14TH ANNUAL MEETING EPIZONE

18-20 MAY 2022
BARCELONA

NEW HORIZONS
NEW CHALLENGES

BOOK OF ABSTRACTS

EPIZONE

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INDEX

WELCOME TO BARCELONA

14th EPIZONE Annual Meeting

"New Horizons, New Challenges"

Dear EPIZONE friends,

We are happy to welcome you to the 14th EPIZONE Annual Meeting in Barcelona!

IRTA-CReSA is hosting this meeting and has done its utmost to develop an inspiring conference program, in the beautiful venue of the Barcelona World Trade Center, close to the city harbour. The theme of this meeting is "New horizons, new challenges", referring to the Epizone goal of searching for innovations in the field of epizootic diseases research and control. Infectious diseases keep on challenging us, animal health researchers, to provide solutions to new threats.

Our organizing and scientific committees developed a program with 9 renowned keynote speakers and about 180 abstracts presented as talks or posters. The focus of the meeting will be on the EPIZONE partner institutes' recent research in the field of epizootic animal diseases to exchange the latest research information and to establish new contacts and collaborations. The Young EPIZONE group has organized an interesting program for young scientists focusing on transversal issues relevant for junior scientists.

Looking at the current challenges to control important epizootic diseases like African swine fever and Avian Influenza, the collaborations established by and maintained within EPIZONE are more important than ever. With this 14th annual meeting, we hope to foster collaborations further, to which the enjoyable social program certainly also will contribute. We are sure that the lively city of Barcelona, Catalunya, with its wonderful Mediterranean sight, will be the perfect place to create a stimulating atmosphere of exciting talks, inspiring discussions, and scientific curiosity.

We wish you a very interesting and fruitful meeting,

Dr. Llilianne Ganges
Chair Local Organizing Committee, IRTA-CReSA

Professor Wim van der Poel
Coordinator of EPIZONE

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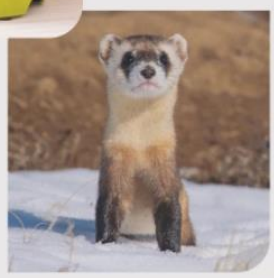

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14th EPIZONE MEETING PROGRAM SUMMARY

MAY 18

TIME	AUDITORIUM	ROOM A2-A3	ROOM A1	ROOM A4	PORT VELL HALL	RECEPTION DESK
08:00 - 09:00						Registration Opening at 8:00
9:00 - 11:30			Young Epizone		Young Epizone Coffe Break	
11:30 - 13:00				EPIZONE Coordinating Forum & Executive Committee Meeting		
13:00 - 14:00					Lunch Break Young Epizone and Coordinating Forum & Executive Committee	
14:00 - 14:30	Opening session: Welcome to Barcelona				Poster and stand viewing available all the afternoon Welcome cocktail	
14:30 - 15:05	Welcome Lecture					
15:10 - 15:45	Keynote Lecture 1					
15:45 - 16:15	Coffe Break					
16:15 - 16:50	Keynote Lecture 2					
17:00 - 18:45	Session 1	Session 2				
19:00 - 21:00	Welcome cocktail in Sala Port Vell					

MAY 19

TIME	AUDITORIUM	ROOM A2-A3	PORT VELL HALL
08:45 - 09:20	Keynote Lecture 3		Poster and stand viewing available all day long Coffee break Lunch
09:25 - 10:00	Keynote Lecture 4		
10:10 - 10:45	Coffe Break POSTER VIEW		
10:45 - 11:15		Flash presentation 1	
11:20 - 12:50	Session 3	Session 4	
12:50 - 13:40	Lunch		
13:40 - 14:15	Keynote Lecture 5		
14:20 - 14:55	Keynote Lecture 6		
15:00 - 16:30	Coffee Break (15:00-15:30) POSTER VIEW (15:30 - 16:30)		
16:30 - 17:00		Flash presentation 2	
17:15 - 18:45	Session 5	Session 6	
19:30 - 23:00	14th EPIZONE DINNER		

MAY 20

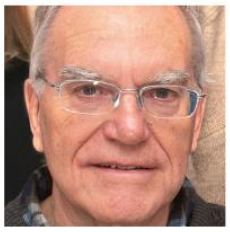
TIME	AUDITORIUM	ROOM A2-A3	PORT VELL HALL
08:45 - 09:20	Keynote Lecture 7		Poster and stand viewing available all day long Coffee break Farewell Cocktail
09:25 - 10:00	Keynote Lecture 8		
10:10 - 10:45	Coffe Break POSTER VIEW		
10:45 - 11:15		Flash presentation 1	
11:30 - 13:00	Session 7	Session 8	
13:15-13:45	Closing Ceremony		
13:45 - 14:45	Lunch and Farewell Cocktail		

KEYNOTE LECTURES

OPENING LECTURE - By Professor Esteban Domingo

Mutant swarms as a challenge for the control of viral disease

AUDITORIUM - MAY 18 - 14:30 - 15:05



Chairs: Dr. Wim van der Poel and Dr. Natàlia Majó

Esteban Domingo is "ad honorem" Professor of Research at the Spanish Research Council, CBMSO, Madrid, Spain. His work documented the high error rates and quasispecies dynamics of RNA viruses, and opened the way to lethal mutagenesis as an antiviral strategy. He is Doctor honoris causa from the Universities of Liège and Bern, and member of EMBO, the European Academy, the US National Academy of Sciences, and the Royal Academy of Sciences of Spain.

Mutant swarms as a challenge for the control of viral disease

High mutation rates of RNA viruses (and several small genome size DNA viruses) result in complex and dynamic mutant swarms referred to as viral quasispecies. Main implications of quasispecies dynamics are: (i) that a "wild type" virus is defined by a set of related genomes, not by a genome with a single nucleotide sequence; (ii) adaptability to different environments, reflected also in a capacity to escape from a vaccine-induced immune response or from antiviral inhibitors; (iii) it has opened prospects for new antiviral strategies such as lethal mutagenesis. Studies with hepatitis C virus (HCV) have documented continuous mutant frequency variations ("mutational waves") even when the virus had been extensively passaged (and presumably partly adapted) to human hepatoma Huh-7.5 cells. The virus did not reach population equilibrium, defined as a steady distribution of variant genomes. Likely related to such absence of equilibrium, residue conservation (nucleotides and amino acids) in mutant swarms did not fit the conservation deduced from the alignment of either consensus sequences or sequences deposited in data banks. This discordance may be relevant to the efficacy of universal antiviral vaccines and pan-genomic antiviral agents whose design is based on data bank alignments. Therefore, it would be valuable to introduce quasispecies information in data banks to provide a repertoire of super-conserved positions. Current studies with SARS-CoV-2 indicate that this emergent pathogen shares swarm complexity with other RNA viruses, with two salient differences: a remarkable abundance of low frequency mutations and the presence of deletions, relative to HCV and other RNA viruses. The possible significance of these differences will be discussed.

KEYNOTE LECTURE 1 - By Dr. Bart Haagmans

Rapid identification and characterization of emerging coronaviruses

AUDITORIUM - MAY 18 - 15:10 - 14:45

Chairs: Dr. Wim van der Poel and Dr. Antonio Lavazza

Bart Haagmans is a virologist working at the Viroscience department, Erasmus Medical Center, Rotterdam. His research line focusses on the pathogenesis of viral infections, especially those viruses that emerge through zoonotic transmission. Studies on MERS and SARS coronaviruses led to intervention strategies to contain the outbreaks. He is an expert consultant of WHO, FAO and OIE, a PI on several Dutch and EU grants and co-coordinated the IMI ZAPI project.

Abstract

During the current coronavirus disease 2019 (COVID-19) pandemic whole-genome sequencing first of all allowed the classification of the causative pathogen as SARS-CoV-2 but also informed public health decision-making. In addition, it allowed detection of mutations that might affect virulence, pathogenesis, host range or immune escape as well as the effectiveness of SARS-CoV-2 diagnostics and therapeutics. However, genotype-to-phenotype predictions cannot be performed at the rapid pace of genomic sequencing. To prepare for the next phase of the pandemic, a systematic approach is needed to link global genomic surveillance and timely assessment of the phenotypic characteristics of novel variants, which will support the development and updating of diagnostics, vaccines, therapeutics and non-pharmaceutical interventions. We used organoids of the human airways and alveoli to investigate Omicron fitness and replicative potential in comparison with earlier SARS-CoV-2 variants. Whereas Omicron replicates more rapidly in the airways and has an increased fitness compared to the early 614G variant and Delta, it did not replicate efficiently in human alveolar type II cells. Mechanistically, we show that Omicron does not efficiently use TMPRSS2 for entry or spread through cell-cell fusion. Omicron BA.1 also almost completely escapes neutralizing antibodies and efficiently infects vaccinated individuals, at least partly explaining why this variant has become the dominant variant globally over the span of a few weeks. We used antigenic cartography to quantify and visualize antigenic differences between SARS-CoV-2 variants using hamster sera obtained after primary infection. Our data show that BA.1 and BA.2 both escape (vaccine induced) antibody responses as a result of different antigenic characteristics. Based on our data we conclude that antigenic cartography and human organoid cell systems are powerful methods to monitor the phenotypic evolution of SARS-CoV-2.

KEYNOTE LECTURE 2 - By Dr. Sofie Dhollander

Prevention and control of ASF in wild boar populations: recommendations based on six annual epidemiological reports of EFSA

AUDITORIUM - MAY 18 - 16:15 - 16:50

Chairs: Dr. Anna Szczotka-Bochniarz and Dr. Sebastian Napp

Sofie Dhollander graduated as veterinarian in 1998, where after she has specialised further in tropical animal health and production and veterinary epidemiology. She started her carrier in Africa, where she worked at the International Trypano-tolerance Centre in The Gambia, with the main focus of her research activities on vector-borne diseases of small ruminants. In 2005, she continued as project coordinator of a veterinary research and development project at the Institute of Agricultural Sciences of South Vietnam in Ho Chi Minh City, Vietnam, until September 2007. Since then, she is employed at the European Food Safety Authority (EFSA) in Parma, Italy, where she works as senior scientific officer for the Biological Hazards, Animal Health and Welfare Unit (BIOHAW). She provides scientific support to the Animal Health and Welfare Panel and its working groups dealing with risk assessments on animal health, including, amongst others, the coordination of working groups and the drafting of scientific reports on emerging vector-borne diseases and African swine fever. Since 2022, she is also the coordinator of the Animal Health and Welfare Panel.

Review of EFSA's risk assessments on African swine fever in wild boar for twelve years: and now?

Dhollander Sofie[1], Abrahantes Cortiñas José[1], Boklund Anette[2], Chinchio Eleonora[1], Gortázar Christian[3], Mur Lina[1], Papanikolaou Alexandra[1], Thulke Hans-Hermann[4] and Ståhl Karl[5]

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3. SaBio research group at IREC (Universidad de Castilla-La Mancha & CSIC), Ronda de Toledo 12, 13003, Ciudad Real, Spain;
4. Helmholtz Centre for Environmental Research GmbH – UFZ, Department of Ecological Modelling, PG EcoEpi, Permoserstr. 15, Leipzig, Germany;
5. National Veterinary Institute, 751 89, Uppsala, Sweden.

African swine fever, wild boar, prevention, control, management, recommendations

Since 2010, EFSA has continuously provided scientific advice on preventing, detecting and controlling ASF in wild boar. In total 45 Scientific Opinions and Reports were delivered based on systematic literature reviews, analysis of surveillance data submitted by ASF-affected countries in the EU and neighbouring areas, and epidemiological models to evaluate the efficacy of measures to control the spread of the disease in wild boar populations. This is a review of the assessments performed, the lessons learned during this last decade, and the recommendations on wild boar management to prevent and control the spread of the disease. Over the years, as the disease has progressed gradually into Europe, with currently 14 countries reporting outbreaks in wild boar or domestic pigs to the World Organisation for Animal Health, more experience has been gained on the efficacy of different measures, applied individually or in combination. In general, the principles of the control measures in wild boar are similar to those in domestic pigs and based on restricting animal movements and culling infectious and susceptible animals in, or near, affected areas to avoid further spread. The efficacy of different prevention and control measures such as culling, carcass removal and fencing depends on the location (far away, adjacent to- or inside affected areas), the type of ASF virus introduction (focal introduction versus adjacent spread) and the timing of intervention (before introduction, after early detection or after several years of virus circulation). For each of the different measures and combinations, recommendations have been summarised based on the information gathered from the different assessments.

Acknowledgments: The authors wish to thank all the ASF working group members, wild boar ecologists, epidemiologists and statisticians from Enetwild and the UFZ, and EFSA colleagues that have contributed, and in particular: Aminalragia Roxani, Broglia Alessandro, Chesnoiu Vasile Theodora, Gogin Andrey, Guberti Vittorio, Desmecht Daniel, Helyes Georgina, Kantere Maria, Korytarova Daniela, Lange Martin, Linden Annick, Masiulis Marius, Miteva Aleksandra, Neghirla Ioana, Olsevskis Edvins, Ostojic Sasa, Petr Satran, Reichold Adam, Staubach Christoph, Van der Stede Yves, Vicente Joaquín, Viltrop Arvo, Wozniakowski Grzegorz and Zancanaro Gabriele.

KEYNOTE LECTURE 3 - By Dr. Gregorio Torres

From Science to Policy - The OIE standards setting process

AUDITORIUM - MAY 19 - 08:45 - 09:20

Chairs: Dr. Natalia Majó and Dr. Llilianne Ganges

Gregorio Torres is the head of the Science Department of the Word Organisation for Animal Health (OIE), based in Paris. He oversees the OIE's systems for identifying, addressing, publishing, and implementing science to develop and review of international standards and global control programmes.
Gregorio supervises the secretariat of the Scientific Commission for Animal Diseases and Biological Standards Commission, being actively involved in the implementation of global disease control strategies, including rabies, African swine fever and zoonotic tuberculosis.
After some years working as a large animal practitioner, he joined the Spanish Veterinary Services where he regularly participated in technical working groups and field missions at national and international level. After a year commissioned to FAO, he joined the OIE in 2014.
Gregorio is a veterinarian graduated from Cordoba University (Spain). He obtained his MSc in Veterinary epidemiology at London University and a MVM at Glasgow University.

Abstract

Animal health risk managers are currently facing difficult and ambiguous problems that require sound scientific advice to be correctly addressed. Animal health science systems are expected to generate evidence and knowledge to help risk managers to better understand the problems, generate policy options and evaluate the impacts of different decision options.

In many circumstances the evidence and knowledge are incomplete or not even available and decisions must rely on knowledge generated by educated experts' opinions. In other circumstances, science systems may generate 'good evidence', but their complexity hinder their use by risk managers.

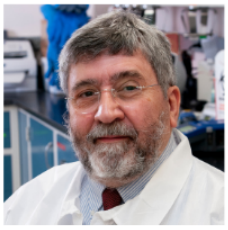
Without claiming that evidence and knowledge generated by animal health science systems should be the only input in the decision-making process, they are crucial to effectively manage animal health risks. The science-policy interface should include an effective communication strategy able to communicate science in a transparent and accountable manner so risk managers can effectively manage the uncertainties and ambiguities inherent in the scientific knowledge.

Using the World Organisation for Animal Health's Science System as a show case, we will explore how the OIE uses scientific information to inform the development and revision of standards, guidelines and opinions to support the 182 OIE's Members in their effort to improve animal health and welfare while ensuring safe trade of animals and animal products.

KEYNOTE LECTURE 4 - By Dr. Manuel Borca

Rational development of live attenuated vaccines via the identification of genetic determinants of virulence

AUDITORIUM - MAY 19 - 09:25 - 10:00



Chairs: Dr. Noemí Sevilla and Dr. Llilianne Ganges

Manuel V. Borca, a DVM and Ph.D. in microbiology, is the Lead Scientist of the "Countermeasures to Control Foreign Animal Diseases of Swine" program for the US Department of Agriculture, Agricultural Research Service (USDA-ARS) at Plum Island Animal Disease Center, since 2001. He has been extensively working with foreign animal disease as foot-and-mouth disease (FMD), African swine fever (ASF) and classical swine fever (CSF). Dr. Borca research focuses on pathogenesis, host immune response and vaccine development for ASF, CSF and FMDV. Using experimental and natural host models, he characterized host immune mechanisms mediating protection to FMDV in infected and vaccinated animals. In CSFV, he discover and characterized most of the reported virus genetic determinant of virulence and use that information to rational develop vaccine candidates. His studies on the genetic basis of ASFV virulence in domestic pigs led to the development of several live attenuated vaccine candidates inducing protection against the virus causing the pandemic currently affecting Eurasian countries. Dr. Borca has authored over 160 peer-reviewed scientific publications and 20 awarded/filed patents mostly covering development of vaccine candidates using recombinant viruses.

KEYNOTE LECTURE 5 - By Dr. Jordi Figuerola

A One Health view of the recent West Nile outbreaks in Spain

AUDITORIUM - MAY 19 - 13:40 - 14:15

Chairs: Dr. Nuria Busquets and Dr. Stefan Zientara

Jordi Figuerola (Barcelona 1971) is Doctor in Biological Sciences and researcher of the Spanish Research Council (CSIC) at Estación Biológica de Doñana (Sevilla, Spain). His research applies the One Health paradigm to better understand the transmission of vector borne pathogens and to identify the main ecological drivers of pathogen emergence, amplification and spillover to humans.

