\gamma$  were determined, but in spleen only IL-10 was higher in infected animals. In HSCs generated from lymph node of leukemic animals only TNF- $\alpha$  level was lower, but all the other cytokines had greater values than in control cows.

The infection of BLV caused statistically significant differences in cytokine expression by hematopoietic stem cells. On the basis of obtained results it is evident, that retroviral infection may influence and disturb the normal haematopoiesis. Viral infection may affect the levels of cytokines and transcription factors. On the other side, this infection may cause apoptosis, destruction of progenitor cells, cytolysis, what in consequence lead to destruction of haematopoiesis and changes in cytokine profiles.

**Keywords:** bovine leukemia, BLV, stem cells

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*Second Parallel Session*

**Pathogen evolution, Pathogenesis  
and Immunology**

*Poster Presentations*

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## P14. Characterization of Toscana virus (TOSV) experimental infection in Type I Interferon Receptor Knock-Out mice

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Toscana virus (TOSV), a phlebovirus of the *Phenuiviridae* family, is transmitted by sandflies and can infect humans during warm seasons. Most cases of infection result in febrile illness as well as neuroinvasive infections affecting the central and peripheral nervous systems. Due to unspecific symptoms similar to other diseases, TOSV cases are usually underestimated. Recently, seroprevalence studies confirmed that TOSV is present in North Africa, in the Balkan Peninsula, and in the Mediterranean islands with percentages of TOSV-specific antibodies between 1-37% in sera from human and domestic animals (dogs, cats, cattle, sheep and goats). Furthermore, in some of these areas the circulation of other closely related phleboviruses may allow genomic reassortments upon coinfection in the same host (human or animal), increasing the chances for the emergence of new viruses with unknown pathogenic properties. Little is known about the pathogenesis of TOSV infection, due to the lack of a suitable animal model. The main goal of this work was to evaluate the susceptibility of interferon (IFN)- $\alpha/\beta$  receptor knock-out mice (IFNAR<sup>-/-</sup>) to TOSV and study viral pathogenesis in several tissues.

IFNAR<sup>-/-</sup> mice were inoculated with different doses of TOSV and clinical signs and mortality were monitored during 15 days. Mice were sacrificed at different times post infection to study pathological/histological lesions. Sera from infected mice were collected and seroconversion was assayed by immunofluorescence. Here it is shown that IFNAR<sup>-/-</sup> mice were susceptible to TOSV and can develop fatal disease between 10 and 15 days post infection. Few mice showed clear clinical signs as early as 3 dpi and, at later stages, hind limb paralysis was also observed in some of the infected mice. Immunofluorescence analysis confirmed the presence of specific anti-TOSV antibodies in serum of all infected animals. Histological examinations of brain confirmed the presence of morphological changes in neurons compatible with neurological affectation. Adult IFNAR<sup>-/-</sup> mice are susceptible to TOSV infection. Therefore, they may constitute a suitable laboratory model to study viral pathogenesis and to test prophylactic measures as well.

**Keywords:** Toscana virus, seroprevalence, reassortments, pathogenesis, animal model

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## P15. Model of foot-and-mouth disease virus (FMDV) infection in primary cells derived from porcine soft palate

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Foot-and-mouth disease (FMD) is a contagious and devastating animal disease affecting artiodactyls. The persistence of FMDV in some ruminants after clinical recovery remains problematic. Such persistence is defined as the presence of infectious virus in the host beyond 28 days post-infection (dpi). Persistent FMDV has been found in dorsal soft palate (DSP) epithelial cells in ruminants. While the DSP is one of the primary sites of virus replication in both ruminants and swine, no persistent infectious virus was found in swine DSP. Only viral RNA could be detected in vivo over 60 dpi. In order to improve FMDV persistence knowledge, we have developed an in vitro model to overcome the lack of porcine suitable models.

Primary epithelial cells were isolated from DSP tissues collected from 4 - 6-month-old swine. Both the presence of epithelial markers in these cells, such as occludin, and the presence of FMDV-specific integrin receptors were assessed by immunofluorescence. Molecular analyses were performed to check that the cells were free of viruses commonly found in swine. Once characterised, the cells were inoculated as monolayers and as multilayers cultivated at the air-liquid interface (ALI), with a type-O FMDV. Samples were regularly collected up to 115 dpi and were tested for the presence of infectious virus, viral RNA and viral antigen.

We confirmed that these cells were epithelial, possessed FMDV-specific receptors and were free of three common porcine viruses. Experimental infections revealed that they were less sensitive to FMDV than the bovine DSP model we previously established. Analysis of collected supernatants identified infectious virus up to 14 dpi, as well as viral RNA up to 60 dpi, consistent with in vivo observations. Multilayer (ALI) cell culture mimicked a multilayered epithelium more similar to the biological system. Although viral RNA was detected up to 35 dpi, no infectious viruses were however detected in the supernatants after infection of the multilayers.

The development and infection of the DSP primary porcine cells confirmed the absence of any evidence of FMDV persistence in this swine cell model as no infectious virus could be detected after 28 days. The relevance of this cellular model having been confirmed by the results obtained, it would be considered to immortalise these cells in view of developing a porcine epithelial cell line that could be used for research and FMD diagnosis.

**Keywords:** foot-and-mouth disease virus (FMDV); FMDV persistence; cellular model; swine

## P16. Translational research of the genetic diversity of key spike epitopes and broadly neutralising antibodies of coronaviruses

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Coronaviruses (CoV) are a family of RNA viruses that cause a variety of diseases in birds and mammals, including humans. Although many CoVs are species specific, in the last 20 years three highly pathogenic CoVs with great zoonotic and cross-species transmission potential have emerged: severe acute respiratory syndrome CoV (SARS-CoV), Middle East respiratory syndrome CoV (MERS-CoV) and SARS-CoV-2. The S protein of CoVs plays an essential role in binding to receptors on the host cell, determining host tropism, and is the major target of neutralising antibodies. Moreover, mutations in the S protein can induce conformational changes that can lead to alterations in antigenicity, enhancing divergence of Spike protein epitopes, inducing change in receptor/antibody binding affinity, affect the transmissibility of CoVs, and negatively impact vaccine and diagnostic test development. The main objective of this project is to develop a translational workflow to improve the comprehension of CoVs mutational patterns and antibody escape.

Pseudovirus constructs based on the S protein will be used to screen already developed in house antibody libraries and clinical serum samples to identify broadly neutralizing antibodies. Pseudoviruses will be further modified by plasmid cloning for expressing Spike of different CoVs. The selected antigen/antibody complexes with the highest neutralisation capacities will be further assessed through biophysical tools for antibody-binding characteristics by surface plasmon resonance for binding kinetics and mass spectrometry for epitope mapping. Moreover, up to five antibody/antigen candidates with the highest capacity will be resolved in three-dimensional complexes/structures using cryo-EM and/or X-ray crystallography.

Pseudovirus constructs have been developed by three-plasmid co-transfection in HEK293 cells using lentiviral backbone, firefly luciferase reporter and S protein expressing plasmids. We are currently working on pseudovirus protocol optimization and establishment of pseudovirus-based neutralisation assays.

The translational nature of the project brings together experts from different fields at Lund University combining expertise in virus evolution, molecular virology, applied biotechnology, and structural biology. The proposed research represents a unique translational initiative assessing how genetic adaptation of CoVs impacts the three-dimensional structure of CoVs S protein epitopes, and the likelihood of these epitopes being targeted by broadly neutralising antibodies. Altogether, the results will provide novel data that could contribute to raised preparedness for future CoV pandemics and have an impact on vaccine and drug development against CoVs.

**Keywords:** Coronavirus, spike, epitope, neutralising antibodies, mutations, structure

**Acknowledgement:** This research is funded by Lund University and the EUGLOH alliance

## P17. A recombinant strain of lumpy skin disease virus displays similar virulence to a cluster 1.2 strain under experimental conditions

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Lumpy skin disease (LSD) is a systemic disease of cattle characterised by multifocal necrotic cutaneous lesions. LSD is caused by lumpy skin disease virus (LSDV), a poxvirus in the genus *Capripoxvirus*. Historically, LSD endemicity was confined to Africa, however since 2014 the disease has spread rapidly through the Middle East, southeastern Europe and Asia, affecting tens of thousands of cattle and causing severe economic losses. Genotypic analysis of LSDV field strains has shown that the disease outbreaks in the Middle East and Europe were caused by cluster 1.2 LSDV strains, whilst outbreaks in Russia and some parts of Asia were caused by recombinant strains of LSDV. These recombinant strains have genotypic features characteristic of both cluster 1.1 and 1.2 strains. To understand the relative virulence of the cluster 1.2 LSDV strains and the newly emerged recombinant LSDV strains, a challenge study was carried out using an experimental bovine model of LSD. Two groups of 8 cattle were inoculated intradermally with  $1 \times 10^6$  plaque forming units of a cluster 1.2 or recombinant strain of LSDV and the clinical and virological outcomes measured. Six out of 8 animals in both groups developed clinical disease, defined as cutaneous nodules distant from the inoculation sites. There was no significant difference between the timing or severity of the clinical disease exhibited by either group, with all six animals inoculated with the recombinant strain and five out of six clinical animals inoculated with the cluster 1.2 strain developing severe disease (over 100 cutaneous nodules). All clinical animals developed a viraemia between 5 and 7 days post inoculation, with no significant difference in the timing or magnitude of the viraemia between the groups. Our findings indicate that the pathogenicity and infection dynamics of cluster 1.2 strains and recombinant strains of LSDV are similar.

**Keywords:** Lumpy skin disease, poxvirus, virus, capripoxvirus, pathogenicity

**Funding source:** BBSRC

## P18. Genomic and evolutionary analysis of swine influenza A virus (H1N2) in challenged vaccinated pigs

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Influenza A viruses (IAVs) are characterized by having a segmented genome, low proofreading function of its polymerases, and a wide host range. Consequently, IAVs are constantly evolving in nature causing a threat to both, animal and human health. In 2009 a new human pandemic IAV strain arose in Mexico because of a reassortment between two strains previously circulating in pigs; Eurasian “avian-like” (EA) swine H1N1 and “human-like” H1N2, highlighting the importance of swine as adaptation host of avian to human IAVs. Nowadays, although limited, trivalent vaccine, which include in its formulation H1N1, H3N2 and, H1N2 swine IAV (SIAV) subtypes, is one of the most applied strategies to mitigate the damage caused by the swine influenza disease in farms. However, due to the genomic distance between farm circulating and vaccine formulation strains, the protection generated during vaccination is not complete, allowing virus circulation, potentially favouring viral evolution.

The evolutionary dynamics of SIAV quasispecies were studied in samples collected from 8 vaccinated and 8 nonvaccinated pigs, previously challenged with H1N2 SIAV. Nasal swab samples were daily collected meanwhile BALFs were recovered at day of each pig necropsy for SIAV detection and whole genome sequencing by NGS. Subsequently, a genomic analysis was carried out to detect single nucleotide variants (SNV) and SIAV intra-host diversity. In the present study, a total of 31 whole SIAV genome sequences were obtained, 18 and 12, from vaccinated and nonvaccinated animals, respectively, as well as the inoculum. Herein, a total of 724 de novo SNV were found along all genetic segments. The impact of those substitutions was hypothesized according to previous literature, particularly in the surface glycoproteins HA and NA, which may be contributing to vaccination scaping by virus. Altogether, the H1N2 SIAV evolution capacity is evidenced, observing different evolutionary trends in vaccinated and nonvaccinated animals.

**Keywords:** Swine influenza virus, virus evolution, vaccination, NGS

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*Third Parallel Session*

**Diagnostic tools and Disease  
Surveillance**

*Oral Presentations*

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## Characterization of classical swine fever virus specific epitopes on the D/A domain of glycoprotein E2

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Classical swine fever virus (CSFV) shares high antigenic homology with other members of the genus *Pestivirus*. Infections of pigs with ruminant pestiviruses can result in the production of cross-reacting antibodies, which may have a significant influence on the serological diagnosis of CSF. Thus, it is all the more important to define CSFV-specific epitopes with regard to improve the serological diagnosis of CSFV. The glycoprotein E2 is responsible for the induction of virus neutralizing antibodies and contains four antigenic domains whereby the domains B and C, as well as D and A represent two structural units.

The specificity of seven monoclonal antibodies (mabs), that detect the antigenic domain D/A of E2, was analysed by using a panel of pestiviruses ( $n = 30$ ). Detailed epitope mapping was performed by analysing the antibody reactivity with different truncated as well as mutated E2 proteins and an overlapping peptide library.

The mabs detected specifically the CSFV strains ( $n = 21$ ) of all tested genotypes, except for two mabs that did not bind to the E2 protein of the CSFV strain Riems. Four CSFV-specific epitopes were identified within the D/A domain of the E2 including the well described linear epitope <sub>829</sub>TAVSPTTLR<sub>837</sub> (Lin et al., 2000). The other three epitopes are located near each other and may form a more complex conformational epitope. Interestingly, the two mabs that did not react with E2 of CSFV strain Riems acquired the ability to bind to the E2 protein after mutating a single amino acid.

The identified epitopes are conserved among the antigenic E2 domain D/A of various CSFV genotypes. Consequently, the mabs of this study represent an important tool to improve the serological differential diagnosis of CSFV.

**Keywords:** classical swine fever virus, glycoprotein E2, epitope mapping

**References:** Lin et al., (2000) J Virol 74, 11619-25.

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## Metagenomics-based protocol for rapid detection of outbreak-causing viruses with ONT technology

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Rapid detection of pathogenic viruses causing an outbreak in animals is vital for an effective and accurate response. Metagenomic methods provide a culture-independent and hypothesis-free approach to know the virome of an organism. The objective of the study was to validate a rapid shotgun metagenomics-based protocol for RNA and DNA virus field detection, developed within the European TELE-Vir project.

Protocol validation was carried out, with slight modifications, using fresh samples (oral swabs and feathers) from red-legged partridges (*Alectoris rufa*) that had been experimentally infected with West Nile virus (WNV) of lineages 1 and 2 (n=4). Animal samples were homogenized and pre-treated to remove host and bacterial DNA. Nucleic acids were extracted and random DNA amplification, including a retrotranscription step, was performed to detect both RNA and DNA viruses. DNA libraries for sequencing with Oxford Nanopore Technology (ONT) were made with the Rapid Sequencing Kit, indexing several samples together for optimization of the protocol and, the resulting reads were analysed using several software, including InsaFlu (developed within the TELE-Vir project by Instituto Nacional de Saúde Doutor Ricardo Jorge). Quantitative PCR for WNV were also performed in order to know the Ct of the samples and to establish a detection limit with this protocol.

Depending on the Ct for WNV of the sample analysed, we obtained WNV reads representing between 0.005% (27 of 576 696 reads/Ct 30) and 5.3% (4 052 of 76 000 reads/Ct 18) of the total reads. Regarding coverage percentage, these reads covered 22% and 99.96% of the WNV genome used as reference, respectively. Although WNV reads represented a very low percentage of the total reads, they were distributed throughout the reference genome, so we were able to confirm, in all samples, the presence of the expected virus. A clear limitation of this virus detection technique was the presence of large amounts of host and bacteria DNA, even after pre-treating the samples, as well as common false positive detection.

Results indicate that the protocol developed in the TELE-Vir project makes it possible to determine the virus causing an outbreak in less than 48 hours, even though the virus reads account for less than 0.01% of the total reads. Furthermore, we can conclude that a Ct of around 30 for West Nile Virus would be the limit for the detection of this virus with this protocol.

**Keywords:** Metagenomics, Outbreak, ONT, Virome, West Nile Virus

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## Improvement of molecular and serological tools for the detection of Venezuelan Encephalitis Virus (VEEV) in equids

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Venezuelan Equine Encephalitis Virus (VEEV) belongs to genus *Alphavirus* within family *Togaviridae*. The virus, transmitted by infected mosquitoes of the genera *Aedes*, *Culex* and *Psorophora*, is responsible for encephalitis in horses but also in humans. Equids serve as amplifying hosts for epizootic strains. VEEV is considered to be highly pathogenic with an equine mortality of between 40 and 90%. Historically, epizootic strains were restricted to North and West South America. However, from 1969 to 1972, an emergence occurred in Central America and the USA. Classified in categories D and E by the new animal health law, which came into force in April 2021, this disease is subject to compulsory declaration and surveillance in Europe. The European Commission is requesting systematic control of horses coming from the American continent. Under scenarios of climate change, we are experiencing, and the intensification of international trade (trade, racing and international horse shows (Olympic Games 2024)), the risk of introducing VEEV into Europe and more particularly into France is not negligible. The serological diagnosis tools currently available prior to international transport or in cases of suspected Venezuelan equine encephalitis are based on time-consuming and tedious techniques that require a high level of technical expertise. In addition, they require the handling of viruses in BSL3 (Laboratory BioSafety Level 3).

As European Union Reference Laboratory (EURL) for Equine Diseases, our main objective is to develop new cost-effective diagnosis tools, easy to implement, that can be deployed by all 26 Member States. For molecular diagnosis, we have optimised a sensitive and specific detection method using the VEEV/ $\beta$ -actin duplex RT-PCR. The specificity of the method was evaluated on different circulating strains of VEEV but also on other equine neurotropic alphaviruses and flaviviruses. The sensitivity of the method was determined. For the serodiagnosis of VEEV, we are currently developing an indirect ELISA using chimeric class 2 Sindbis virus, expressing the envelop, membrane and capsid structural proteins of VEEV on the Sindbis backbone. The performance, sensibility and sensitivity of the ELISA will be compared to that of seroneutralisation using sera from horses vaccinated or naturally infected with VEEV. The EURL will propose to the member states a range of efficient serological and molecular tools to diagnose equines infected by VEEV and therefore to alert as soon as possible public authorities in order to deploy management measures.

**Keywords:** diagnosis, Venezuelan Equine Encephalitis Virus, horses, risk of introduction, preparedness

## Evaluation of a differential detection ELISA test for the presence of antibodies against West Nile virus and Usutu virus in birds

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West Nile virus (WNV) and Usutu virus (USUV) are two mosquito-borne flavivirus frequently co-circulating in Europe. Serosurveillance of wild birds is suitable for early detection of WNV circulation in prevention programs. However, cross-reaction between these two flaviviruses is a well-known problem in serological tests, making difficult their differential diagnosis, which is crucial for surveillance and control. Current diagnostic protocols involve a confirmatory test over the positive samples in a first screening test round (usually ELISA), by a comparative virus-neutralization titration (VNT) against the viruses known to circulate in the study area. However, the VNT confirmation method still shows some degree of cross-neutralization, is cumbersome, time-consuming and requires biosafety level-3 (BSL3) facilities and maintenance of cell culture lines. In order to solve some of these issues, a pan-avian differential ELISA test has been developed.

In-house ELISA test was performed coating Maxisorp micro-plates with recombinant WNV and USUV EDIII proteins as antigens. After serum incubation, a HRP-conjugated monoclonal antibody (mAb 1A3) with reactivity for Igs from a broad range of avian species was used to detect the antigen-antibody reaction. To validate the method, sera obtained from different avian species experimentally infected with WNV, USUV or a third flavivirus cross-reacting in commercial ELISA tests (Bagaza virus, BAGV) were analysed in parallel by the in-house ELISA test and VNT.

Although some differences were observed for the different avian species, the performance of the ELISA test to determine the presence of specific WNV and USUV antibodies (diagnostic sensitivity of 52% for WNV and 36% for USUV and specificity over 99% for both viruses) was similar to that observed by the VNT reference confirmation method.

The ELISA test described in this work is an easier and less time-consuming alternative to VNT that can be carried out without special biosecurity facilities (non BSL3) allowing a broader range of laboratories to perform serological surveillance and implement this method in animal and Public Health prevention programs.

**Keywords:** ELISA, West Nile virus, Usutu virus, birds, serosurveillance

**Funding source:** Spanish Research State Agency project PID 2020-116768RR-C2

## Newcastle Disease virus containing PCR-targets for West Nile virus as a positive control to validate and implement a Real-time PCR.

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West Nile Virus (WNV) is a virus species within the family *Flaviviridae* and transmission occurs via *Culex*-mosquitos, while mostly affecting birds. Humans and horses are considered as dead-end host, but infection can manifest in disease and neurological symptoms. In 2020, WNV was detected in the Netherlands for the first time in wild bird species and seroconversion was also observed in humans. WBVR is prepared for incursions of WNV with operational ELISAs and PCR-diagnostics. However, extensive validation of PCR-diagnostics requires significant numbers of well-defined WNV-positive samples. In addition, transportation of infectious WNV-containing material is cumbersome, expensive and requires specialized biosafety facilities for the entire logistics process. Therefore, the aim of this research is to develop alternative WNV-positive material that can be applied for validation purposes and as positive control in real-time PCR runs.

Literature was screened for PCR-diagnostics of WNV, and the best primer-probesets were selected by *in silico* validation. Then, the DNA-sequence of the best two PCR-targets was introduced in full-length cDNA of Newcastle Disease Virus (NDV) using reverse genetics. The recombinant virus (WNV-rNDV) was rescued by transfection of VeroE6-cells followed by inoculation of embryonated chicken eggs. WNV-rNDV was titrated and applied as positive control and as WNV-positive material to spike 52 EDTA-blood samples from horses. In addition, the WNV proficiency test of 2020 was re-analyzed.

In total, nine primer-probesets were found in the literature of which Linke *et al.* and Eiden *et al.* showed the best results with respect to *in silico* sensitivity and specificity. These PCR-targets were introduced in NDV and WNV-rNDV was successfully rescued with a viral titre of  $10^{9.21}$  TCID<sub>50</sub>/ml. The analytical sensitivity was an equivalent of  $10^{1.21}$  TCID<sub>50</sub>/mL, while PCR testing of the spiked EDTA-bloods showed no inhibition during amplification. Finally, testing of the WNV proficiency test with the optimized real-time PCR test showed no cross reactivity with other related Flaviviruses.

Because of the lack of WNV-positive samples, we have developed an alternative in the form of a recombinant NDV containing two PCR-targets. The WNV-rNDV was applied as positive control in the real-time PCR and as positive material to spike samples in the matrix of interest. By the use of WNV-rNDV, we have validated and implemented a previously published PCR-test in our routine diagnostics in the view of an outbreak of WNV in the Netherlands.

**Keywords:** West Nile virus, Real-time PCR, Newcastle Disease Virus, Reverse genetics

**Funding source:** This research was funded by the Dutch ministry of Agriculture, Nature and Food quality under project number: WOT-01-002-041.

## Novel diagnostic platform based on LAMP assays for the efficient detection of African and classical swine fever viruses using minimal equipment

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Classical swine fever (CSF) and African swine fever (ASF) are devastating infectious diseases caused by classical swine fever virus (CSFV) and African swine fever virus (ASFV). ASFV currently represents the biggest threat to the porcine industry worldwide, with high economic impact and severe animal health and welfare concerns. Outbreaks have occurred in Europe and Asia and recently in Hispaniola, Caribbean. CSFV continues to be prevalent in many regions of the world. This work aimed to develop and validate a novel diagnostic platform based on LAMP assays for the accurate and rapid detection of these two infectious agents. Isothermal PCR techniques, such as LAMP, have become increasingly attractive as point-of-care diagnostic tools given the minimal material expense, equipment, and training required. Two LAMP primer sets for ASFV and CSFV, were designed and validated. Both primer sets showed thermal stability, amplifying the ASFV or CSFV target genome at temperatures between 60-70°C using both fluorometric and colorimetric methods. The selected primers did not yield false positive or cross-reactive results with other common swine pathogens. Panels from diverse types of samples collected from pigs experimentally infected with ASFV or CSFV were used in the validation of the assay, in which the reference techniques accredited by the WOA for the molecular diagnosis of these viruses were used. The results show that both LAMP tests would be a useful tool for rapid, highly sensitive and on-site ASF and CSF diagnostic and will easily transfer to other laboratories, improving the diagnostic capacity for both diseases.

**Keywords:** African swine fever, classical swine fever, LAMP assay, molecular diagnosis, on-site diagnostic

**Funding source:** This research was funded under the grants AP21VSD&B000C011 by the National Animal Health Laboratory Network from the United States Department of Agriculture, PID2019-107616RB-I00 and PID2021-125599OB-I00 from the Spanish Science Ministry

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## P19. Molecular Pen-Side test for the detection of the Peste des Petits Ruminants Virus

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Peste des Petits Ruminants (PPR) is a contagious and in most cases severe disease of small domestic ruminants caused by Peste des Petits Ruminants virus (PPRV). Next to small domestic ruminants different species of wild small ruminants show to be susceptible to PPRV. Infection with PPRV is mostly characterized by fever, mucopurulent ocular and nasal discharge, mucosal lesion, pneumonia and diarrhea of different intensity. Early detection of the virus, with methods moving the process closer to the field, play a crucial role in providing precious time for immediate implementation of control measures in order to halt virus transmission concerning envisaged disease eradication.