Abstract

West Nile virus (WNV) is the main cause of viral encefalitis in humans in the world and is transmitted by mosquitoes and birds are the main reservoirs. In Europe, the incidence and geographic occurrence of this virus has increased in the last years. Despite WNV is endemic in Spain at least since 2003, only six clinical infections in humans were recorded between 2004 and 2016. However, an important WNV outbreak occurred in southern Spain in 2020, with 77 clinical cases and 8 deaths recorded in Andalucía and Extremadura. The entomological data collected at the main area of the outbreak detected an important proliferation of *Culex perexiguus*. Virological analyses of mosquitos identified *Cx. perexiguus* as the main amplifier of WNV and also the presence of WNV *Culex pipiens*. Genomic analyses suggest this virus strain was already circulating in the area in the last years and the outbreak was not the result of a new virus introduction. Serological analyses of resident wild birds confirmed an important circulation of WNV inside the urban areas of Puebla and Coria del Río, where most of the human cases occurred. Blackbirds (*Turdus merula*) presented the higher antibody prevalences. An intensive entomological and virological surveillance effort was done in 2021 that allowed to detect the first infected mosquito pools approximately one month before the detection of the first human cases. While virus infection rates in mosquitoes were also very high in 2021, the vector control programs in place at the urban areas probably reduced WNV transmission to humans as only six cases of disease were detected in 2021. Both virological and serological data confirm that Usutu is also circulating in the area. Entomological/virological surveillance may provide a powerful early warning tool to reduce the risk of WNV transmission in areas with WNV circulation in previous years.

KEYNOTE LECTURE 6 - By Professor Wim Van der Poel

SARS-CoV2 zoonosis and reverse zoonosis

AUDITORIUM - MAY 19 - 14:20 - 14:55

Chairs: Dr. Esther Blanco and Dr. Antonio Lavazza

Wim H. M. van der Poel, DVM, PhD, dipl. ECMV, is a senior scientist at Wageningen Bioveterinary Research and special Professor of 'Emerging and Zoonotic viruses' at Wageningen University. He is also coordinator of the EPIZONE European Research Group, the network on epizootic animal diseases research. The research work of Prof. Van der Poel involves at least three main areas: New and emerging viruses, Foodborne and Zoonotic viruses 'Global One Health'.

KEYNOTE LECTURE 7 - By Dr. Sandra Blome

African swine fever and its challenges in Europe

AUDITORIUM - MAY 20 - 08:45 - 09:20

Dr. Linda Dixon and Dr. Fernando Rodriguez

Sandra BLOME studied veterinary medicine at the University of Leipzig, Germany, and has a doctorate degree in veterinary medicine. Since 2008 she is senior scientist at the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health (FLI), Germany, and responsible for the national reference laboratories for classical and African swine fever.

African swine fever and its challenges in Europe

Sandra Blome[1]

[1)] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald, Insel Riems – Germany

Keywords: African swine fever, situation, specific challenges, wild boar, domestic pigs

It is not without reason that African swine fever has recently been called a forgotten pandemic that has transformed from an exotic disease to one of the greatest threats to domestic and wild pigs world-wide.

Europe is currently facing different scenarios with front and point introductions, disease in wild boar and domestic pigs, and areas with long-lasting endemicities. This diversity calls for tailored approaches to surveillance, diagnosis, and control. While our proven, traditional control strategies work well for industrial pig farms, we quickly reach our limits when we have to control the disease with the means available to date in the abundant wild boar population (now also including wild boar in cities) or in regions with a majority of backyard farms. In addition, we face conflicts of interest that arise between disease control and conservation or between hunting and farming, respectively. To turn the tide, vaccines could be the missing tool. Here, benefit-risk-analyses will have to precede the decisions to implement vaccination strategies with the vaccines available to date (that are not licensed so far). Furthermore, we have to stay open-minded when it comes to alternative approaches.

Beyond the aforementioned challenges, the virus itself also holds surprises. With the introduction into Germany in 2020, viral variants were observed that showed local distribution and could be followed by genomic epidemiology.

To combat the disease, we will have to tie strong bonds between countries and disciplines. Furthermore, we have to work on closing critical knowledge gaps.

KEYNOTE LECTURE 8 - By Professor Jerry Wells

Organoids: new tools to meet future challenges in epizootic and zoonotic disease research

AUDITORIUM - MAY 20 - 09:25 - 10:10

Chairs: Dr. Falko Steinbach and Dr. Natalia Majó

Jerry Wells is full Professor and Chair of the Host-Microbe Interactions Group at Wageningen University and Research and holds a part-time position as Professor at the Department of veterinary Medicine, University of Cambridge, U.K. His major research interests are in the field of host-microbial interactions, mucosal immunology, bacterial infection and immunity and applications of stem cell technologies. Moreover, he is interested in research on surveillance and vaccines against Streptococcus suis.

ORAL COMMUNICATIONS

SESSION 1 - Emerging and re-emerging diseases

HPAIV H5 in Germany 2020-2022 - A "Thriller" about Diversity, Genotypes, Persistence and Distribution

Jacqueline King[1] , Timm Harder[1] , Anja Globig[2] , Lina Stacker[1] , Anne Guenther[1] , Christian Grund[1], Martin Beer[1] , Anne Pohlmann[1]

1) Friedrich-Loeffler-Institut, Institute of Diagnostic Virology
2) Friedrich-Loeffler-Institut, Institute of International Animal Health/One Health

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Since 2016, repeated incursions of clade 2.3.4.4b highly pathogenic avian influenza viruses (HPAIV) of the H5 goose/Guangdong lineage into Germany have caused devastating losses in wild and domestic birds. Regarding the plethora of sub- and genotypes detected, viruses of this clade are characterized by high reassortment propensity. This study aims to portray a comprehensive genetic analysis of detected sub- and genotypes from 2016-2022 with emphasis on the 2020/2021 and ongoing 2021/2022 HPAIV season in Germany, equating similarities and variances between both epizootics by amplicon nanopore sequencing.

During the initial H5 clade 2.3.4.4b epizootic in 2016-2018, infections with HPAIV H5N8, H5N5, and H5N6 occurred, unprecedented at the time in size and genetic variability. Genetic characterization from 2016-2018 using second-generation sequencing detected reassortant HPAIV. Experience regarding the genetic variability of clade 2.3.4.4b HPAI outbreaks transpired the need for increased efficiency in full-genome acquisition. An amplicon-based third-generation sequencing method using nanopore technology was chosen for fast AIV genome generation. The all-encompassing 2016-2018 epizootic was followed by a new incursion of a clade 2.3.4.4b HPAI H5N8 strain (February 2020) that led to a minor outbreak. Here, nanopore sequencing proved successful and enabled phylogenetic studies indicating a new introduction distinct from previous HPAI.

The next novel incursion was observed in October 2020 as HPAIV of subtype H5N8 and H5N5 (clade 2.3.4.4b) were reported in wild birds. The 2020-2021 influenza season went on to surpass all prior recorded epizootics in case numbers and genetic diversity. By utilization of amplicon-based nanopore sequencing, 178 HPAIV full genomes were collected. Five subtypes (H5N8, H5N5, H5N1, H5N4, H5N3) and seven varying genotypes were detected in a timely manner from October 2020 to July 2021. In addition, three dead harbor seals carrying previously detected H5N8 genotypes were reported in August 2021, highlighting potential zoonotic characteristics.

In October 2021, a new clade 2.3.4.4b epizootic took its course, this time encompassing nearly only one subtype HP H5N1 in a variety of different genotypes alongside few cases of HP H5N2. For the first time, the H5N1 virus showed high similarity to the previously circulating HPAIV H5N1 detected in the 2020/2021 season, pointing towards a re-introduction from Northern Europe. In comparison to previous outbreaks and with only one dominant subtype (H5N1), the discovery of over a dozen genotypes in the 161 full genomes achieved since October 2021 has exceeded all previous epizootics. This HPAIV season is on a similar course as the previous 2020/2021 epizootic, yet shows variances in geographic, host and spatiotemporal progression.

The diversity and reassortment propensity of these viruses suggests increasing variability of future clade 2.3.4.4b HPAIV. This may give rise to new viruses with modified virulence and increased zoonotic potential. Given the current extensiveness of virus dispersion in Europe, it can no longer be ruled out that pockets of endemic infection will become established in Europe. Therefore, ongoing and intensified surveillance and detection of outbreaks using molecular methods is all the more important and has become a significant component of HPAI disease control.

Evidence of SARS-Coronavirus-2 infection in cats, dogs and ferrets in Northeastern Spain

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of Coronavirus Infectious Disease 2019 (COVID-19), is considered a zoonotic pathogen that is mainly transmitted human-to-human. Back to early 2020, few reports indicated that pets can be exposed to the virus, and experimental infections of cats, ferrets and hamsters showed potential for transmitting the virus to a direct contact mate. At that time, the frequency of exposure of pets was virtually unknown and based on few worldwide descriptions of infection. Therefore, the objective of the present work was to establish the SARS-CoV-2 infection frequency in pet animals during April 2020-December 2021 in Catalonia, Northeastern Spain.

A total of 850 animals (431 dogs, 363 cats and 56 ferrets) of different COVID-19 household environments (positive (+), negative (-) or unknown) were screened. Samples were taken by veterinarians from multiple veterinary clinics as well as from animals necropsied at the Veterinary School of Barcelona (Spain). Samples included nasopharyngeal or oropharyngeal swabs (NS/OS), rectal swabs (RS), lung tissue (from necropsied cases) and/or blood. Detection of SARS-CoV-2 RNA was performed on NS/OS, RS and lung tissue following an RT-qPCR method. For the positive samples, viral RNA was extracted, sequenced and SARS-CoV-2 variant type established. Neutralizing antibodies (NAb) targeting RBD of the SARS-CoV-2 were measured with a commercial receptor- binding inhibition assay.

From all the animals analyzed by RT-qPCR (n = 838), only three tested positive: one cat and two dogs. These three animals were living in a COVID-19(+) household. The SARS-CoV-2 detected in the cat (April 2020) corresponded to a classical variant (B.1 pango lineage, D614G), while one dog was infected with the Alpha (B.1.1.7) variant (February 2021), whereas the other dog was infected with the Delta (B.1.617.2) variant of concern (July 2021). Regarding the serological analysis of available sera (n=635), 12/280 (4.3%) cats, 13/313 (4.15%) dogs and 2/42 (4.76%) ferrets had NAb. Importantly, 6/12 seropositive cat sera (50%), 8/13 seropositive dog sera (61.5%) and both seropositive ferret sera (100%) corresponded to animals in COVID-19 affected households.

This investigation allowed the screening of present or past SARS-CoV-2 infection (through viral RNA or antibody detection, respectively) in pets, further emphasizing the susceptibility of several animal species to different variants of SARS-CoV-2. Since positive animals for viral RNA or NAb detection came from COVID-19 environments, a high likelihood of SARS-CoV-2 exposure of pets from diseased owners is suggested. Moreover, existing sequencing data further support human to animal virus exposure of the dominant variants at each pandemic wave.

A New Emerging Ovine Pestivirus Related to Classical Swine Fever Virus: Pathogenesis in Pregnant Ewes and Pigs

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Pestiviruses, a member of the *Flaviviridae* family, are an ever-expanding viral genus, with several new candidates being reported lately in a wide range of species. One of these newly reported *Pestivirus* species, ovine pestivirus (OVPV), was isolated in 2017 from aborted lambs in northern Italy. This virus showed the highest homology with classical swine fever virus (CSFV) out of all pestiviruses, one of the most important pathogens for the swine industry, which causes devastating economic losses worldwide. However, the capacity of OVPV to induce disease had not been established. This study aimed to elucidate the origin of OVPV, as well as to stablish its role as a pathogenic agent in pregnant ewes. The capacity of OVPV to infect and induce disease in swine, as well as the induction of immune response in both species were also evaluated.

Eight ewes at 68 days of gestation were infected with OVPV. The virus caused abortions and stillbirths after infection in seven out of eight pregnant ewes and showed high capacity for trans-placental transmission as well as the birth of lambs suffering OVPV-persistent infection. The OVPV infection induced early antibody response in the ewes, detected by the specific ELISA against CSFV. The neutralizing antibody response was similar against genotype 2 CSFV strains and OVPV. These viruses showed high identity in the B/C domain of the E2-glycoprotein. Additionally, the molecular diagnostic test for CSFV showed cross-reactivity between CSFV and OVPV, and a new molecular assay specific for OVPV had to be developed.

To assess the capacity of OVPV to infect swine, twelve piglets were infected either by intranasal or intramuscular route. Daily clinical evaluation and weekly samplings were performed to determine pathogenicity, viral replication and excretion and induction of immune response. Five weeks later, two pigs from each group were euthanized and tissue samples were collected to study viral replication and distribution. OVPV generated only mild clinical signs in the piglets, including wasting and polyarthritis. The virus was able to replicate, as shown by the RNA levels found in sera and swabs and persisted in tonsil for at least 5 weeks. Viral replication activated the innate and adaptive immunity, evidenced by the induction of interferon-alpha levels early after infection and cross-neutralizing antibodies against CSFV, including humoral response against CSFV E2 and Erns glycoproteins. Close antigenic relation between OVPV and CSFV genotype 2.3 was detected. To determine the protection against CSFV, the OVPV-infected pigs were challenged with a highly virulent strain. Strong clinical, virological and immunological protection was generated in the OVPV-infected pigs, in direct contrast with the infection control group. Our findings show, for the first time, the OVPV capacity to infect swine, activate

immunity, and the robust protection conferred against CSFV. The phylodynamic analysis showed that CSFV seems to have emerged as the result of an inter- species jump of Tunisian sheep virus (TSV) from sheep to pigs. OVPV and CSFV share the TSV as a common ancestor, emerging around 300 years ago. This suggests that the differentiation of TSV into two dangerous new viruses for animal health (CSFV and OVPV) was likely favored by human intervention for the close housing of multiple species for intensive livestock production.

Coronavirus surveillance in bats in the Covid-19 era, Italy 2020-21

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Varieties of pathological conditions in domestic and wild animals have been attributed to coronavirus (CoV) infections. Since 2002, three highly virulent strains classified in two subgenera (Sarbecovirus and Merbecovirus) of the genus Beta-Coronavirus (BetaCoV) have caused severe respiratory diseases causing thousands of infections and deaths worldwide. Two species belonging to the Sarbecovirus subgenus that cause severe acute respiratory syndrome are SARS-CoV and SARS-CoV-2 that emerged in China in 2002 and December 2019, respectively.

Bats, with their wide geographical distribution and flight capabilities, have been documented as natural hosts of a large number of different viruses, such as *lyssavirus, paramyxovirus, filovirus* and *coronavirus*. The genetic diversity of CoVs in bats exceeds that known from other hosts, which is consistent with bats being the main reservoir of mammalian CoVs. In this study, coronavirus surveillance were carry out on 100 bat carcasses and 20 faecal samples collected from a Wildlife Recovery Centre and from two bat colonies in Northern Italy during 2020-21.

Identification of bat species was achieved by using a PCR targeted the COI gene and subsequent sequencing; the most represented species were *Pipistrellus kuhlii* (38.3%), *Hypsugo savii* (29.2%), *Rhinolophus hipposideros* (16.7%) and *Myotis daubentonii* (6. 7%) followed by other species (*Pipistrellus pipistrellus, Pipistrellus nathusii, Plecotus auritus, Myotis crypticus, Myotis mystacinus* and *Tadarida teniotis*) in low percentages from 1.7 to 0.8%. In 1.7% of cases, no species could be identified. For CoV detection, a pancoronavirus nested PCR was employed, targeting the RNA-dependent RNA polymerase (RdRp) sequence (PanCoV-PCR). Phylogenetic analysis was performed using MEGA6 including CoVs sequences retrieved from public databases. Eighteen samples from 14 animals (11.6%) were positive for PanCoV-PCR. Phylogenetic analysis showed a wide diversity of CoVs with sequences grouped in the alpha and beta CoVs. Most sequences belong to BetaCoV and culminate in two groups. The first 9 sequences, all from *R. hipposideros,* form a homogeneous group with other sequences from European bats within the Sarbecovirus genus (SARS-like viruses). Other 5 sequences from 3 bats (2 from *H. savii* and 1 *P. auritus*) were highly correlated with other Italian sequences and were placed in the Merbecovirus genus (MERS-like). Finally, 2 sequences from *P. kuhlii* and *M. cryticus* belonged to alphaCoVs and were highly correlated with sequences from bats collected in Germany.

Among the four CoV genera, only alpha- and betaCoVs were found in bats worldwide, with higher detection rates for alphaCoVs. In our studies, however, a higher percentage of BetaCoV was always detected. Of these, all the SARS-like sequences came from the same two bat colonies that have been sampled periodically and shown to harbour the viruses for more than 10 years. It should also be noted that, as already observed in other European studies, the percentage of identity of Italian sequences with SARS-CoV and SARS-CoV-2 is lower than that of Chinese bats. A greater variability was instead observed among the MERS-like sequences of Italian bats where two groups can be observed within the cluster, one for *Pipistrellus* and *Hypsugo* sequences and another for *Pleotus* sequences. These results highlight the need for intensive surveillance in bats to contribute to a more complete and detailed picture of the global epidemiology of CoVs.

Comparative pathobiology of clade 2.3.4.4b H5N8 HPAIVs from the European 2017 and 2020 epizootics in domestic ducks

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In the past years, highly pathogenic avian influenza (HPAI) has been occurring with increasing frequency in Europe. A total of 487 and 492 outbreaks of H5 clade 2.3.4.4b HPAIVs were registered in France between 2016-17 and 2020-21 respectively, and a third epizootic is currently ongoing, resulting in increased mortality and severe neurological signs in domestic ducks. Viral tissue tropism is defined as the ability of a viral strain to infect susceptible and permissive cells. This results from the combination of host factors and viral genetic determinants. Due to their high evolution potential, HPAIVs can consequently lead to changes in the clinico-pathological presentation and viral shedding, impacting both infection dynamics and on-field diagnosis. The aim of the study was to compare the pathological profiles, viral biodistribution and viral shedding between 2016-17 and 2020-21 H5 HPAIVs on naturally- and experimentally-infected ducks.

A total of 10 H5 HPAIV-positive flocks originated from 2016-17 and 2020-21 epizootics, which included mule ducks (8 flocks), and Muscovy ducks (2 flocks), were submitted to necropsy, histopathology and viral antigen detection. To investigate both pathobiological timeline and viral shedding, a total of 32 5-week-old mule ducks were then experimentally infected through intrachoanal inoculation with 10^5 EID_{50} of either H5(2017) or H5(2020). Six additional birds were introduced as contact groups. Ducks were monitored during 14 days post-challenge (dpc) to compare mortality, clinico-pathological findings, viral antigen and RNA biodistributions, and viral shedding assessed on cloacal, oropharyngeal, conjunctival swabs and feathers collected at 2, 4, 7, 10 and 14 dpc. Six birds per virus were used for necropsies at 3 dpc and 5 dpc.

Naturally-infected ducks of both epizootics presented increased mortality, depression, torticolis, and paralysis. Necrotizing and non-suppurative inflammation with concurrent viral antigen detection was identified in brain, heart, pancreas, hepatobiliary system, growing feathers, nasal and Harderian glands. Histopathological scores were significantly higher in the heart of H5(2017)-infected birds compared to the H5(2020) ones. Cholangio-hepatitis and pancreatitis were significantly higher for Muscovy ducks compared to mule ducks.

The experimental infection reproduced the disease: depression, torticolis and death. Mortality rates were 100% and 33% for H5(2017) and H5(2020) contact birds respectively, and 30% and 20% for inoculated ones. Viral shedding was observed as early as 2 dpc with a peak of excretion at 4 dpc. Viral titers were higher in H5(2017) inoculated birds compared to H5(2020) ones in cloacal swabs at 4 dpc, and in feathers and conjunctival swabs at 7 dpc.

Pathological investigation confirmed the lesions observed in the field. They were detected histologically and grossly nd 5 dpc, respectively. In H5(2017)-infected birds, myocardial lesions were frequent, severe, and associated with a significantly higher viral load. Interestingly, Harderian gland adenitis was severe, detected as early as 3 dpc and associated with accumulation of nucleoprotein-positive debris in collecting ducts. Our results also confirmed a massive viral detection in barb ridges and pulp of growing feathers as early as 3 dpc and up to 6 dpc.

Altogether, these results suggest that the H5(2017) HPAIV is more virulent for domestic ducks than the H5(2020) HPAIV. While both strains shared marked nervous and epithelial tropism, cardiomyotropism was predominant for H5(2017) HPAIV.

Detection of zoonotic Borna Disease Virus 1 (BoDV-1) in shrew species of the Genus Crocidura in Germany

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Borna disease virus 1 (BoDV-1, species *Mammalian 1 orthobornavirus*), the long known causative agent of Borna disease in horses, sheep and new world camelids, has recently regained attention due to its detection in dozens of lethal human encephalitis cases. So far, only the bicoloured white-toothed shrew (*Crocidura leucodon*) has been described as a natural reservoir of BoDV-1. The objective of this study was the characterization of BoDV-1 infections in putative small mammal reservoir hosts.

Small mammals were collected all over central Europe. Primary screening of brain tissue for bornavirus RNA was performed using a BoDV-1-specific and a broad range panBorna RT-qPCR. Positive results were confirmed by partial or whole genome sequencing. For BoDV-1-positive individuals, viral antigen and RNA tissue distribution was analyzed via immunohistochemistry and a quantitative RT-qPCR.

Screening of about 9,000 small mammals from 23 different rodent and insectivore species resulted in the detection of 28 BoDV-1 RNA-positive shrews: the highest detection rate was observed in bicoloured white-toothed shrews 23/95 (24.2%), but BoDV-1 RNA was also detected in 4/38 (10.5%) lesser white-toothed shrews, *Crocidura suaveolens* and 1/243 (0.4%) greater white-toothed shrew, *Crocidura russula*. The positive shrews originate from three different German federal states (Brandenburg, Saxony-Anhalt, Bavaria) which belong to the known BoDV-1 endemic area. We did not detect BoDV-1 RNA in any other species, even when originating from the same locality as an infected shrew. Furthermore, mammalian bornaviruses other than BoDV-1 were not detected. All three species of shrews show a similarly broad viral RNA and antigen tissue tropism with the highest loads in neuronal tissue and lower amounts in non-neuronal tissue. Phylogenetic analysis of three human-derived BoDV-1 sequences matches sequences from shrews collected nearby residences of human cases, supporting the risk of peridomestic infection risk.

A better understanding of the transmission within and between shrew populations and to accidental dead-end hosts, gained by the performed viral RNA distribution analyses, will enable us to improve public health measures. The present study confirms the reservoir function of bicoloured white-toothed shrews and reveals two closely related shrew species, the lesser and greater white-toothed shrew, as potential additional reservoir species. Future studies should evaluate reasons for the limited distribution of BoDV-1 in parts of Europe, although *C. leucodon* has a broader range.

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Susceptibility of Golden Syrian hamsters to extremely low doses of SARS-CoV-2 may enable zoonotic transmission

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Golden Syrian hamsters (*Mesocricetus auratus*) are now a well established small animal model for human infections with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated Coronavirus Disease 19 (COVID-19), and are widely used in various fields of research. Worldwide, millions of Golden Syrian hamsters are also kept as pets in very close contact to humans. We therefore decided to determine the minimal infectious dose for this species, which is essential for an evidence-based risk assessment of a potential human- hamster zoonotic transmission cycle. At the same time, the results of this study are basic for the definition of the optimal infection dose for future infection studies. We inoculated hamsters by the orotracheal route with SARS- CoV-2 doses ranging from $1*10^5$ tissue culture infection dose $(TCID)_{50}$ to $1*10^{-4}$ $TCID_{50}$, and monitored the body weight, clinical behavior, and virus shedding daily for up to 10 days. We found that an infection dose of $1*10^{-2}$ $TCID_{50}$ was sufficient to induce clinical disease and virus shedding in infected hamsters up to 10^4 $TCID_{50}$/ml, which is higher than the estimated minimal infectious dose for humans. Interestingly, even a dose of $1*10^{-4}$ $TCID_{50}$ (equivalent to 0.7 genomic RNA copies per infection dose) was sufficient to induce a subclinical SARS-CoV-2 infection without detectable shedding, but with a clear involvement of the tissues of the respiratory tract. Onset of virus shedding occurred with a delay of 2-3 days in the groups infected with doses below $1*10^{-1}$ $TCID_{50}$, but viral loads in the tissue samples reached comparable levels in all infected groups. All uninfected control animals remained negative. This astonishingly high susceptibility of Golden Syrian hamsters to extremely low infection doses is relevant for the assessment of a transmission risk between humans and pet Golden Syrian hamsters.

SESSION 2 - Pathogen evolution

Genetic tracing of an historic outbreak of classical swine fever in the UK

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The last outbreak of classical swine fever (CSF) in the UK occurred 22 years ago in 2000. A total of 16 domestic pig holdings in the East Anglia region were confirmed as infected over a 3-month period. Technology developments now allow full length viral genome sequences to be obtained in real-time and has accordingly been applied to trace viral transmission events for viruses such as SARS-Cov2 and FMDV.

To examine if genetic changes over the course of any future CSF outbreak would allow to track virus movements and assist in control efforts, we sequenced the E2 gene and full genome of virus present in archived tonsil samples from 14 of these infected premises. Insufficient changes occurred in the full E2 gene to discriminate between the viruses from the different premises. However, phylogenetic analysis of almost full genome sequences allowed to either corroborate or question some of the transmission pathways inferred by epidemiological investigations at the time. We are thus confident that in future outbreaks of similar size and duration, real-time monitoring of the outbreak via full genome sequencing will be beneficial.

For sequence data to provide the most useful information to aid disease control efforts genetic diversity needs to occur to distinguish between closely related viruses. The rate of genetic evolution varies for different viruses and is influenced by different transmission events, which will vary according to the epidemiology of an outbreak. Our data provides information on the extent of genetic change that can occur between infected premises in the absence of CSFV replication in a feral wild boar population. Further knowledge on the extent of virus evolution of specific viruses under different situations, such as mutations per passage and by different infection routes, is important to assist in interpreting the significance of genetic changes.

Evolution of 14 Bluetongue and 3 epizootic hemorrhagic disease virus serotypes in French Guiana from 2011 to 2020

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In French Guiana (South America), many serotypes of BTV and EHDV were identified in 2011 - 2012 (BTV1, 2, 6, 10, 12, 13, 17 and 24, EHDV1 and 6). This study was aimed to further identify strains/serotypes of these 2 viruses circulating in this area and to follow their genomic temporal evolution. Thus, between 2013 and 2020, we received field samples from 840 young cattle without clinical signs. The BTV and EHDV genomes were tested by RT-qPCR and isolation performed. The serotype was determined by conventional RT-PCR and Sanger sequencing. We assessed the seroprevalence of these viruses by ELISA. Finally, 43 BTV or EHDV strains were fully sequenced.

In the period 2013 - 20, 21.7% of the tested animals were EHDV RNA positive by RT-qPCR and 54.5% were positive by EHDV ELISA. For BTV, 70.4% of the blood samples were RNA positive by RT-qPCR and 99.2% positive by ELISA. Sixty isolates of BTV and 8 of EHDV were obtained. Thus, 6 BTV serotypes (3, 11, 14, 18, 19, 22) were identified in addition to the 8 BTV serotypes previously described. An EHDV2 was isolated.

NGS analysis shows that a large number of BTV and EHDV strains of the same serotype are present in French Guiana, due to recombination phenomena. The results obtained with 9 BTV1 genomes, isolated from 2011 to 2018, illustrate this point. Indeed, if we consider that, within a single serotype, a BTV strain is genetically different from another if at least 1 segment (seg) out of the 10 is reassorted, 7 out of 9 sequenced BTV1 strains are different. Three BTV1 genomes from 3 cattle collected on the same day from the same farm shows 3 different genomes with 1 to 6 seg reassorted. The seg 2 and 6 sequences of these 9 BTV1 strains show 99.39-100% homology to each other, demonstrating a common origin for these 9 BTV1 strains with respect to the 2 seg coding for the outer capsid. The other 8 seg reassort more or less frequently. We observe the same phenomena when we compare the seg 2 and 6 of 31 other BTV strains belonging to 10 different serotypes: the seg 2 and 6 (in each serotype) are identical, showing at least 99.34% homology. An exception is observed for a BTV12 isolated in 2020 showing only 89 to 91 % homology, respectively, with seg 2 and 6 of the 6 BTV12 strains isolated between 2011 and 2018, suggesting a new introduction of a BTV12 strain. Similar observations are reported with EHDV. Alignment of 2 sequenced EHDV1 genomes showed that they have 2 distinct origins: their seg 2 and 6 show only 83 and 86% homology, respectively. Interestingly, EHDV2 isolated in 2018 has 5 seg showing more than 99% homology with the EHDV1 isolated in 2011, suggesting reassortments with an EHDV1 strain similar to those isolated in 2011. The results also show that a same VP5 can be present in different BTV serotypes of the same nucleotype (i.e. BTV strains with less than 35% difference in their seg 2 nucleotide sequences).

The frequency of orbivirus antibodies/genomes detection in cattle demonstrate an intense circulation of the 2 viruses, with 14 BTV and 3 EHDV serotypes circulating in this area. Overall, the viral sequences show a distant homology to homologous seg of viruses detected in North and Central America or the Caribbean. The sequencing results suggest that new strains have been introduced into this region during the period of our study and that many reassortments are observed between the different serotypes. For each serotype, the seg 2 and 6 coding for the BTV outer capsid are highly conserved

over time and this pair is always found while the other 8 seg reassort. The VP2 - VP5 pair seems stable and would therefore be the marker allowing the origin of a BTV strain to be characterized.

Subclinical ferret hepatitis E virus infection in laboratory ferrets in the UK

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Ferrets are widely used for experimental modelling of viral infections. However, background disease in ferrets could potentially confound intended experimental interpretation. Here we report the detection of a subclinical infection of ferret hepatitis E virus (FRHEV) within a colony sub-group of female laboratory ferrets that were had been enrolled on an experimental viral infection study (non-hepatitis). Lymphoplasmacytic cuffing of periportal spaces were identified on histopathology but were negative for the administered viral agent RNA and antigens by virological and microscopic assessments. Follow up viral metagenomic analysis conducted on liver specimens revealed sequences attributed to FRHEV and were confirmed by reverse-transcriptase polymerase chain reaction. Further genomic analysis revealed contiguous sequences spanning 79-95% of the FRHEV genome and that the sequences were closely related to those reported previously in Europe but not of North America or Asia origins.

Using *in situ hybridisation* by RNAScope®, we confirmed the presence of the open reading frame 2 (ORF2) in the hepatocytes. The protein ORF2 was also detected by IHC in the hepatocytes and the biliary canaliculi. Whilst the viral infection was subclinical, our results highlighted a background infection in laboratory ferrets that should be recognised, such as through virological surveillance or hepatic enzyme monitoring, to enable better evaluation of *in vivo* studies in the future.

Insight into the Virome of Nigerian Fruit Bats

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The emergence of the coronavirus 2019 (COVID-19) pandemic has been a major health, economic and social disaster of the 21st century. The ecology and anthropological mechanism of emergence is still being investigated, but early evidence suggests bat coronaviruses as the probable origin of SARS-CoV-2. Although bats have been identified as reservoir of many zoonotic pathogens and as a source of spill-over infections to other species, their role in Nigeria, a 'hot-spot' for zoonotic diseases, is (not?) well documented. Through an increased animal-human interface triggered by, high population density, as well as human activity involved in bat processing and consumption, the spread of zoonotic diseases is facilitated. Early surveillance of potentially zoonotic pathogens at the source of origin is recommended as a primary pandemic prevention action.

In order to estimate the diversity of viral pathogens with zoonotic potential in mixed-species colonies of migratory fruit bats native to Nigeria, we will determine the virome in bat tissue, urine and fecal samples. In different ecological sampling locations, bat roosting sites amid human settlements, employing invasive and non-invasive sampling methods will be adopted in Ondo and Plateau State of Nigeria, respectively. Nucleic acid will be extracted and cDNA synthesised for shot-gun sequencing and further metagenomic analysis. From the sequence data, the contiguous sequences of viral pathogens that are of potential zoonotic and public health threat will be characterized in detail. The outcome of this investigation will support effective surveillance and early detection of viral zoonoses in the region. Ideally, this will result in preventive measures such as the adoption of stronger surveillance and conservation of bats as part of national and global policy for One Health.

First findings from this research will be presented at the conference.

Evolution of Swine influenza virus H3N2 in Vaccinated pigs after previous natural H1N1 infection

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Swine influenza viruses (SIV) produce a highly contagious and worldwide distributed disease that could cause important economic losses in pig industry. Currently, the application of trivalent vaccine, which includes the most circulating subtypes H1N1, H1N2 and H3N2, is one of the most extended strategies to control SIV. The vaccine could potentially favor the evolution of the SIV, as it does not provide sterilizing immunity. Consequently, viral escape mutants could be generated during SIV infection. In the present study, the viral variants generated in vaccinates and nonvaccinated H3N2 challenged pigs with a previous natural infection with H1N1 SIV subtype were determined and analyzed. For this purpose, sixteen domestic pigs were selected and divided in two groups: Eight animals were twice intramuscularly vaccinated against SIV with trivalent vaccine and the rest of the animals were intramuscular vaccinated with PBS. Three weeks after vaccination, all animals were challenged with A/Swine/Spain/SF32071/2007 (H3N2) and nasal swab samples were collected periodically to detect and sequence SIV to analyze the viral evolution. Here, fourteen whole SIV genome were determined, 5 from vaccinated pigs, 8 from nonvaccinated and the inoculum, by next generation sequencing. The proportion of nonsynonymous substitutions was greater than synonymous one. Therefore a natural selection pressure may be driven SIV evolution in both scenarios. In total 180 variants with an allele frequency greater than 1% were detected along all the genomic segments, 70 and 110 in samples collected from vaccinated and nonvaccinated animals respectively.

The highest number of nonsynonymous substitutions were reported in polymerases segments (PB2, PB1 and PA), although most of these substitutions were not detected with an allele frequency greater than 5%. However, 18 and 15 nonsynonymous substitutions with an allele frequency greater than 5% were reported from vaccinated and nonvaccinated animals respectively and further analyzed. No nonsynonymous substitutions were found on NP protein, meanwhile, much of these substitutions were allocated in both surface glycoproteins HA and NA and in the NS1 protein. These proteins are involved in relevant mechanism for SIV infection, such as, the binding to the cell receptor, viral release from cells and viral replication. The present study highlights the continuous SIV evolution despite vaccination and/or previous natural infection with a different viral subtype.

Different impact of bovine CD46 as a cellular receptor for members of the species Pestivirus H and Pestivirus G

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Bovine viral diarrhea virus 1 and 2 (BVDV-1 and -2), classified as *Pestivirus A* and *Pestivirus B*, respectively, are members of the genus *Pestivirus* within the family *Flaviviridae*. As causative agents of the notifiable animal disease bovine viral diarrhea (BVD), which may result in reproductive disorders or manifest in lethal mucosal disease (MD), BVDV-1 and -2 represent highly relevant animal pathogens. HoBi-like pestiviruses (HoBiPeV; *Pestivirus H*), cattle pathogens first described in 2004, can induce a disease similar to BVD. As contaminants of fetal bovine serum (FBS), HoBiPeV also pose a threat to biosafety of products containing FBS, e.g. vaccines. Giraffe pestivirus (GPeV; *Pestivirus G*) was first isolated from a dead giraffe showing MD-like lesions. Both strains known to date replicate efficiently on bovine cells, indicating that cattle are a susceptible host of GPeV.

To enter the host cell, BVDV-1 and -2 use complement regulatory protein 46 (CD46) as a receptor, but are also able to escape CD46-dependency by increased binding to cellular heparan sulfate (HS) *in vitro*. The entry mechanism of HoBiPeV and GPeV has not been investigated yet. Therefore, this study addressed the questions whether members of the distinct pestivirus species *Pestivirus H* and *Pestivirus G* also use CD46 as a receptor and whether they are also able to circumvent receptor usage by adaption to HS.