Cell culture isolated PPRV lineage IV originated from Gazelle from an outbreak in the United Arab Emirates (UAE) was used for intranasal infection of six goats and two goats were kept in-contact. Daily goats were monitored, and clinical score was recorded. Conjunctiva, nasal and rectal swab were collected for the detection of the PPRV genome. A standard RT-qPCR method on BioRad CFX96 cycler, a short RT-qPCR method using transportable Real-Time Thermocycler Genechecker and a commercially available ID Rapid Peste des Petits Ruminants Antigen test (rapid pen-side test) were used to compare the detection of PPRV genome in sample material. The NucleoMag VET kit with half-automated King Fisher platform and IndiMag Pathogen kit with manual easy express extraction method (TripleE) was used for the extraction of viral nucleic acid from swab sample material.

All infected and in-contact goats in the study developed clinical signs typical for PPR indicating successful infection and vertical transmission of the virus. Following clinical reaction score, all infected goats reached human end point before the end of the experiment. Detection of PPRV genome using all three methods was feasible in all three different matrices. According to obtained results, standard RT-qPCR method showed highest sensitivity, slightly lower sensitivity had short RT-qPCR method and lowest sensitivity was observed in rapid pen-side test.

The investigated PPRV strain originated from Gazelle from the UAE shows to be highly virulent and easily transmittable in domestic goats causing severe disease. Standard RT-qPCR method using standard viral nucleic acid extraction method will provide the most reliable results but requires more time. On the other hand, the short RT-qPCR method showed to be reliable and highly sensitive, providing results in shorter period of time (1/4 of standard) and can be performed directly in the field.

**Keywords:** Peste des Petits Ruminants Virus, RT-qPCR, TripleE, Pen-Side test, field detection

## P20. Improvement of point of care diagnosis of African Swine Fever

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African Swine Fever (ASF) is a highly infectious disease of swine, caused by an enveloped double-stranded DNA virus, the ASF virus (ASFV). Infection with ASFV correlates with a wide range of clinical syndromes from almost unapparent disease to haemorrhagic fever with high fatality rates (95–100%). To date, disease is actively being transmitted all over the world, and there are no licensed vaccines. Therefore, ASF control is based on early diagnosis and the enforcement of sanitary measures.

Due to their characteristics, lateral flow assays are one of the most widely used techniques for point-of-care testing, accelerating the final diagnosis. In this work, we improved the diagnostic performance of rapid tests for both, antibody and antigen detection, through the application of new reagents and cassettes.

For the improvement of antigen detection, a new recombinant monoclonal antibody produced at Eurofins-Ingenasa was used to develop a lateral flow assay using latex nanoparticles. This new assay was compared to the commercial INgezim<sup>®</sup> ASF CROM Ag and the PCR described under the WOAHP manual, evaluating a total of 124 experimental positive and 160 negative bloods.

For antibody detection, a new version of the recombinant VP72 was employed to develop a colorimetric assay employing colloidal gold. This assay was compared to other commercial assays evaluating a total of 107 experimental positive sera, 73 field negative bloods and 50 field negative sera.

In both assays, new double cassettes (one antigen and one antibody strip per cassette), with an additional window for sample addition were implemented.

The new assay for antigen detection exhibited the same sensitivity as the commercial assay evaluated but with improved specificity. Out of the 160 samples evaluated, no false positive results were detected. The new assay for antibody detection exhibited better sensitivity than the commercial rapid test INgezim<sup>®</sup> PPA CROM Anticuerpo, but also when compared to the reference ELISA INgezim<sup>®</sup> PPA Compac, and with the INgezim<sup>®</sup> ASFV-R ELISA, based on early expressed antigens.

Concerning a highly contagious disease like ASFV, rapid tests are of great interest to control the epidemic due to their fast interpretation, that accelerate the identification of infected pigs and wild board. The assays described in this work improve ASF diagnosis, giving more accurate results for antigen detection and earlier antibody detection. Therefore, the combination of these assays in a single cassette represents a precise tool for the rapid detection of ASF.

**Keywords:** African Swine Fever, Rapid Test, Antigen detection, Antibody detection

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## P21. Utilization of ONT NGS in the workflow of veterinary diagnostics

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Next generation sequencing (NGS) has been applied to the diagnosis and molecular epidemiology of infectious diseases impacting the poultry and swine industries. NGS provides a shortcut to obtain sequence information needed for identification and typing of pathogens – compared to the traditional workflows.

Sample types, among other factors, may profoundly affect the outcome of diagnostic procedures. We demonstrate that Oxford Nanopore Technologies (ONT) approach based NGS protocols enabled us to successfully detect and characterize important veterinary pathogens responsible for direct or indirect economic losses to animal husbandry. The samples represented different types/matrices and originated from different geographic regions.

The applied protocols were as follows: 1) chicken infectious bronchitis virus (IBV) whole S1 gene sequencing from dry swabs, allantoic fluid, DNA/RNA shield (Zymo), and FTA card; 2) fowl adenovirus (FAdV) whole genome sequencing (WGS) from cell culture supernatants and tissue homogenate; 3) avian influenza virus (AIV) WGS from allantoic fluid and 4) swine influenza virus (SIV) WGS from nasal and tracheobronchial swabs into virus transport media, DNA/RNA shield, organs and allantoic fluid.

Finally, it must be emphasized that our protocols and analysis pipelines are constantly being verified and validated, and improved, if necessary, in order to deal with the different sample type panels, and to deliver effective, cost-efficient, and time-saving protocols.

**Keywords:** veterinary diagnostics, sequencing, ONT, sample type

## P22. Lateral flow device: a promising tool for field diagnosis of Lumpy skin disease

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Lumpy skin disease (LSD) is a transboundary cattle disease caused by the lumpy skin disease virus (LSDV), a dsDNA virus that belongs to the *Capripoxvirus* genus, *Poxviridae* family. Since LSD is a notifiable disease according to WOA, innovative and effective tools for detecting on-site virus are of great interest to expedite diagnosis and implementation of control measures. Lateral flow devices (LFD) could contribute to this goal given their extreme simplicity which make them feasible in field conditions. This study aimed to preliminarily evaluate the performance of an LFD produced using monoclonal antibodies (mAbs) to detect LSDV.

Two mAbs (2C6 and 2F10) were selected to set up the LFD; they recognize two epitopes of the LSDV p32 protein and cross-reacted with GTPV and SPPV. To evaluate the analytical sensitivity, serial dilutions of an *in vitro* culture of LSDV Neethling strain were tested, and the results were compared to those obtained with a sandwich ELISA based on the same mAbs. Analytical specificity was investigated by testing other viruses causing similar infectious diseases (*e.g.* Bovine papular stomatitis virus and Pseudocowpox virus, both belonging to *Parapoxvirus* genus). Eleven skin nodules from an experimental infection with the LSDV-Macedonia2016 strain, collected nine days post-infection, were also analysed. The results were compared to those obtained with the qPCR.

The evaluation of analytical sensitivity showed that the test detection line was still visible at a 400-fold dilution of the viral culture, corresponding to  $10^{3.4}$  TCID<sub>50</sub>/mL, whereas the ELISA detection limit was set at a 1/250 dilution. No cross-reactivity was identified with viruses from other genera. Skin nodules from experimental infection with Ct values in the range of 14-17 showed a strong signal (8/11), while a weaker signal was present when samples with a lower viral load were tested (Ct values 22-25). By analysing isolates with similar genome loads, we identified a reduced analytical sensitivity for GTPV and SPPV compared to LSDV.

LFDs represent the simplest tool for rapid on-site diagnosis. For all the tested samples, the sensitivity of the developed LFD correlated with the viral load. Ongoing analyses involving field samples will provide a better understanding of the test performance. Further investigation is needed to evaluate the LFD's performance for detecting GTPV and SPPV.

**Keywords:** Lumpy skin disease, Capripoxvirus, lateral flow device, field test, monoclonal antibodies

## P23. Recombinant Bovine Ephemeral Fever Virus (BEFV) N protein production: evaluation in a competitive ELISA

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Bovine Ephemeral Fever virus (BEFV), member of the genus *Ephemerovirus* within the family *Rhabdoviridae*, is an arthropod-borne virus transmitted by many species of biting midges and mosquitoes. It can have serious economic impacts due to losses in milk production and of general condition in beef cattle and water buffalo. Although in Europe this disease has not been reported yet, the main threat is represented by possible spreading through neighbouring countries (especially Middle East). The viral genome encodes five structural and four accessory proteins. In this work, a recombinant structural nucleoprotein (N), was expressed and its use in competitive ELISA was evaluated.

The sequence JX234572.1 was used as a template to design the primers set. The complete N coding region was inserted into a pET45b plasmid through double digestion, cloned in OneShot TOP10 cells and expressed in BL21(DE3) cells. The recombinant protein was purified through Ni-NTA Agarose and checked in SDS page. It was tested and characterized in western blotting, indirect, and competitive ELISA with a panel of 19 mAbs previously produced against the inactivated virus through the hybridoma technology. A total of six BEFV naturally infected bovine sera (VNT titre ranging from 1/8 to 1/256) and 50 negative sera were tested with the recombinant N in a competitive ELISA prototype, previously set up with the inactivated viral antigen.

The recombinant protein was successfully expressed and purified with yield of 20 mg/l in bacterial culture. The SDS page showed a band at 50 kDa, consistent with the expected 49 kDa product weight. Ten out of 19 available mAbs were able to recognise a linear epitope in western blotting. The recombinant N was then tested in indirect ELISA and 16 out of 19 mAbs were likely recognising it. Two mAbs, namely 3E6 and 3A2-HRP, previously selected to design a competitive ELISA with the complete viral antigen, showed to work well also with the recombinant N, allowing to discriminate between BEF positive and negative sera.

Considering that viral antigens are difficult and expensive to produce, heightening the biosecurity issues, a valid alternative to their use in diagnostic assays is fundamental. The aim of this study, therefore, was to develop a more convenient and cost-effective competitive ELISA with a recombinant protein. Our preliminary results suggest that the recombinant N reproduces the antigenic and immunogenic features of the viral one, showing to be suitable for the set-up of methods usable for the serological BEF diagnosis.

**Keywords:** Bovine Ephemeral Fever, vector-borne disease, recombinant protein, ELISA, monoclonal antibodies

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*Third Parallel Session*

**Diagnostic tools and Disease  
Surveillance**

*Poster Presentations*

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## P24. Enhancement and extension of WNV and USUV surveillance in France through a national serosurvey conducted in wild captive birds and improvement of molecular diagnostic tools

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West Nile virus (WNV) and Usutu virus (USUV) are emerging vector-borne zoonotic flaviviruses. Both viruses are transmitted through an enzootic cycle involving *Culex* mosquitoes and a range of avian hosts but they occasionally infect mammals such as equines or humans. Vertebrate hosts can develop diseases ranging from mild illness to severe neurological disorder. Humans and horses are accidental hosts and epidemiological dead ends. Since their first introduction through migratory birds from Africa in the 1960's and 1990's respectively, WNV and USUV have been (re)emerging through Europe with a high epidemic year witnessed in 2018, and geographical spread with novel introductions and sometimes conversion to an endemic status in several European countries. Control mainly relies on surveillance and preventive measures in case of detection. Passive surveillance programmes in equids and birds are implemented in most European countries. Where prevalence is high, active surveillance programmes that involve yearly screening of a significant number of individuals (birds and mosquitoes) are implemented. Strengthening/improving surveillance strategies for WNV and USUV is a key concern for National and European reference laboratories. In France, circulation of both viruses are currently monitored through passive surveillance that encompasses (i) serological testing of clinically symptomatic horses or captive birds manifesting neurological signs and (ii) molecular detection of viral genome in targeted organs by RT-qPCR. We aim to reinforce and extend the integrated surveillance system in place. As an initial approach, we conducted a serosurvey in captive birds from zoos distributed across the country. In order to strengthen the surveillance of these two viruses, we have extended our surveillance network throughout the country and optimised molecular diagnostic tools that can be adapted according to epidemiological criteria (region, period, samples). Thus, we will use wild birds as sentinel and propose an efficient, simple, and cost-effective strategy that is adaptable depending on the intensity of circulation of WNV and USUV. During the transmission season, from June to November, when viruses prevalence is high, we will use a multiplex RT-qPCR assay for the simultaneous detection of these two viruses as well as the house keeping gene  $\beta$ -actin. Apart from this season we will screen bird samples using, a Panflavi RT-qPCR as first intention diagnostic test.

The development of a low cost active surveillance system should allow earlier detection of WNV and USUV infections and guide policy makers to adapt prevention and control measures.

**Keywords:** West Nile virus, Usutu virus, surveillance, diagnostic

## P25. Diagnostic tools in laboratory for *Brucella suis*

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Brucellosis is one of the most important zoonotic diseases in the world. The overall laboratory capacity, as well as available diagnostic tools are equally important in the diagnostic process. In Serbia there have been outbreaks of swine brucellosis caused by *Brucella suis* (*B. suis*) in the past and the last one occurred in 2022. *Brucella suis* severely affects pigs with abortion in pregnant sows and orchitis as the most common symptom. The outbreaks of swine brucellosis result in high economic losses due to both abortions and stamping out policy.

For this survey 50 samples of swine blood sera were examined, taken from a farm with suspected *B. suis* outbreak. In total six diagnostic tools were used and compared: Rose Bengal test (RBT), competitive ELISA (c-ELISA) and indirect ELISA (i-ELISA) kits. Three ELISA kits were specific for diagnostic of *B. suis*, and two were multispecies kits. One of the multispecies kits had plates coated with *Brucella abortus*.

From the total of 50 samples, 6 were negative with RBT (12%). With i-ELISA specialised for *B. suis*, the same samples were negative plus one more sample (which was suspicious with RBT), in total 7 (14%) negative samples. With c-ELISA specialised for *B. suis*, the same samples were negative, plus two more (which were suspicious with RBT) so in total 8 (16%). With multispecies c-ELISA, the same samples were negative plus three more samples (which were suspicious with RBT) so in total 9 (18%). With multispecies ELISA coated with *B. abortus*, all of the samples were negative.

The authorised laboratories for diagnostics of *B. suis* have to be very cautious in choosing the kits. Rose Bengal test is sufficient as screening test for *B. suis* outbreaks. According to our first results, it is not enough to use just two tests for diagnostic of *B. suis* (screening and conformation) because it might lead to missing of positive cases.

**Keywords:** *Brucella suis*, diagnostics, ELISA, Rose Bengal, swine

**Funding source:** This work was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract No: 451-03-47/2023-01/200031.

## P26. Seroprevalence of selected infectious diseases in red deer population in Serbia

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While domestic and wild animals share the same pathogens, wildlife is recognized as the reservoir of many diseases. Considering their role as indicators, victims, bridge hosts, or maintenance hosts, the monitoring of wildlife should be an integrated part of country surveillance and disease control strategy. Thus, the main aim of this study was to determine the presence and the spread of selected infectious diseases in red deer population in Serbia through seroprevalence estimation.

A random geographical collection of samples from 123 shot red deer was organized during the hunting season from August 2022 to February 2023. The blood was collected directly from the heart using a syringe and needle. For each sample, data on geolocation, species, age, and sex were collected. Sera samples were decanted after centrifugation at 2000 g for 10 minutes and tested for the presence of specific antibodies against Capripox virus (ELISA: ID Screen® Capripox Double Antigen Multi-species, IDvet), West Nile fever virus (ELISA: INgezim West Nile Compac, Ingenasa), Schmallenberg virus (ELISA: ID Screen® Schmallenberg virus Indirect Multi-species, IDvet), Bluetongue disease virus (ELISA: INgezim BTV DR, Ingenasa), Bovine viral diarrhoea virus (ELISA: PrioCHECK™ Ruminant BVD p80 Ab Serum & Milk, Prionics), Bovine Herpesvirus-1 (ELISA: ID Screen® IBR gB Competition, IDvet), Q fever (ELISA: ID Screen® Q Fever Indirect Multi-species, IDvet), *Brucella* spp. (RBT; ELISA: INgezim® Brucella Compac 2.0 Ingenasa) and *Leptospira* spp. (MAT).

Specific antibodies against Capripox virus, Bovine Viral Diarrhoea Virus and *Leptospira* spp. were not detected in any of tested samples. Seroprevalence of *Brucella* spp. infection was 0.8%. Q fever infection was indirectly confirmed in 3 red deer (2.4%) from the same hunting ground. Seroprevalence of vector-borne diseases in the red deer population was 18.5% for Schmallenberg disease, 39% for Bluetongue disease, and 58.5% for West Nile fever. Bovine Herpesvirus-1 antibodies were detected in 43.1% of samples.

The red deer population can be considered the reservoir of vector-borne viral diseases such as Bluetongue disease, West Nile fever and Schmallenberg disease. Based on estimated seroprevalence, red deer should not be considered a risk for maintaining Q fever, Brucellosis, Capripox virus, Bovine Viral Diarrhoea Virus and *Leptospira* spp. infection. The results of the present study indicate that the red deer population should be thoughtfully considered during livestock disease eradication programs, particularly infectious bovine rhinotracheitis and Bluetongue disease.

**Keywords:** red deer, seroprevalence, Serbia

**Funding source:** The study was funded by the Serbian Ministry of Science, Technological Development and Innovation (Contract No 451- 03-68/2022- 14/200030)

## P27. Survey of antibodies to Crimean-Congo haemorrhagic fever virus in livestock from Georgia, Kosovo, Macedonia and Montenegro, using screening and confirmatory diagnostic tests

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Crimean-Congo haemorrhagic fever is a severe infectious disease causing up to 40% mortality in humans, which is emerging in different European countries. The etiological agent, Crimean-Congo hemorrhagic fever virus (CCHFV) is transmitted in nature by tick bites, and a number of mammal species (including livestock) act as reservoir hosts. Animals are not clinically affected.

Serological surveillance in animals provides important information about local circulation of this pathogen. This, together with monitoring of ticks (including infected ticks) and surveillance of human cases, constitutes an optimal One Health surveillance enabling prevention and control measures adjusted to public health risk. However, reliable diagnostic tools for monitoring of CCHFV antibodies in animals with sound validation data are still scarce. Indeed, lack of reference materials and gold standard tests make this task even more difficult. The objective of this study was to evaluate the suitability of a “screening-confirmatory test” approach to detect serum antibodies against CCHFV in different reservoir animal species, and to apply this approach to perform serological surveys in animals in different Eastern European countries with known CCHFV circulation or at risk of it.

After examination of different serological tools we selected a double antigen (DA) ELISA (ID Screen CCHF Double Antigen Multispecies, IDVet, France) as screening test and an indirect immunofluorescence assay (IFA) (CCHFV Mosaic 2, IgG, Euroimmun, Germany) adapted to animals, as confirmatory test. Panels of serum samples from Georgia (n=60), Kosovo (n=80), Macedonia (n=110) and Montenegro (n=44), mostly from domestic ruminants, were analyzed.

Antibodies to CCHFV were found in livestock from all countries examined. Overall, 56% of the DA ELISA-positive samples were confirmed as positive by IFA, while none of the DA ELISA negative samples was IFA-positive. No relevant differences between species (cattle, sheep, goat, horses) or countries were found.

In the absence of alternative tests, the assayed strategy provides laboratories with a diagnostic system to detect and confirm CCHFV seropositive livestock species.

**Keywords:** ELISA, IFA, Crimean-Congo hemorrhagic fever virus virus, livestock, serosurveillance,

**Funding source:** MediLabSecure project (EU DG DEVCO: IFS/21010/23/-194 and 2018/402-247)

## P28. Use of environmental samples for laboratory zebrafish health monitoring

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Implementing a microbiological monitoring program for research animals, such as zebrafish, guarantees researchers the reliability and reproducibility of experimental data as well as greater protection of animal welfare. In accordance with 3Rs principle (1), the development of molecular methodologies for safeguarding the health of housed animals falls under the concepts of Refinement and Reduction. The Federation of Animal Science Laboratory Associations (FELASA) recommends, for good surveillance, to monitor fish performance, mortality, morbidity, determine diseases of interest, establish a screening pattern, use specific tools to diagnose pathologies and report results (2). The present study aimed to create a health monitoring plan for zebrafish, investigating pathogens circulating in aquarium systems. The diagnostic program was based on the molecular analysis of environmental samples collected from animals. Environmental samples can be considered an adjunct to animal tests, which could improve the conditions of housing and, at the same time, limit the number of sentinel subjects sacrificed.

Sequences of interest, specific primers and probes have been identified for the detection of the pathogens. Sampling was performed at the IZSLER animal facility, and the filtering system of the aquariums was analysed: sludge, contained in the pre-filter, and the water cartridge filter of the recirculation system were collected. DNA was isolated by extraction kits provided by different companies (Qiagen, Promega, Zymo Research, Macherey-Nagel™) and evaluated in this study. DNA extracted was quantified and analysed by PCR, qPCR for the detection of *Mycobacterium spp.*, *Mycobacterium chelonae*, *Mycobacterium marinum*, *Pseudocapillaria tomentosa*, *Pseudoloma neurophilia*, *Ichthyophthiriu multifilis*, *Infectious Spleen and kidney necrosis virus*, *Infectious pancreatic necrosis virus*.

Commercial kits showed good extraction efficiency. The tests performed detected *Mycobacterium spp.*, more specifically, *Mycobacterium chelonae* and *Pseudocapillaria tomentosa*. By these preliminary tests, it can be deduced that the starting matrix, the extraction kits used, and the PCR conditions adopted are suitable for the work to be performed.

This type of approach aims to realize screening for pathogenic microorganisms, improving control of the animal health status and, at the same time, limiting the number of subjects used in the experiments. In addition, testing environmental samples it is possible to avoid the stress and suffering of the animals.

**Keywords:** Health monitoring, 3Rs principle, environmental sample

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## P29. A new RT- PCR for Classical Swine Fever Virus detection in Swine and Wild boar, allowing for parallel testing with the ID Gene ASF qPCRs

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Classical swine fever (CSF) is a contagious viral disease of domestic and wild swine, caused by the CSF Virus (CSFV), a single-stranded RNA virus, member of genus pestivirus like Bovine Viral Diarrhea Virus (BVDV) and the Border Disease virus (BDV) which affects Sheep.

Reliable and accurate diagnostics are the key for rapid implementation of control measures to detect and prevent the spread of CSFV.

Innovative Diagnostics has developed a rapid and specific RT-qPCR for detection of CSFV RNA, the ID GENE™ Classical swine fever Virus duplex. Its protocol is compatible with those of our two ID Gene™ African Swine Fever qPCR kits, allowing, from the same extracts, for a parallel testing on the same qPCR run of CSFV and ASFV, which share similar clinical patterns.

The test, which targets the non 5' UTR region, was performed as per kit's instructions. Results are obtained in 65min with a rapid amplification protocol.

Analytical specificity was evaluated on 21 Classical swine fever virus (CSFV) strains provided by the Friedrich-Loeffler-Institut (FLI), 11 CSFV isolates from National Reference Laboratory (ANSES - Ploufragan, France) and 62 other pathogens involved in animal disease including BVDV and Porcine reproductive and respiratory syndrome virus (PRRSV).

Limit of detection of the PCR ( $LD_{PCR}$ ) was determined with a synthetic RNA fragment and Method Detection Limit (MDL) was determined by using negative swine blood, serum and spleen samples spiked with the modified live virus vaccine (PESTIFFA - Boehringer).

Diagnostic specificity and sensitivity were evaluated on 66 samples.

All tested CSFV strains were successfully detected by the ID Gene qPCR, without any cross reaction with other pathogens, giving 100% inclusivity and exclusivity.

The  $LD_{PCR}$  (95%) was 4 copies/PCR and the MDL obtained on swine blood was  $4.10^3$  copies/ml with automated extraction method (ID Gene™ Mag Fast Extraction Kit).

The measured diagnostic sensitivity and specificity were 100%.

The RT-PCR kit shows high analytical performance. It is easy-to-use, with a single reaction mix containing an internal control. If differential diagnosis is needed, samples can be processed on the same plate in parallel with our ASF PCRs (ID Gene® African Swine Fever Duplex and ID Gene® African Swine Fever Triplex). The kit was Approved in Germany by the Friedrich-Loeffler-Institut (FLI C-106).

**Keywords:** Diagnostic, Classical Swine Fever, RT-PCR, Duplex

## P30. A new indirect ELISA for the discrimination of anti-Equine Herpes Virus Type 1 and Type 4 antibodies in horse sera

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Equine herpes virus type 1 and 4 (EHV-1 and EHV-4) infections are widespread in horse population around the world. Both viruses are responsible for respiratory syndromes, but EHV-1 is more of concern for horse industry because of severe symptoms, such as neurological disorders and abortions, that do not occur during EHV-4 infections. Performances of sport horses are affected as much as equine breeding, resulting in considerable economic loss.

These two alphaherpesviruses are closely related, making serological diagnosis difficult by conventional methods.