The role of CD46 in entry of representatives of the species *Pestivirus H* and *Pestivirus G* was investigated by *in vitro* infection experiments. Using CRISPR/Cas9 genome editing, a bovine CD46-knockout cell line was generated to prove loss-of-function. In order to show gain-of-function, the CD46-knockout cell line was subsequently trans- complemented with either of two different CD46 variants to generate CD46-rescue cell lines. Furthermore, entry via CD46 was blocked by a CD46-specific antibody. HS-mediated entry was investigated by blocking of putative HS binding sites on the viral glycoproteins. The mechanism of HS adaptation was analyzed by comparison of sequences from native and adapted viruses.

CD46-knockout cells as well as cells blocked with a CD46-specific antibody displayed a dramatically decreased permissivity to HoBiPeV strain HaVi-20 comparable to infections performed with BVDV-1 and -2. Moreover, CD46-rescue completely restored susceptibility to these viruses. Interestingly, both CD46-deficiency and

CD46-rescue had only a minor impact on infection with GPeV strain PG-2. Other members of *Pestivirus H* and *Pestivirus G* strongly bound to HS and were thus able to enter cells independently of CD46. HS-adapted GPeV particles exhibited an G475R amino acid exchange corresponding to BVDV G479R described to be responsible for HS-adaptation.

In conclusion, the relevance of CD46 as a cellular receptor differs between members of the species *Pestivirus H* and *Pestivirus G*. While it was demonstrated that HoBiPeV (*Pestivirus H*) strongly depend on CD46 as an entry factor, GPeV (*Pestivirus G*) revealed to be rather independent of CD46, pointing to the use of a different molecular determinant that is unknown so far. As it might fulfill an interesting role also in entry of other pestiviruses, identification of this factor will be the topic of future research.

Furthermore, it was shown that members of both *Pestivirus H* and *Pestivirus G* are able to adapt to HS under *in vitro* conditions, apparently by a similar mechanism, as it is known for *Pestivirus A* and *Pestivirus B*. Overall, the entry mechanisms of pestiviruses turn out to be more diverse than previously assumed.

SESSION 3 - Vaccine development

Bluetongue Disabled Infectious Single Animal (DISA)/DIVA vaccines provide sterile immunity in cattle

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Bluetongue (BT) is a midge-borne OIE-notifiable disease of ruminants caused by bluetongue virus (BTV). There are at least 29 BTV serotypes as determined by serum neutralization tests and genetic analyses of genome segment 2 encoding serotype immunodominant VP2 protein. Large parts of the world are endemic for multiple serotypes. Sheep are the most susceptible to Bluetongue, while cattle are an important source for onward spread by midges because of a higher and longer viremia. The most effective control measure of BT is vaccination. Live- attenuated vaccines (LAVs) and inactivated BT vaccines are available, but have their specific pros and cons and are not DIVA compatible. The profile of veterinary vaccines is defined by safety, efficacy, affordability, "Differentiating Infected from VAccinated animals" (DIVA principle), and acceptance. Previously, we have developed the Disabled Infectious Single Animal (DISA)/DIVA vaccine platform based on LAV without NS3/NS3a expression and exchange of genome segment 2. DISA vaccine for serotype 8 fulfils all criteria for modern veterinary vaccines, including serotype specific protection of sheep against virulent serotype 8, reviewed in van Rijn[1]. The DISA/DIVA vaccine platform is completely innocuous for sheep, and transmission of vaccine virus to and by midges is double blocked; no viremia in the ruminant host and no replication in midges (DISA principle)[2].

Further, the DISA vaccine platform is DIVA compatible with OIE-recommended PCR-diagnostics and enables DIVA because of lack of antibodies against immunogenic NS3/NS3a protein (DIVA principle).

Here, we show that deletion of 72 amino acids in NS3/NS3a also resulted in the ideal vaccine profile. Even more, the prototype DISA/DIVA vaccine for serotype 8 induced sterile immunity in cattle at three weeks post single vaccination[3]. The DISA/DIVA vaccine platform was explored for more serotypes by exchange of genome segments 2 and 6 encoding serotype specific outer shell proteins VP2 and VP5. Pentavalent DISA/DIVA vaccine for "European" serotypes 1, 2, 3, 4, 8 also provides sterile immunity in cattle for two studied serotypes. Neutralization antibody titres indicated protection against other included serotypes[4]. In conclusion, the BT DISA/DIVA vaccine platform generates safe, fast DISA/DIVA vaccines for emergency vaccination, multivalent DISA/DIVA vaccines to combat several serotypes, and the development of broad protective DISA/DIVA vaccine.

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Advances in the characterization of the RVFV vaccine candidate 40Fp8

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Rift Valley fever (RVF) is an important zoonotic viral disease affecting several species of domestic and wild ruminants that causes high economic losses and dozens of human deaths in different geographical areas in Africa, where it is endemic. Although not present in Europe, there is some risk of introduction and spread linked to globalization and climatic change. To date the only measure that could help to prevent the disease is vaccination of flocks in RVF risk areas. Available live-attenuated vaccines are an effective way to control the disease, but in certain conditions their use is limited because of residual virulence. On the other hand, no vaccine for human use is currently licensed. Therefore, the development of safe, effective vaccines is an active area of research.

In previous studies, we described the obtention by random mutagenesis of a highly attenuated RVFV 56/74 virus variant termed 40Fp8. This virus displayed a reduced virulence phenotype even in immunosuppressed mice (A129) that did not preclude the induction of immune responses in inoculated animals, therefore supporting its potential use as a live attenuated vaccine. 40Fp8 carries 47 mutations along the three genomic segments that are not present in the parental strain, and some of them have already proven to be strong contributors to viral attenuation.

To further explore the potential of this virus as a safe vaccine candidate, in this work we have analysed some other features of 40Fp8, such as its attenuation level in immunosuppressed mice (A129) compared to other RVFV attenuated viruses, its immunological properties in a target livestock species (sheep), as well as its genetic stability both in vitro an in vivo. Our results confirm its higher attenuation when compared to other attenuated vaccine prototypes, while still able to induce in inoculated sheep a potent humoral neutralizing antibody response whose titers correlate well with protection. Furthermore, the growth pattern of the selected 40Fp8 virus in Vero cells shows no alterations, rendering high virus yields at 3-4 dpi, a result supporting its high scale production. All these data underscores the potential of this virus as a candidate for the development of a safe live attenuated RVF vaccine.

Abrogation of the Erns RNase activity in a low virulence classical swine fever virus reduces viral virulence

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Classical swine fever virus (CSFV) remains one of the most important pathogens that affects swine health and the pork industry worldwide. The emergence of less virulent CSF virus (CSFV) strains that could induce chronic persistent and subclinical forms of the disease further difficult CSF eradication. Despite the importance of the CSFV virulence determinants for the outcome of CSF, the molecular basis behind the CSFV attenuation remains a topic under study. It is known that the E^{rns} glycoprotein, an exclusive protein of pestiviruses, has a unique RNase activity. This function of E^{rns} is not only required to block the type I IFN induction but also played an important role for pestiviruses virulence *in vivo*. Considering this, the aim of the present study was to assess the potential role of the RNase activity in the low virulent CSFV Pinar de Rio (PdR) strain isolated from an endemic country, for the viral pathogenicity, persistence, and transmission in pigs. We constructed a functional cDNA clone of CSFV PdR abrogating the E^{rns} RNase activity (pPdR-H_{30}K-36U) based on the PdR wild-type clone(pPdR-36U). Eighteen five- day-old piglets were infected with the cDNA-derived virus vPdR-H_{30}K-36U. Nine piglets were introduced as contact animals. Disease progression, virus replication and immune responses were monitored. The vPdR-H_{30}K-36U virus was clearly attenuated in the piglets compared to the parental vPdR-36U, with only RNA traces being found and no virus being isolated from serum and body secretions. The vPdR-H_{30}K-36U mutant strongly activated the interferon-? (IFN-?) production in plasmacytoid dendritic cells. While the infected piglets only showed transient IFN-? responses in the serum after one week of infection being consistent with virus replication capability *in vivo*. Infection with vPdR-H_{30}K-36U resulted in higher antibody levels against the E2 and E^{rns} glycoproteins and in enhanced neutralizing antibody responses when compared with vPdR-36U. 94% of the piglets developed neutralizing antibodies after 3 weeks of infection. This virus lost its transmissibility to the contact piglets. Our findings give a better understanding of the CSFV attenuation related to E^{rns} RNase activity, also provide new insights relevant for the development of DIVA vaccines against CSFV with appropriate accompanying diagnostic tests for CSF eradication.

Expression of NS1 and NS2-Nt (NS2 1-180) by ChAdox1 and MVA confers protection in sheep against bluetongue

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Bluetongue, caused by bluetongue virus (BTV), is a widespread arthropod-borne disease of ruminants that entails a recurrent threat to the primary sector of developed and developing countries. Current BTV vaccines are effective but they do not allow to distinguish between vaccinated and infected animals (DIVA strategy) and are serotype specific. In this work, we report modified vaccinia Ankara virus (MVA) and chimpanzee adenovirus Oxford 1 (ChAdOx1) vectored vaccines designed to simultaneously express the immunogenic NS1 protein and/or NS2-Nt, the N-terminal half of protein NS2 ($NS2_{1-180}$). A single dose of MVA or ChAdOx1 expressing NS1-NS2-Nt improved the protection conferred by NS1 alone in IFNAR(-/-) mice. Moreover, mice immunized with ChAdOx1/MVA-NS1, ChAdOx1/MVA-NS2-Nt or ChAdOx1/MVA-NS1-NS2-Nt developed strong cytotoxic CD8+ T-cell responses against NS1, NS2-Nt or both proteins and were fully protected against a lethal infection with BTV serotypes 1, 4 and 8.

Furthermore, although a single immunization with ChAdOx1-NS1-NS2-Nt partially protected sheep against BTV-4, the administration of a booster dose of MVA-NS1-NS2-Nt promoted a faster viral clearance, reduction of the period and level of viremia and also protected from the pathology produced by BTV infection.

The Importance of Quality Control of LSDV Live Attenuated Vaccines for Its Safe Application in the Field

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Vaccination is an effective approach to prevent, control and eradicate diseases, including lumpy skin disease (LSD). This has been demonstrated during the LSDV outbreaks in in Israel (2012–2013), in the northern part of Cyprus (2014–2015) and in the Balkan region (2016–2017). This observed protection efficacy in the field was similarly confirmed by a recent comparison of several commercial LSDV-based LAVs under highly controlled and standardized laboratory setting [Haegeman et al 2021)]. Notwithstanding these successes vaccination is not without opposition. One of the measures to address farmer hesitation to vaccinate is guaranteeing the quality of vaccine batches. The purpose of this study was to demonstrate the importance of such a quality procedure via the evaluation of the LSD vaccine, Lumpivax (Kevevapi). The initial PCR screening revealed the presence of field type LSD virus (LSDV) and goatpox virus (GTPV), in addition to vaccine LSDV. New phylogenetic PCRs were developed to characterize in detail the genomic content. This confirmed the presence of LSDV field-, vaccine- and GTPV-like sequences in the vaccine vial. The analysis was also suggestive for the presence of GTPV-LSDV (vaccine/field) recombinants. The absence of such field, GTPV-like and recombination sequences in 2 other commercially available LSDV vaccines (namely OBP and Caprivac) showed that the observations for the Lumpivax vaccine were not due to the applied methodology. Subsequently, the impact on efficacy and diagnostics of the use of the Lumpivax in the field was investigated by a vaccination/challenge experiment. Notwithstanding the fact that the vaccine proved to be protective against a LSDV challenge, the use of the vaccine resulted in contradictive DIVA PCRs results and could lead to misinterpretation since signals indicative for infection with wild type strains were found in some tests. When combining the results from our in vitro and in vivo studies, the presence of LSDV recombinants better explains the observed results than the potential presence of different Capx strains in the vaccine. Further confirmation of our findings by whole genome sequencing would be advisable. Whether the recombinant nature of this LSDV vaccine strain influences its potential for further transmission needs further investigation. Finally, the Capripox heterogeneity observed in the vaccine is troublesome as it is unclear how this will vary, batch to batch, and evolve with the creation of new or additional recombinations. Therefore, quality control of each new batch is warranted.

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Evaluation of the immunogenicity of RHDV VLPs with increased mechanical stiffness

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Virus-like particles (VLPs) are protein complexes which mimic virion well-defined geometry, while being devoid of genetic material. They are outstanding and safe immunogens combining a highly ordered and particulate nature with lack of replication ability.

Rabbit Hemorrhagic Disease Virus (RHDV) VLPs represent a promising platform for multimeric antigen display due to their intrinsic characteristics: i) RHDV VLPs are composed of a single capsid subunit (protein VP60), easing the production and engineering of chimeric VLPs; ii) they are highly immunogenic, inducing complete protection of rabbits against RHDV lethal challenge; iii) RHDV is restricted to lagomorphs (rabbits and hares), hence no pre- existing immunity is expected in humans or livestock species, avoiding potential detrimental effects of anti-carrier immunity; iv) RHDV VLPs have proven to be very tolerant, accepting simultaneous insertion of target epitopes at different insertion sites, as well as incorporation of tandem copies of foreign epitopes at surface-exposed loops; and v) chimeric RHDV VLPs displaying foreign epitopes have been shown to be excellent immunogens, inducing cellular immune responses against inserted target cytotoxic T-cell epitopes, as well as potent protective humoral responses against foreign B-cell epitopes in the mouse and pig models (1-3).

One important feature of VLPs intended to be used for biotechnological applications is the physical stability of the assembled particles. Generating VLPs with increased physical stability might be desirable for the development of improved biotechnological nanodevices (i.e. particles able to resist relatively harsh physical and/or chemical conditions during production processes and/or increased storage duration). Currently, no information is available on the mechanical properties of the RHDV capsid. Here, we report the analysis of the mechanical properties of RHDV capsid protein (virions and VLPs) using atomic force microscopy (AFM), and the generation of rationally modified VLPs with increased mechanical stiffness. These engineered VLPs were subjected to immunogenic evaluation in the mouse model.

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SESSION 4 - Vector-borne diseases

Pathogenesis of West Nile virus in domestic geese in Germany

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Since its introduction into Germany in 2018, West Nile virus (WNV) has become an endemic mosquito-borne flavivirus with annually reoccurring outbreaks throughout the avifauna. With its vast vector and host range, WNV can be transmitted by numerous mosquito species and equine as well as human infections are becoming increasingly frequent. On a global scale, domestic waterfowl has repeatedly been described as a possible amplifying host for WNV and outbreaks with high mortality rates have occurred in Israel, United States of America, Canada, and Hungary. The question, however, persists: What role does free-ranging domestic waterfowl, such as geese, play in the transmission of WNV throughout Germany?

To investigate the pathogenesis in waterfowl, domestic geese (juvenile; 3-week-old) were infected via a subcutaneous needle injection either with a lineage 1 strain from Italy (GenBank accession no. HM991273/HM641225) or a lineage 2 strain isolated in Germany (GenBank accession no. MH924836). Subsequently, the birds were monitored daily for clinical signs and blood and swab samples as well as body weights were collected at set time points. Genome virus and antibody loads were quantified via RT-qPCR and ELISA and VNT, respectively. Depending on the experiment, the geese were either all euthanized 21 days post infection (dpi) or throughout the experiment at various time points (3, 6, 10, 14, and 21 dpi), followed by a subsequent pathological examination.

Juvenile geese were susceptible to a WNV infection and viral loads in the blood, swabs, and tissues of the birds were similar independent of the virus strain. All geese developed viremia levels that peaked 2 or 3 dpi, shed virus from 2 to 7 or 8 dpi, and rapidly seroconverted. At 21 dpi, histological lesions were confined to the brain (encephalitis) and heart (myocarditis) and low genome loads were only isolated from brain tissues. The only observed difference in the pathogenesis between the two European strains was linked to the clinical signs; these were more frequent with an earlier onset after an infection with the German lineage 2 strain. Two geese, however, stood out: One goose developed a severe clinical manifestation after infection with the German WNV lineage 2 strain (i.e., was highly susceptible) and had to be euthanized. Another goose developed a delayed course of infection after inoculation with the Italian WNV lineage 1 strain with a later onset of viremia and viral shedding.

In conclusion, geese are susceptible to WNV with only minor, if any, differences in pathogenesis between an Italian WNV Lineage 1 and a German WNV Lineage 2 strain. Even though high susceptibility could be observed in individual cases, geese in general are less susceptible than for example passerine species and do not succumb to an infection with European WNV strains. The majority of infected geese produce viremia levels that are too low to transmit the virus to mosquitoes and, therefore, probably do not constitute as primary amplifying hosts for WNV.

Part of the experiments are already published:

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Stomoxys calcitrans feeding activity influences the kinetics of lumpy skin disease

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The poxvirus lumpy skin disease virus (LSDV) causes lumpy skin disease (LSD), a severe, systemic transboundary disease in cattle. LSDV is a rapidly emerging pathogen, spreading into the Middle East, eastern Europe and Asia in the past decade, causing substantial production and economic loss to rural communities. LSDV is mechanically transmitted by haematophagous arthropods including stable flies (*Stomoxys calcitrans)*. Cutaneous host responses to arthropod feeding activity have been shown to influence systemic disease following inoculation with a range of biologically transmitted viruses including dengue and west Nile virus. We examined whether this was also true for mechanically transmitted pathogens by studying *S. calcitrans* and LSDV in an experimental bovine model of disease. Three groups of ten cattle were inoculated intradermally with $1x10^6$ pfu of LSDV via multiple intradermal microdoses. Group 1 received virus alone, group 2 received virus mixed with S. *calcitrans* saliva that had been collected from laboratory-reared *S. calcitrans* using an artificial membrane feeding system, and group 3 received virus inoculated into a site that *S. calcitrans* had previously fed on (spot feeding). Fewer cattle in group 2 developed LSD (3/10) compared to group 1 (6/10) and group 3 (7/10), indicating that *S. calcitrans* saliva inhibits LSDV infectivity *in vivo*. Cutaneous lesions and viraemia appeared more rapidly in group 3 compared to group 1, revealing that pre-feeding *S. calcitrans* on the inoculation site accelerates disease kinetics. *S. calcitrans* are therefore more than a simple means for deposition of virus into the skin of a cow. Through the co-incident bite trauma and/or saliva deposition *S. calcitrans* also influence the resultant disease.