Therefore, the **ID Screen® EHV-1/EHV-4 Discrimination test Indirect** ELISA kit was developed as a bivalent ELISA in order to distinguish between antibodies against EHV-1 or EHV-4 in equine serum / plasma, without cross-reactions.

Diagnostic specificity and sensitivity of this tool was evaluated using horse sera from France and Iceland. Results were compared to another commercial ELISA.

Seroconversion of 7 experimentally infected horses (EHV-1 strain FR-56628) was followed-up. Horses were sampled between day 0 and 20 dpi. Sera were tested in parallel by VNT test and ID Screen® ELISA.

For EHV-4, eight horses were monitored during a natural EHV-4 infection (France). Sera and nasal swabs were collected regularly from these 8 horses, from the appearance of the first clinical signs in the stable (day 0) and until 77 days. All sera were tested with the ID Screen® ELISA.

Correlation with the other commercial ELISA was good with a correlation of 83% for EHV-1 ( $k = 0.76$ ;  $CI_{95\%} = [0.73; 0.90]$ ), and 92% for EHV-4 ( $k = 0.65$ ;  $CI_{95\%} = [0.51; 0.93]$ ).

Regarding EHV-1 seroconversion, EHV-1-antibodies were detected between 13 and 17 dpi by the ID Screen® ELISA.

During EHV-4 outbreak, EHV-4 antibodies were detected by the ID Screen® ELISA between 6 and 10 days following first clinical signs.

No cross-reactivity was observed between EHV-1 and EHV-4 antibodies, allowing for distinction between both viral infections.

The ID Screen® ELISA is easy-to-use with both EHV-1 and EHV-4 testing performed within the same analytical run, thanks to its biwell plate format. It presents excellent discriminatory capacity between EHV-1 and EHV-4 infected horses and shows good correlation with another commercial ELISA Kit, based on field samples.

**Keywords:** equine herpesvirus, discrimination ELISA, serology

## P31. A new pan RT-PCR for the detection of *Epizootic Haemorrhagic Disease Virus* in cattle and small ruminants

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Epizootic hemorrhagic disease (EHD) is a Culicoides-borne viral disease caused by the EHD virus (EHDV), associated with clinical manifestations in domestic and wild ruminants, primarily white-tailed deer, and cattle. Along with BlueTongue Virus (BTV), EHDV is classified within the genus *Orbivirus*. EHDV has a 10-segmented RNA genome encoding for seven structural proteins (VP1-VP7) and five nonstructural proteins (NS1-NS4, NS3a).

In late September 2021, EHDV was reported in cattle farms in central/western Tunisia. It rapidly spread throughout the country with more than 200 confirmed outbreaks. For the first time in 2022, EHDV was detected in continental Europe.

Innovative Diagnostics has developed a qualitative duplex real-time PCR (RT-PCR) for pan EHDV detection. The test can be used on whole blood, spleen, or organs from bovine or small ruminants' species. It simultaneously amplifies a specific target from the EHDV genome as well as an endogenous non-target positive (ruminant housekeeping gene).

After RNA denaturation (heating at 95°C for 3 minutes), 5 µl of denatured RNA was added to the ready-to-use mastermix and the amplifications were carried out using a rapid amplification program (65 min).

Specific synthetic RNA was designed to estimate the limit of detection of PCR (LD<sub>PCR</sub>) and to determine the RT-PCR efficiency. RNAs panels (provided by ANSES, Maisons-Alfort, France) from the 7 EHDV known serotypes, and 27 BTV serotypes or from other pathogens affecting cattle were tested to assess test inclusivity and exclusivity, respectively. Samples were also tested in parallel on a commercially available PCR kit.

The LDPCR (95%) was estimated around 10 copies/PCR and PCR efficiency was determined above 98% with the synthetic RNA. The analytical specificity of RT-PCR assay was evaluated in silico by aligning the target PCR systems with the databases available on NCBI (National Center for Biotechnology Information). The alignments show 100% in silico specificity for the EHDV targeted region, and no homology with other pathogens.

This was confirmed experimentally, as the panEHDV RT-PCR successfully detected all 7 EHDV serotypes, including Tunisia-2021 strains which emerged in Europe, without showing any cross-reactions with neither BTV nor other pathogens.

Compared to a commercially available ELISA, IDvet's RT-PCR gave earlier Cq values with a mean difference gain of 2.8 Cq.

Along with the ID Screen® EHDV Competition ELISA, which is available for specific EHDV antibody detection, the new panEHDV RT-PCR allows for rapid and accurate detection of EHDV, regardless of the serotype.

**Keywords:** Diagnostic, EHDV, RT-PCR, duplex

## P32. A new Classical Swine Fever indirect ELISA for herd profiling and vaccination monitoring

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Classical swine fever (CSF) is a highly contagious swine disease caused by the CSF virus (CSFV, genus *Pestivirus*), closely related to the ruminant pestiviruses which cause Bovine Viral Diarrhea and Border Disease. In its acute form, the disease is characterized by fever, depression, superficial and internal hemorrhages, high morbidity and mortality. In its chronic form, the clinical signs are less severe, and recovery is occasionally seen in mature animals. Transplacental infection with low virulence viral strains often results in persistently infected piglets, responsible for virus dissemination.

Serological tests such as serum neutralization or enzyme-linked immunosorbent assay (ELISA) are commonly used for CSFV antibodies monitoring. IDvet already offers a competitive ELISA (cELISA), the ID Screen® CSF Competition ELISA for anti-CSFV E2-glycoprotein antibody detection. In this study, we describe a new kit, the ID Screen® Classical Swine Fever E2 Indirect ELISA (iELISA) which will allow to assess the anti-CSFV E2-glycoprotein antibodies titer in swine serum or plasma, better quantifying the E2 response after vaccination or natural infection.

Diagnostic specificity was evaluated on 247 swine samples from CSFV-free herds in France, without history of CSFV vaccination or infection.

Diagnostic sensitivity was assessed using 179 swine sera samples from vaccinated herds in Asian countries. Out of the 179 samples, 92 were tested in parallel using the iELISA, the cELISA and Kit A, an indirect commercially available ELISA.

Two serum samples from vaccinated pigs, one with a C-strain vaccine and one with a recombinant E2 subunit vaccine, were serially diluted and tested in parallel with all ELISAs.

Measured specificity was 98.4% (CI95%: 95.9% - 99.4%), n=247. Measured sensitivity was 99.4% (CI95%: 96.8% - 99.9%), n=179. Out of the 92 vaccinated samples, 90, 89 and 60 samples were found positive with the cELISA, the iELISA, and Kit A, respectively.

The C-strain vaccinated sample were found positive until dilution 1:32 with the 3 ELISAs. The recombinant E2 subunit vaccinated sample were found positive until dilution 1:256, 1:128 and 1:32 with the cELISA, the iELISA and Kit A, respectively.

The ID Screen® CSF indirect ELISA demonstrates good specificity efficiently detects anti-CSFV E2 antibodies and shows high analytical sensitivity, delivering improved results compared to Kit A.

The new iELISA is a reliable tool for the detection of pig antibodies directed against CSFV. Further work is now ongoing to finalize a quantitative approach allowing antibody titer determination. Any collaboration welcomed!

**Keywords:** Classical Swine Fever, indirect ELISA, pigs, serology

### **P33. A new highly performant competitive ELISA for the detection of *Besnoitia besnoiti* antibodies in cattle**

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Besnoitiosis is a vector-borne disease due to an apicomplexan parasite, *Besnoitia besnoiti* (*Bb*), causing economic losses and increasing mortality. Besnoitiosis was classified as an emerging disease in Europe. As identification of seropositive animals is a key to control the disease, serological tools such as ELISA, western blot (WB) or IFAT play a crucial role for *Bb* diagnosis. Most of the commercially available ELISA kits are based on an indirect method. To improve *Bb* serological diagnosis, Innovative Diagnostics has generated several monoclonal antibodies and the most promising one was used to develop a new blocking ELISA (cELISA).

ELISA testing was performed as described in the kit insert. Diagnostic specificity was evaluated on 500 cattle sera samples from bovine besnoitiosis-free herds without history of besnoitiosis and/or with regular negative serological results.

Diagnostic sensitivity was evaluated on 200 cattle sera samples from France, which positive status had been determined by WB.

To check the absence of cross-reactions with other apicomplexan protozoa, 10 and 5 cattle sera (France) having a seropositive status confirmed by the ID Screen® *Neospora caninum* Indirect ELISA and the ID Screen® Toxoplasmosis Indirect ELISA, respectively, were tested with the ID Screen® *Besnoitia* Competition.

Repeatability, stability, robustness and interlaboratory reproducibility were also evaluated.

Measured specificity was 100.0 % (CI95%: 99.2 % - 100.0 %). Measured sensitivity was 100.0 % (CI95%: 98.1 % - 100.0 %). The percentage of correlation with the WB was 100%, indicating perfect agreement.

All *Neospora caninum* or *Toxoplasma gondii* seropositive samples were found negative with the cELISA, indicating good exclusivity. Repeatability, stability, and robustness were validated. The inter-laboratory reproducibility was good, as the coefficients of variation were inferior to 15%.

The ID Screen® *Besnoitia* Competition ELISA efficiently detects positive animals, demonstrates excellent specificity and excellent correlation with the WB. Furthermore, the kit performances comply with the requirements of the French Expert Laboratory for Besnoitiosis (ANSES).

The new ID Screen® *Bb* cELISA is a reliable tool for the detection of cattle antibodies directed against *Besnoitia besnoiti*. This new kit will reinforce IDvet's unique expertise in Besnoitiosis diagnostics, offering the most complete range, with kits for bulk tank milk surveillance, milk or serum testing.

**Keywords:** Besnoitiosis, Competition ELISA, cattle, serology

## P34. A new pen-side test to confirm RVF clinical suspicions in the field

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Rift Valley fever (RVF) is a zoonotic disease causing an acute infection in domestic ruminants, with a high mortality rate among young animals. IDvet developed a multispecies cELISA for the detection of RVFV specific antibodies, and ruminant RVFV specific IgM antibodies. Fast and reliable tools are needed to rapidly diagnose suspected clinical cases.

Diagnostic performances of a new pen-side test to detect RVFV in less than 15 minutes: the ID Rapid® Rift Valley fever is presented here.

The test is based on biological materials raised at CIRAD (Cêtre-Sossah C. et al., 2019). Anti-RVFV nucleocapsid-specific antibodies are bound to colloid gold particles or immobilized onto the membrane test line. A control line validates the migration. The kit contains a migration buffer dropper and micropipettes for sample transfer (15 µl serum, 30 µl blood).

Diagnostic specificity was assessed by testing 580 negative sera (292 cattle, 96 small ruminants and 192 camelids) and 184 heparinized whole blood (cattle) from RVF free areas.

Analytical sensitivity was evaluated using a titrated RVFV strain (Smithburn) spiked in negative whole blood or serum (bovine and caprine).

Measured specificity on serum was 99.7% (98.1 – 99.9%) for cattle, 99.0% (94.3 – 99.1%) for small ruminants and 100% (98.0 – 100%) for camelids; on bovine whole blood was 98.9% (96.1% – 99.7%).

For this Mab pair, inclusivity had been previously assessed using different RVFV viral strains, all successfully detected, indicating 100% inclusivity. Exclusivity with flaviviruses and alphaviruses were also documented, excluding possible cross-reactivity with other viral genera with same clinical features. The limit of detection (LOD) was  $3.5 \times 10^3$  pfu for serum, and  $7.5 \times 10^4$  pfu for whole blood. Diagnostic sensitivity on serum of a previous similar test having the same LOD was previously assessed by testing 25 isolated strains of from different geographical origins mimicking clinical specimens and 10 clinical samples from the 2019 Mayotte outbreak, which were positive by RTqPCR (Bird et al., 2007). Diagnostic sensitivity was 100% (CI<sub>95%</sub>: 90,1 – 100 n = 35).

The ID Rapid® Rift Valley fever antigen demonstrates very high levels of specificity and inclusivity, and a sensitivity level adapted to a specific, accurate and rapid detection of RVFV in serum or whole blood. It is a reliable tool able to confirm a RVF clinical suspicion and enhance virus detection during an outbreak in less than 15 minutes directly on the field, without any specific lab equipment.

**Keywords:** Rift Valley fever, pen-side test, antigen detection

## P35. The importance of endemic bacterial zoonoses in the territory of the Republic of Croatia in the period from 2017-2022.

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This research presents the current state of the most important bacterial zoonoses, tuberculosis, brucellosis, Q fever and leptospirosis in domestic animals in Croatia. Tuberculosis, together with brucellosis, represent the world's most widespread bacterial zoonosis, and in addition to being a public health problem they also cause large economic losses. Q fever as an endemic disease of the coastal area and leptospirosis which represents one large natural focus of almost entire continental part of Croatia are constantly present diseases in our country, causing economical and health problems.

The animals had been sampled between 2017 and 2022 as part of programs run by the Croatian Ministry of Agriculture to eradicate and monitor the mentioned diseases. Disease diagnosis was carried out by serological, bacteriological and molecular methods specific to each disease.

The results for each disease are shown below in Table 1.

Regarding to brucellosis, we can see the successful implementation of the national program for disease control. There are still some cases of tuberculosis, mainly in fattening herds. Implementation of eradication programme for tuberculosis is in line with the new EU requirements, which now imposes additional testing and includes fattening animals, should accelerate disease eradication. Control of Q fever is necessary to prevent the uncontrolled spread of the disease to people in endemic areas. In the case of leptospirosis, we search only cattle abortions which leads to incomplete information about the prevalence.

**Keywords:** tuberculosis, brucellosis, Q fever, leptospirosis, zoonosis

**Table 1.** Number of confirmed infections (establishments) in Croatia in the period 2017-2022

Year	Bovine tuberculosis	<i>Brucella melitensis</i>	<i>Brucella suis</i>	Leptospirosis (no. of animals)	Q fever
2017.	1	1	4	685	7
2018.	3	1	6	712	16
2019.	8	1	2	386	13
2020.	4	0	5	537	12
2021.	2	0	0	408	20
2022.	7	0	1	312	25

## P36. Genetic diversity of rotaviruses in healthy and diarrheic pigs in the Philippines

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Rotavirus (RV) is one of the major diarrhea-causing agents in pig populations worldwide. In the Philippines, the prevalence of rotavirus infections in pig farms is not monitored and the economic impact of the pathogen is poorly described. In this study, the fecal viral composition of healthy and diarrheic pigs from the Philippines was characterized using high-throughput sequencing to generate the full genome of rotaviruses circulating in the country.

Stool samples were collected from adult pigs in one commercial and one backyard farm. RNA was extracted from individual samples and pooled according to concentration. A total of 6 diarrheic and 5 healthy pools were sequenced using the NovaSeq platform and subsequent viral metagenomic bioinformatics analysis was performed using an in-house pipeline. Rotavirus-associated reads were subjected to downstream analysis and phylogenetic trees for VP4 and VP7 were constructed using the MEGA software.

While initial analysis showed that RV and other porcine enteric viruses were found in both cohorts, these viruses dominated the viral metagenome of diarrheic pigs. Moreover, only RV had a higher detection rate in diarrheic pigs than the healthy cohort. RVA, RVB, and RVC were found across all pools, however RVH-associated reads were only detected in the healthy samples. Genotype characterization of the infecting RVA strains using VP7 and VP4 identified G3, G5, G9, G26, P[13] and P[23] as the predominating genotypes in the samples. The presence of RV reads in both diarrheic and healthy samples support previous reports on the asymptomatic circulation of the virus.

This work provides the first full genome characterization of RV species present in Philippine pig samples. Information on the circulating genotypes will also benefit both animal and public health stakeholders as they develop measures to control RV circulation in pig and human populations. Given its detection in healthy pools, the effect of RVA infection in the pig industry must be further studied to establish its role in the causation of diarrhea.

**Keywords:** diarrhea, rotavirus, viral metagenomics, Philippines

**Funding source or Acknowledgement:** University of the Philippines Manila – National Institutes of Health (UPM-NIH) Faculty Grant

## P37. Surveillance and Detection of Lyssavirus in the UK Bat Population

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Rabies is a zoonotic disease caused by viruses in the genus *Lyssavirus*. Whilst classical rabies virus (RABV) is no longer present in the UK, European bat lyssavirus 1 (EBLV-1) and European bat lyssavirus 2 (EBLV-2) have been detected in UK bat species. The UK passive bat surveillance programme has been ongoing since 1987. Following the death of a bat conservationist in Scotland 2002 the number of bats submitted to the programme increased by over 70% within a year. Out of the 17 resident and breeding bat species found in the UK, lyssavirus has only ever been isolated in two species: namely EBLV-2 in Daubenton's bats (*Myotis daubentonii*) and EBLV-1 in serotine bats (*Eptesicus serotinus*). The programme prioritises the testing of any bats submitted that have bitten, licked or scratched humans or other susceptible animal species.

Testing of submitted bats is done using a combination of fluorescent antibody test, reverse transcription-PCR and the rabies tissue culture inoculation test.

Since 1987 over 40 cases of lyssavirus have been detected in the UK. With 28 being EBLV-2 over 26 years and 20 EBLV-1 in just 4 years. EBLV-1 has exclusively been detected in the southwest of the UK whereas EBLV-2 cases have been distributed throughout Great Britain.

There has been a consistent detection of EBLV-2 in the UK for over 20 years, but the numbers detected have increased since 2014. The recent incursion of EBLV-1 into the UK suggests that there could be detection of other lyssavirus found in other European countries. The scheme is expanding to testing bat species that are considered a low risk of lyssavirus infection, with the aim of detecting other circulating lyssavirus, including novel viral species, potentially present in the UK bat population. Continued detection of lyssavirus is key in notifying the public, veterinarians and doctors in high-risk areas as well as informing the UK health security agency to act upon possible cases of human exposure.

**Keywords:** Lyssavirus, disease surveillance, emerging infectious diseases

**Funding source:** The passive bat surveillance programme is funded by Defra, the Scottish Government and Welsh Government through grant SV3500

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*Forth Parallel Session*

**African swine fever**

*Oral Presentations*

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## A multi gene-approach genotyping identifies 24 genetic clusters of European African swine fever genotype II virus isolates circulating from 2007 to 2022.

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African swine fever (ASF) is an infectious disease caused by the ASF virus (ASFV), a big complex virus with big complex genome, that affects both domestic and wild suidae. ASF is a devastating disease with up to 100% mortality, and the most important challenge to the global swine industry. The genotype II ASFV entered in Europe from East African regions in 2007, and since then up to 19 European countries have been affected. To understand the ASF epidemiology, genome sequencing of the circulating viruses is essential. While whole genome sequencing remains as gold standard for the identification of new genetic markers, sequencing of multiple loci with significant variations could be used as a rapid and cost-effective alternative to track outbreaks and virus origin, as well as to follow up the movement of ASFV isolates and disease evolution in endemic areas.

The study initially was based on sequencing of the central variable region (CVR), the intergenic region (IGR) between the I73R and I329L genes and the O174L and K145R genes of 382 European ASFV isolates collected from 2007 to 2022. For further discrimination, two new PCRs were designed to amplify the tandem repeat sequences (TRS) located in the IGR between the 9R and 10R genes of the multigene family 505 (MGF505) and the IGR between the I329L and I215L genes.

The combination of the results obtained by sequencing the six variable genome regions allowed to differentiate the European II-ASFV genotypes into 24 different groups. In addition, the SNP identified in the I215L gene, grouped the viruses from North Macedonia that caused the 2022 outbreaks with viruses from Romania, Bulgaria, Serbia and Greece, differentiating from other genotype II isolates. Furthermore, TRS within the 9R-10R genes of the MGF505 revealed eight different variants circulating. These findings describe a new multi-gene approach sequencing method that can be used in routine genotyping to determine the origin of new introductions in ASF-free areas and track infection dynamics in endemic areas.

**Keywords:** ASFV, genotyping, TRS, SNP, genetic groups

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## Detection of African swine fever virus (ASFV) and blood meals of porcine origin in hematophagous insects collected on a Lithuanian pig farm without ASFV-infected pigs

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A seasonal trend of African swine fever (ASF) outbreaks in domestic pig farms has been observed in Eastern Europe, with outbreaks during the summer period coinciding with the seasonal activity of blood-feeding insects. ASFV DNA has previously been detected in hematophagous insects on outbreak farms, but it remains unclear whether the ASFV DNA in these insects originated from blood meals from pigs inside or outside the outbreak farm. It is not unlikely, however, that hematophagous insects after feeding on an ASFV-infected wild boar or domestic pig could introduce the virus into pig farms by circumventing even strict biosecurity measures. Recently, ASFV DNA was detected in a pool of insects, more specifically *Haematopota* spp., caught in a trap just outside a high biosecurity pig farm in Lithuania, which did not have ASFV-infected animals (Stelder et al., 2023). Based on this finding, additional pools of insects collected inside the protective wildlife fences of the same farm were screened for the presence of ASFV DNA and for the source of mammalian blood.

The insects were collected in the summer of 2020 using H-traps located in the surroundings of a high-biosecurity pig farm in the Southern Šiauliai County, Lithuania, with no evidence of ASF in the herd. Collected insects were pooled according to location, date, trap number and genus, and DNA was extracted from the pools. DNA from 48 pools were tested for the presence of ASFV and mammalian blood meals using qPCR and Sanger sequencing.

Using qPCR, ASFV DNA was detected in six insect pools (containing either (*Haematopota* spp., *Tabanus* spp. or *Stomoxys calcitrans*) and in four of these pools, DNA of suid origin was identified. In the two last pools, DNA of bovine origin was detected. These findings coincided with ASFV being reported in the wild boar population within a 10 km radius from the pig farm.

These results show that blood from ASFV-infected suids was present within hematophagous insects on the premises of a pig farm without ASFV-infected domestic pigs and demonstrates that blood-feeding insects might potentially act as a mechanical bridge vector between wild boar and domestic pigs.

**Keywords:** African swine fever virus, ASF, hematophagous insects, high biosecurity farm, virus transmission

Reference: Stelder JJ, Olesen AS, Belsham GJ, Rasmussen TB, Bøtner A, Kjær LJ, Boklund A, Bødker R (2023). Introduction of African swine fever virus and blood from mammals into high biosecurity pig farms by hematophagous insects in Lithuania. In revision for *Transbound. Emerg. Dis*

## An ASFV-host interaction mapping reveals the role of protein CP204L in the regulation of endosomal trafficking

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African swine fever virus (ASFV) was first identified over a hundred years ago. Since then, much effort has been made to understand the pathogenesis of ASFV. However, the specific roles of many individual ASFV proteins during the infection remain enigmatic.

To gain insight into ASFV protein functions, we performed virus-host protein-protein interaction mapping using an affinity tag purification-mass spectrometry approach.

Our study shows that the essential viral protein CP204L, also known as P30 or P32, is a multifunctional protein engaged in endosomal trafficking and localizes to virus replication sites. We identify VPS39, a component of the cellular homotypic fusion and protein sorting (HOPS) complex, as a direct host interactor of CP204L. The virus protocomplex and the VPS39 domain responsible for its recruitment to endosomal membranes, prevents its integration into the HOPS complex, and promotes the clustering of lysosomes. Moreover, we show that VPS39 is an important factor in the early phase of infection, as virus replication and protein synthesis in VPS39 knockout cells are delayed.

These results indicate the potential mechanism by which ASFV escapes degradation and establishes its virus factories. In summary, our study gains new insights into ASFV-host protein interaction and virus biology. Furthermore, additional characterization of other protein-protein interactions revealed by this study is expected to identify more potential targets for novel therapeutic strategies.

**Keywords:** African swine fever virus, CP204L, VPS39, virus-host interaction, endosomal trafficking

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## Duration of immunity upon infection with African swine fever virus

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African swine fever (ASF) has gone pandemic and is reaching endemicity in wild boar populations in Europe. To understand disease dynamics and tailor control strategies, knowledge on the long-term effects of ASF virus (ASFV) infection is needed. Moreover, data sets are needed to inform regulatory authorities to define performance characteristics asked from licensable vaccines against ASF. Against this background, we set out to assess duration of field virus induced ASF immunity.

To induce ASFV specific immunity, ten domestic pigs were oro-nasally inoculated with the moderately virulent ASFV field strain "Estonia 2014" (genotype II). After six months, ten age-matched control animals were added to the experiment and all animals were inoculated with the highly virulent ASFV strain "Armenia 2008" (genotype II) by the oro-nasal route. All animals were monitored for clinical and pathomorphological signs and were further investigated using routine virological, serological and immunological methods.

Upon primary infection with ASFV "Estonia 2014", one out of ten animals reached humane endpoint criteria and had to be euthanized showing severe ASF signs. All other animal recovered after transient disease and showed swift seroconversion and declining viremia. All survivors were negative for ASFV genome in blood after post infection day (PID) 126. Antibody levels reached a high plateau after PID 28. Upon challenge infection, all control animals had to be euthanized seven or eight days after infection showing severe clinical signs and high viral loads in all sampled organs. In contrast, all animals that had recovered from the ASFV "Estonia 2014" infection were protected against lethal disease courses. Two animals showed mild and transient clinical signs upon challenge. Four animals were found positive for viral genome and infectious virus in several organs. Two additional animal showed low viral genome loads in one or two organs. Antibody and T-cell responses are currently investigated in more detail.