Remote identification of mosquitoes: new insights into vector surveillance

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Every year, more than one billion people are infected, and almost one million die as a result of vector-borne diseases (VBDs). Mosquitoes (Diptera: Culicidae) and particularly those belonging to *Aedes*, *Anopheles* and *Culex* genera, are one of the deadliest vectors worldwide. Mosquito species can transmit pathogens that cause diseases such as malaria, dengue, yellow fever, West Nile fever, Zika, Chikungunya and others, which are responsible of hundreds of thousands of deaths every year. Mosquito surveillance is of key importance to monitor tendencies in vector populations, to identify virus circulation and to design risk analysis programs in order to control possible outbreaks of VBDs. Traditional entomological surveillance has always been performed by means of physical traps, which implied the installation and collection of the traps in the field and the subsequent taxonomical identification of the samples in the laboratory by expert entomologists. This routine is expensive in terms of time and professionals involved in the task. Also, there is a time lapse between the moment the trap is set up in the field and the moment the sample is processed, and the data is analysed, thus possibly affecting the proper modelling of the temporal dynamics of the vector. In this context, the emergence of the so-called e-traps introduce the concept of automatic real time monitoring of mosquito populations which is a great advantage in the state of art of mosquito surveillance. Here, we present a novel optical sensor prototype attached to a commercial suction trap which has been trained in laboratory conditions with machine learning techniques for the automatic classification of mosquito genus and sex with high levels of accuracy. The sensor has also been tested in the field for the automatic count of mosquitoes (differentiating them from other arthropods) and the distinction of females and males, again with high accuracy. The strong positive correlation between the real mosquito counts and the predicted by the sensor demonstrates that the sensor may be a realistic complement to traditional surveillance methods and could be introduced in vector surveillance plans in Europe.

The impact of BTV-4 infection on the volatilome of cattle and sheep: a proof of concept.

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Infectious diseases alter the host metabolome either directly through pathogen-induced tissue damage or indirectly through the host immune response. Some metabolites are volatile and these, along with microbiota-associated volatile compounds and environmental contaminants, make up the host volatilome, which is the aura of volatile molecules that surrounds each organism. It is of major interest, yet a major challenge, to elucidate how the emission of these volatile organic compounds (VOCs) changes in response to vector-borne viral infections. From an evolutionary perspective, VOCs contain specific cues that arthropod vectors employ to locate their hosts and these cues could potentially interfere with the transmission of vector-borne pathogens, such as the bluetongue virus (BTV). In mammalian hosts, vector-borne viral infections can be acute and characterized by a defined and short-term period of high viraemia, which raises significant challenges to collecting VOCs in a controlled and repeatable manner from naturally infected hosts across rapidly changing stages of infection and disease. Here, we utilised a well-established BTV infection system of cattle and sheep to establish methodologies to collect VOCs pre- and post-infection from natural mammalian hosts within high containment facilities.

We investigated the following hypothesises: (1) infection with vector-borne viruses can alter the host volatilome in a systematic and repeatable way, that this change is (2) tissue or system-specific related to the pathogen replication site, (3) infection is host-specific related to species level resistance and tolerance to a given pathogen, and (4) varies depending on the stage of infection. We selected a strain of BTV-4 which leads to mostly asymptomatic infection of cattle, while sheep develop typical bluetongue disease. Animals were experimentally infected with

BTV-4MOR2009/07 through the natural infectious route, a bite from infected *Culicoides* midges. One animal was sham infected in each replicate. We captured the volatile compounds emitted by cattle and sheep maintained in a high containment facility using entrainment over two types of sorbent polymers three times before and three times after BTV infection (or sham treatment). We characterized the volatile chemicals emitted using gas chromatography and also thermal desorption-coupled gas chromatography – time of flight - mass spectrometry and we applied different analytical approaches to distinguish infection-related processes from normal volatilome and environmental background. We discuss the challenges of measuring VOCs in high-containment environments. Our two-host experimental approach with poly-systemic sampling provides new insight into BTV infection pathophysiology while highlighting potential future opportunities for non-invasive diagnosis of BTV and for the identification of allelochemicals involved in insect attraction.

Recent genomic epidemiology of western BTV-4 strains in the Mediterranean basin

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Bluetongue (BT) is a non-contagious, viral disease of domestic and wild ruminants caused by bluetongue virus (BTV), an Orbivirus with a double strand RNA segmented genome, mostly transmitted from one animal to another by biting midges (Culicoides spp.). Since 1998, Southern Europe has experienced multiple incursions of different serotypes and topotypes (western (w) or eastern (e)) of BTV. Strains of BTV-1e, BTV-4w, BTV-9e and BTV-16e have all entered the eastern Mediterranean region. In addition, strains of BTV-1w, BTV-2w, BTV-3w and BTV-4w have entered Southern Europe because of wind-driven dissemination of infected midges from Northern African countries. Specifically, the virus had been likely introduced to Europe from Northern Africa via two major gateways: (i) from Morocco to Spain through the Straits of Gibraltar, (ii) from Tunisia or Algeria to Italy and Spain through Sardinia, Sicily, and Balearic Islands, respectively. In recent years (2012-2021), multiple BT outbreaks caused by BTV-4 strains have been notified in several countries facing the Mediterranean basin including Tunisia, Spain (mainland and Balearic Islands), France (mainland and Corsica), and Italy (mainland, Sardinia, and Sicily). In particular, between 2019 and 2021, these outbreaks have caused severe economic losses and a surge in demand for vaccines. However, recent genomic analysis of BTV-4 circulating strains was limited and hampered by the COVID-19 pandemic crisis. Therefore, in the present work, a collaborative effort of several Institutions, operating in these countries and active in Orbivirus surveillance, has been put in place to disentangle the recent molecular epidemiology of BTV-4. To do so, viral RNA was purified from a total of 60 BTV-4-positive biological samples (whole blood, spleen homogenates) collected throughout the years (2012-2021) in Tunisia, France, Italy, Morocco and Spain and sequenced either by NGS or Sanger sequencing to get the genome constellations of the involved strains. The obtained sequences were compared with those available on GenBank. All strains identified in Northern Africa and Southern Europe throughout the years (2012-2021) belonged to the western topotype. Moreover, results suggest that evolution shaped the recent genomic epidemiology of BTV-4w as strains, characterized by different genome constellations and drift mutations, were identified. In regards to recent years, BTV-4w strains collected in 2021 in Italy (mainland and Sardinia), France (Corsica) and Spain (Balearic island) were remarkably close (>99.56 % of nucleotide identity in all genome segments) to homologous strains collected in Tunisia in 2019, 2020 and 2021. These novel BTV-4w, along with a different genome constellation, were slightly divergent in Seg-2 (97.85% of nt identity) with respect to Balkanic BTV-4w strains isolated from 2014 onward in Europe including also recent French (Corsica, 2020) and Italian (Sicily, 2021) BTV-4w strains. The novel BTV-4w differed also from Spanish BTV-4w strains which have circulated in mainland Spain since 2010 as these latter were related to BTV-4w strains collected in Morocco and Tunisia in 2012 and 2013, respectively. Overall, combined results suggest that the novel BTV-4w strains from 2021 had likely originated in southern Europe as a consequence of a novel wind-driven dissemination of infected midges from Northern

African countries. Only combined efforts may disentangle the epidemiology of a transboundary disease such as BT.

West Nile virus lineage 2 overwinters in north-eastern Spain.

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West Nile virus lineage 2 (WNV-L2) first appeared in Europe in 2004; since then, it has spread across the continent, causing significant outbreaks in humans and animals. In Spain, WNV-L2 was first found in 2017 in Lleida province (Catalonia), particularly in a northern goshawk with neurological symptoms. This event indicated the westward expansion of WNV-L2 in Europe, which had previously been limited to the Central/South and Eastern regions of the continent. In 2020 and 2021, WNV-L2 was again detected in Catalonia, in wild birds (most of them northern goshawks with WN disease symptoms) and mosquitos. Complete genome sequencing and phylogenetic studies were carried out to characterize these first Spanish WNV-L2 isolates and to evaluate the possibility of virus overwintering in this Mediterranean location. According to our findings, the Spanish WNV-L2 isolates belonged to the central-southern WNV-L2 clade. In more detail, they were related to the Lombardy cluster that emerged in Italy in 2013 and has been able to spread westwards, causing outbreaks in France and Spain. Our work shows that the WNV-L2 has circulated and overwintered in the region for several years. The circulating WNV-L2 strain is highly pathogenic for northern goshawks, which appear to be highly susceptible to WNV infection, and consequently, may be used as a good indicator of virus circulation. Because the frequency of human and animal cases in Europe has increased in recent years, this zoonotic flavivirus should be closely monitored using a One-Health approach.

SESSION 5 - Pathogenesis and immunology

MERS-CoV internalized by llama alveolar macrophages do not result in replication and induction of inflammatory cytokines

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The Middle East respiratory syndrome coronavirus (MERS-CoV) can cause severe pneumonia and even fatal infection in humans. Severe MERS is characterized by diffuse alveolar damage and acute respiratory distress syndrome, which mainly occur due to the massive infiltration of immune cells into the lungs that exacerbate disease by producing an inflammatory cytokine storm. In fact, MERS-CoV replicates in human monocyte-derived macrophages (MDMs) *in vitro*, which secrete high levels of inflammatory cytokines. In contrast, camelids are the main reservoir of MERS-CoV and their infection cause an asymptomatic infection. The action of robust and timely innate immune responses at the nasal mucosa of camelids is thought to play a key role in MERS-CoV infection clearance. Furthermore, infiltration of leukocytes at the lower respiratory tract has been observed in camelids upon MERS-CoV experimental inoculation, but their role during the infection course remains unknown. The present work aimed to elucidate if llama alveolar macrophages are susceptible to MERS-CoV-2 infection and can elicit a pro- inflammatory response.

Bronchoalveolar fluid lavages were performed after the euthanasia of three llamas. These animals were firstly utilized for MERS-CoV transmission studies; specifically, they were inoculated with MERS-CoV Qatar15/2015 or Egypt/2013 strain three weeks before euthanasia. Alveolar macrophages were isolated and cultured in triplicates with media containing 1 µg/mL PolyI:C, exposed to an M.O.I. of 0.1 of MERS-CoV (the same isolate used for inoculation procedure), or only cultured in media for 48 h. Culture supernatants and cells were collected at 0, 24 and 48 h post viral exposure (hpe). Conventional RT-qPCR and infectious virus titration in cell culture allowed to quantify viral titers, while transmission electron microscopy (TEM) was used to locate MERS-CoV in alveolar macrophages. Microfluidic RT-qPCR was used to detect the expression of pro- and anti-inflammatory cytokines.

MERS-CoV clade B (Qatar15/2015) and C (Egypt/2013) did not replicate in camelid alveolar macrophages, as evidenced by RT-qPCR targeting genomic and subgenomic viral RNA, as well as infectious virus titration in Vero E6 cell culture. Indeed, TEM analyses of cells pulsed with the Qatar15/2015 isolate demonstrated that alveolar macrophages engulf viral particles that are transported to degradation organelles. While no viral structures were observed at 0 and 24 hpe, cells contained virus-like particles inside vesicles, cisternae, vacuoles and dense globular compartments at 48 hpe. Non-exposed cells also contained vacuoles and dense globular compartments but did not contain virus-like particles. Double-membrane vesicles embracing the replication of some coronaviruses inside the cell were not observed at any time. Gene expression analyses of the alveolar macrophages capturing MERS-CoV within cellular compartments showed no evident differences in the transcriptomic levels of type I, II and III interferons, pattern recognition receptors, transcription factors, IFN-stimulated genes, pro- and anti-inflammatory cytokines compared to the cells non-exposed to the virus at any time point of the study.

Overall, we show that alveolar macrophages of llamas are resistant to MERS-CoV infection, although these antigen- presenting cells effectively capture, internalize and degrade viral particles. Moreover, contrary to human MDMs, these cells do not release pro-inflammatory cytokines upon viral sensing.

PPRV infection hinders ovine monocyte-derived dendritic cells maturation: functionality and transcriptomics analysis

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Peste des Petits Ruminants (PPR) is an economically important disease, especially in developing countries, affecting sheep and goats. Peste des Petits Ruminants virus (PPRV) is the causative agent of PPR, closely related to other Morbillivirus like measles, rinderpest virus and canine distemper virus, within the *Paramyxoviridae* family. PPRV targets immune cells, including dendritic cells (DC). Our goal was to assess whether PPRV infection affects the maturation process from immature DC to mature DC, an essential step for the development of adequate adaptive immunity to the infection. To do so, a number of functional assays and RNAseq analysis were performed. Immature monocyte-derived DC (iMoDC) were obtained by a 48-hours differentiation process of positive-selected $CD14^+$ cells (monocytes) from peripheral blood mononuclear cells (PBMCs), employing GM-CSF and IL-4 cytokines. iMoDC were then PPRV- or mock-infected and after 24 hours cultures were transfected overnight with Poly I:C for maturation. PPRV-infected mature monocyte-derived DC (mMoDC) showed an increased expression of CD14, CD11b, CD11c and CD209 cell markers, while CD80, CD86, MHC-I and MHC-II expression levels were reduced, compared to mock-infected mMoDCs. PPRV-infected mMoDC showed as well a decrease in antigen presentation, as detected by allogeneic MLR assays. RNAseq analysis was performed using RNA extracted from PPRV and mock-infected mMoDCs cultures from four different sheep. PPRV-infected mMoDCs showed 453 up- regulated and 179 down-regulated genes compared to mock-infected counterparts. KEGG analysis revealed 31 different up-regulated pathways, including some involving autophagy or mitophagy mechanisms and signalling pathways related to viral infection responses like TNF, mTOR or IL-17 pathways, among others. Taken together, these data indicate that PPRV can target DC maturation to hinder adaptive immunity and thus contribute to the immunosuppressive effects of PPRV infection on its natural hosts.

Comprehensive immune profiling of bluetongue virus infection reveals acute immunosuppressive effects on T cell responses

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Bluetongue is a viral disease transmitted by the bite of infected *Culicoides* midges that affects domestic and wild ruminants. It causes an acute disease with high morbidity and mortality that produces great economic losses. Bluetongue virus (BTV) infection induces an IFN response in the host that is followed by an adaptive immune response that plays a critical role in disease clearance. There is nonetheless evidence that BTV can also impair IFN and humoral responses. The main goal of this study was to gain a more detailed understanding in BTV pathogenesis and its effects on immune cell populations in order to provide potential new targets for interfering with the virus at key points during infection. To this end, we used flow cytometry and transcriptomic analyses of several immune cells at different times post-infection (pi). Four sheep were infected with BTV serotype 8 and blood samples were collected at different times to isolate peripheral blood mononuclear cells (PBMCs). Transcriptomic analysis of B-cell marker$^+$, CD4$^+$, and CD14$^+$ sorted cells showed that the maximum number of differentially expressed genes (DEG) occurred at day 7pi, which coincided with the peak of infection. The maximum DEG in CD8$^+$ cells was observed at day 15pi. KEGG pathway enrichment analysis in B-cell marker$^+$, CD4$^+$, and CD14$^+$ cells indicated that genes belonging to virus sensing and immune response initiation pathways were enriched and day 3 and 7 post-infection. Concomitant to the enrichment in the immune response initiation pathways, we observed an increase in the percentage of non-classical CD14$^+$ CD16$^+$ monocytes, indicating that these cells could be important in the antiviral response. Flow cytometry analysis indicated that CD8$^+$ T cell expanded at day 15pi and ELISpot assays also showed that T cell responses to BTV became detectable by day 15pi. Curiously, T cell exhaustion pathway was enriched in CD4$^+$ cells at day 7pi, while CD8$^+$ cells showed downregulated immune response initiation pathways at this timepoint. We therefore assessed T cell functionality during the course of the infection. ELISpot assays and intracellular cytokine staining demonstrated that, at day 7pi, BTV impaired CD4$^+$ and CD8$^+$ T cell responses to the mitogen concanavalin-A and the potent activation cocktail of PMA and ionomycin. These data indicate that BTV produces an acute inhibition of T cell activation at the peak of replication. These findings identify several mechanisms behind the immune response against BTV, which could help develop better tools to combat the disease.

Deciphering the immune mechanisms underlying cross-protection against African swine fever virus

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Introduction: African swine fever (ASF) currently represents the number one threat for the swine industry. No commercial vaccine is available against the ASF virus (ASFV), and their rational development is hampered by the poor knowledge of ASFV immunity. The experimental use of live attenuated viruses (LAVs) is a valuable tool to analyze ASFV-specific immune responses. In this regard, we previously generated a recombinant LAV, namely BA71?CD2, by deleting the CD2v gene (encoding the ASFV hemagglutinin) from the BA71 virulent strain (genotype I). Notably, BA71?CD2 confers heterologous protection against the genotype II ASFV currently circulating worldwide. Here we used this vaccine prototype to investigate the cross-protective cellular recall response against ASFV.

Methods: Pigs were intranasally vaccinated with BA71?CD2, and blood and submandibular lymph node (LN) samples were harvested three weeks later. PBMC were stimulated *in vitro* with ASFV and their transcriptomic signature was analyzed by RNA-sequencing. Similarly, submandibular LN cells were stimulated *in vitro* with ASFV and analyzed by single-cell RNA-sequencing. The most relevant results from transcriptomic data were further validated by flow cytometry.

Results: Bulk RNA-seq analysis of PBMC revealed a robust Th1 response in cells from BA71?CD2-vaccinated pigs. Concomitant with this adaptive immune response, we also distinguished an innate immune response marked by the expression of macrophage-related inflammatory genes. Immunophenotyping by flow cytometry demonstrated the presence of polyfunctional CD4+CD8+ memory T cells, and an IFN?-dependent inflammatory response mediated by TNF-producing macrophages. scRNA-seq analysis of submandibular LN cells further validated and extended these results: (i) we observed a rapid inflammatory response characterized by the upregulation of interferon-stimulated genes and the Th1 chemokine CXCL10 in several cell subsets; and (ii) we found cytotoxic CD8+ T cells within the cell clusters that were only present or overrepresented in the vaccinated animal. Indeed, flow cytometry analysis of perforin-expressing PBMC revealed the presence of cytotoxic CD4+CD8+ and ?? memory T cells as well as increased levels of nonspecific cytotoxic CD8+ T and NK cells in vaccinated animals after *in vitro* stimulation with ASFV.

Conclusions: Altogether, this study allowed elucidating the complex cellular response associated with cross- protection against ASFV. Our findings represent a step forward in the understanding of ASF immunology and provide important clues on the functional immune mechanisms that should be considered to more rationally design future ASF vaccines.

Identification of differential immune responses to PPRV virulence in infected goats using a multi-omics approach

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Peste des petits ruminants (PPR) is an acute disease of small ruminants caused by a morbillivirus. Clinical observation of the disease in the field has revealed that a wide variability in the effect of the disease across host species and breeds. This difference could depend either on the host or on the virulence of the virus strain. In this study, we used a multiomics approach based on flow cytometry, RNA sequencing and mass spectrometry to identify the immune factors underlying the observed differences in virulence between vaccine, low-virulent, and high virulent PPRV strains used to infect Saanen goats. Assessment of lymphocyte numbers by flow cytometry revealed no decrease in subpopulations during vaccination, whereas there is a transient decrease in CD4+ cells during infection with the low virulent strain. Infection with the highly virulent strain induced a decrease in all lymphocyte subpopulations and these cells carry virus particles, reflecting viremia. The appearance of antibody responses highlighted mechanisms that could potentially induce B cell activation even in the absence of T cells during lymphopenia. Data from RNA sequencing of cells collected at different stages of the disease, analysed by an approach involving blood translational modules, reveal the activation of a stronger innate immune response when animals are infected with the highly virulent strain. However, the IFN-I response is commonly activated by all infections or vaccination. The detection of proteins accumulated in cells during infections or vaccination confirmed the patterns of RNA modulation. Taken together, these results suggest that the induction of a strong innate immune response coupled with replication capacity of a viral strain underlies the development of clinical signs associated with PPR.

Pigs fed SDPP and exposed to ASFV improved the immune responses and the efficiency of live attenuated ASFV vaccine

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Introduction. African swine fever virus (ASFV) causes high mortality in pigs of all ages. Spray-dried plasma (SDP) is used in feed because it benefits growth, gut function and immunity[1]. The main objective of this study was to evaluate the potential benefits of feeding SDP to either naïve (Study 1) or ASFV-vaccinated pigs (Study 2) exposed to direct contact with pigs infected with the Georgia 2007/01 ASFV virus strain.

Material and Methods. Study 1: 24day-old, weaned pigs were assigned to a control or 8% SDP feed treatment group of 12 pigs each and fed their respective diets the entire study. Two pigs (trojans)/group (1:5 trojans:naïve pig ratio) were injected i.m. with 10^3 GEC of ASFV (Georgia 2007/01) and were comingled with the rest of the pigs to simulate a natural rout of transmission. Transmission did not occur by d23 so 3 more trojan pigs/group were prepared as described before to have a second exposure using a 3:7 trojan:naïve pig ratio. Study 1 ended on d35. Study 2: 24day-old, weaned pigs were assigned to a control (n=16) or SDP (n=8) feed treatment group for the entire study. Eight pigs fed the control diet served as trojans. On d24 all naïve pigs were intranasally vaccinated (V) with 2 mL of 10^5 PFU of the BA71?CD2 ASFV vaccine[2]. At d19 post-vaccination (pv) the non-V trojan pigs were ASFV infected as described for study 1 and 2d later 4 trojans/group were placed with V pigs to expose them to ASFV by direct contact. Trojans in both studies were euthanized when clinical ASFV signs developed. Blood, nasal (NS) and rectal swabs (RS) were collected weekly in both studies (d35 study 1; d41 pv study 2). Submaxillary, retropharyngeal, and gastro-hepatic lymph nodes, spleen, and tonsil samples were collected from all pigs at end of both studies. All samples were analyzed by RT-PCR and in study 2 all samples were also analyzed by a new DIVA- PCR to differentiate between ASFV vaccine and wild strain.

Results: In study 1 all pigs fed with SDP enriched diet showed detectable ASFV-specific T-cells in their blood before second in contact challenge, while only 2 responded in the control diet group. After the second trojan exposure at d23, rectal temperature (RT) of control contact pigs increased >40.5?C by d30, but a RT increase was not observed in SDP contact pigs until d34. PCR Ct values in blood, NS, RS, and tissue samples were lower ($P< 0.05$) for control vs SDP contact pigs. In study 2, two pigs/group died because of reasons not related to ASFV, thus 6 pigs/group finished study 2. During the exposure period 4/6 control V pigs did not have fever while the other 2 pigs showed a peak of fever (RT >40.5?C) by the end of study 2. None of the V pigs fed SDP had fever and all were PCR negative in blood and rectal swab. Tissue samples from 5/6 control V pigs were PCR+ at a given time point for ASFV, while 0/6 SDP V pigs were PCR+ for ASFV in tissue samples after contact exposure ($P< 0.05$). As expected, the few PCR+ samples detected before exposure were DNA from the ASFV vaccine (BA71?CD2) strain, but all post exposure samples detected as PCR+ were DNA from the Georgia 2007/01 strain.

Discussion and Conclusions: Under these study conditions contact exposed non-V pigs fed SDP had delayed ASFV transmission and progression, likely by enhanced specific T-cell priming after the first week in contact ASFV- exposure. Confirming this finding, feeding SDP to contact exposed V pigs improved ASFV vaccine efficacy.

Feeding SDP can possibly be a nutritional intervention to improve protection and reduce and delay transmission by direct contact, especially under endemic ASFV situations with less virulent strains.

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SESSION 6 - Diagnostic tools and Disease surveillance

Improving African Swine Fever surveillance using fluorescent rapid tests

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INTRODUCTION

African Swine Fever (ASF) is a highly infectious disease of swine population, 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 a haemorrhagic fever with high fatality rates (95–100%), and it has a huge impact to the industry and animal wellbeing. To date, there are no commercially available vaccines and therefore, ASF control is based on early diagnosis and the enforcement of strict sanitary measures. ASF was first described in Kenia in 1921 and, despite all the efforts carried out, the disease if far from being eradicated. To date, new outbreaks are continuously being reported in different countries around Europe, Africa, Asia, and America.

Due to its fast spread and its high economic impact, access to highly sensitive diagnostic tests is crucial to control the disease. Due to its characteristics: user-friendly, low cost, rapid results, and long-term stability over a wide range of climates, lateral flow assays are one of the most widely used techniques for the point-of-care testing, accelerating the final diagnosis.

In this work, we described the optimization of ASFV detection through the implementation of highly sensitive fluorescent rapid tests for the direct detection of the virus and for ASF surveillance through the detection of specific antibodies.

MATERIALS AND METHODS

Using specific monoclonal antibodies, a fluorescent lateral flow assay has been developed employing europium- labelled nanoparticles. Assay was evaluated using the target antigen p72, one of the most conserved antigens among different AFSV strains, and its analytical sensitivity was determined using spiked-blood samples. For the development of the rapid test for the detection of antibodies, an ASFV antigen was also bounded to europium- labelled nanoparticles. In both cases, commercial colorimetric assays (INgezim ASF CROM Ag and INgezim PPA CROM Anticuerpo) were used as reference. For the reading of assays' result, an UV-lamp or rapid test reader (Pacific Image Electronics) could be used indistinctly.

RESULTS

Both assays were optimized using different particle brands, sizes, and materials. Final assays for both antigen or antibodies tests exhibited a significant increase in their sensitivity compared to the colorimetric assays, reaching up to a 10-fold increase employing recombinant protein or reference sera, respectively. Afterwards, the developed antigen assays were tested with p72-spiked blood samples, detecting 10 ng/ml of antigen in blood without matrix interferences. Antibody assays were evaluated with a reference sera panel. Finally, assays' performance is determined using field samples.

CONCLUSIONS

Rapid tests are of great interest to control epidemics evolution due to their fast diagnosis. Concerning a highly contagious disease like ASFV, its surveillance is important not only for the identification of infected pigs and wild boards, but also for the maintenance of swine industry. The assays described in this work exhibited a high increase in their sensitivity compared to colorimetric assays, what would improve ASFV surveillance through a fast and highly sensitive identification of infected animals, and an active surveillance of the serological status of a given region. Making these assays an accurate tool for the control and tracing of ASF evolution.

FlagDIVA: A novel DIVA test for the Classical swine fever virus FlagT4G vaccine

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Classical swine fever virus (CSFV) causes a viral disease of high epidemiological and economical significance that affects domestic and wild swine. Currently, control of the disease in endemic countries is based on live-attenuated vaccines (LAVs) that induce an early protective immune response against highly virulent CSFV strains. The main disadvantage of these vaccines is the lack of serological techniques to differentiate between vaccinated and infected animals (DIVA concept). The FlagT4G vaccine is a highly promising marker LAV prototype. This vaccine candidate is based on the CSFV Brescia strain, with a mutation in a highly immunogenic epitope in the E2 glycoprotein, as well as the insertion of a FLAG® sequence. FlagT4G has shown to be highly effective, inducing sterilizing immune response as early as 3 days after vaccination. However, it still lacks a reliable DIVA diagnostic test

Here, we describe the development of the FlagDIVA test, a serological diagnostic tool allowing to differentiate animals vaccinated with the FlagT4G candidate from those infected with CSFV field strains. FlagDIVA is an ELISA test based on the use of a dendrimeric peptide construct as a capture antigen. This peptide contains two copies of the wild type sequence of the conserved CSFV epitope in the structural E2 glycoprotein, that has been mutated in the FlagT4G vaccine, accompanied by one copy of an epitope found in the NS2-3.

In order to test the capacity of the FlagDIVA assay to detect antibody response against the FlagT4G, 15 pigs at six weeks of age were vaccinated with FlagT4G and sera samples were collected weekly until 28 days post vaccination (dpv). A boost immunization with the same vaccine dose was also carried out at 18 dpv. Sera samples from these pigs were evaluated for antibodies against CSFV by the FlagDIVA test and a commercial ELISA. Neutralizing antibody response was also assessed by neutralization peroxidase-linked assay (NPLA). The FlagDIVA was also used to evaluate the antibody response in 177 samples from a serum collection, including naïve pigs and animals subjected to experimental infection with different field CSFV strains. All these serum samples had previously been tested for antibodies against CSFV by the commercial ELISA.

Specific anti-E2 antibody response was detected in all the FlagT4G-vaccinated pigs between 13 and 28 dpv by the commercial ELISA. Meanwhile, the FlagDIVA assay did not recognize the antibody response induced in the FlagT4G-vaccinated animals, showing the efficacy of FlagDIVA as a negative-selection DIVA assay. In the samples from the serum collection, the FlagDIVA assay showed similar performance to the commercial ELISA test.

Interestingly, FlagDIVA outperformed the commercial ELISA in antibody detection from samples of pigs infected with low-virulence CSFV strains. However, detection at early times post-infection remains to

be improved. These results show the efficacy of FlagDIVA for detecting anti-CSFV antibodies in infected animals, while it does not recognize the antibody response of FlagT4G-vaccinated animals. Therefore, the FlagDIVA test constitutes a valuable DIVA tool in implementing vaccination with the FlagT4G candidate.

Environmental sampling as a surveillance tool for transboundary animal diseases in Nigeria

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Environmental sampling enables disease surveillance beyond regular investigation of clinical cases, extending available data on the circulation of a pathogen relevant to a specific area. Viruses can be shed into the environment via the secretions and excretions of infected animals. Whilst the contaminated environment can represent a transmission risk, it also provides the opportunity for non-invasive, low-cost sample collection methods which can be implemented to supplement diagnostic and surveillance efforts.

A longitudinal study was conducted, and electrostatic dust cloths were used to swab the environment (e.g. food troughs, hard floor surfaces, boots and ropes) at livestock markets, households and transhumance sites. Swabs were collected monthly from four local government areas (Bassa, Jos South, Wase and Kanke) in Plateau State, Nigeria, where different transboundary diseases affecting livestock are endemic. A total of 476 samples were collected, stored in lysis buffer and transported to The Pirbright Institute (UK) for analysis. The samples were tested for the presence of three transboundary viruses of livestock: foot-and-mouth disease virus (FMDV), peste des petits ruminants virus (PPRV) and capripox viruses (CaPV) using real time PCR. FMDV RNA was detected in 6% (n=28) of samples, with the majority of positive samples coming from Jos South (17/28). PPRV RNA and CaPV DNA was detected in 2% (11/476) and 1% (6/476) of samples, respectively. Sequence data will be generated from the FMDV-positive samples using probe enrichment techniques.

The methods presented here provide an opportunity for large scale surveillance of livestock diseases which could improve capability for the rapid detection of outbreaks and provide important information regarding virus circulation in an area.

WGS- versus ORF5-Based Typing of PRRSV: A Belgian Case Study

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of one of the most widespread and economically devastating diseases in the swine industry. Typing circulating PRRSV strains by means of sequencing is crucial for developing adequate control strategies. Most genetic studies only target the highly variable open reading frame (ORF) 5, for which an extensive database is available. In this study, we performed whole-genome sequencing (WGS) on a collection of 124 PRRSV-1 positive serum samples that were collected over a 5-year period (2015–2019) in Belgium. Our results show that (nearly) complete PRRSV genomes can be obtained directly from serum samples with a high success rate. Analysis of the coding regions confirmed the exceptionally high genetic diversity, even among Belgian PRRSV-1 strains. To gain more insight into the added value of WGS, we performed phylogenetic cluster analyses on separate ORF datasets as well as on a single, concatenated dataset (CDS) containing all ORFs. A comparison between the CDS and ORF clustering schemes revealed numerous discrepancies. To explain these differences, we performed a large-scale recombination analysis, which allowed us to identify a large number of potential recombination events that were scattered across the genome. As PRRSV does not contain typical recombination hot-spots, typing PRRSV strains based on a single ORF is not recommended. Although the typing accuracy can be improved by including multiple regions, our results show that the full genetic diversity among PRRSV strains can only be captured by analysing (nearly) complete genomes. Finally, we also identified several vaccine-derived recombinant strains, which once more raises the question of the safety of these vaccines.

New insight on highly pathogenic avian influenza H5 viruses environmental dispersion and surveillance strategies

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Avian influenza is a viral airborne disease caused by influenza A viruses, segmented negative single-stranded RNA viruses part of the *Orthomyxoviridae* family. Wild aquatic birds are considered as the virus reservoir and their winter migration can occasionally lead to highly pathogenic avian influenza (HPAI) panzootics. HPAI epizootics are characterized by high mortality rates in wild and farm birds causing major economic losses for the poultry industry and threat to public health due to their zoonotic potential. Even with past HPAI outbreaks, little is known about farm- to-farm transmission which is fast and happens despite drastic biosecurity measures. A better understanding of virus transmission as well as the development of novel surveillance and early detection strategies are essential to limit viral dispersion.

Therefore, to study HPAI virus environmental dispersion, we sampled dust (using wipes) and aerosols (with two aerosol collectors) during both 2020/2021 H5N8 and 2021/2022 H5N1 HPAI France epizootics. Sampling was performed in more than 70 HPAI virus-infected or suspected animal houses, in addition to tracheal swabs collected for official surveillance. All samples were analyzed by RT-qPCR using officially-approved commercial kit to compare sampling detection sensitivity by class latent model. Model outputs suggest that dust sampling enables higher detection sensitivity in non-clinically HPAI-infected flocks while dust and aerosol samples present equivalent detection sensitivity in clinically flocks. In addition, egg isolation assays confirmed the presence of infectious particles in all environmental samples. These findings make dust and aerosols reliable for early detection of HPAI infection and highlight their role in environmental dispersion.

In order to further investigate the viral shedding and environmental dispersion in the course of infection, two groups of conventional mule ducks, known to be highly susceptible to HPAI viruses, were housed in biosafety level 3 animal facilities and experimentally inoculated with H5N8 HPAI 20216/2017 and 2020/2021 viruses, respectively. Environmental samples (aerosols, dust and drinking water) were collected at 1, 2, 3, 4, 5, 7, 10 and 14 days post- challenge (DPC) alongside feather, oropharyngeal, cloacal and conjunctival swabs at 2, 4, 7, 10 and 14 DPC. Viral RNA was detected from environmental samples (aerosols or drinking water) as early as 1 DPC for the H5N8 HPAI 2020/2021 virus. The H5N8 HPAI 2016/2017 virus from aerosol and dust sampling was detected from 2 DPC onward. These results indicate that viral shedding and environmental dispersion happened extremely quickly after the birds' inoculation. The overall RNA viral load in all environmental samples were in line with pathological findings and individual swab results. Finally, viral RNA from both viruses was detected in all the collected samples until the end of the experiment with relative consistency from 10 to 14 DPC.

Altogether, field and experimental data suggest (1) that dust and aerosols seem to play a major role in the environmental dispersion of HPAI viruses, and (2) the high potential of environmental (dust, aerosols, water) sampling for early detection of HPAIV and surveillance. These non-invasive sampling strategies are faster, easier and at least as sensitive as the officially-approved method. Environmental surveillance strategies based on dust and aerosols could be a valid alternative, as they can assess the presence of the virus in animal houses at very early stages of infection, even before clinical signs appear.