Animals recovered from ASFV infection were protected against lethal challenge for at least six months. However, immunity was not "sterile" and limited transmission would be likely. Apart from providing data on ASF immunity in general, the presented data can aid discussion regarding vaccine efficacy requirements.

**Keywords:** African swine fever, antibodies, protection, duration of immunity

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## Taking a promising vaccine candidate against African swine fever further: Safety and efficacy tests using “ASFV-G-ΔMGF”

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African swine fever (ASF) is a pandemic threat to the global pig industry and wild suids. A safe and efficacious vaccine could tremendously assist in disease eradication. In the past years, promising live attenuated vaccine candidates emerged in proof-of-concept experiments, among which was “ASFV-G-ΔMGF”. Taking this candidate further, we conducted safety and efficacy trials in domestic pigs and wild boar.

Two trials were designed to assess safety and efficacy of a double intramuscular vaccination scheme of domestic pigs. In the first trial, a macrophage-grown virus was used, while in the second, the vaccine virus master seed was grown on a commercial permanent cell line. The third trial was performed as a proof-of-concept study with a single oral immunization of wild boar. Challenge was performed by the oro-nasal route using highly virulent genotype II viruses. For an *in vivo* reversion to virulence study, the vaccine virus was passaged five times in domestic pigs (n=10 in each passage). Passaging was done by the intramuscular route. Animals of all trial parts were monitored for clinical and pathomorphological signs and were investigated using accredited routine virological and serological methods.

Two-dose intramuscular immunization could induce close-to-sterile immunity with full clinical protection irrespective of the production system of the vaccine virus. After oral immunization, 50% of the vaccines seroconverted and all responders were completely protected against subsequent challenge. In the reversion to virulence study, a virus variant emerged after forced animal passaging that was associated with transient rise in body temperature and an increased replication and shedding. However, all animals were healthy upon completion of the study and reversion to significant virulence was not observed. The genomic changes did not affect the recombination site but involved deletions and reorganizations in the terminal regions of the genome.

“ASFV-G-ΔMGF” is fully efficacious when administered intramuscularly, and like-wise, all responders to oral immunization are protected. However, efficiency of oral immunization has room for improvement. The reversion to virulence underscores that in-depth safety characterization is needed for live ASF vaccines. For this particular candidate, additional studies should target transmission characteristics before thorough benefit risk analysis can be carried out.

**Keywords:** African swine fever virus, vaccine candidate, ASFV-G-ΔMGF, safety, efficacy

**Acknowledgement:** The authors would like to thank all animal caretakers and technicians for their excellent support

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*Forth Parallel Session*

**African swine fever**

*Posters with Flash Presentation*

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## P54. High-throughput mapping of virus-host interactions to identify new factors of virulence and pathogenicity for ASFV

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

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

In parallel, we were interested in the ability of ASFV proteins to individually inhibit the IFN-I pathway. For this purpose, we have screened 100 ASFV proteins using an IFN-luciferase reporter gene system. We showed that at least seven viral proteins (I267L, MGF360-11L, DP96R, MGF505-3R, R298L, DP71L, C962R) contribute to the inhibition of the IFN-I induction pathway. In order to characterize their antagonist effect, a split-nanoluciferase approach was used to screen these viral proteins with a library of 16 major proteins of the IFN-I response. This approach led us to identify IRF3, IRF7, NEMO as new putative targets of ASFV proteins. By combining different screening approaches, we have already highlighted new mechanisms by which ASFV hijacks cellular pathway for replication and escapes the vigilance of the immune system. Later on, by comparing virus-host interactions that have been obtained with attenuated strains of ASFV, we should identify specific targets that could explain the attenuation process at the molecular level.

**Keywords:** ASFV, interactomic, innate immunity

**Funding source:** ICRAD, an ERA-NET co-funded under EU Horizon 2020 research and innovation programme (<https://ec.europa.eu/programmes/horizon2020/en>), under Grant Agreement n°862605, and co-funding, under the grant codes: ANR-21-ICRD-0001-01 for ANSES and ANR-21-ICRD-0001-02 for INRAE

## P55. Evaluation of African Swine Fever on East Balkan pigs and backyards in Bulgaria: a qualitative assessment

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African Swine Fever (ASF) has become a serious challenge for the pig production sector. Besides the direct damages related to its lethality and resistance, ASF management and control (e.g. early detection, animal movement restrictions and implementation of slaughter policies) is heavily affecting backyards and smallholder farms, which represent a sustainable way to produce animal proteins and support the local communities, especially in rural areas that cannot be farmed intensively. The East Balkan swine (EBS), Bulgaria's last indigenous pig breed, has been deeply affected by the ASF epidemic, being at high risk of extinction and inbreeding. This study analysed ASF's direct and indirect consequences on EBS and backyard farmers in Bulgaria. In 2022 two field missions have been organised in Bulgaria, collecting information through semi-structured interviews with backyards and EBS farmers, farmers representatives and veterinarians. Six ex-EBS farmers and the president of their association were interviewed. Veterinarian Authorities were involved in discussing possible strategies to support smallholders and EBS farmers during epidemics. Both backyards and EBS farmers have been heavily affected by ASF consequences in Bulgaria. The backyard sector has been almost destroyed, while the EBS farmers interviewed have lost their entire herds. In 2016 59 EBS herds were registered, while nowadays ten herds are left. Many factors limited the possibility of restarting the activity of EBS farms. In fact, areas for farming in the allowed regions (Burgas, Varna and Shumen) are part of a public resource and cannot be fenced, in opposition to the biosafety requirements. Moreover, the same areas are often used by hunters and cannot be allocated only to EBS farmers. In addition, due to ASF measures, to date, no legal options exist for selling EBS meat and no slaughterhouses accept EBS (before ASF, 60% of EBS farmers annual income resulted from EBS meat selling). In general, the financial support offered was insufficient to restore an economically viable herd, re-gaining revenue. Based on the EBS experience, ASF control measures will, in the long term, i) pose at risk the chains of local/own consumption production, ii) the cultural heritage (e.g. traditional products), iii) it will reduce biodiversity, and iv) contribute to the land abandonment phenomenon. Financial support of these realities, collaboration among Veterinary Authorities and rural development parties, possible focused derogation and identification of indicators which quantify the economic, social and cultural value of these traditional farms would be fundamental to preserving these sectors.

**Keywords:** African Swine Fever, East Balkan pigs, backyards, smallholder farms, impact

**Funding source or Acknowledgement:** Italian society of pig pathology and production (SIPAS), DEFEND project No. 773701

## P56. A deep sequencing strategy for investigation of virus variants within ASFV infected pigs

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African swine fever virus (ASFV) is the causative agent of African swine fever, an economically important and highly contagious disease of domestic pigs, wild boar and other members of the family *Suidae*. The ASFV genome is a large linear double stranded DNA molecule that ranges from 170 to 194 kb depending on the virus strain. ASFV has demonstrated a high degree of genetic stability, with low genetic diversity among isolates in Eurasia (Forth *et al.* 2019). To explore the influence of viral variants on clinical outcome and infection-dynamics, we have designed a deep sequencing strategy with 18 overlapping PCR fragments, ranging in size from approx. 9600 bp to 12800 bp, covering almost the entirety of a European ASFV reference genome (Olesen *et al.* 2018). The overlapping PCRs are multiplexed in non-overlapping pools on DNA extracted from ASFV infected pigs. The PCR pools are then merged for each sample, barcoded and sequenced by Nanopore or Illumina, resulting in coverage of above 1000x for each amplified region. This allows for variant calling and haplotyping with high confidence. Comprehensive deep sequencing is critical in following ASFV molecular evolution, especially for identification of modifications that can affect virulence. The current method can be used for the current European ASFVs and adapted to other ASFV strains and genotypes simply by alterations of primers in regions with low compatibility.

**Keywords:** ASFV, deep sequencing, virus variants, haplotyping

**Funding source or Acknowledgement:** This work was supported by the Horizon 2020 ERA-NET Cofund International Coordination of Research of Infectious Animal Diseases (ICRAD), project ASF-RASH.

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*Forth Parallel Session*

**African swine fever**

*Poster Presentations*

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## P58. Differences in replication kinetics and cell tropism for genotype I and II ASFv strains

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African swine fever is one of the most important viral diseases in domestic pigs due to its relevance for animal health and pig industry. The causative agent, African Swine Fever Virus (ASFv) is a large and complex DNA virus, single member of the genus *Asfivirus* in the *Asfarviridae* family (Alonso et al., 2018). The infection of domestic pigs (*Sus scrofa domesticus*) and European wild boars (*Sus scrofa*) results in a wide range of clinical pictures, varying from subclinical disease to severe haemorrhagic disease with up to 100% lethality depending on the virulence of the isolate (Blome et al., 2013). The different genotypes of African Swine Fever present large difference in their virulence, pathogenicity and transmission capacity. ASFv mainly targets immune cells of the myeloid lineage, especially monocytes and macrophages, which are thought to be crucial for viral persistence and dissemination (Franzoni et al., 2017). In this project, the replication kinetics of ASFv genotype 1 (E70, NHV) and genotype 2 (Belgium2018/1, Estonia) will be compared in different macrophage subpopulations.

Peripheral blood mononuclear cells are collected from heparinized blood by a Ficoll Paque density gradient. Afterwards, the monocytes are isolated by plastic adhesion. Porcine alveolar macrophages are collected by lung lavage. Splenic macrophages are collected after physical cell separation and plastic adhesion enrichment. Isolated cell populations are inoculated with genotype 1 and genotype 2 ASFv strains of various virulence at a m.o.i. of 1. At 0, 3, 6, 9, 12, 18, 24, 48 and 72 hours post inoculation, supernatants and cells are collected. In the supernatants and cells, the number of viral genome copies and virus titre are determined by qPCR and titration by IPMA on porcine alveolar macrophages respectively. Infected cells are fixed and stained for ASFv viral antigens in order to determine the percentage of infected cells by immunofluorescence microscopy. Differences observed in the kinetics and cell tropism of genotype 1 and genotype 2 strains may explain the different in vivo characteristics of both genotypes.

**Keywords:** ASFv, virulence, replication kinetics, monocyte/macrophages

## P59. An attempt to determine the effect of temperature on the virucidal activity of selected active substances used in disinfectants against African swine fever virus (ASFV)

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African swine fever (ASF) is one of the most dangerous and fatal swine diseases, described for the first time roughly a hundred years ago. Even now, there are neither a commercially approved vaccine nor treatment available. The only way to hinder further spread of the disease is by culling the affected herds and applying prevention methods based mainly on proper biosecurity using effective disinfectant. Nevertheless, it is important that the disinfectant maintains its virucidal activity over a wide range of temperatures. It should show effectiveness in both summer and winter, despite low temperatures. Previously we have proven that several generally approved active compounds are capable to inactivate enveloped ASFV. Here we present the study on the effect of temperature on virucidal activity of selected active substances used in disinfectants against ASFV, in order to verify the effectiveness of these substances exposed to wide range of temperatures. In the present study three active substance were selected to represent the main groups of chemical compounds, *i.e.*: sodium hypochlorite (1.0%), glutaraldehyde (0.1%) and potassium peroxymonosulfate (0.5%). The examination was carried out using the suspension test inspired by the PN-EN 14675: 2015 European Standard procedure. All tested active substances were analysed in three different temperature conditions (21°C; -10°C; -20°C) in triplicate, diluted with water of standardized hardness. The tested mixture contained two types of interfering substance simulating low or high soiling level (BSA - bovine serum albumin or BSA + YE - bovine serum albumin with a yeast extract, respectively). The presence of cytopathic effect was observed in 96-well plates (in quadruplicate) containing Vero cell culture after 7 day-incubation in 37°C. If the difference in ASF titre between tested active substance and virus control was  $\geq 4$  log (TCID<sub>50</sub>/ml), it was considered as virucidal against ASFV.

Exposure to range of tested temperatures, did not negatively affect the virucidal efficacy of the tested active substances against ASFV. All three chemical compounds reduced virus titres by 4log, which confirms their virucidal activity against ASFV in a wide range of temperatures, including freezing temperatures.

However, the biggest problem of biosecurity in winter is the fact that the disinfection solution freezes, *e.g.* in disinfection mats or troughs, which prevents proper penetration and can be the direct cause of ASF introduction into a farm. Therefore, it is necessary to find a substance that prevents from freezing of disinfection solutions, and preferably the one that will enhance its virucidal properties through synergistic action.

**Keywords:** African swine fever; disinfection; suspension test; virucidal activity

**Funding source:** S/548 "Effect of temperature on the virucidal activity of chemical compounds commonly used in the production of commercial disinfectants against African swine fever virus (ASFV)."

## P60. African swine fever – preventive and control measures undertaken in the first disease outbreak in the backyards population (Serbia)

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African swine fever (ASF) continues to spread in Europe and in 2019 the first outbreak was detected in Serbia, Western Balkans. The first ASF case was detected in domestic swine backyard population in central part of the country. After disease confirmation, the Veterinary Directorate established the infected and protection zones, implemented the stamping out procedure in infected as well as in contact yards, as an urgent measure to stop the virus spreading. According to the results of the epidemiological field investigation, within two municipalities, i.e. 3 villages, 17 infected backyards were identified, with a total population of 279 domestic pigs. The clinical signs of disease were detected in only 21 domestic pigs. Having in mind the very low or total absence of biosecurity measures in the backyard's population, the preventive depopulation in the selected villages was implemented as the final control measure. According to the decision, the preventive depopulation (stamping out) in the surrounding villages included 339 domestic pigs in the contact yards. The total number of euthanized domestic pigs in the backyards was 618. It is well known that backyards with their low biosecurity standards are considered prone to ASF and thus are of particular interest in disease prevention and control strategy. According to Serbian experience, in the case of ASF outbreak in one backyard, the whole village has to be considered as one epidemiological unit and urgent control and preventive measures according to the Law need to be applied.

**Keywords:** African swine fever, control measures, backyards, Serbia

**Funding source:** This study was funded by Ministry of Education, Science and Technological development of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2022, Contract No: 451-03-68/2022-14/200031.

## P61. Evaluation of surveillance strategies for African Swine Fever (ASF) in wild boar

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The continuous monitoring of animal diseases is of great importance in order to minimize health and economic impacts on livestock, animal owners and consumers. To assess the risk of disease transmission from wild animals to livestock, wildlife surveillance is particularly crucial. African swine fever (ASF) is present in Germany since 2020. Similar to many other affected countries, mainly the wild boar population is affected. Thus, in order to protect the domestic pig sector, it is vital to control the disease in wild boar and to implement sufficient surveillance activities.

Large parts of Germany are still free from ASF. The main surveillance objective in those areas is to detect the entry of the disease as early as possible.

The aim of the project is to conduct a comprehensive evaluation of the currently implemented surveillance strategies for ASF in German wild boar in ASF free areas, and to compare these strategies to alternative, risk-based surveillance strategies.

Risk factors for ASF infection in wild boar and for detection of ASF infected wild boar have been identified and were used to inform and develop alternative surveillance strategies. The currently applied surveillance strategy was defined as the reference strategy. To evaluate the performance of the surveillance strategies, sensitivity and timeliness of the strategies were examined utilizing a simulation model previously developed for the evaluation of classical swine fever surveillance in wild boar. Within the project, the model was adapted and parameterized according to the epidemiological characteristics of ASF. The sensitivity was determined by measuring the probability of detection of an ASF infection within six months. Timeliness was defined as the time between introduction and detection of an ASF infection.

The project is still in progress. The poster will show some potential preliminary results, but the main focus will be on the approach of the project and to inform the scientific community and exchange ideas.

**Keywords:** African swine fever, surveillance, simulation model, sensitivity, timeliness

**Funding source or Acknowledgement:** The authors want to thank the IDOH+ master coordinators for their support.

## P62. Genomic analysis of an early African swine fever virus isolate from the Philippines reveals significant genetic variations

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African swine fever (ASF) is highly contagious haemorrhagic infection and devastating mortality in domestic pigs. It was first reported in the Philippines in July 2019 and have since spread nationwide, reportedly reducing 30% of the local swine population. The present study aims to optimize a pipeline to analyze next-generation sequencing data of African Swine Fever virus (ASFV) isolates from the Philippines.

An ASFV qPCR-positive porcine blood sample (Philippines/Ifugao/Pig/2020-1) was collected from Ifugao Province, Philippines in May 2020. We optimized a pipeline for the analysis of ASFV from metagenomic short-read data. To maximize depth of coverage and alignment rate, raw reads were iteratively mapped three times to consensus sequences generated from cleaning and resolving scaffolds obtained from various de novo assembly tools.

Genomic analysis of common molecular targets revealed that the Philippine isolate shared 100% homology with other Asian isolates, with capsid protein p72 as genotype II, p54 envelope protein as subtype IIa, central variable region (CVR) as variant I, and CD2v haemadsorption protein as serotype 8. Analysis of intergenic region (IGR) confirmed the presence of one set of 10-nucleotide tandem repeat sequence (TRS) insertion distinctive of IGR variant type II. We also detected 31 genetic variations in the Philippine isolate compared to the reference sequence ASFV Georgia 2007/1. Of these, 8 are intrahost variants; 11 were non-coding homopolymer tract variations; 6 homopolymer tract variations occurred at coding regions; 6 other indel changes caused open reading frame (ORF) mutations and one occurred at an intergenic region. Genetic variations that caused coding changes were observed in KP93L, B125R, NP1450L, I267L, I9R, and DP60R genes. Variations in ORF range from single codon substitutions to frameshift causing large truncations.

Sequencing of ASFV is important to provide insights on transmission dynamics and viral evolution. Data from the ASFV Philippines/Ifugao/Pig/2020-1 provide a helpful baseline for genetic variations that exist in Philippine ASFVs. Moving forward, we aim to expand analysis of more isolates and verify variations in TRS and homopolymer regions by utilizing long- and short-read sequences in a hybrid genome assembly workflow.

**Keywords:** African swine fever, genomic characterization, next-generation sequencing

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***Fifth Parallel Session***

**Avian influenza**

***Oral Presentation***

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## Natural selection of H5N1 avian influenza A viruses with increased PA-X and NS1 shutoff activity

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Influenza A virus (IAV) can infect a broad range of mammalian and avian species representing an important challenge both in poultry farming and human health. In addition, some IAV subtypes, such as H5N1, preferentially infecting avians, occasionally infect humans, having zoonotic potential. The host innate immune system provides defences that restrict IAV replication and infection. However, IAV have evolved to develop efficient mechanisms to counteract host antiviral responses. The IAV PA-X and NS1 non-structural proteins are key virulence factors that modulate innate immune responses and virus pathogenicity during infection. Previously, we have shown a functional coevolution of NS1 and PA-X proteins in pH1N1. Here, we assessed the functional coevolution of NS1 and PA-X proteins from early and more recent H5N1 IAV, as well as the role of specific amino acid mutations in the PA-X protein of H5N1 IAV on their ability to inhibit host gene expression, viral replication, and pathogenesis.

***In silico* analysis.** Human and avian H5N1 PA-X and NS1 sequences available at the Influenza Research Database ([www.fludb.org](http://www.fludb.org) (accessed on 23 June 2020)) were downloaded and aligned using the “analyze sequence variation (SNP)” function available in this webpage. ***In vitro* assays.** Canine MDCK, human A549 or HEK293T, and chicken DF-1 cells were used in this study. ***In vivo* studies.** A129 mice were housed at the BSL3 animal facilities in the Animal Health Research Centre (CISA-INIA/CSIC), Madrid (Spain). All animal experimental protocols were approved (Permit number: PROEX 116/19).

Our results indicate that NS1 and PA-X proteins from currently circulating H5N1 IAV have evolved to increase their ability to inhibit host gene expression and innate immune responses. Notably, viruses encoding the NS1 protein from early H5N1 strains were more virulent in mice and replicated slightly more efficiently in the lungs than the viruses encoding the NS1 protein from recent H5N1 strains. However, changes in the H5N1 PA did not significantly affect viral pathogenesis. Moreover, we found that IAVs encoding circulating H5N1 NS1 and PA-X proteins, induce less inflammatory responses than IAV encoding the original genes, most likely due to their increased ability to inhibit host gene expression.

Consistent with our previous findings showing a functional coevolution of IAV NS1 and PA-X genes, our data suggest that circulating H5N1 IAV have adapted to induce less inflammatory responses and be less virulent than original circulating H5N1 IAV, highlighting the importance to monitor the evolution of virulence factors. However, additional experiments are needed to further analyze these differences.

**Keywords:** H5N1 HPAIV; NS1; PA-X; influenza A virus; shutoff

## Avian influenza surveillance in wild birds in Lombardy and Emilia Romagna regions (Italy) in 2022

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Avian Influenza viruses (AIV) infect a broad spectrum of animal species, preventing the eradication of the disease worldwide. Each year, genetically different strains circulate abundantly in wild birds, natural reservoirs that can spread these viruses to domestic birds and numerous incidental hosts, including humans. During migration periods, AIV can spread to free regions, making Avian Influenza a significant threat to public health as well as an economical burden. From 10 September to 2 December 2022, EFSA reported 613 cases of clade 2.3.4.4b H5N1 HPAIVs in wild birds in Europe, during the greatest HPAI pandemic ever recorded. Considering the importance of monitoring this notifiable disease, a surveillance study for Alphainfluenzavirus was conducted on 2,398 Anatidae hunted in the Lombardy and Emilia Romagna regions (northern Italy) during the period from January to December 2022, covering two incomplete hunting seasons.

Cloacal swabs or feathers were collected through active surveillance within the framework of the Emilia Romagna and Lombardy wildlife monitoring plans. Samples were tested by real time RT-PCR targeting the M gene and positive samples were fully sequenced by Illumina NGS.

74 out of 2,398 wild birds tested positive for AIV (3.1%). The most frequently affected species were mallard (n=37/1,042) and teal (n=21/832), followed by pintail (n=6/29), wigeon (n=5/127) and other Anatidae. Several subtypes were recognised in positive animals. The most frequently detected were H5N1 (HPAI) (22.9%) and H3N8 (13.5%), mainly in mallards and teals. These two species in particular hosted the highest variety of subtypes, confirming their role as the main reservoirs of AIV. In addition to these, subtypes H2N3, H3N1, H5N2, H5N3, H6N1 and H11N9 were also isolated in mallards, while subtypes H1N1, H2N2, H2N3, H6N1, H8N4 and H12N2 were detected in teals. Moreover, subtype H12N8 was identified in a pintail. The H5N1 subtype was the most widespread and affected the largest number of wild birds. Complete sequencing of the positive samples was also conducted, and phylogenetic analysis is ongoing.

This study highlights the large circulation of different subtypes of AIV in wild ducks; this certainly represents a concern for both public health and economic losses from its introduction into poultry farms. Therefore, continuous monitoring in wild birds is of crucial importance.

**Keywords:** Avian influenza, H5, Anatidae, Sequencing

**Funding source:** Study partially funded by NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (project no. PE00000007INF-ACT)

## Identification of amino acid residues required for inhibition of host gene expression by influenza virus A/Viet Nam/1203/2004 H5N1 PA-X

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PA-X is a nonstructural protein of influenza A virus (IAV), which is encoded by the polymerase acidic (PA) mRNA and contains an N-terminal sequence common to the PA gene and a C-terminal +1 frameshifted sequence. PA-X protein modulates IAV-induced host innate immune responses and viral pathogenicity via suppression of host gene expression or cellular shutoff, through cellular mRNA cleavage. Highly pathogenic avian influenza viruses (HPAIV) of the H5N1 subtype naturally infect different avian species, they have an enormous economic impact in the poultry farming, and they also have zoonotic and pandemic potential, representing a risk to human public health. In the present study, we describe a novel bacterium-based approach to identify amino acid residues in the PA-X protein of the HPAIV A/Viet Nam/1203/2004 H5N1 that are important for its ability to inhibit host protein expression.

**Bacterial screening assay:** The PA-X gene of A/Viet Nam/1203/2004 H5N1 was cloned into pGBKT7 plasmid, under the control of T7 polymerase promoter. DH5 $\alpha$  competent E. coli bacteria were then transformed and plated on agar plates supplemented with the antibiotic kanamycin. Then, single bacterial colonies were isolated and sequenced. Leaked PA-X expression from the T7 promoter within the bacteria results in inhibition of host gene expression and therefore no bacterial growth. However, PA-X mutants affected in inhibition of host gene expression allow bacteria to grow. **In vitro assays:** Shutoff, minigenome, Interferon-stimulated response element (ISRE) promoter activation and immunofluorescence assays were performed in human 293T or chicken DF-1 cells. **Structural analysis.** Amino acid positions were plotted on the crystal structure of the N-terminal region of PA protein (PDB accession: 4E5E) from A/Vietnam/1203/2004 H5N1 (AY818132) using PyMOL.

Identified PA-X mutants displayed a reduced shutoff activity compared to that of the wild-type A/Viet Nam/1203/2004 H5N1 PA-X protein. Notably, this new bacterium-based screening allowed us to identify amino acid residues widely distributed over the entire N-terminal region of PA-X. Furthermore, we found that some of the residues affecting A/Viet Nam/1203/2004 H5N1 PA-X host shutoff activity also affect PA polymerase activity in a minigenome assay. This information could be used for the rational design of new and more effective compounds with antiviral activity against IAV.

Our results demonstrate the feasibility of using this bacterium-based approach to identify amino acid residues important for the activity of viral proteins to inhibit host gene expression. In fact, this approach was also used to identify residues important for A/Brevig Mission/1/1918 H1N1 (Spanish flu) PA-X's shutoff activity.

**Keywords:** PA-X protein; H5N1 avian influenza viruses; baloxavir; minigenome; shutoff

## Poultry vector vaccines: innovative serological assays for vaccination monitoring and DIVA testing for H5 avian Influenza A

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Influenza viruses belong to the family Orthomyxoviridae. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further divided into subtypes based on their Hemagglutinin and Neuraminidase antigens. Some subtypes containing H5 or H7 are associated with highly pathogenic forms of the disease and high rate of mortality. A current H5 HPAI lineage has been circulating worldwide since 2004 and has been responsible for important poultry losses. To control poultry disease, vaccination is more and more used, especially with recombinant vaccine technology. In the last 5 years, successive waves of H5 Influenza in Europe pushed the health authorities to review their vaccination strategy concerning this virus. Given the need for rapid and reliable serological tools for monitoring of vaccination, IDvet has developed unique indirect ELISAs: one for the monitoring of recombinant vaccines, the ID Screen<sup>®</sup> Influenza H5 Indirect ELISA, and one for DIVA strategy (differentiated Infected from Vaccinated Animals), the ID Screen<sup>®</sup> Influenza A Nucleoprotein Indirect.

Layer and broiler flocks vaccinated with different technology of vaccines (H5 RNA, r-HVT-AI(H5) or inactivated sub-unit AIV-H5 vaccines) were tested. Antibody titers for H5 were evaluated using the ID Screen<sup>®</sup> Influenza H5 Indirect ELISA, based on a H5 recombinant protein. Samples were also tested with the ID Screen<sup>®</sup> Influenza A Nucleoprotein Indirect, based on a nucleoprotein, to monitor field challenge. For each tested flock, the following parameters were measured: mean titers, minimum, maximum, and CV%. All samples with titers higher than 732 for FLUH5S, and higher than 668 for FLUNPS, were considered positive.

All the flocks vaccinated with H5 vaccines were found positive with the H5 iELISA. Some of them were also found positive with the nucleoprotein iELISA. Therefore, the positivity of the H5 iELISA, belonging to negative flocks with the NP iELISA, demonstrated the detection of seroconversion induced by vaccines. The positivity of the NP ELISA in some flocks highlighted the presence of challenge with one HxNy strain.

The ID Screen<sup>®</sup> Influenza H5 Indirect is the only commercial quantitative ELISA for the specific detection of H5 antibodies, and the only test which allows for H5 vaccination monitoring. The ID Screen<sup>®</sup> Influenza A Nucleoprotein Indirect is an excellent tool for the detection of wild virus in populations vaccinated with recombinant H5 vaccine.

**Keywords:** vector vaccines, vaccination monitoring, innovative ELISAs

## Development of a 3d Models of Swine Respiratory Cells for The Study of In Vitro Pathogenesis of Influenza A Virus

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Influenza A viruses (Orthomyxoviridae) can infect humans and a large variety of animals including pigs and birds. Even if they are species-specific viruses, there are increasing reports of the virus spillover with the consequent risk of reassortment between different strains with possible pandemic potential. In vivo experimental models are able to provide relevant elements for the study of IAV (Influenza A Virus), but they involve numerous ethical and animal welfare implications. It is necessary to standardize alternative models, such as 3D culture, which allow to evaluate the infectivity and replication capacity of the viruses in systems that maintain the anatomical and functional integrity of the target tissues.

In this study, we used the New Born Pig Trachea (NPTr) cell line, suitable for the study of swine, avian and human influenza viruses, and we developed both 2D and 3D cultures. In 2D cultures, the morphological aspect, the presence and the type of the cytopathic effect were evaluated when inoculated with swine influenza virus (H3N2 A/swine/1523/98, H1N1 A/swine/1523/98 and H1N2 A/swine/1523/98). Cells grow in MEM added with 10% FBS at 37 ° C and 5% CO<sub>2</sub>. Two different matrices were being tested for the 3D model: TissueSpec® Lung ECM Hydrogel (Xylyx, NY, USA) and PeptiGel® (Manchester BIOGEL, UK).

The most promising results were obtained with PeptiGel®: for growth in inclusion, a cell suspension was prepared at a concentration of 1.2x10<sup>6</sup> cells/ml. The cellularized matrix was seeded on 12 mm inserts with a transparent PET membrane and 0.4 µm pores (ThinCert®, Greiner bio-one, Austria) suitable for 12-well plates. The best compositional and stiffness formulation of PeptiGel® were found for the cell line we used, and growth was observed for a few weeks.

These preliminary tests will be useful to set up air-liquid interface cultures and dynamic 3D systems that can reproduce even more faithfully the organization and functionality of tissues. These methods can represent an ideal system for the study of infectious diseases, since they allow to deepen knowledge on the mechanisms of microorganism-host interaction and viral replication.

**Keywords:** Influenza virus; Non animal based method; Health monitoring

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## Highly Pathogenic H5N1 Avian Influenza in Free-Living Griffon Vultures

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Spain houses approximately 75% of the world's population of griffon vultures (*Gyps fulvus*). While vultures are generally resistant to infectious disease agents, during the 2022 breeding season highly pathogenic avian influenza virus H5N1 affected griffon and bearded (*Gypaetus barbatus*) vultures. We report epidemiologic information gathered in the context of a program on movement ecology of Spanish griffon vultures that could help to understand risks in future breeding seasons.

A total of 91 nestling and adult griffon vultures were ringed and radiotagged in 2021 (n = 50) and 2022 (n= 41) and vascular feather and blood samples were taken to study exposure to Flaviviruses. Tissue including feather pulp or bone marrow from decomposed or previously frozen (-20°C) carcasses (n=11) from northern and southern Spain were subjected to Real Time RTPCRs for influenza A matrix and H5 AIV. Samples from positive birds were sent to the Central veterinary laboratory for confirmation.

We confirmed highly pathogenic H5N1 AIV in feather pulp and bone of deceased, highly decomposed, individuals. Feathers of all other vultures except a nestling that died after tagging were negative by Real time RTPCR. None of the vultures sampled in 2021 had antibodies against H5 AIV, while three ELISA positive nestlings from 2022 had high titres of antibodies against H5N1 by haemagglutination inhibition test, showing that at least two nestlings had survived the infection and fledged. Retrospective analysis of stored tissues from adult griffon vultures thought to have died from poisoning confirmed HPAIV H5N1 in at least one individual. Our results suggest that the current H5N1 AIV is highly pathogenic for griffon vultures but that not all individuals with systemic infections succumb. Further investigations should determine whether such survivors could become persistently infected and spread the disease.

Our results suggest that the current H5N1 AIV is highly pathogenic for griffon vultures but that not all individuals with systemic infections succumb. Further investigations should determine whether such survivors could become persistently infected and spread the disease.

**Keywords:** HPAIV, H5N1, vultures, epidemiology.

**Funding source:** Funded by Project PID2020-114060RR-C32

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*Fifth Parallel Session*

**Avian influenza**

*Posters with Flash Presentation*

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## P63. Pathology associated with highly pathogenic avian influenza H5N1 in naturally infected birds in Serbia in the 2021/2022 epidemiological year

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The highly pathogenic avian influenza (HPAI) epidemic in the 2021/2022 epidemiological year was the most widespread in Europe, and HPAI H5N1 was by far the predominant virus type reported. In Serbia, since early 2021 and 2022, multiple outbreaks of HPAI have occurred. The H5N1 subtype was also dominant during this epizootic, although a few cases of H5N8 subtype infection were reported in mute swans. This epizootic affected backyard chickens (3 outbreaks), and the virus was also detected in wild birds, mostly in mute swans (4 outbreaks). All cases were reported in the north of Serbia. Here we describe the pathological findings of natural HPAI H5N1 infection in poultry species (chickens and turkeys) and wild birds (mute swans) that died during this epizootic. Routine necropsies were carried out on the carcasses of 15 mute swans, 15 chickens, and four turkeys. The external body and internal organs were examined grossly, and the gross pathology was recorded and photographed. Body condition was estimated based on the amount of body fat and musculature. Tissue samples of the brain, pancreas, spleen, and lungs were collected, and the supernatants of the tissue homogenates were used for molecular diagnosis by RT-qPCR method. The affected birds showed nervous manifestations (abnormal head position, tremors, leg paralysis) and all birds were in good condition. The presence of influenza virus was detected in tissue samples of all tested animals. The external macroscopic changes included cyanosis and necrosis in the crest and wattle, and these lesions were more pronounced in chickens. In mute swans, there were no external lesions. The H5N1 HPAI virus produced several consistent gross lesions among the species investigated. Foremost among these lesions was: multifocal pancreatic necrosis and hemorrhages, petechial hemorrhages in coelomic and epicardial fat and epicardial petechiae. The lungs showed moderate (turkeys) to severe (chickens and mute swans) diffuse congestion and oedema. In most cases, mild splenomegaly and spleen necrosis were noted. Additionally, gizzard or proventricular lesions were not observed in any bird. The natural HPAI H5N1 infection in poultry and mute swans showed similar clinical disease, including neurological disorders, as well as similar pathologic presentation involving necrotic lesions and vascular damage, primarily affecting the pancreas and myocardium. Evaluating the pathological presentation of natural disease is particularly important in emerging infectious diseases such as influenza A virus, in which different strains can have different pathogenicity and clinical presentations.

**Keywords:** avian influenza, H5N1, pathology, Serbia

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## P64. Within-host genetic diversity of wild bird-origin LPAIV H7N7 in poultry

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The evolution of low pathogenic (LP) avian influenza viruses (AIV) is determined by the genetic variability of these pathogens as well as by ecology and migratory behaviour of their main reservoirs and natural hosts i.e. anseriform and charadriiform birds. However, the exact mechanisms shaping the intrahost genetic diversity of AIV during infection of different bird species remain unknown. Both interspecies differences in susceptibility to infection and the presence of various AIV shedding patterns in poultry may suggest that the selection pressure affecting the virus diversity during infection varies between the hosts. Our study attempts to elucidate the role of various poultry species in AIV evolution, especially in terms of the possible emergence of adaptive variants following wild bird-to-poultry virus transmission.

For this purpose, two galliform species (chickens and turkeys) and one representative of aquatic poultry i.e. domestic duck were experimentally infected with  $10^6$  EID<sub>50</sub> wild bird-origin H7N7 LPAIV via the oculonasal and oral route. During 7-day long animal trials, oropharyngeal and cloacal swabs were collected on specified sampling days (2-4-7dpi). Swabs tested positive for AIV in RT-qPCR directed against the M gene were further processed in high-throughput sequencing (Illumina NextSeq). The bioinformatic analysis included minority variant calling, identification of molecular markers affecting AIV biological properties and basic characteristics of virus populations present in both replication sites i.e. respiratory and digestive tract.

No significant interspecies differences in the cumulative number of single nucleotide polymorphisms (SNPs) detected in the oropharynx and cloaca during the entire animal experiment period (2-7dpi) were noticed. However, ducks showed the highest virus complexity in the respiratory system and its comparison to turkeys disclosed a statistical difference ( $p=0,01$ ). Complex variant analysis revealed unevenness of viral populations in swabs that consisted mainly of unique minority variants. The vast majority of non-unique SNPs were present in the same individuals on different sampling days or were inoculum-derived SNPs found in most bird samples except for cloaca swabs in turkeys. Sporadically mutations associated with zoonotic potential were identified.

Preliminary results suggest that the main determinant of AIV diversity in the course of infection may be genetic drift without noticeable interspecies differences in the selection pressure between the respiratory and digestive tract in chickens, turkeys and ducks. A more powerful approach is needed to describe within-host evolutionary dynamics and selection processes occurring during AIV infection.

**Keywords:** avian influenza, within-host diversity, adaptation

**Funding source::** National Science Center in Poland (grant no. 2018/30/Q/NZ6/00216)

## P65. Characterization of High Pathogenicity Avian Influenza Virus H5Nx Detected in Wild Mammals in Denmark, 2021 and 2022

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Since autumn 2020, high pathogenicity avian influenza virus (HPAIV) has caused massive die-offs within the wild bird population as well as widespread outbreaks on poultry premises across Europe. Findings of these viruses have also been reported in a number of wild mammals in several European countries. So far, most findings have been in foxes.

In connection with the Danish influenza surveillance, mammals have been tested for infection with AIV. The brain and/or lung of the mammals were sampled at necropsy for virological and pathological evaluation. Full-genome sequencing of the detected HPAIVs was performed and phylogenetically compared.

In 2021 and 2022, five mammals tested positive for clade 2.3.4.4b H5Nx high pathogenicity avian influenza virus (HPAIV) in Denmark. The first mammalian case was a harbor seal (*Phoca vitulina*) found dead on a beach at Southwest Funen in September 2021. The seal was emaciated with pronounced skin changes on large parts of the body. Influenza virus was detected in the lung of the seal. No other disease-causing pathogens were detected that could otherwise explain the cause of death. Upon further investigation, the virus was elucidated to be a clade 2.3.4.4b H5N8 HPAIV. In 2022, clade 2.3.4.4b HPAIV H5N1 was detected in four red foxes (*Vulpes vulpes*). HPAIV H5N1 was detected in the lung of an adult fox that was found dead on 17 January 2022 close to a fox pit. In a separate event, three fox cubs were found dead on 28 April 2022 together with the remains of a duck carcass. HPAIV H5N1 was detected in brain tissue of one of the fox cubs and in the lung of the two other fox cubs. All three fox cubs were previously observed alive on 25 April. Full-genome analysis revealed that the viruses from the cubs and the adult fox belonged to two different genotypes. The viruses were closely related to contemporary viruses. Analysis of the amino acid sequence revealed substitutions related to mammalian adaptation.

Within the last couple of years, an unprecedented number of mammals have been found infected with HPAIVs. For pandemic preparedness, it is crucial to increase disease surveillance of the wild animal population to monitor potential mammal-to-mammal transmission and the emergence of zoonotic mutations.

**Keywords:** influenza A virus, mammals, epizootic, high pathogenicity avian influenza virus

**Funding source:** The study was financially supported by the Danish Veterinary and Food Administration and the Ministry of Environment of Denmark, Environmental Protection Agency

## P66. Whole genome sequencing of Avian Influenza virus on the Illumina MiniSeq platform

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Active global surveillance and characterization of the Avian Influenza virus (AIV) is crucial for improving preparedness against potential pandemic events. Given the virus's ability to evolve rapidly through mutation and reassortment, obtaining comprehensive information about its genome can improve the understanding of the evolution of the influenza virus, emergence of new strains and improve vaccine design strategies. This work investigated the use of high-throughput sequencing on the Illumina MiniSeq platform to obtain complete genome information from AIV from samples collected in Serbia in 2021. Viral RNA was extracted from clinical samples from wild birds found dead and backyard poultry, followed by reverse transcription and genome amplification using universal primers and SuperScript™ IV OneStep-RT-PCR System (ThermoFisher Scientific, Waltham, Massachusetts, USA, cat. no. 12594025). PCR amplicons were visualized by electrophoresis on 1.5% agarose gels. Illumina-based library were prepared, pooled together and sequenced (2x150 paired-end) simultaneously, following the manufacturers' protocols. Total information of 2.6 Gbp was obtained from 321±3 K/mm<sup>2</sup> density with 78.56±0.42% of the clusters passing quality control (QC) filters. Approximately 87.16% of all Read1 and 92.43 % from Read2 sequences contained high quality bases that were ≥Q30, a base calling QC standard. Raw reads' quality was checked with FastQC (v0.11.9), raw reads statistical data was generated using Prinseq (v0.20.4), low quality bases, primers and adapters were removed with Trim Galore (v0.6.7), BWA (v0.6), Samtools (v1.16.1) and iVar (v1.3.1) were used for reference guided genome assembly and weeSAM (v1.6) was used for coverage statistics. Custom reference genome was built with the Vapor tool (v1.0.2) by using custom-built database containing all AIV genomes from the NCBI GenBank. Exploratory maximum-likelihood phylogenetic analysis was performed with the MEGA 11 software. BLAST and phylogenetic analysis of the AIV hemagglutinin and neuraminidase sequences revealed that three of the viruses belong to H5N1, while the fourth belonged to the H5N8 subtype. All AIV belonged to clade 2.3.4.4b. High pathogenicity of the viruses was confirmed by the presence of the multi-basic hemagglutinin cleavage sites, using the EMBOSS Transeq tool. The complete genomes of all four virus samples yielded equal or greater than 3000-fold sequence coverage depth. This study presented the successful use of the MiniSeq Platform for AIV whole genome sequencing and demonstrated the continuous presence and circulation of highly pathogenic AI in wild and domestic birds. It also highlights the significance of establishing sequencing and bioinformatics capacities for continuous surveillance and tracking of animal and zoonotic viruses.

**Keywords:** virus, high-throughput sequencing, bioinformatics, MiniSeq

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*Fifth Parallel Session*

**Avian influenza**

*Poster Presentations*

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## P67. Results of avian influenza surveillance in the Vojvodina Province of Serbia in 2022

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Avian influenza (AI) is a highly contagious infectious disease of birds that causes high health and economic losses in the poultry industry, and as zoonotic disease, represents a constant threat to human health. During the seasons 2020/2021, and 2021/2022, whole Europe was affected by many outbreaks of AI in both wild birds and domestic poultry, mostly caused by highly pathogenic AI virus strains (HPAI) of subtypes H5N8 and H5N1 as well as some other strains of the H5 virus subtype.

In Serbia, according to the Program of Measures of Animal Health, passive surveillance of the occurrence of AI is carried out continuously, which includes examination of the presence of AIV in cases of wild birds found dead, as well as in flocks of domestic poultry with an increased number of deaths. As in the previous season, an active surveillance program for AI (based on testing the cloacal swabs of domestic poultry from backyards and small village open markets, and on testing of cloacal swabs of trapped or hunted wild birds or their fresh faeces) was implemented in 2022 on the whole territory of Serbia. The detection of AIV presence and further subtyping of detected AI virus isolates were done by molecular diagnostic methods (by real-time RT-PCR, conventional RT-PCR and genome sequencing).

In Vojvodina, a northern Province of Serbia, during the implementation of the active surveillance in 2022, in total 381 pooled samples of cloacal swabs of backyard poultry (chickens, ducks, poultry, turkey, geese etc.) from 381 localities, then 60 pooled samples of cloacal swabs of backyard poultry and ornamental birds collected from local birds' markets, as well as 181 fresh faeces and 70 cloacal swabs of hunted or trapped wild birds from high risk localities, distributed in all 7 districts of the Vojvodina province, had been tested. The presence of HPAI H5N1 was detected in cloacal swabs of 3 *Larus ridibundus* sampled near the city of Novi Sad in South Bačka District in March 2022. The AI virus, but not of H5 or H7 subtype, was also detected in cloacal swabs of 3 *Anas platyrhynchos* sampled in municipality of Bela Crkva, South Banat District in November 2022.

In passive surveillance, suspected domestic poultry for AI, mainly broilers from 2 localities and the backyard chicken from three localities were tested in February, May and Decembar 2022. HPAI H5N1 was detected in February 2022 in 2 nearby localities in 2 backyards in North Bačka District and in May 2022 in backyard chickens at one locality in Central Banat District. Dead wild birds were collected from February until November 2022 in 16 occasions from 18 localities in 12 municipalities from all 7 Districts of Vojvodina Province. In total, 46 wild birds (mainly of species: *Cygnus olor*, *Anas Platyrhynchos*, *Ardea alba*, *Circus aeruginosus*, *Larus ridibundus*, *Buteo buteo*, *Haliaeetus albicilla*, *Corvus frugilegus* etc.) were tested. HPAI H5N1 virus was detected in 2 *Anas Platyrhynchos* from one locality and in 1 *Ardea alba* from one locality in February 2022; in 1 *Buteo buteo* from one locality in March 2022; and in 5 *Cygnus olor* from 3 localities in November 2022. During 2022 no large poultry farm was infected, and so far, there have been no significant economic losses. Considering the obtained results, as well as the still unfavorable epizootic situation of AI in Europe, it is necessary to continuously implement a program-based surveillance and early detection system for influenza virus in the territory of the Republic of Serbia in the future.

**Keywords:** avian influenza, active and passive surveillance, wild birds, domestic poultry, Serbia

**Funding source:** This study was supported by Ministry of Agriculture, Forestry and Water Management, as well as the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Contract No 451-03-47/2023-01/200031).

## P68. Molecular pathotyping and characterization of Avian Influenza viruses from Serbia during 2022

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Influenza in birds is caused by infection with viruses from the Orthomyxoviridae family, genus *Alphainfluenzavirus*. Influenza A viruses are the only orthomyxoviruses known to naturally affect birds. It is considered that aquatic birds are the major reservoir of influenza A viruses, which are, in majority of cases, low pathogenic for chickens and turkeys. Highly pathogenic strains that produce clinical disease and economic impact are associated with H5 and H7 subtypes. The cases of low pathogenic strains of H5 and H7 subtypes turning to highly pathogenic by mutation are also recorded. Some avian influenza strains can also cause sporadic infection in humans. In 2022 AI was reported in 37 European countries including Serbia. In this abstract we describe the results of Serbian National Reference Laboratory for AI and ND in 2022.

Samples of backyard poultry (4) and wild birds (8) were tested. Presence of AIV in the samples and subtyping and pathotyping was determined by RT-qPCR and RT-PCR assays recommended by EU reference laboratory, IZSV, Italy. Sanger sequencing of cleavage site was performed using Kha primer set. Phylogenetic analysis was performed using MEGA X software.

Totally 12 samples from 9 locations were found positive on influenza A RT-qPCR assay. All of them were typed as H5N1 subtypes. Amino acid motif on cleavage site (PLREKRRKRGFLF) revealed that they belong to highly pathogenic avian influenza viruses. Phylogenetic analysis of part of HA gene sequences showed that they can be sided with clade 2.3.4.4.b viruses. Further analysis shows that there is slightly difference in sequences between cases from the beginning of the year until April, and those registered after suggesting that they belong to two different sub-clades.

Although the epizootiological situation in the Europe was unfavorable and wave of AI in 2022 was one of the worst in recent history, due to import bans, strict movement and trade control, and increased biosecurity measures imposed by the Government, there was no outbreak of HPAI on big poultry farms in Serbia. Active monitoring program is also introduced by Government measures since constant monitoring of presence of avian influenza viruses and their timely pathotyping and characterization at National Reference Laboratory are crucial to promptly detect the emergence in or introduction of viruses relevant for animal and public health.

**Keywords:** Avian influenza, virus, PCR, sequencing

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*Sixth Parallel Session*

**Epidemiology  
and Risk assessment**

*Oral Presentations*

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**Invited lecture**

## **Molecular epidemiology as a tool for understanding the viral infections in honeybees**

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Slovenia is a country with a very long beekeeping tradition and an extremely high density of apiaries and bee colonies. Several positive-stranded RNA viruses, found in managed honeybees (*Apis mellifera carnica*) may represent a complex of emerging infectious viral diseases, together with other pathogens, as a part of the global problem of decreasing pollinators. Some viruses may be present in different species living in the environment closely with honeybees and meet through the direct or indirect contacts.

Between 2010 and 2022 the direct sequencing of positive samples of acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), deforming wing virus (DWV), sacbrood bee virus (SBV) and Lake Sinai virus (LSV) was performed from obtained RT-PCR products in different regions of genome, detected in honeybees, bumblebees, and butterflies in Slovenia. The genetic comparison of identified positive samples of bumblebees and detected honeybee field strains of ABPV, BQCV, SBV and LSV has shown from 98.74 to 100 % nucleotide identity between both species. The evidence that honeybees and bumblebees are infected with genetically identical or closely related viral strains of four endemically present honeybee viruses explain the transmission of different viruses between apiaries and some other pollinators. High diversity of circulating strains was observed among honeybees, similar was detected also in bumblebees. The presence of ABPV, BQCV, LSV3 and SBV was detected for the first time also in butterflies in Slovenia. The viral load in the positive butterfly samples was much lower than in the positive bee samples, which could indicate that they are passive carriers of bee viruses. Molecular epidemiology represents an excellent tool for understanding viral infections in different pollinators. An important new genetic data for endemic strains circulating in honeybees and bumblebees in Slovenia will be presented.