Comparison of diagnostic techniques for the detection and quantification of SARS-CoV-2 in different experimental studies

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Animal models have proven instrumental in the characterization and understanding of human diseases, COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) being no exception. Rodents have been some of the most used organisms of choice for the study of this novel disease under experimental conditions. For these studies, different laboratory techniques to detect and quantify the virus have been used in the literature, but there is not a side-by-side comparison of their performance. Therefore, the objective of this study was to assess the degree of agreement between different diagnostic techniques for the detection of SARS-CoV-2 (RT-PCR detecting genomic and subgenomic RNA [gRNA and sgRNA], viral titration [VT] and immunohistochemistry [IHC]) performed on samples from golden Syrian hamsters experimentally infected with SARS-CoV-2.

Results from 240 hamsters (125 females, 115 males) corresponding to multiple experimental studies were considered. All animals were between 5 and 13 weeks of age at the beginning of the experiments and were intranasally inoculated with a dose of 10^3 to $10^{5.2}$ $TCID_{50}$/animal depending on the study of SARS-CoV-2 D614G variant. In all studies, animals were sacrificed either at 2-, 4-, or 7-days post infection (dpi). At necropsy, samples from oropharyngeal swabs (OS), nasal turbinates (NT) and lungs were collected gRNA and sgRNA were quantified by RT-qPCR. The presence and quantity of replicating viral particles were assessed by VT. The presence of the nucleoprotein antigen (NP) was detected by IHC.

A non-parametric correlation test was performed to determine the agreement between results obtained with the different techniques, first including data from all time points, and then considering different time points separately. The variables included in the analysis were the Ct values for gRNA and sgRNA, the $\log_{10}$ of the $TCID_{50}$ for VT, and the IHC semi-quantitative score for the determination of viral antigen in tissues.

Analysing the entire dataset, we observed a statistically significant correlation between most of the results obtained with the different techniques, with the exception of VT from OS, which did not correlate with sgRNA from any of the tissues, nor with the IHC scores assigned to lung samples. On the other hand, VT from OS did correlate with VT obtained from both NT and lung samples. Similar results were obtained analysing male and female animals separately. When considering the different time

points at which animals were sacrificed, we observed that the correlation between different techniques were not always maintained, and that their agreement changed with time and/or animal sex. Interestingly, fewer results show correlation at 2 or 4 dpi, while most of the agreements were restored in animals sacrificed at 7 dpi. In particular, the correlation between VT from OS and the results obtained from other tissues or techniques was lost 2 dpi. On the other hand, the correlation between VT obtained from NT samples and the gRNA quantified in the same tissue was maintained across all considered time points, even when analysing male and female animals separately.

SESSION 7 – African Swine Fever

Biological and Genetic Characteristics of non-HAD genotype II- ASFV Isolated in the EU (2018-2020)

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African swine fever (ASF) is considered one of the most devastating diseases of pigs and wild boar. Since its introduction in 2007 in Georgia, the virus has spread throughout Eastern Europe, affecting both domestic pigs and wild boar. The first occurrence of ASF was reported in the EU in 2014 and since then, numerous countries have been affected by ASF that continues to be reported in 16 countries (during 2020/2022). Belgium (event resolved in March 2020) and Czech Republic (event resolved in April 2018) have managed to eradicate the disease. In August 2018, the virus leapt to China (People's Rep. of), which represented the first occurrence of ASF in Asia. Since then, the disease continued to spread in the Region, affecting 17 countries as of 2022. In September 2019, the first occurrence of ASF in Oceania was reported by Timor-Leste, followed by Papua New Guinea (March 2020), and recently in Thailand in 2022. And in July 2021 the disease reappeared in the Americas after an absence of almost 40 years, having been introduced in Dominican Republic and later in Haiti. In January 2022, ASF genotype II was notified on the Italian mainland after around 40 years of absence. ASF currently considered the greatest threat to the global pig industry.

The causal agent, the African swine fever virus (ASFV), the only member of the *Asfaviridae* family, is a DNA virus with a size that varies between 170 and 193 bp depending on the isolate. Genetically, ASFVs are separated into 24 genotypes, classified by the sequencing of a small portion of the p72 protein. Genotype II is responsible for the current epidemic wave of ASFV in Europe, Asia and recently in America. These wild-type ASFVs cause very acute disease with almost 100% mortality in affected countries. However, both in the EU and in China, the presence of moderately virulent or attenuated viruses has emerged over time, capable of inducing subacute, chronic or even subclinical forms, difficult to recognize in the field (Gallardo et al., 2019, 2021; Sun, Zhang, et al., 2021). This makes ASF control and eradication programs difficult due to the existence of infected animals that cannot be detected clinically. The emergence of these viruses makes molecular and serological diagnosis and control of ASFV more complex and challenging.

In the present study, we describe the genetic and biological properties of nine non-haemadsorbing (HAD) genotype II ASFVs isolated from wild boar in the EU in 2018, 2019 and 2020. A common feature in non-HAD viruses is mutations in the *EP402R* gene (which encodes the CD2v protein). We found that all nine non-HAD ASFV isolates had different types of mutations or deletions in the *EP402R* gene that prevent the viruses from translating the intact CD2v protein and result in a non-HAD phenotype, which is consistent with the results of the analysis of virus isolation. To determine whether the natural mutations and deletion in the *EP402R* gene attenuate the field isolates from the EU, 3 of these non-HAD viruses were tested in domestic pigs, two from Poland and one from Latvia. Non- HAD ASFV isolates caused sub-acute or chronic disease, or even some pigs remained asymptomatic. Disease signs, lesions, viremia, excretion, contact transmission, and viral loads in different organs and tissues were assessed. The results were compared with those obtained with two non-HAD strains isolated in 2017 in Latvia (Gallardo et al., 2019; personal communication 2021). Implications of these disease parameters for early detection and transmission of disease are discussed in this study.

Intranasal immunization with BA71?CD2 confers solid cross-protection against direct-contact ASFV experimental challenge

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Introduction: African swine fever (ASF) is number one threat to the swine industry worldwide. In the absence of commercial vaccines against the ASF virus (ASFV), early diagnosis and slaughtering of affected and in-contact animals is the OIE-recommended measure to control virus spread. So far, experimental vaccines based on inactivated ASFV and subunit vaccine formulations have failed to induce solid protection. Conversely, and despite their inherent biosafety issues, live attenuated viruses (LAVs) are the most advanced vaccine prototypes available today. Previous collaborative work demonstrated the ability of BA71?CD2, a recombinant live attenuated ASFV lacking the CD2v gene (the ASFV hemagglutinin of the genotype I BA71 virulent strain), to confer experimental protection against intramuscular challenge with homologous and heterologous virulent ASFV strains, including the genotype II pandemic virus.

Methods: Aiming to optimize the vaccine efficacy of BA71?CD2, here we tested its immunogenicity after intranasal delivery, and we evaluated its cross-protective efficacy against an experimental challenge mimicking natural conditions. Three groups of six pigs each were intranasally inoculated with three different doses of BA71?CD2 [10^6, $3.3x10^4$ and 10^3 plaque forming units (pfu) per pig], and three weeks later animals were challenged by direct contact with pigs infected with the virulent Georgia2007/1 strain (genotype II). A group of unvaccinated pigs served as control.

Results: All unvaccinated animals died during the second week after ASFV exposure showing clinical signs characteristic of ASF. In contrast, vaccinated pigs were protected in a dose-dependent manner. All the animals receiving the high vaccine dose and five out of six pigs receiving the intermediate dose were protected from the lethal challenge without showing major clinical signs. In the case of the low dose, only half of the pigs survived, and the surviving animals were only partially protected. Surviving animals vaccinated with the high and intermediate doses controlled virus expansion as demonstrated by the lack of high virus titers in sera and nasal cavities after challenge. As expected, the vaccine-induced systemic AFSV-specific humoral and cellular responses were also dose-dependent. All surviving pigs vaccinated with the high and intermediate doses showed high levels of virus- specific antibodies as well as IFN?-secreting peripheral blood mononuclear cells (PBMC) responding to *in vitro* stimulation with both the BA71?CD2 and Georgia2007/1 ASFV, and overall vaccine-specific immune responses correlated with protection.

Conclusion: Intranasal immunization with BA71?CD2 confers a dose-dependent cross-protection against ASFV lethal challenge by direct contact. Furthermore, immunity induced by intranasal

vaccination with BA71?CD2 proved effective for the control of ASFV spread. Although field implementation will require biosafety guarantees, BA71?CD2 is a promising ASF vaccine candidate to be used in the near future.

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

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

First, we used an unbiased screen to search for cellular partners of 18 viral proteins considered as important virulence factors. 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, KRT15, CENPF) and the innate immunity (COPA, TNIP2, TRIM7, CALCOCO2).

In parallel, we were interested in the ability of ASFV proteins to individually inhibit the IFN-I pathway. For this purpose, we have screened 105 ASFV proteins using an IFN-luciferase reporter gene system. We showed that at least five viral proteins (I267L, MGF360-11L, DP96R, MGF505-3R and R298L) contribute to the inhibition of the IFN-I induction pathway. In order to characterize their antagonist effect, an original 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 and TRIF as new putative targets of ASFV proteins.

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

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African Swine Fever in Italy: Fighting the virus in different scenarios.

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Introduction and Objectives

The current African Swine Fever (ASF) epidemiological wave, caused by genotype II virus, reached Europe in 2014 and spread westward. Likely, the human factor allowed this virus to jump in mainland Italy. The first ASF virus (ASFV) positive wild boar was detected in the North West Italy on January 2022. At present the infected zone involved 2,811 km² and more than 100 municipalities by February 2022, across Liguria and Piedmont regions, covering a mountainous landscape and the urban territory as well. Indeed, Italy has been experiencing ASF since 1978 in Sardinia, where genotype I virus reached the island and became endemic, involving 3 animal populations: domestic pigs, wild boar and illegal free-range pigs. However, the goal of eradication is closer, due to efforts of authorities. Our aim is to describe the current ASF unique epidemiological scenario in Italy.

Material and Methods

The national surveillance plan for ASF early detection, in force since 2020, requires carrying out passive surveillance activities in the wild boar population and in domestic pig farms, verifying the implementation of biosecurity measures, applying management measures for wild boar population and performing an awareness campaign. Following the notification of the Italian index case, the national expert group to prevent and control the ASF in wild boar identified the infected zone as provided by EU legislation. Here slaughtering in backyard farms, reinforcement in applying biosecurity measures, active research/removal of wild boar carcasses, hypothesis for fencing and considerations about eradication plan have been carrying out. At the same time, in Sardinia a straight eradication plan, in place since 2015, focused on measures dealing with all swine populations and on the re- organisation of command chain; moreover, animal welfare conditions, reproductive parameters and biosecurity measures were evaluated.

Results

The passive surveillance activities allowed detecting the first ASFV incursion in mainland Italy. Implementation of control measures in the North Italy infected zone has been prompt, due to collected knowledge acquired from the application of ASFV national surveillance plan. Finally, in Sardinia, eradication efforts allowed a decrease in virus circulation: the last RT-PCR positive kept pig, illegal free-range pig and wild boar were in September 2018, January and April 2019, respectively. Illegal free-range pigs have been massively depopulated. By joining welfare requirements with sanitary ones, the epidemiological situation improved, rendering the goal of eradication possible.

Discussion and Conclusions

Currently Italy is the only country out of Africa where ASFV genotypes I and II co-exist, with different phases of the infection dynamics. In mainland Italy, the initial virus spread in a susceptible wild boar population is on-going (invasion phase), with probably a high number of still unobserved cases.

Unfortunately, the long distance between detected cases, size of infected area and its geographical characteristics are not favourable conditions to control ASFV spread in the wild boar population. In Sardinia, a new strategy is in force, by applying passive surveillance activities in order to demonstrate freedom of infection. Sardinia could be the paradigm case for ASF eradication in a not well-disposed socio-cultural context, after some decades of endemic. Italy is dealing with a double ASF challenge, to control the disease spread in mainland Italy and to eradicate the disease in Sardinia Island.

Host factors influence the severity of disease caused by attenuated African swine fever virus

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The immune response to African swine fever virus (ASFV) infections is still poorly understood. With the long-term goal of identifying host factors influencing the immune response to ASFV, we established a baseline of viral characteristics and host responses in pigs of different hygiene and immune status. For this purpose, Large White SPF pigs with a naïve immune system bred at the IVI and conventional Large White farm pigs were infected with either a highly virulent (Armenia 08) or an attenuated (Estonia 14) ASFV isolate. Body temperature and clinical scores were monitored daily. Whole blood, serum, rectal and nasal swabs were taken at different times post- infection and the full blood count, viral load and cytokine profile were analyzed. Samples from selected organs were taken at necropsy for qPCR and titration of virus.

At steady state, SPF pigs showed higher platelet but lower white blood cell counts and reduced basal inflammatory and antiviral activity in circulating leukocytes. There were also profound differences in the microbiota composition between the two groups. All animals infected with the virulent Armenia 08 isolate showed severe disease and had to be euthanized on day 7 post-infection according to the discontinuation criteria, with no significant difference in clinical score, body temperature and viremia between the two types of pigs. In contrast, during infection with the attenuated Estonia 14 field isolate, SPF pigs presented a milder and shorter clinical disease with full recovery and seroconversion, whereas farm pigs had a more severe and prolonged disease, and some of them had to be euthanized. Farm pigs showed higher production of inflammatory cytokines, whereas SPF pigs produced more anti-inflammatory cytokines early after infection with the Estonia 14 virus. Altogether, our data indicate that the immune status and hygienic background have a major influence on the severity of ASF. While a higher baseline innate immune activity helps the host in reducing initial replication of a highly virulent ASFV causing acute disease and early mortality, it promotes immunopathological inflammatory cytokine responses, and delays lymphocyte proliferation when infection occurs with a less virulent virus causing acute to chronic ASF. Such effects should be considered for live attenuated vaccine development.

Challenging the Genetic Stability - Emerging African Swine Fever Virus Variants in Germany

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Since its introduction into Georgia in 2007, the African swine fever virus (ASFV) incessantly paved its way through numerous European countries, threatening domestic and wild suid populations likewise. During this period, the ASFV genome, a double stranded DNA molecule of 190 kbp, maintained a high genetic stability compared to other viruses that prevented any meaningful genomic epidemiology. With the introduction of ASFV into Germany in 2020, however, the situation changed. By now, at least ten distinct ASFV variants were identified via whole-genome sequencing revealing point mutations and indels affecting several viral genes, and the numbers keeps raising.

Based on these findings, a variant-specific Sanger sequencing routine was implemented for a fast and feasible assignment of virus variants, revealing the formation of regional ASFV variant clusters in Germany and enabling the tracing of outbreak strains. Moreover, first biological studies were conducted to evaluate the pathogenic impact of the virus variants compared to the original ASFV GT II wildtype strain from 2007. In order to identify key drivers for the unprecedented increase of emerging ASFV variants, investigations are focused on a potential mutator-gene coding for a truncated variant of the ASFV DNA polymerase X. Therefore, structural modeling was performed to assess the influence of the observed mutations on the enzyme's catalytic functionality. Although the connection between the mutated ASFV polymerase X gene and the emergence of virus variants remains to be proven, the herein presented data support the hypothesis of an increased mutation rate leading to the observed ASFV cluster in Germany. In conclusion, this variant emergence is a worrying development and the situation must be monitored closely.

SESSION 8 – Risk Assessment and Epidemiology

Transdisciplinary research to tackle current and future pandemic risks at the site of probable emergence

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The emergence of SARS-CoV-2 as a One Health threat has shaken the whole planet. Even though scientists have notoriously predicted such an event for several years, policy makers worldwide were still caught more or less unprepared. Thanks to immense scientific and financial efforts, especially high-income countries were able to develop and distribute vaccines at an unprecedented velocity. In contrast, middle- to low income countries, including Nigeria the most populous country on the African continent, are still facing enormous difficulties in obtaining vaccines for their population. Concomitantly, Nigeria is regarded as an emergence hotspot of zoonotic pathogens, not least because of the progressing human encroachment leading to broad interfaces between wildlife, domestic animals and humans. These may give SARS-CoV-2 as well as so far unidentified zoonotic pathogens the opportunity for spilling back and forth between human and animal hosts, possibly leading to altered variants with unknown pandemic potential.

Identifying and understanding processes at the human-animal interface in regards to the risk of bidirectional transmission and the role of environment on those viral exchanges is key to pandemic preparedness and response. In a joint effort, an international and national team of anthropologists, physicians and veterinarians will exemplarily bring a rural and an urban community settings located in Nigeria into focus. Radiant from households of previously SARS-CoV-2 infected health care workers, the multispecies networks of contact interfaces in the community will be explored and investigated under epidemiological and socio-cultural aspects. Swabs and serum samples from domestic animals, wildlife as well as environmental samples will be taken and analysed for the presence of SARS- CoV-2 or former exposure to it. Interactions with people through interviews and participatory observation will capture potential drivers for contamination. All results will be jointly interpreted and are supposed to lead to a broadly adopted risk communication adapted to the people's actual perception and needs.

Here, we would like to present the general multi-sectoral and holistic study approach and give insights to the preliminary results results obtained during the first field mission in spring 2022.