The identification of genetically closely related honeybee viruses during several consecutive years of sample collection on different locations in Slovenia was confirmed by sequencing of detected strains. For the better control of viral infections in honeybees, such studies should be extended to other types of pollinators. Through this approach, we will obtain a more complete picture of the actual prevalence and the transmission of different strains of viruses in the environment.

**Keywords:** *Apis mellifera carnica*, bumblebees, viruses, transmission, epidemiology

## Pigeon paramyxovirus-1 outbreak in captive pigeons in Denmark, 2022

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Pigeon paramyxovirus-1 (PPMV-1) forms a separate group of viruses within Avian orthoavulavirus-1 (AOaV-1) of which virulent strains are the causative agent of Newcastle disease in poultry. Since its introduction to Europe in the 1980s, PPMV-1 has spread throughout the continent, causing both fatal and asymptomatic infections. In June 2022, an outbreak of PPMV-1 occurred in a vacation home community in South Zealand, Denmark. Here we present a case report of the Danish outbreak together with the results of the genetic and virological characterization of the virus.

The outbreak was detected in a vacation home community housing hundreds of captive birds, most of which were not vaccinated against PPMV-1. Viral detection was achieved through RT-qPCR analysis of cloacal and oropharyngeal swabs and virus isolation in embryonated chicken eggs. Full genome sequence analysis was performed to determine the genotype of the virus. Pathogenicity assessments were conducted using molecular characterization of the cleavage site and by *in vivo* intracerebral pathogenicity index (ICPI) test.

The virus was determined to belong to genotype VI.2.1.1.2.2, a PPMV-1-specific genotype currently circulating in wild pigeons in several European countries. The virus exhibited the characteristic sequence of virulent AOaV-1 at the F-gene cleavage site, indicating high virulence. However, the ICPI index was 0.650, suggesting that the virus was not highly virulent for chickens. Similar results have been observed with other PPMV-1 viruses, demonstrating the uncertainty of genetic analysis of PPMV-1 for the assessments of virulence in chicken.

This study presents a case report of the PPMV-1 outbreak in Denmark and the genetic and virological characteristics of the virus. The clinical presentation of the outbreak combined with the pathogenicity assessments indicate that the outbreak-causing virus was virulent for pigeons but not for chickens. These findings highlight that challenges associated with assessing virulence of PPMV-1 viruses, and that relying on a single method for the assessment of virulence, may not provide a comprehensive understanding of the viral pathogenicity.

**Keywords:** Newcastle disease, PPMV-1, outbreak, pathogenicity, virulence

**Funding source:** Danish Veterinary and Food Administration

## Novel diagnostic and statistical tool combinations accurately predict time since bluetongue virus infection in ruminants for assessing transmission risk

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Bluetongue virus (BTV) has a segmented, dsRNA genome which encodes structural (VP1-7) and non-structural (NS1-4) proteins. It causes haemorrhagic disease, bluetongue, in susceptible wild and domestic ruminants. BTV infection is typically diagnosed through presence of viral RNA in the blood (qRT-PCR) or serum antibodies against the group-specific, immune-dominant VP7 protein (cELISA). However, this only confirms BTV exposure (not presence of infectious virus) and does not provide information on time since infection, which is crucial for estimating infectiousness and potential transmission risk in BTV-positive ruminants. We aimed to develop serological assays to investigate isotype-specific anti-BTV antibody dynamics during infection and assess their ability, alongside current BTV diagnostics, to delineate and predict time since infection.

Novel ELISAs were developed to detect IgM-, IgG Fc-, IgG1- and IgG2-specific anti-NS2 antibodies in cattle and sheep serum using recombinant, bacterially-expressed NS2 protein. Isotype-specific VP7 protein ELISAs were also adapted from the ID Screen® cELISA platform. Time-course serum from BTV-infected sheep (n=43) and cattle (n=12) were screened to determine anti-VP7/NS2 antibody dynamics during infection. Bivariate normal distributions were fitted to data, creating two-parameter (cELISA/qRT-PCR) and nine-parameter (cELISA/qRT-PCR/IgM VP7/IgG VP7/IgG1 VP7/IgG2 VP7/IgM NS2/IgG NS2/IgM:IgG VP7) statistical models and leave-one-out cross validation performed to determine their suitability for predicting days post-infection (dpi).

BTV infection was characterised by consistently earlier IgM-specific anti-VP7/-NS2 responses, followed later by IgG, allowing ruminant antibody profiles of high, moderate, and low transmission risk to be delineated. IgG1-specific anti-VP7 antibodies were in greater abundance than IgG2, however provided no further differentiation. Whilst anti-VP7 antibodies were consistently detected in BTV-infected ruminants, those targeting NS2 were not. Sheep had low detectable IgM/high IgG anti-NS2 antibodies, which was reversed in cattle. Statistical models were able to accurately predict early infection (<7dpi) to within two-days, whilst novel isotype ELISAs enhanced predictions between 7-12 dpi.

Novel isotype-specific ELISAs targeting BTV structural and non-structural proteins complement current diagnostics in accurately predicting time since infection and transmission risk in BTV-infected ruminants. These novel diagnostic and statistical tools are critical for helping prevent/control future BTV outbreaks.

**Keywords:** BTV, diagnostics, transmission, infectiousness

**Funding source:** UK Government Department for Environment, Food and Rural Affairs (SE2622) and BBSRC (BBS/E/I/00007039, BBS/E/I/00007037).

## A Machine Learning Approach for Predicting the Risk of WNV in Fine Spatiotemporal Scale Relying on Earth Observation Data

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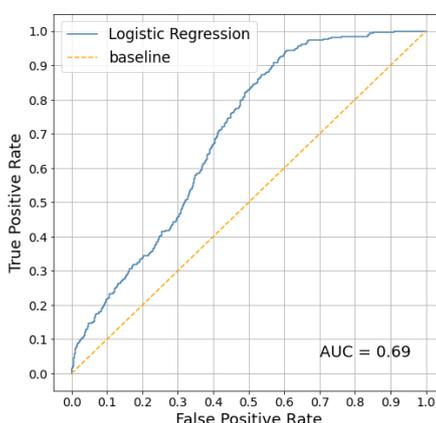
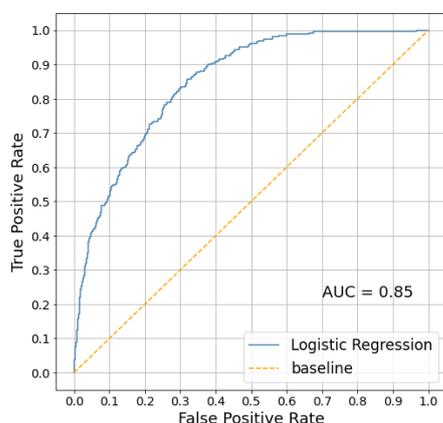
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Mosquitos carry and transmit Mosquito-Borne Diseases which affect an estimated 700 million annually and are responsible for almost a million deaths. In the absence of available vaccines and treatments the best countermeasure against MBDs is the development of an Early Warning System (EWS) to accurately estimate the probability of MBD occurrence, so that health authorities can take preventive action. This work proposes the development of EWS based on historical data of WNV cases, combined with Earth Observation (EO) data, synthetic entomological information (mosquito populations), and socioeconomical data. The proposed system learns the patterns between input features and the epidemiological situation in order to predict the probability of WNV occurrence at LAU1 (municipality) spatial scale and with a time lead of two weeks. The EWS developed in this study was tested using historical WNV data from two areas of interest: Veneto region in Italy and Central Macedonia region in Greece. The model's performance was evaluated based on its goodness of fit and operational effectiveness as an EWS. In terms of goodness of fit, the model achieved a ROC AUC of 0.85 in Veneto and 0.69 in Central Macedonia, while its PR AUC was 0.10 and 0.15 respectively, compared to a baseline of 0.01 and 0.07. As an operational EWS, the model correctly predicted 17% of all infected units in Veneto and 44% in Central Macedonia for the years 2018 and 2019. This study is the first to develop a data-driven EWS for MBDs that makes predictions at such a fine spatiotemporal scale (municipality and biweekly). The results are promising, indicating a strong dependence between EO data, mosquito populations, socioeconomic factors, and WNV occurrence that the model manage to capture. As a probability estimator, the model performs ten times better than the baseline estimator in Veneto and two times in Central Macedonia, and as an operational EWS, it performs two to three times better than random depending on the region. The average temperature and precipitation during the winter months of the current year, along with two remotely-sensed indices, NDVI and NDWI, were identified as variables with strong predictive power for WNV case occurrence in both instances

**Keywords:** mosquito-borne diseases, West Nile virus, early warning system, machine learning, earth observation

**Funding source:** EYWA, EIC Horizon Prize



## Wild boar as a reservoir of Aujeszky disease virus, coronaviruses and circoviruses – a study in African swine fever affected population in Poland

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Wild boars are a reservoir of many infectious diseases of pigs and humans. As the most important reservoir of African swine fever (ASF), they are responsible for the spread of the second wave of the disease in Europe, first identified in 2007 in Georgia. In Poland ASF was first diagnosed in wild boar in 2014 and since then intensive monitoring of the disease in this species has been carried out. However, there are no current data on the occurrence of other, epizootic and economically significant diseases in wild boar, e.g. Aujeszky's disease (AD), for which a national eradication program has been carried out since 2008. The aim of the study was to verify if wild boar in Poland is a reservoir of swine viruses, namely: Aujeszky's disease virus (ADV), coronaviruses: transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV) as well as circoviruses: PCV2, PCV3 and PCV4. The study was conducted in wild boar population in Poland, positive for African swine fever (ASF) by serology, molecular detection or by both methods.

The study was conducted on 300 samples of blood/serum from wild boar shot in Poland. The samples were initially recognized as ASF-positive by real-time PCR: 105 samples; by immunoperoxidase technique: 163 samples or by both methods: 32 samples. This material was analyzed by ELISA tests for the presence of antibodies against ADV, TGEV and PRCV, according to the manufacturers' instructions. Identification of circoviruses was also performed by the real-time PCR test.

As a result of the tests, 58 (19.33%) samples were found to contain antibodies to ADV. Only 1 sample was positive in the TGEV/PRCV ELISA. The presence of the circoviruses: PCV2 and PCV3 was confirmed in 96 (32.00%) samples, with PCV2 being identified in 57 (19.00%) and PCV3 in 39 (13.00%) samples. The presence of PCV4 was not confirmed. Most ADV, PCV2 and PCV3 positive results were identified in the ASF serologically positive group.

The results of the project confirm the circulation of ADV, PCV2 and PCV3 in the wild boar population in Poland, but do not confirm the presence of coronaviruses: TGEV and PRCV, or PCV4. These findings are of particular importance in connection with the implementation of the national program for the eradication and monitoring of AD in pigs, as well as compliance with the principles of biosecurity in pig farms.

**Keywords:** wild boar, Aujeszky's disease virus, coronaviruses, circoviruses, African swine fever

**Funding source:** This study was supported by the Polish Ministry of Agriculture and Rural Development Project no. S/492 "Determination of the spread of important infectious diseases in the wild boar population in Poland"

## African Swine Fever in Italy: effectiveness of eradication measures in newly affected regions and in Sardinia region.

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The current genotype II African Swine Fever (ASF) epidemiological wave reached wild boar populations in mainland Italy in 2022, firstly in the Piemonte/Liguria regions, then in the Latium region. In Sardinia region genotype I ASF endemic involved domestic pigs, wild boar and illegal free-range pigs since 1978. Our aim is to describe the effectiveness of the eradication measures applied in Italy.

Following ASF notification in mainland Italy, emergency measures have been carried out immediately. The eradication plan provided for closing ASF core area and for establishing a white zone around the infected zone through wild boar depopulation; in the mountainous territory of Piemonte/Liguria, fencing was required, whereas in the urban affected zone in Latium, trapping was the unique suitable tool. In Sardinia an advanced eradication plan focused on measures dealing with involved swine populations since 2015; culling of illegal free ranging pigs was the most relevant activity.

Application of the contingency plan allowed to front ASF emergency in mainland Italy, later measures to contain infected wild boar populations were implemented in different ways. In Piemonte/Liguria, local authorities gradually showed increasing reluctance to share ASF restriction measures. The lack of available resources affected the strengthening of surveillance activities and the timing of the early installation of fences: the strategy 'chased' the spread of infection and the route of fences had to be changed several times. In Latium, disease containment inside the highway surrounding the affected zone, wild boar depopulation activities, carcasses search, and removal have effectively prevented the further viral spread. In Sardinia, eradication efforts allowed a decrease in virus circulation. Moreover, the European Commission allowed an amendment of ASF risk level by reshaping the restricted zones recently. To date some seropositive cases have been detected only.

The current situation in Piedmont/Liguria is the most worrying, since the restriction zones have more than doubled after one year, with the risk of further expansion. In Latium, no new cases have been recorded since September and the representativeness of passive surveillance results demonstrates the effectiveness of the disease control on the territory. In Sardinia, the latest efforts are being directed towards achieving definitive ASF-free status.

**Keywords:** African Swine Fever, Surveillance, Eradication

**Funding source or Acknowledgement:** RC IZS VE 10/21

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*Sixth Parallel Session*

**Epidemiology  
and Risk assessment**

*Posters with Flash Presentation*

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### P43. Risk assessment of foot-and-mouth disease and biosecurity measures on a cattle farm in Serbia

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Periodic outbreaks of foot-and-mouth disease (FMD) in the countries of the Middle East and Africa pose a serious threat to European countries, especially the countries of the Mediterranean and the Balkan Peninsula. Due to insufficient development of veterinary services, diagnostic capacities, and socioeconomic and political reasons, the disease is endemic. Serbia is on a transit route between the Middle East and Central Europe and is at risk of introducing FMD which is considered the most devastating disease in cattle production. Experience with African swine fever outbreak in Serbia has shown that agent's transmission through man is very likely, that awareness raising about biosecurity measures at many stockbreeders is a slow process, and that even apparently good biosecurity measures on farms have gaps that allow agents breakthrough in population. As for FMD, the current epidemiological situation in the cattle population in Serbia is stable, the most common diseases detected on farms are BVDV, BRSV and *Mycoplasma bovis* infections, and sporadic cases of paratuberculosis. In such circumstances, attention to biosecurity measures among farmers and veterinarians is declining. That fact makes farms vulnerable to introducing new infectious agents such as FMD. The main risk factors for possible FMD outbreaks on farms are the proximity of international highways that connect the Middle East and Central Europe in the northern part of Serbia. The lowland geographical area, occasionally windy, allows the FMD virus to spread easily. Many farmers do not cooperate with local veterinarians, rather they prefer to use the services of specialists who visit many farms over long distances. Biosecurity measures on farms are at a very low level or non-existent, while dairy trucks daily entering into farms. The geopolitical migrant crisis and the illegal traffic of migrants across state borders also pose a risk of the introduction of the FMD virus into the country. Taking into account the mentioned risk factors, as well as the circumstance of the constant threat of the endemic presence of FMD virus in the Middle East, it is necessary to constantly maintain readiness and vigilance. Although the current epidemiological situation is favourable there is a need for significant improvement of biosecurity measures on cattle farms, as well as awareness campaigns and surveillance measures.

**Keywords:** risk assessment, cattle farm, FMD, biosecurity

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*Sixth Parallel Session*

**Epidemiology  
and Risk assessment**

*Poster Presentations*

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## P44. Thirteen-year review of brucellosis of cattle and sheep in the Republic of Srpska

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Brucellosis is one of the most common natural foci of zoonotic infections. It significantly endangers human health and causes great economic damage to livestock production. The cause is bacteria from the genus *Brucella*. The species *B. melitensis*, *B. abortus* and *B. suis* biovar (bv.) 1 and 3 have the greatest zoonotic significance. Brucellosis is primarily a disease of domestic animals, but it also has zoonotic character with people with acute and chronic courses. At the beginning of the 1990s, brucellosis of animals and humans was diagnosed in Bosnia and Herzegovina at the Manjača region (farm of the Military Institution). After 2001, brucellosis became more prevalent in both sheep and humans.

In the Republic of Srpska, in accordance with the regulations, "mandatory reporting and reporting on the occurrence of infectious diseases" is performed, on the basis of which a veterinary-epizootic analysis is performed, which includes planning measures and activities in the following period. The monthly report of the Ministry of Agriculture of the RS - Department of Veterinary Service on the occurrence of infectious diseases (bulletin) was used as a source of data. During 2009, mass vaccination of all sheep and goats that are not pregnant and young animals older than three months began, with the Rev-1 vaccine, which is applied conjunctively. From 2010-2022 (table 1), brucellosis was constantly present in cattle and sheep. The highest number of cases of sheep brucellosis was recorded in 2015 in 14 foci with 398 infected sheep and the lowest number in 2013 in 3 foci with 3 infected sheep. Observed in the same period, the highest number of infected cattle from brucellosis was recorded in 2016 with 48 infected cattle in 32 foci, and the lowest number in 2013 with 1 infected and 1 focus. Control and eradication of sheep and goat brucellosis implies consistent control of the movement of animals and complete control over the nomadic way of animal husbandry (which is otherwise prohibited "only on paper"). Based on these data, it is evident that brucellosis in cattle and sheep is still present and that in the following period the authorities must take this problem most seriously, analyse past shortcomings and find the best solution for future programs, with mandatory consultation with the profession!

**Keywords:** Brucellosis, epizootic situation, disease

Table.1

Year	Total per year		
	Total foci	Cases of sheep	Cases of cattle
2010	30	32	20
2011	21	108	14
2012	15	42	10
2013	7	32	1
2014	7	97	2
2015	20	398	6
2016	39	105	48
2017	29	152	22
2018	42	138	23
2019	20	67	15
2020	10	3	10
2021	8	18	4
2022	9	54	6
Total	257	1198	229

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*Seventh Parallel Session*

**Vector-borne diseases**

*Oral Presentations*

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*Invited lecture*

## Companion Animals as Sentinels for Viral Zoonoses in Urban Areas

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In recent decades, the emergence and re-emergence of viral zoonoses, including COVID-19, has become a major global threat. This growing public health challenge needs intensive studies of zoonotic pathogens in animals, in line with the One Health approach.

Various surveillance systems are implemented in order to obtain information for public health sectors. Active surveillance, by testing of sick or dead wild and domestic animals, is successfully used for emerging zoonotic viral pathogens like West Nile virus (WNV), Usutu virus (USUV), tick-borne encephalitis virus (TBEV), or hepatitis E virus (HEV). A major limitation of active surveillance is that some emerging viral zoonoses are often asymptomatic in animals. Furthermore, the reporting of clinical symptoms in animals depends on the owner's public awareness of zoonoses, which could compromise surveillance results. Also, the location and time of sampling are random and unmanageable.

In contrast, passive surveillance, using domestic animals as sentinels, has the advantage of controlled sampling in specific geographic areas at an optimal time. In addition, the limited and controlled movement of domestic animals and the availability of samples increase the value of this type of surveillance. A good example is the active surveillance of the WNV using horses and poultry as sentinel animals. Despite all the advantages, a surveillance system using livestock, horses or poultry as sentinels has limitations due to the limited number of these animals in urban areas.

Nowadays, the number of households owning pets increased significantly. Pets are part of everyday family lives sharing the same space and environment. In line with this, pet animals could serve as an optimal sentinel species in urban areas.

Our research group has investigated the possibility of using pets as sentinel animals in active surveillance for different viral zoonoses. Recent results show that testing pet animals could be a valuable surveillance system for the presence and activity of viral zoonotic pathogens such as the human influenza virus and emerging zoonotic pathogens such as WNV, TBEV, USUV and HEV. The correlation between infection in pets and humans is also clearly confirmed during the COVID-19 pandemic. Even more, surveillance of the pet animal population was a valuable source of public health data showing the presence of neglected viral zoonosis like *Tahyna orthobunyavirus* in the capital of Croatia.

It is clear that the emergence of viral zoonoses will continue to occur, which requires introducing new and adjustment of existing surveillance systems. The trend of urbanization and a growing number of pets is giving us new surveillance tools for the protection of human health in accordance with the One Health approach.

**Keywords:** viral zoonosis, surveillance, pets, One Health

## Automated remote real-time mosquito surveillance by the means of an intelligent sensor trained with machine learning

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Mosquitoes (Diptera, Culicidae) are vectors of important pathogens than cause neglected diseases such as malaria, dengue, West Nile Virus, Zika, Yellow Fever or Chikungunya, among others, leading to hundreds of thousands of deaths every year. Vector surveillance is on the focus of Public Health programs to prevent and manage potential outbreaks of these diseases. Traditional surveillance methods can be very time consuming, require expert entomologists to analyse the samples and may not adequately reflect the temporal dynamics of mosquito populations. New methods based on remote sensing and artificial intelligence that allow automated real-time characterization of mosquito populations have the opportunity of truly improve this field. A new machine learning model has been developed for mosquito genus and sex classification based on the analysis of thousands of *Aedes* and *Culex* mosquito flight recordings obtained with a pseudo-acoustic sensor coupled to a standard mosquito suction trap in the laboratory for different temperature regimes. This system was implemented for two consecutive summers in two different localities of the province of Barcelona (Spain) covering the period of mosquito activity in the area and a variety of environmental conditions. The sensor + trap system was installed for periods of 24-72 hours in the field and the results obtained from the manual inspection of the catch bags inside the traps were compared with the results predicted by the system with two classification purposes: mosquito identification and genus and sex classification. The model was able to distinguish *Aedes* and *Culex* mosquitoes from other insects that entered the trap with 95.3% of accuracy; and to classify these mosquitoes by genus and sex with 92.6% of accuracy. In both cases, the predicted and the observed values showed a significant strong positive correlation. The accuracy of the predictions was not correlated neither with the proportion of mosquitoes over other insects in the samples nor with the hours of sampling. The system presented here has both predictive power to monitor tendencies of *Aedes* and *Culex* mosquito populations and it is accurate to predict the actual number of these mosquitoes that enter a trap in real-time. It is easy to be incorporated into the routine of vector surveillance since it works attached to a conventional mosquito trap, it can be working remotely for several days without the need of constantly revising the trap, it can be implemented in a variety of temperature environments, and it generates real-time data with high accuracy. The combination of artificial intelligence and the Internet of Things introduces a step-forward in the field of vector surveillance by offering a potential scalability that has not been achieved until now.

**Keywords:** Mosquitoes, Vector surveillance, Remote sensing, Machine learning, Artificial Intelligence

**Funding source:** VECTRACK project, European Union's Horizon 2020 research and innovation programme under grant agreement no. 853758

## Transmission of West Nile virus by *Culex pipiens* Infected with *Culex Flavivirus*

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West Nile virus (WNV) is a mosquito-borne flavivirus that is kept in an enzootic cycle mainly between birds and mosquitoes. Occasionally, it affects mammals including humans and equines causing encephalopathies. On the other hand, *Culex flavivirus* (CxFV) is an insect-specific virus within the *Flavivirus* genus globally distributed among *Culex* populations. Some studies point to an interaction between both viruses. Here, we assess the ability of a Spanish CxFV to alter vector competence of *Culex pipiens* mosquitoes for WNV. We seek for an effective control tool for WNV, which could be used in the future.

3-to-5 days old *Cx. pipiens* females were inoculated intrathoracically with Spanish CxFV. After a week, CxFV positive and CxFV negative females were fed with blood doped with WNV. Seven and 14 days post-exposure females were sacrificed and head, body and saliva were extracted to assess infection, dissemination and transmission rates and transmission efficiency.

CxFV was able to infect *Cx. pipiens* after intrathoracical inoculation and had not impact on WNV infection, dissemination, and transmission in *Cx. pipiens* mosquitoes, as WNV was detected in head, body and saliva of both CxFV positive and negative groups without significant differences.

In the present study, a sequential infection experiment, CxFV does not seem to affect the vector competence of *Culex pipiens* for WNV, so that CxFV may not be used as a biotool for WNV control in mosquitoes.

**Keywords:** *Culex flavivirus*, West Nile virus, *Culex pipiens*, vector competence

## Confirmation of seasonal flavivirus activity at suspect localities through targeted mosquito trapping and testing

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Over the past 20 years, the seasonal activity of West Nile Virus (WNV) in Hungary has been monitored through the combination of passive and active surveillance focused on the detection of wild bird, horse, and human cases. In the absence of resources for a country-wide vector surveillance system, mosquito trapping and testing had only been performed intermittently in a focal and targeted manner. In 2022 we have utilised our capacity for mosquito trapping and testing developed recently for the monitoring of invasive mosquito species ([www.mosquitosurveillance.hu](http://www.mosquitosurveillance.hu)) to confirm local WNV circulation in mosquitoes at localities with laboratory-confirmed host cases of WNV infection, simultaneously monitor for Usutu virus (USUV) presence, identify the affected vector species, and check for the involvement of invasive mosquito species.

Mosquito trapping sites were selected at ten settlements with reported human, horse or, bird WNV cases between weeks 34 (22 August) and 42 (18 October) 2022. Two to three BG-Sentinel mosquito traps were deployed at each settlement overnight for one or two days. Collected mosquitoes were identified and pooled according to species, sex, and location. Following RNA extraction, pooled samples of max. 20 mosquitoes were tested for the presence of WNV lin. 1, WNV lin. 2, and USUV in a multiplex qRT-PCR assay.

We collected a total of 67 pools comprising 723 individuals of 9 mosquito species. 10 pools tested positive for flaviviruses. From the 10 suspect outbreak sites, flaviviruses were detected in mosquitoes at 4 localities. Flavivirus circulation was confirmed in mosquitoes at Győr (USUV), Jánossomorja (WNV lin. 2 and USUV), Szeged (WNV lin. 2), Mórahalom (WNV lin. 2), and Vác (USUV). Mosquitoes from Szentes, Dunakeszi, Nyíregyháza, Tiszabercel, Eger and Szerencs tested negative. Mosquito species found infected were *Culex pipiens* (WNV, USUV) and *Aedes vexans* (WNV).

Considering the limited trapping effort, the confirmation of WNV circulation at 3 sites and detection of USUV circulation at 3 locations still provided convincing evidence of favourable conditions for local flavivirus circulation in at least half of the study sites. With some modifications, this approach could efficiently complement the evidence of seasonal flavivirus circulation obtained from permanent mosquito trapping.

**Keywords:** mosquito, trapping, WNV, USUV, surveillance

**Funding source:** The study was supported by the Hungarian National Research, Development and Innovation Office grants RRF-2.3.1-21-2022-00006, PD135143, K135841 and K120118

## T lymphocytes contribute to protective immunity and pathogenesis in bluetongue virus-infected sheep but not viral replication or transmission

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Bluetongue virus (BTV) is an arthropod-borne orbivirus which causes haemorrhagic disease, bluetongue, and is transmitted to susceptible ruminants through blood-feeding of infected *Culicoides* midges. BTV infection is characterised by immunosuppression and lymphopenia around peak viremia. However, the relative importance of T lymphocytes for immune protection, BTV pathogenesis and transmission are largely unknown. Here, we utilised a natural *in vivo* *Culicoides*-borne BTV infection model to study the role of specific T cell subsets in BTV pathogenesis, viral replication dynamics, immune protection and onwards transmission to a susceptible *Culicoides* vector.

Sheep were intravenously administered specific monoclonal antibodies (mAbs) to deplete CD4<sup>+</sup> (n=7), CD8<sup>+</sup> (n=7) or WC1<sup>+</sup>  $\gamma\delta$  (n=7) T cells. Seven additional sheep were administered an isotype-matched depletion control mAb. Adult female *Culicoides sonorensis* were fed on blood containing BTV-4 MOR2009/07 and, after the extrinsic incubation period, were allowed to blood-feed on the T cell-depleted sheep to establish infection. Uninfected *C. sonorensis* were blood-fed on sheep at peak viremia to assess onwards BTV transmission to the arthropod vector.

The natural infection model resulted in transmission of BTV-4 to all 28 sheep. T lymphocyte depletion and changes to cellular populations in whole blood were confirmed through multi-colour flow cytometry. CD8<sup>+</sup> cytotoxic T cell absence was consistently associated with lower clinical scores, higher survival and more favourable anti-BTV antibody responses. CD4<sup>+</sup> T cell absence resulted in higher clinical scores (along with WC1<sup>+</sup>  $\gamma\delta$  T cells), impairment of protective neutralising antibody responses and delayed class-switching of antibodies targeting non-structural BTV protein, NS2, identifying a likely role in protective immunity. BTV also appeared to target downstream effector functions of specific T cell subsets as a likely pathogenic mechanism. T cells did not impact viral replication, shedding or onwards *Culicoides* transmission.

This study highlights the multifaceted role of T lymphocytes during BTV infection, emphasising their contribution to protective immunity and disease pathogenesis.

**Keywords:** bluetongue, BTV, T lymphocytes, *Culicoides*

**Funding source:** Biotechnology and Biological Sciences Research Council (BB/P006841/1, BBS/E/I/00007039, BBS/E/I/00007037) and Defra (SE:2622).

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*Seventh Parallel Session*

**Vector-borne diseases**

*Posters with Flash Presentation*

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## P45. Vectors responsible for the transmission of Epizootic Haemorrhagic Disease virus (EHDV) in Italy

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Epizootic Hemorrhagic Disease (EHD) is an infectious, economic important viral disease transmitted by *Culicoides* that affects wild and domestic ruminants. The causative agent, EHD virus (EHDV), belongs to the family *Sedoreoviridae*, genus *Orbivirus*. Seven serotypes have been officially identified. In November 2022, outbreaks caused by EHDV-8 were reported in Sicily and Sardinia islands. This was the first incursion of EHDV ever recorded in Italy and Europe. Herein we report the detection of EHDV in European species of *Culicoides*, as a result of a field entomological investigation during the Sardinian outbreaks. Due to the simultaneous circulation of bluetongue virus (BTV) in the area, the midges were also tested for BTV.

An intensive entomological field activity was performed in four EHDV outbreaks located in the south-western part of Sardinia (Sud Sardinia province). Entomological activities were set up immediately after the first outbreak was reported and are currently in progress. UV blacklight suction traps were used. They are operating all night long from at least one hour before dusk. All *Culicoides* collected were identified, age graded, and sorted in pools of maximum twenty-five midges. Each pool was tested by serogroup/serotype specific real-time RT-PCRs for EHDV/EHDV-8 and BTV/BTV-3, respectively.

A total of 22 pools (consisting of 391 parous females) resulted positive to EHDV and EHDV-8 out of 411 tested pools (6,062 midges). Of these, 11 pools were composed of *Culicoides imicola*, four of *Culicoides obsoletus/scoticus*, two of *Culicoides newsteadi*, four of *Culicoides pulicaris ss*, and one pool of *Culicoides bysta*. Two pools consisting of 43 *C. imicola* resulted positive to both, BTV/BTV-3 and EHD/EHDV-8.

Our study reports for the first time the detection of EHDV/EHDV-8 in *Culicoides* biting midges in Europe. EHDV-8 was detected in five species caught in affected farms, namely *C. imicola* and *C. obsoletus/scoticus* (subgenus *Avaritia*), and *C. newsteadi*, *C. pulicaris ss*, and *C. bysta* (subgenus *Culicoides*).

*Culicoides imicola*, *C. obsoletus/scoticus*, *C. newsteadi* and *C. pulicaris* are also known vectors of BTV in Italy, with *C. imicola* playing a pivotal role in Sardinia, while *C. obsoletus/scoticus* acting as main vector in Northern Italy. According to our results, EHDV seems to use the same transmission patterns as BTV. In turn, this means that EHDV-8 has the potential to spread in Europe, with *C. imicola* and *C. obsoletus/scoticus* being responsible for its diffusion in the Mediterranean countries, while *C. obsoletus/scoticus* acting as the main vector in Central-Northern Europe.

**Keywords:** Epizootic Hemorrhagic Disease, *Culicoides*, Sardinia, Italy

## P46. Epizootic haemorrhagic disease in Spain, November 2022

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Epizootic haemorrhagic disease (EHD) is a *Culicoides*-borne viral disease caused by an Orbivirus. Until 2022, it had never been detected in Europe, but in November 2022 EHD was reported for the first time on cattle farms in Sardinia and Sicily, Italy. On November 18, 2022, EHD was also notified in cattle in Andalusia, Spain. We describe the detection of the EHD virus in Spain, focusing on diagnostics and viral transmission dynamics. EDTA blood samples from suspect bovines were analysed by three rRT-PCR methods (Maan *et al.*, 2017; Viarouge *et al.*, 2015; in-house method, 2007) and a gel-based RT-PCR method (Aradaib *et al.*, 1994) to compare the results and assess performance of the methods. Some positive samples were analysed by serotype-specific rRT-PCR and Sanger sequenced to confirm serotype and inoculated in cell culture for virus isolation. Serum samples from some affected bovines were analysed by cELISA. In addition, by protocol starting one month after the last outbreak, samples were periodically taken from affected bovines for ELISA and rRT-PCR assays, respectively, to collect data about dynamics of the disease. Moreover, other ruminants in close proximity to affected bovine farms were sampled to study susceptibility in other species.

EHDV-8 was confirmed using RT-PCR methods, virus isolation and detection of specific antibodies by ELISA. Partial sequence of segments 2 (557 nt) and 5 (348 nt) are identical to that of EHDV-8 circulating in Italy and Tunisia. Animals with positive RT-PCR results in November 2022 were still positive in February 2023, although infectivity has not been evaluated. All of them also had positive antibody ELISA results, including two calves born from symptomatic mothers (maternal antibodies). Ovine have shown negative ELISA results to date.

Some days after its notification in Italy, EHDV-8 was found in the southwest of Spain. This virus is identical to that found in Italy and the north of Africa at least in two partial segments of its genome. The rRT-PCR technique, especially Viarouge *et al.* (2015), is the best option for the detection of viraemic animals. Preliminary results show that virus genome can be detected in blood for at least two months after the onset of the infection. Data available until now have not demonstrated that ovine are susceptible to infection. Further studies are necessary to better understand the disease, especially the overwintering looking ahead to the next circulation season associated to vector activity.

**Keywords:** EHDV, outbreak, diagnostic, Europe, Spain

## P47. Screening of Mosquitoes for Usutu Virus in Lombardy region (Northern Italy) 2014–2022

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Usutu virus (USUV) is a single strand RNA virus included in the mosquito-borne cluster of the genus *Flavivirus*, within the Family *Flaviviridae*. As all the members of this group, including human pathogens like Japanese encephalitis virus (JEV), Murray Valley encephalitis virus (MVEV), Saint Louis encephalitis virus (SLEV) and West Nile virus (WNV), USUV is maintained in the environment through a bird–mosquito life cycle.

Programs performing environmental surveillance of arboviruses, mainly targeting West Nile virus (WNV), were established in Lombardy region (Northern Italy) starting from 2014.

During a six-year period (2014–2022) the surveillance system was activated in the period June 1<sup>st</sup>–September 30<sup>th</sup> with bi-weekly collection of mosquitoes in fixed stations. Mosquitoes were trapped using modified CDC traps baited with CO<sub>2</sub> (CO<sub>2</sub>–CDC traps). Mosquitoes were pooled, with a maximum number of 100 individuals per pool, according to date, location, and species, and tested by real-time RT-PCR for WNV and USUV RNA. Samples positive for USUV were subjected to sequencing targets E and NS5. The minimum infection rate (MIR) was calculated as the ratio of positive pools to the total number of mosquitoes tested.

Observations of daily average temperature and daily total amount of precipitation were obtained from ARPAL (Agenzia Regionale per la Protezione dell’Ambiente della Lombardia).

A total of 519,148 mosquitoes were sampled and identified. The *Culex* species was grouped in 353,342 pools and tested by real-time RT-PCR for WNV and USUV RNA. The presence of USUV RNA was confirmed in 87 pools of *Culex pipiens* mosquitoes collected during the period July–September. The highest bi-weekly MIR of USUV infection rates of *Cx. pipiens* estimated from pooled samples per year was detected in 2017 (MIR=5.4). Seasonal distribution of positive mosquito’s pool shows that: i) the earliest USUV-positive pool typically was sampled before or contemporarily to the collection of the first WNV-positive pool; ii) the weekly average precipitation and temperature were not a good predictor of Usutu circulation; iii) the seasonal exploratory data analysis revealed two important peaks of positive mosquito’s pool number in 2016 and 2017. To characterise the genomic distribution an evolution of USUV different lineages, sequencing has been conducted and results will be ready ahead.

In conclusion, our findings prove a cocirculation of USUV and WNV in Lombardy region in the summer season. Our study suggests that are necessary comprehensive data on the interaction between USUV and its vectors, hosts, and environmental parameters. Since USUV, as WNV is able to cause neurological disease in humans in particular conditions, the circulation of USUV poses a new potential threat to human health in this area.

**Keywords:** USUV, Mosquitoes, Surveillance, Seasonal Distribution, Lombardy

## P48. Seroprevalence of West Nile virus in domestic donkeys in the Special Nature Reserve “Zasavica”, Serbia

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West Nile virus (WNV) is a Flavivirus that is currently the most spread arbovirus in the world. The virus is maintained in nature in a bird-mosquito enzootic cycle. Humans and horses, as well as donkeys and other equids, are susceptible to WNV infection. There is not many available data on WNV infection in donkeys, since this is still a neglected area of research. The aim of this study was to assess the presence of specific antibodies against WNV in blood sera of domestic donkeys in the Special Nature Reserve “Zasavica”, in the Vojvodina Province, in the northern part of Serbia. In total, 53 randomly selected blood samples (51 females and 2 males) were collected during July 2022. The age of the donkeys varied from 3 to 10 years and all animals were apparently healthy with no evidence of clinical signs of the disease. Serological testing was conducted with WNV IgG enzyme-linked immunosorbent assay (ELISA) and all obtained positive results were further verified by virus neutralisation test (VNT). For this purpose, anti-WNV IgG were analysed by commercial blocking ELISA (Ingezim West Nile Compac, Ingenasa, Spain), according to the manufacturer instructions. Furthermore, virus neutralization test was performed following the World Organization of Animal Health (WOAH) guidelines. The VNT was performed on MDBK cell lines (ATCC-CCL-22, LGG Standards) using virus strain lineage 2 WNV SRB - Novi Sad/12 (GenBank: KC407673.1), positive and negative control sera. The neutralizing titre of 1:2 and higher was considered positive. West Nile virus IgG antibodies were detected by ELISA in 46 (86.79 %) tested specimens while 7 (13.21 %) sera reacted negative. All ELISA seropositive samples were confirmed by VNT with the titer varying from <1:2 to >1:256. Both ELISA and VNT results were fully correlated. Based on these serological findings, we can conclude that WNV is intensively circulating in the population of donkeys in Serbia. Many researchers believe that donkeys are resistant to West Nile virus infection and thus rarely get sick, which indicates that donkeys can serve as sentinel animals for WNV monitoring. Therefore, it is necessary to implement a risk assessment study and include donkeys in the national WNV surveillance program.

**Keywords:** West Nile virus, donkeys, ELISA, virus neutralisation test, Serbia

**Funding source:** This work was funded by the Ministry of Agriculture, Forestry and Water Management of Republic of Serbia, Directorate for Agricultural Payments, by the Contract No: 680-00-00042/1/2021 from 24 September 2021

## P49. Amplicon-based complete genome sequencing of the West Nile virus

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With the advent of sequencing technologies and novel bioinformatics tools and analysis, new insights into the epidemiology and improved surveillance have been established in the field of virology. Whole genome sequencing (WGS) of WNV from clinical samples can provide tremendous insight into the epidemiology and evolution of the virus. However, there are some common difficulties in obtaining quality and sufficient sequence information: low copy number of viral genomes in samples and the error introduced by WGS techniques. To optimize detection and minimize error in WGS of WNV, we tested an amplicon-based approach using the Illumina MiniSeq platform. Viral RNA was extracted from a wild bird (rook) found dead and five pools of mosquitoes, followed by reverse transcription with LunaScript® RT SuperMix Kit (New England Biolabs, Ipswich, Massachusetts, USA, cat. no. E3010). Custom primers for amplicon generation were designed with the PrimalScheme tool by Tešović and Vidanović based on WNV sequences retrieved from NCBI GenBank, which generated two pools of approximately 850bp overlapping amplicons that cover the whole genome. Amplicon cDNA was generated with Q5® HotStart High-Fidelity 2X MasterMix (New England Biolabs, Ipswich, Massachusetts, USA, cat. no. M0494S). Electrophoresis on 1.5% agarose gels was used for amplicon visualization. The pools were mixed together, followed by library preparation and sequencing on the MiniSeq platform following the manufacturers' protocols. Total information of 3.2 Gbp was obtained from 345±5 K/mm<sup>2</sup> density with 81.56±0.42% of the clusters passing quality control (QC) filters. Around 90.16% of all Read1 and 92.43% from Read2 sequences contained high quality bases that were ≥ Q30 (base calling QC standard). Quality of raw reads was checked with MultiQC (v1.12.0) and additional statistical raw read data was obtained with AfterQC (v0.20.4), low quality bases, primers and adapters were removed with Cutadapt (v1.8.3), BWA (v0.6), Samtools (v1.16.1) and iVar (v1.3.1) were used for reference guided genome assembly and weeSAM (v1.6) was used for coverage statistics. The MEGA 11 software was used for maximum-likelihood phylogenetic analysis. The complete genomes of all samples yielded equal or greater than 28000 sequence coverage depth. BLAST and phylogenetic analysis showed that all WN viruses belonged to lineage 2. Through this work, the successful use of the MiniSeq Platform for WNV amplicon-based whole genome sequencing has been demonstrated for samples of various origins and underlines the contribution of sequencing technologies for WNV surveillance and diagnostics. It also shows the ongoing presence and circulation of WNV in birds and mosquitoes in Serbia.

**Keywords:** West Nile virus, next generation sequencing, bioinformatics, Illumina

**Funding source:** This work was funded by the Ministry of Science, Technological development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2023, Contract No: 451-03-47/2023-01/200031.

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*Seventh Parallel Session*

**Vector-borne diseases**

*Poster Presentations*

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## P50. Pathogenesis of Bagaza virus infection in experimentally infected red-legged partridges

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Bagaza virus (BAGV) is a mosquito-borne flavivirus of the Ntaya serocomplex that emerged in 2010 in Spain in an outbreak in gamebirds, primarily red-legged partridges. It reappeared in 2019 and again in 2021 when it emerged also in Portugal. An experimental infection confirmed the high susceptibility of red-legged partridges to the virus and direct transmission to uninfected contacts. Here we study pathogenesis and viral antigen distribution in experimentally infected partridges in comparison to what was found in field cases. Sixteen five-month-old red-legged partridges were inoculated with BAGV (strain Spain RLP-Hcc1/2010). Four additional partridges were kept as contacts in the same cage, and ten sham-inoculated partridges as controls. Two partridges were euthanized at 4, 7 and 10 days post-infection (dpi) and another three birds died on days 6, 7 and 10 dpi, respectively. Tissues (brain, lung, pancreas, liver, kidney, spleen, heart, bursa of Fabricius, large and small intestines) were fixed in 10% neutral buffered formalin and processed for histopathology and immunohistochemistry. Slides were scored for lesion severity and number and type of viral antigen positive cells per 0.2mm<sup>2</sup>.

Partridges euthanized at 4 dpi only had congestion and inflammatory infiltrates in spleen and heart, while from 7dpi on, the main findings were congestion and endothelial hypertrophy in addition to mononuclear inflammatory infiltrates in the spleen, kidney, liver and heart. Perivascular cuffs of inflammatory cells, endothelial cell swelling, and neuronal necrosis and gliosis were observed in the brain, especially of birds euthanized/dead on 10 dpi. Hemosiderosis, one of the hallmarks of BAGV infection in free-living red-legged partridges, was only observed sporadically. BAGV antigen was found on 4 dpi in the caeca and spleen. On 7 and 10 dpi, BAGV antigen were mostly identified in the spleen, bursa, heart, and kidney. No BAGV antigen was detected in the brain except for two Purkinje cells in the cerebellum of a bird that died on 7dpi.

Viral distribution of experimentally BAGV infected red-legged partridges shows viral tropism for endothelia, inflammatory cells and cardiomyocytes, and differs from the pathology of field infected birds in the lack of hemosiderosis and very scarce presence of the antigen in the brain. Both may be related to additional factors in the field or the timepoint of death after infection.

**Keywords:** Bagaza virus, gamebird, histopathology, immunohistochemistry

**Funding source:** Funded by AGL2011-13634-E and Project 261391 EuroWestNile

## P51. West Nile virus (WNV) surveillance in Denmark

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West Nile Fever (WNF) is a vector borne infection caused by West Nile Virus (WNV), a single-stranded RNA virus belonging to the *Flaviviridae* family. The virus is circulating in Europe and has spread from South towards North during the last decades, reaching Germany and the Netherlands in 2018 and 2020, respectively. Circulation of virus within the host (bird) and vector (mosquito) population occasionally gives rise to spill-over infection in mammals. Humans and horses are dead-end hosts, most often giving rise to either asymptomatic infections or mild clinical cases including flu-like symptoms, but in few cases also leading to severe neuro invasive disease with a fatal outcome. For veterinary preparedness and within a one-health perspective, Denmark has performed annual WNV surveillance since 2011 in birds and mosquitoes.

The annual surveillance program for WNV includes samples from other disease surveillance programs in Denmark and blood collected from migratory birds when handled for registration and ringing. *Passive surveillance* was performed in dead wild birds with focus on species that are highly sensitive to infection with WNV including raptors, corvids, passerines and owls. *Active surveillance* was performed on serum samples collected from free-ranging poultry and on blood samples from migratory passerines coming to Denmark in the spring after overwintering in Africa or by the Mediterranean Sea. *Surveillance in mosquitoes* was performed on *Culex* species collected during the mosquito season, in Denmark from June to November. Bats were also included in surveillance in 2022, as Usutu virus, a virus closely related to WNV, has previously been detected in bats within Europe (Benzarti et al, 2019). Detection of antibodies against WNV (WNV-ab) was performed on serum samples using ELISA, while WNV RNA analysis by RT-qPCR was performed on brain material from dead wild birds and bats, on blood from migratory birds and on pools of mosquitoes.

The results from the last 12 years of annual surveillance shows that WNV have not been detected in host or vector populations in Denmark. Migratory birds, normally show a seroprevalence of WNV-ab in 1 - 6% of total tested birds, however in 2022, blood samples from 31 out of 243 birds (~12%) showed positive reactions.

WNV is not yet detected in Denmark, despite circulating in our neighbouring countries to the South. The amount of WNV-ab positive migratory birds coming to Denmark has dramatically increased in 2022, a further indication on this virus increasing prevalence.

**Keywords:** West Nile virus, WNV, surveillance, vector borne

### Reference:

Benzarti E, Sarlet M, Franssen M, Cadar D, Schmidt-Chanasit J, Rivas JF, Linden A, Desmecht D, Garigliany M. Usutu Virus Epizootic in Belgium in 2017 and 2018: Evidence of Virus Endemization and Ongoing Introduction Events. *Vector Borne Zoonotic Dis.* 2020 Jan;20(1):43-50. doi: 10.1089/vbz.2019.2469. Epub 2019 Sep 3. PMID: 31479400.

## P52. Dog babesiosis at Belgrade area: retrospective analysis of registrate clinical cases at last ten years

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Babesiosis is a tick-borne disease of dogs caused by protozoan parasite belonging to genus *Babesia*. In Serbia the presence of *Babesia canis* and *B.gibsoni* has been recorded in past decades. Spread of babesiosis in dogs in Belgrade area has been continuously examined since 1997. In this paper we present the results of prevalence for babesiosis in dogs in the period 2010-2019.

In total 3085 dogs' blood samples with clinical signs of babesiosis (anemia, haemoglobinuria, fever, pale of mucous membranes etc.) or infested with ticks were examined. We used capillary blood for examination, blood films were air-dried, fixed in absolute methanol (>99.8%) for 1 minute and stained with 10% Giemsa stain for 20-30 minutes. *Babesia* species were identified by morphometric characteristics.

The positive results were found in 36.69% (1132/3085) of suspected animals. Throughout years the following results were obtained: in 2010 babesiosis was established in 35.47%; in 2011 in 36.26%; 2012 in 41.04%; 2013 in 33.74%; 2014 in 34.96%; in 2015 in 31.88%; in 2016 in 33,77%; 2017 in 41.71%; 2018 in 37.97% and in 2019 in 35.97%. Dominant *Babesia* species occurred during our examination was *B.canis* established in more than 95% of positive cases. The dynamics of occurrence of dog babesiosis was monitored from January to December. They showed that babesiosis occurs throughout the year in dogs. It was noted that the increase in incidence of dog babesiosis started in the interval March-April. May was the month of infection maximum, decreasing gradually until July. The autumn infection peak occurred in September and the lowest level in December.

The number of positive findings of *Babesia* spp. in dogs in period 2010-2019 was about the same level, with small deviations, within the investigated period. The appearance of babesiosis in dogs coincided with the seasonal dynamic of vector ticks especially *Rhipicephalus sanguineus*, *Dermacentor reticulatus* and *D. marginatus*.

**Keywords:** babesiosis, dogs, Belgrade, ticks

**Funding source:** The study was funded by the Serbian Ministry of Science, Technological Development and Innovation (Contract No. 451-03-47/2023-01/200030)

## P53. Molecular characterization of West Nile Virus from mosquitoes using NGS protocol for Oxford Nanopore platform in Serbia 2022

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West Nile virus (WNV) is single-strained RNA virus which can affect birds, humans and horses and causes asymptomatic infection, mild febrile disease, neurological and/or systemic disease and death. This virus belongs to family Flaviviridae, genus Flavivirus. Nine genetic lineages have been described, while lineage 1 and lineage 2 are the most important for human disease and they are the most pathogenic lineages. It can replicate in birds, mosquitoes, ticks, reptiles, amphibians, and mammals. The virus circulates between mosquitoes as vectors and birds as primary hosts. Horses and humans are dead end host. Mosquitoes are responsible for the enzootic circulation of the virus. Since the beginning of the 2022 transmission season and as of 7 September 2022, Serbia has reported 130 human cases of WNV infection and seven deaths. The aim of this study is identification and molecular characterization of WNV detected in mosquito during monitoring program in Serbia in 2022.

Pool of mosquitoes was collected in Šumadija district in July 2022. RNA extraction was performed using an automatic extraction device and tested using real-time protocol described in OIE manual. In order to determine genotype of the virus, whole genome sequencing was performed using a multiplex PCR based high-throughput sequencing protocol on the Oxford Nanopore MinION platform. The homology of obtained nucleotide sequence with known isolates identified in Serbia and neighbour countries in previous years was assessed.

BLAST analysis was performed with the obtained sequence. Our sequence showed 100% homology with sequence of the West Nile virus isolate Sad/12, 99,97% homology with the sequence of West Nile virus isolate WNV/SRB/Curug-5871/2018, 99,61% homology with the sequence of West Nile virus strain Austria/2008\_gh and 99,47% homology with the sequence of West Nile virus isolate Nea Santa-Greece-2010. Our phylogenetic analysis of the whole genome sequence along with the corresponding sequences from several other strains obtained from GenBank showed that our isolate from mosquitoes in 2022 grouped together with lineage 2 WNV strains.

Results obtained during the whole genome sequencing of WNV, and consequential phylogenetic analysis are important for the understanding of molecular epizootiology and could be used for the determining the origins and geographical distribution of the virus. At the same time, we can track changes in viral sequence during the time.

**Keywords:** West Nile virus, multiplex PCR, next generation sequencing, MinION, Oxford Nanopore platform

**Funding source:** This work is completely financed by Veterinary Specialized Institute "Kraljevo"

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*Eighth Parallel Session*

**Vaccine development and  
Disease control**

*Oral Presentations*

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## Adaptation of the ASFV-989 live attenuated virus on continuous cell line, a step forward to become a vaccine candidate

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African swine fever since its reintroduction in Georgia in 2007 has spread across Europe, Southeast Asia and Caribbean. ANSES Ploufragan laboratory has recently developed an experimental vaccine candidate, ASFV-989, by thermo-attenuation of the highly virulent pandemic ASFV strain Georgia 2007/1. ASFV-989 has proven to be safe and highly efficacious in challenge studies using the parental strain, either by intramuscular (IM) or oronasal (ON) routes. The next step to pass for further vaccine development is the adaptation of the ASFV-989-PAM grown on porcine alveolar macrophages, to a continuous cell line.

Here, we will present data demonstrating the successful adaptation of ASFV-989 to grow on Swine Testis (ST) cells, with a replication kinetics similar to the ASFV-989-PAM.

*In vivo*, 21 specific pathogen free pigs inoculated with the ASFV-989-ST strain, either IM (13) or ON (8), exhibited only a few cases of hyperthermia from D4 to D9 post-inoculation (pi) and a small decrease of growth performances during the first week pi. Animals immunized with the ASFV-989-ST strain showed a low vaccine viremia between D3 to D27pi in all IM inoculated and in 7/8 ON inoculated pigs. ASFV specific antibodies were detected from D11pi, and ASFV specific cell mediated response at D13pi.

In pigs immunized with the ASFV-989-ST strain then challenged 4 weeks later with the Georgia 2007/1 strain, 1/6 pigs immunized IM developed ASF symptoms from D6 post challenge (pc) and had to be euthanatized at D8pc, when the 5 other pigs only displayed late and light hyperthermia. Only one ON immunized pig displayed hyperthermia during one day pc. In contrast, the 3 non-immunized pigs presented a fatal evolution in less than 1 week.

In ASFV-989-ST immunized pigs, we saw a strong control of the Georgia strain viremia compared to non-immunized pigs. Georgia strain viremia was detected in 5/6 pigs immunized IM, and only in 1/6 pigs (1 time point) for the ON immunized pigs.

In summary, the ASFV-989-ST presented a very good safety and efficacy for ON immunization. For IM immunization, the ASF-989-ST was safer than the original ASFV-989-PAM but less efficient, probably due to a suboptimal dose.

Therefore, the ASFV-989-ST strain looks like a very good vaccine candidate that can be administered oronasally, a critical attribute for potential vaccination of wild boars populations. Further studies are needed to evaluate its stability and increase its efficacy for IM pig vaccination.

**Keywords:** African swine fever, vaccine, live attenuated virus, cell line

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## (Autonomous) Live cell visualization of African Swine Fever Virus DNA replication for the discovery of antiviral compounds

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African swine fever (ASF) is a reemerging swine disease that has severely disturbed the global pig farming industry due to its uncontrolled spread throughout several countries in Europe and Asia and, more recently, the Americas. Efficient vaccines or specific treatments are not available yet. Therefore, development of methods for the rapid screening of novel antiviral compounds are of relevance and may contribute in the effort to contain this disease. Here, we have adapted a system derived from bacterial DNA-binding protein (ANCHOR™) for the live cell fluorescent detection of ASF virus replication and show its efficiency for the discovery of compounds displaying antiviral activity in cell culture.

A plasmid bearing all genetic elements required for specific DNA tagging *in vivo* and for site specific integration into the ASFV-Ba71V strain genome was constructed and used to generate and isolate the corresponding virus (ASFV\_Ba71V\_ANCHOR) by homologous recombination and consecutive rounds of plaque selection on Vero cells. NGS, Western blot, immunofluorescence and electron microscopy assays along with standard growth assays on Vero cell monolayers were used to characterize the virus. Vero cells were infected with ASFV\_Ba71V\_ANCHOR and incubated with bisbenzimidides Hoechst 33342 and Hoechst 33258 and fluorescence intensity was determined by flow cytometry or confocal laser scanning microscopy as a direct measure of virus replication.

ASFV\_Ba71V\_ANCHOR shows a specific labelling of viral replication sites at late times of infection, starting at approximately 6 hours post infection with increasing number and intensity of fluorescent spots during virus replication. Correlative light electron microscopy together with immunofluorescence assays showed that the staining is mostly restricted to replicative centres within the viral factory. To show that this live tagged system can be used to evaluate putative antiviral compounds, we tested two bisbenzimidides which have been shown to inhibit replication of the distantly related Vaccinia Virus. H33258 showed a specific dose dependent effect on the number of fluorescent cells and mean intensity of detected fluorescence, which perfectly correlated with the antiviral effect as assessed by standard virus growth assays.

We have developed an AFSV genome where infection, replication and propagation can visualized directly in living cells. Our system is compatible with high-content fluorescence analyses and can be easily adapted to virulent, non-attenuated strains. Using image-based readout, our system allows rapid determination of the activity of novel antiviral compounds with minimum hands-on investment.

**Keywords:** African swine fever virus, antiviral compound, live cell visualization

## Prime-boost strategies using Adeno and MVA vectors for vaccination against PPRV

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Peste des petits ruminants (PPR) is a highly contagious disease affecting small ruminants, mainly goats and sheep, whose causal agent is the Peste des petits ruminants virus (PPRV). Due to the economic, social and health impact of PPR, the Food and Agriculture Organization of the United Nations (FAO) and the World Organization for Animal Health (WOAH), set the goal in 2011 of eradicating PPR by 2030, following the eradication model used for rinderpest virus (RPV), which is closely related to PPR.

The vaccines currently used, live attenuated vaccines, allow the control of the disease. However, they have several drawbacks. The aim of this work is to study the immunogenicity against PPRV induced by vaccination with a first immunization based on adenoviral vectors, which have shown great potential as vaccine candidates, and a subsequent booster dose using recombinant MVAs, since these show better results when used as booster doses. As the F and H proteins of PPRV are the most immunogenic proteins, they have been cloned into different vaccine vehicles to be used as antigens and to induce an immune response against the virus.

8-week-old female C57BL/6 mice were inoculated intramuscularly with the vaccine vectors. Adenovirus vectors were used in the first inoculation (D0). After 21 days (D21), they received a booster dose based on the recombinant MVAs. Different vaccination strategies were performed, designing the following groups: Ad-F + MVA-F (N=10); Ad-H + MVA-H (N=10); (Ad-H + Ad-F) + (MVA-H + MVA-F) (N=10); (Ad-H + Ad-F) + MVA-H/F (N=10); Ad-RFP + MVA-Control (N=10).

Our data indicate that vaccination with the Ad-F+MVA-F and Ad-H+MVA-H groups, mono-antigenic vaccination, is able to induce a better specific humoral response against PPRV. Also, the data suggest that Ad-H is more antigenic than Ad-F as antibodies are detected with a single inoculation of Ad-H. Moreover, H mono-antigenic and bi-antigenic groups induced the highest neutralizing antibody titers.

Groups immunized with both antigens [(Ad-H+Ad-F) + (MVA-H+MVA-F)] and [(Ad-H+Ad-F) + MVA-H/F] developed a better cellular response against F protein. By contrast, the mono-antigenic immunization (Ad-H+MVA-H) induced a better cellular response against H protein than the one obtained with the expression of a combination of the two proteins.

The Ad-MVA vaccination strategy showed a good humoral and cellular response. Mono-antigenic vaccination with either F or H produced the highest titers of PPRV-specific IgGs. However, the antigenic combination expressing the proteins separately induced the highest titers of neutralizing antibodies. The mono-antigenic vaccination (Ad-H+MVA-H) induced the more potent cellular response against H protein, while the cellular response against F protein is improved with bi-antigenic vaccination.

**Keywords:** PPRV, adenovirus, MVA, vaccination strategy, immune response

## Safety and efficacy upon infection in sheep with Rift Valley fever virus ZH548-rA2, a triple mutant rescued virus

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Rift Valley fever (RVF) is an arboviral zoonotic disease affecting many African countries with the potential to spread to other geographical areas. Both live-attenuated and inactivated vaccines are available in Africa for veterinary use. Those based on live-attenuated RVFV allow a more successful immunization, but their use for more susceptible animals is limited because of risks associated to their residual virulence.

In a previous work, we used a reverse genetics system based on the virulent RVFV ZH548 strain to rescue a triple mutant virus termed rZH548-A2. This virus carries G924S and A1303T substitutions in the viral polymerase (RdRp) and P82L in the non-structural NSs protein. Experiments with immunocompetent 129Sv mice showed that these changes fully attenuated the virus without affecting its immunogenicity. In this work, we have assayed the sheep response to rZH548-A2 in order to test its efficacy and safety as vaccine candidate in a natural host of RVFV infection.

We run a small pilot vaccination/challenge experiment, in which sheep were inoculated sc with rZH548-A2 ( $4 \times 10^6$  pfu), and challenged three weeks later with the virulent isolate rZH548. At days 3 and 4 post-challenge animals were euthanized for necropsy examination and histopathological sampling.

Upon rZH548-A2 inoculation, no fever or any other clinical sign was observed. All whole-blood samples collected in the early days after immunization (d3 to d9) scored negative by RT-qPCR except for one single 5 dpi sample, in agreement with the expected attenuated nature of rZH548-A2 that was further confirmed by histopathological studies. Neutralizing antibodies were detected as early as 4 dpi, reaching levels correlating with protection. After challenge, rZH548-A2 immunized sheep remained healthy without histopathological findings upon necropsy showing that in spite of its limited replication, rZH548-A2 was still able to induce a protective immune response in adult sheep.

Our data show that the triple combination of amino acid substitutions carried by rZH548-A2 leads to a strong attenuation in sheep without affecting its capacity to induce an immune protective response, suggesting that their addition could be an alternative approach to improve the safety of live-attenuated-vaccine prototypes.

**Keywords:** RVFV, attenuation, live vaccines, triple mutant, reverse genetics

**Funding source** [This work was supported in part by MICIN grants AGL-2017-83226R, Pdc2021121717-I00 and by PID2021-122567OB-I00, MCIN/AEI/10.13039/501100011033/ FEDER, UE]:

## Perspectives of Sterile Insect Technique in control of the Asian Tiger Mosquito: Experiences gained from the first pilot study in Serbia

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The Asian Tiger Mosquito (ATM), *Aedes (Stegomyia) albopictus* (Skuse, 1894) was recorded in Serbia for the first time in 2009, at the border crossing to Croatia. During the following years, this invasive species of medical and veterinary importance gradually spread to neighboring villages. In 2019, ATM has successfully established in Novi Sad, the second largest city in Serbia. Due to the bio-ecological and behavioral complexity of the species, conventional mosquito control measures alone cannot provide significant reduction of population density. Therefore, efforts in direction of integrating Sterile Insect Technique (SIT) in control of ATM were undertaken in 2022, in collaboration with International Atomic Energy Agency (IAEA) and Centro Agricoltura e Ambiente (CAA), Bologna Italy. The objective of the study was to determine the longevity and flight range of ATM released sterile marked males, according to the Mark-Release-Recapture (MRR) protocol of IAEA.

Serbian strain of ATM was reared at CAA mass rearing facility. Sterilized male mosquitoes were shipped to Novi Sad and released on weekly basis in four rounds during August 2022 (about 50,000 males per each round, 200,000 males in total), following mark-release-recapture protocol. Two areas (20 ha each) were selected for the study: the first was located in the city center where sterile marked male mosquitoes were released and the second was located in suburban area where only conventional control measures were conducted by the local mosquito control company. Sterile males, marked by fluorescent powder dye were released in the treatment area from two selected points, 50m distanced and recaptured by human landing captures (HLC) at 40 sampling stations, on the 1<sup>st</sup>, 2<sup>nd</sup>, 4<sup>th</sup>, and 6<sup>th</sup> day after each release. In the control area HLC was conducted once a week at 12 sampling stations. Moreover, 20 ovitraps were employed on a weekly basis at both areas in order to check if induced sterility of eggs could be detected.

The first results of the pilot study demonstrated that sterile males of *Ae. albopictus* could successfully survive the shipment. After the release, they could live up to 6 days and they were able to fly at distances up to 216 m from the release point.

**Keywords:** *Aedes albopictus*, Control, Mark-Release-Recapture, Sterile Insect Technique

**Funding source:** The study represents the result of the regional project: Enhancing the Capacity to Integrate Sterile Insect Technique in the Effective Management of Invasive Aedes Mosquitoes (RER5026) supported by IAEA

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*Eighth Parallel Session*

**Vaccine development and  
Disease control**

*Posters with Flash Presentation*

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## P38. MVA vectors carrying chimeric VP2 of African horse sickness virus increased cross-protection against a heterologous serotype

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African horse sickness (AHS) is one of the most lethal viral diseases that affect equids, which could reach a mortality rate of 90% in horses. AHS virus (AHSV) is transmitted between equid hosts by the bite of *Culicoides* midges and the major vector *C. imicola* is distributed worldwide. Nine different serotypes have been described so far. VP2 protein is the main target of neutralizing antibodies, with low cross-reactivity since VP2 is highly variable. As cross-protective immune responses are crucial in the control of this virus, our objective was to develop recombinant MVA (Modified Vaccinia Ankara) vector expressing a chimeric AHSV-4 VP2 protein carrying the conserved central domain of AHSV-7 (MVA-A7VP2) in order to increase protective immunity against heterologous AHSV. Then, a dual MVA expressing also the conserved non-structural NS1 protein (MVA-A7VP2-NS1) was constructed and both vectors were evaluated in mice against the infection with different AHSV serotypes.

Sequence of AHSV-4 VP2 with the AHSV-7 VP2 tip domain was cloned into pSC11 plasmid. Homologous recombination between VP2 of the transfer plasmid and TK locus of wt-MVA allowed the generation of a recombinant MVA-A7VP2. In addition, NS1 sequence was inserted in FL13 locus of MVA-A7VP2 to obtain a bivalent MVA vector expressing these two proteins of AHSV. Groups of IFNAR -/- mice (n=4) were inoculated twice (days 0 and 28) with these constructions and immune responses/protection evaluated at two weeks after vaccination.

The immunization of mice with MVA-A7VP2 or MVA-A7VP2-NS1 vectors triggered humoral and cellular responses against VP2 and NS1. Humoral response was characterized by induction of neutralizing antibodies with a highest titre against AHSV-7 (log PRNT<sub>50</sub> 2.25) and AHSV-4 (log PRNT<sub>50</sub> 1.31). Detectable cross-neutralizing antibody responses were observed against AHSV-1 and AHSV-2 (log PRNT<sub>50</sub> 0.85 and 0.92, respectively). Regarding to cell-mediated immune responses, induction of specific T CD8+CD107+ and IFN-γ+ cells was detected against proteins VP2 and NS1. Two weeks after boost, animals were infected with AHSV-4 (homologous) or AHSV-9 (heterologous challenge). Control mice developed high viremia and died after infection with AHSV-4, while vaccinated animals survived and just one animal developed almost undetectable viremia at 11 dpi. Infection of AHSV-9 resulted in high viral loads and clinical disease in control mice, whereas MVA-A7VP2 vaccinated mice had a significant reduction of viremia and clinical score. Protection was slightly higher in MVA-A7VP2-NS1 vaccinates.

The chimeric VP2 proteins made up from two serotypes increase cross-protective immunity against heterologous AHSV serotypes.

**Keywords:** African horse sickness, MVA, vaccine, cross-protection

**Funding source or Acknowledgement:** 101059924-SPIDVAC HE

## P39. Functional analysis of the programmed -1 frameshift signal of porcine respiratory coronavirus (PRCV)

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A range of different RNA viruses, including coronaviruses, use programmed -1 ribosomal frameshifting (-1 PRF) to achieve the expression of some of their non-structural proteins. Two elements, the “slippery sequence” and an RNA pseudoknot play a vital role in the process of frameshifting in coronaviruses. Mutations in these elements and small molecules that inhibit -1 PRF activity can have deleterious effects on virus replication. Various studies have been conducted previously to study the structural and functional analysis of -1 PRF activity in betacoronaviruses, such as severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2. However, no studies have been reported for porcine respiratory coronavirus (PRCV), an alphacoronavirus. The objective of this study is to understand the structural and functional activity of -1 PRF in alphacoronaviruses using PRCV as a model.

The recombinant plasmids were designed and constructed containing different lengths (100-130 nucleotides) and mutants of the PRCV cDNA sequence (including both the slippery and pseudoknot sequence) within the DNA-launched RNA replicon (DREP) vector (based on Semliki Forest virus) that contains renilla luciferase (RLuc) and chloramphenicol acetyltransferase (CAT) reporter genes. The recombinant plasmids were used to transfect BHK-21 cells. The replicon produces RNA transcripts within the cytoplasm of the cells. After 48 h, the transfected cells were harvested and the expression of RLuc and CAT were analyzed using RLuc, CAT ELISA, and western blot assays.

We compared the “slippery” and RNA pseudoknot sequences of different coronaviruses and identified the slippery sequence of PRCV (UUUAAAC). Furthermore, the PRCV RNA pseudoknot sequence and structure were predicted based on the models for other coronaviruses. Initial experiments verified that the expression of the reporter proteins was dependent on the presence of a functional RNA-dependent RNA polymerase within the replicon. Furthermore, the expression of reporter proteins indicated that the PRCV frameshift activity changed in parallel with similar constructs containing the SARS-CoV-2 frameshift element. Single nucleotide changes can abolish the activity of the frameshift signal. Further studies will seek to understand the role of different features of the PRCV frameshift signal in determining the efficiency of -1 PRF activity.

**Keywords:** -1PRF, coronaviruses, slippery, pseudoknot, expression proteins

**Funding source or Acknowledgement:** Internal resources of the University of Copenhagen supported this work.

## P40. Mapping biosecurity measures applied on different animal production systems across Europe – Progress of COST Action BETTER

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Biosecurity is of paramount importance to prevent the introduction and spread of pathogens and to preserve the animal health. The new COST Action BETTER has started in 2021, with the aim to evaluate how biosecurity is currently used across Europe and will use participative approaches to understand motivators and barriers for biosecurity implementation. The overall aim of the COST Action is to reduce the risk of infectious disease introduction and spread by improving the implementation of biosecurity measures in animal production systems.

At present, the COST Action is composed of 265 participants from 46 different countries. Within the four different working groups, working group 1 (WG1) has the task to map how biosecurity is applied across Europe and neighbouring countries in different animal production systems. This working group will address the challenge of understanding how biosecurity measures are applied in the different cattle, pig and poultry production systems across Europe in order to identify existing knowledge gaps and ways for improvement. At the end of 2022 a data collection tool based on excel has been created to capture biosecurity practices applied in different animal production systems (poultry, cattle and pigs). The created database is related to biosecurity measures (mandatory by law and by other than law) and their implementation rate in different countries. At the moment, data collection tool for terrestrial animals has been initiated and the phase of active data collection is in progress. Besides addressing how biosecurity measures are applied in Europe and neighbouring countries, in the frame of COST Action network, WG1 members will try to identify ways for biosecurity improvement, especially in production systems where there is a lower level of implementation or in settings where biosecurity is more challenging to carry out.

**Keywords:** Biosecurity, COST Action, network, BETTER

**Funding source or Acknowledgement:** This abstract is based upon work from COST Action 20103 Biosecurity Enhanced Through Training Evaluation and Raising Awareness (BETTER), supported by COST (European Cooperation in Science and Technology)

## P41. Developing methodologies to profile humoral immune responses against Crimean Congo haemorrhagic fever virus to support effective vaccine design

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Nairoviruses, including Crimean-Congo Haemorrhagic fever virus (CCHFV) can cause viral haemorrhagic diseases in humans and animals. CCHFV is associated with high mortality and morbidity in humans, while animals do not experience significant disease, they are a viral reservoir, perpetuating disease transmission to humans. There is an urgent need to develop vaccines and accompanying methodologies for CCHFV due to the wide geographic spread of this virus. We have developed a range of assays that can be used to detect reactivity towards various nairoviruses and to support vaccine design.

Evaluation of the assays was carried out using a range of field sheep serum samples from endemic CCHFV region (Bulgaria; n=1200) and from a CCHFV free country (UK; n=213). Sero-reactivity towards CCHFV glycoprotein (Gc) and nucleoprotein (NP) was assessed by antigen-specific in-house ELISAs. These assays along the other were used to profile humoral responses induced by replication-deficient adenoviral-vectored CCHFV vaccine (ChAdOx2 CCHF), encoding CCHFV glycoprotein precursor (GPC) in BALB/c mice. Parameters measured include antigen-specific ELISAs, IgG avidity profiling and assessment of neutralisation by using transcription and entry-competent virus like particles (tecVLP).

We demonstrate that anti-CCHFV Gc and anti-CCHFV NP ELISAs were able to detect antigen-specific IgG in sheep from endemic CCHFV areas, showing different profiles of the antibody response to the two CCHFV antigens. Vaccination with ChAdOx2 CCHF induces a humoral immune response in BALB/c mice towards the CCHFV GPC, in particular the Gc protein with mixed IgG2a/IgG1 subclass profile-high-avidity antibodies with neutralisation capacity.

Anti-CCHFV Gc and -NP IgG ELISAs are an important advance on currently available CCHFV tests. The in-house ELISAs allow antigen-specific response to be detected robustly with high specificity and are costs effective. Using these assays, we provide new insight into the heterogeneity of the immune response induced by natural infection in livestock. Furthermore, profiling the humoral immune response in mice highlight the potential of the ChAdOx platform as a vaccine against CCHFV infection.

**Keywords:** CCHFV, humoral immune response, livestock, vaccine

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*Eighth Parallel Session*

**Vaccine development and  
Disease control**

*Poster Presentations*

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## P42. Genotyping of avian infectious bronchitis virus in Afghanistan

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Avian infectious bronchitis (IB) is a highly contagious viral disease which affects the poultry industry. The virus exists in a wide variety of genotypes, and phylogenetic analysis has been used to classify infectious bronchitis virus (IBV) strains. The tracheal tissue specimens from 100 different commercial broiler flocks with respiratory distress in Afghanistan were collected during 2016-2017. After real-time reverse transcriptase-polymerase chain reaction (RRT-PCR), IBV-positive samples were further characterized. A 390 bp hypervariable spike glycoprotein gene segment was amplified using Nested PCR, sequenced, and analyzed. It is the first study focusing on the molecular epidemiology of IBV in Afghanistan, extending our understanding of IB in the region. These results showed the high rate of IB infection in Afghanistan broiler farms and confirm the continuing monitoring of IBVs to modify the vaccination program.

**Keywords:** Afghanistan, Avian infectious bronchitis, Genotyping, GI-19, GI-23

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