Mapping the Risk of Spread of Peste des Petits Ruminants in the Black Sea Basin – a Knowledge-driven Approach

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Peste des Petits Ruminants (PPR) is a highly contagious viral disease affecting small ruminants and threatening farmers' food security and sustainable livelihoods across Africa, South East Asia, and the Middle East. After the successful eradication of Rinderpest, PPR was targeted as the next animal disease to be eradicated under the PPR Global Control and Eradication Strategy (PPR GCES) coordinated by FAO and OIE. Nonetheless, in the last two decades, PPR has continued spreading, including into countries where it had never been reported and in others that had already been recognised as PPR free. This happened in the Black Sea Basin where PPR became endemic in Turkey and emerged for the first time in Georgia and Bulgaria, in 2016 and 2018, respectively. These and other occurrences confirm the transboundary nature and rapid spread of PPRV, which has led to increasing concerns over the reintroduction of PPR into Europe. In this project, we aim to identify areas with a high risk of PPR spread within the Black Sea region (including Armenia, Azerbaijan, Belarus, Bulgaria, Georgia, Moldova, Romania, Ukraine and Turkey). Our ultimate goal is to support decision-makers in the implementation of prevention, control and surveillance activities, in those areas at a higher risk of disease spread, thus facilitating more efficient risk- based use of resources. In this study, we applied a knowledge-driven approach (Spatial Multi-Criteria Decision Analysis) to identify the most suitable areas for PPR spread in the region and produce risk maps. This method uses the opinion of experts (personnel with knowledge of small ruminant production in the country and/or PPR epidemiology) to weigh the importance of risk factors (RFs). To gather such information, we opted for the elicitation of a pair-wise comparison matrix. Firstly, we conducted an extensive literature review to identify the RFs for PPR spread within the study region and, jointly with FAO and PPR experts, defined the most relevant RFs to include in the pair-wise comparison matrix. The selected RFs included: small ruminant density, smallholder/commercial farming, movements to seasonal pastures, proximity to animal markets, proximity to previous (or current) outbreaks and PPR Monitoring and Assessment Tool score (a companion tool of the PPR GCES) as a proxy for the technical capacity of countries to combat PPR. Spatial data for each of the RFs were gathered by national consultants from each study country and the FAO database FAOSTAT. In a regional PPR workshop organized by the FAO Regional Office for Europe and Central Asia and FAO members of the PPR Secretariat, participants from the animal health sector and PPR epidemiology experts filled in the RFs pair-wise comparison matrix. Each of the 25 matrices obtained was assessed for its consistency, the resulting values were standardised and the RF weight was calculated. Corresponding geographical data and RFs weights will be combined based on a weighted linear combination to obtain PPR spread suitability maps. This will follow the application of sensitivity analysis and further results validation. The final outcomes of this study will be presented at the conference. We believe the resulting risk maps will provide a decision support tool for the implementation of disease surveillance and control measures within this region thus contributing to the PPR eradication goal of OIE and FAO and global partners by 2030.

Low prevalence of hepatitis E virus in the liver of Corsican pigs over 12 months of age

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Hepatitis E virus (HEV) infection can be acute and benign or evolve to chronic hepatitis with rapid progression toward cirrhosis or liver failure in humans. Hence, Hepatitis E (HE) disease is a major public health concern. In countries where pig populations are highly contaminated with HEV, cases of HE are mainly foodborne, occurring frequently after consumption of raw or undercooked pork products. In fact, the prevalence of HEV RNA in pig livers entering the food chain can reach up to 43%. Among factors associated to the presence of HEV in pork livers in intensive rearing systems, early slaughter (?6 months) seems to be a major factor. In Corsica, local breed of pigs are raised in extensive free-range breeding systems and pigs are slaughtered at later age (>12 months).

In order to evaluate if slaughter of pigs over 12 months reduces the risk of HEV presence in livers used for human consumption, 1 197 samples were collected at the slaughterhouse. Presence of HEV RNA was analyzed and HEV seroprevalence was determined in paired serum. The sampling included 1,083 livers from animals between 12 months and 48 months of age and 114 livers from younger animals (< 12 months). The samples were in majority from semi-extensive and extensive farms (n= 1 154). Estimated HEV seroprevalence was high, > 88% and HEV RNA prevalence in adult pig livers (>12-month-old) was very low 0.18 %. However, in livers from younger animals (< 12 months), including piglets below 6-month-old, 5.3% (6/114) of the samples were positive for HEV RNA. Sequences recovered from positive livers belonged to HEV genotype 3c and 3f. The presence of infectious HEV was confirmed by virus replication in HepaRG cells.

Thus, this study, demonstrates the low prevalence of HEV in livers of pigs over 12 months, even in farms with high HEV circulation. This observation may open new perspectives on the preferential use of livers from older animals for production of raw pork liver products as well as for improving pig farming systems.

Risk analysis at farm-level: a tool to improve biosecurity applied for Tuberculosis in cattle

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On-farm biosecurity plays a key role in reducing the risk of introduction and spread of infection diseases. The adoption of biosecurity measures (BSMs) can be beneficial to prevent exotic diseases, but also diseases affecting production, or other endemic diseases subject to control programs, as in the case of bovine tuberculosis (bTB).

Nevertheless, in the cattle industry, the implementation of BSMs remains limited, especially in extensive, pasture- based, or organic systems. The objective of this study was to develop a quantitative risk assessment model to identify the most effective BSMs in reducing the risk of bTB introduction into extensive beef cattle farms. To do so, the risk pathway proposed by Benavides et al. (2020) for animal introductions were adapted to the epidemiological characteristics of bTB. The probability of bTB introduction through this pathway was calculated considering the characteristics of each studied farm and the already implemented biosecurity measures. Farm-level data were collected through a face-to-face questionnaire. Moreover, to identify BSMs that should be prioritized in each farm to reduce such risk, the model was run under several hypothetical scenarios, assuming the implementation of additional BSMs. Measures considered within the model were selected among those recommended against bTB, which were identified through a narrative review. Estimations of the impact of the additional BSMs on the farm- specific risk were obtained by calculating the risk reduction before and after their application. This allowed to identify what measures should be implemented to get a significant risk reduction. The stochastic analysis was performed using the mc2d package in R, performing 10,000 iterations of Monte Carlo simulations. All non-fixed parameters were included as uncertain parameters. The model was tested on 14 farms from North-East Spain (Catalonia). The estimated annual risk of bTB introduction into the herd by purchasing cattle was low for most of the farms, with an overall median value of 0.02%. However, it was highly variable among farms, ranging between 7.80×10^{-11}% and 0.13% (lowest and highest median risk, respectively). In most of the farms, recommended biosecurity measures to reduce the risk were a combination of quarantine together with adequate isolation and testing, especially if cattle had not been tested before transport. This analysis will be extended to other pathways (i.e., movements to pastures, interaction with wildlife, interaction with neighbouring infected farms, etc) to obtain a quantitative characterization of the risk of bTB introduction at farm-level and the identification of most critical points, prioritising the BSMs that should be implemented to mitigate such risk. The developed model can be a powerful tool to optimise the risk management on farms and support the development of farm-specific biosecurity plans.

Moreover, it can contribute to educate and raise awareness on the benefits of BSMs by demonstrating the quantitative impact of their adoption.

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The incursion risk of African swine fever for The Netherlands by human-mediated routes

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African swine fever (ASF) is a viral disease with serious health effects for both domestic pigs and wild boar. ASF was introduced in Georgia in 2007, and subsequently it spread to Europe and Asia. So far (January 2022), ASF has not been detected in pigs or wild boar in The Netherlands, but the risk of introduction is deemed to increase with more countries in Europe reporting ASF outbreaks. Although local extensions of infected areas can be attributed to movement of infected wild boar, new outbreaks at larger distance are usually linked to human contacts. More insight into the potential introduction routes of ASF in new areas at larger distances from known infected areas, especially via human action, is needed to take effective measures to prevent introduction of ASF. The current study was initiated with the aim to assess the risk of exposure of domestic pigs and wild boar in The Netherlands, via four human-mediated introduction routes: (1) Hunters hunting in ASF-infected countries and returning with a wild boar carcass or meat, (2) travelers taking pork products from ASF-infected countries, (3) truck drivers discarding pork products from ASF-infected countries on parking places, and (4) farm workers at pig farms carrying pork products from ASF-infected countries. A quantitative risk model was built, that estimated the expected annual number of entries of ASF-infected pork products into The Netherlands for each introduction route.

Furthermore, the model estimated the expected annual number of exposures of domestic pigs and wild boar to these ASF-infected materials. Results of the model indicate which introduction route is most important for introduction of ASF and which pig populations are most at risk. The model can also be used to estimate the effectiveness of preventive measures.

First results show that truck drivers and travelers pose a higher risk for entry and exposure of ASF in the Netherlands than farm workers and hunters. The majority of the exposures is expected in wild boar. Some input parameters in the model had high uncertainty, resulting also in high uncertainty on the estimated values for the number of ASF entries and exposures in the Netherlands. Uncertainty analysis indicated that the model output was, however, robust in ranking the four introduction routes. We therefore conclude that the model is a useful tool to prioritize introduction routes for risk management.

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Pathotyping and genotypic characterization of avian orthoavulavirus 1 viruses in Denmark

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Newcastle disease is a notifiable disease in poultry. It is caused by infection with virulent forms of avian orthoavulavirus 1 (AOaV-1), also known as Newcastle disease virus (NDV). Based on the severity of clinical disease in chickens, the disease caused by AOaV-1 appears in a lentogenic (mild), mesogenic (moderate) or velogenic (very virulent) form. AOaV-1 viruses cause infection in a range of wild birds and poultry worldwide, and the velogenic strains can cause up to 100% mortality in poultry and is therefore a serious threat to animal welfare and poultry production. Even lentogenic strains are potentially pathogenic as they may mutate and become velogenic when infecting poultry. Many countries, including Denmark, use mandatory vaccination to control Newcastle disease. However, there is an increasing concern that the commonly used vaccines may not be effective against AOaV-1 strains emerging in Europe. Surveillance and knowledge of currently circulating strains is therefore of outmost importance in order to predict and prevent new outbreaks.

In this study, we pathotyped and genotyped AOaV-1 viruses isolated from wild and domestic birds in Denmark during the last decade. The viruses were identified in samples from apparently healthy wild birds as part of the national avian influenza virus wild bird surveillance program and from farmed ducks. The viruses were isolated in 8-10-day-old embryonated chicken eggs by inoculation of cloacal swab material that tested positive for AOaV-1 by real-time RT-PCR. RNA from the isolates was extracted and the full-length fusion (F) gene was amplified by RT- PCR and sequenced using the Illumina MiSeq platform. The viruses were genotyped by phylogenetical analysis of the F-gene sequence and pathotyped according to their F-gene cleavage site sequence. Preliminary results show that Danish AOaV-1 viruses are avirulent strains belonging to Class 2 genotype I.2 and II. Genotyping and pathotyping of further Danish AOaV-1 viruses is ongoing to characterize both previously and currently circulating Danish strains.

FLASH PRESENTATIONS AND POSTERS

TOPIC 1 - Emerging and re-emerging diseases

1. Experimental infection of pigs with recent European porcine epidemic diarrhea viruses

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Porcine epidemic diarrhea virus (PEDV), belonging to the genus *Alphacoronavirus*, can cause disease in pigs of all ages, and especially in suckling pigs. However, differences in virulence have been observed between various strains of the virus e.g. the INDEL strain characterized by the presence of certain insertions and deletions in the spike (S) protein gene, compared to the non-INDEL strain (Vlasova et al., 2014) and between outbreaks with the same strains.

Previously, we have described experimental infections of pigs with a non-INDEL PEDV from the USA and with an older European cell-culture adapted strain (Br1/87) (Lohse et al., 2017). For this new study, the objective was to characterize the properties of recent European porcine coronaviruses, under defined experimental conditions, and to observe clinical signs, describe pathological lesions, generate infectious organ material (for subsequent virus isolation) and to produce antisera in the infected pigs.

In this study, four pigs of 4.5 weeks of age were infected with PEDV from Germany (intestine/intestinal content collected from pigs in 2016) and four pigs of 6 weeks of age with PEDV from Italy (intestine/intestinal material collected from pigs in 2016). The pigs were inoculated with the same virus on multiple occasions to create a more robust infection and enhance antibody responses.

The clinical signs and pathological changes observed were generally mild. Surprisingly, a second distinct peak of virus excretion was seen following the re-inoculation in the group of pigs inoculated with the PEDV from Germany, while only one strong peak was seen for the group of pigs that received the virus from Italy. Seroconversion was seen by 18 and 10 days post inoculation with PEDV in the pigs from the groups that received the inoculums from Germany and Italy, respectively.

Attempts were also made to infect pigs with a swine enteric coronavirus (SeCoV) from Slovakia (Belsham et al. 2016) in a similar way. SeCoV is a naturally occurring recombinant virus derived from transmissible gastroenteritis virus (TGEV) or porcine respiratory coronavirus (PRCV) with an S gene resembling PEDV. Unfortunately, these attempts were unsuccessful, and no signs of virus infection were observed in the inoculated animals.

Acknowledgements

This study was performed as part of a CoVetLab project (CoVetLab is a partnership of veterinary public health institutes from Denmark, France, The Netherlands, Sweden and the United Kingdom). PEDV inoculation materials were kindly provided by our colleagues: Sandra Blome at Friedrich-Loeffler-Institut, Germany and Antonio Lavazza and Davide Lelli at Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna, Italy.

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2. A methodology to identify socio-economic factors and movements impacting on ASF and LSD in rural and insecure areas

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In recent years, the EU and neighbouring countries have experienced the progressive spreading of several infectious diseases, including African Swine Fever and Lumpy Skin Disease, with a high impact on animal health and countries' economy, and a threat on European integration, trade and mobility patterns, historical rural livelihoods, and local gastronomic traditions.

Policies, socio-economic factors, and related human and animal movements could facilitate disease spread, especially in those areas where conflicts, insecurity, and poverty are present. The aim of this work, carried out within the H2020 DEFEND project (No 773701), is to analyse these factors and increase the knowledge on their role in animal disease emergence and spread.

Border zones in the Middle East region, East Europe, and in Balkan area were explored (Figure 1). To collect information and data an extensive literature review including press/grey/local literature at the national level and in languages other than English has been carried out. To facilitate the collection of information at the local level, five local assistants (LAs) from different countries (Figure 1) and with different backgrounds (socio-economic,animal and anthropological sciences) have been trained and involved. The data retrieved from the literature/press played an essential role in understanding local dynamics, behaviour and identifying the areas where focus the fieldwork.

Between 2019 and 2021 three field missions have been performed in four countries (Romania, Serbia, Greece, and Bulgaria). Participatory methodological tools based on focus groups discussions, interviews with key informants, participatory maps, and field inspection of strategic points have been used. Further missions are expected in 2022 to complete the data collection.

To date, 63 people among vets, hunters, farmers, researchers, and staff working in humanitarian/migration associations have been involved in single or groups semi-structured interviews, exploring the cross-border movements of people (both local and transnational), live animals, and animal products mainly related to informal market, socio-economic factors affecting rural livelihoods, socio-cultural dynamics affecting hunting, wildlife and interaction with forest, policy frame affecting local dynamics in rural/poor areas and policy frame affecting mixed migrants. Finally, the ASF and LSD knowledge, perceived risk and spreading/limiting factors have been discussed. All data collected were then compared with official data obtained from main dedicated platforms and

notification systems. Representatives of national bodies/institutes (DEFEND partners) have also been involved in the discussion and validation of the results.

Preliminary results indicate the need to consider specific borderland contexts and local dynamics (including social inequality) to effectively face animal diseases. Where limited livelihood opportunities exist, cross-border activities, including the use of forests and other natural resources, are essential coping strategies for the local population. On the other hand, these dynamics are in many cases informal, not regulated, and not monitored.

Recent studies and policies on One Health strongly suggest a closer involvement of the local stakeholders and the population facing a disease event in the international decisional bodies, to scale up local knowledge on one side, and to increase the effectiveness of the implemented strategies on the other. Filling these knowledge gaps will be a key element for the prevention and control of diseases in the future.

3. A biosafety framework to categorise intrinsic and procedural risks for Coronaviruses during diagnostics and research.

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In the UK coronaviruses are classified according to their public health risk into hazard groups by the Advisory Committee on Dangerous Pathogens (ACDP) and/or are also given a Specified Animal Pathogens Order (SAPO) category based on their presence or absence in UK animal populations and the potential economic consequence of a release. Both the ACDP hazard group and SAPO level, determine the biosafety/biosecurity (BSL/BCL) and containment level(s) at which the organisms can be handled. However, there are many different lineages and strains of coronaviruses, all of which elicit different disease outcomes, from sub-clinical to high morbidity and mortality, and infect a wide range of species. In addition, coronaviruses continually evolve, emerging variants and adapting to new hosts e.g., SARS-CoV-2 in animals. Traditional viral/host characterization, genome sequencing for lineage and key mutations and susceptibility predictions mean that we need accurate and proportional risk assessments which are essential but challenging and complicated.

We present the APHA Coronavirus Risk Assessment Matrix (CoVRAM) framework aimed to assess the phenotypic and genotypic properties of these viruses and robustly risk assess case studies e.g., SARS-CoV-2, porcine epidemic diarrhoea virus (PEDV) and infectious bronchitis virus (IBV), as examples of zoonotic/reverse zoonotic and livestock pathogens. The framework utilises experimental and real-world information on pathogenicity, transmissibility, antiviral resistance, vaccination status, receptor preference, disinfection/inactivation/destruction of pathogen, and other factors to predict risk to human and animal health, as well as the economic consequence of potential release. The framework also outlines a matrix of how specific experimental and diagnostic procedures, both *in vitro* and *in vivo*, can be safely performed with viruses in each category.

The risk assessment platform used at APHA to determine the virus hazard category based on sample type, sample shipping security, viral phenotypic, legislative, procedural and genetic characteristics; these characteristics determine the risk level (A, B or C), the ACDP and SAPO level and procedural countermeasures. This allows mitigation of field / laboratory occupational exposure threats and risks through utilising engineering, procedural and health protection measures.

The use of the CoVRAM framework has facilitated the rapid, accurate and proportionate risk assessments for a range of human and animal CoVs from experimental and diagnostic backgrounds. The CoVRAM framework is dynamic, allowing for revisions based on up-to-date information on emerging CoV hazards, and can be adapted to local requirements at the level of institute or from advisory bodies – national and international (WHO/OIE), safety executives, etc.

The CoVRAM framework provides methodology to robustly and proportionally assess the threats and define parameters for safe working practices for coronaviruses during *in vitro* and *in vivo* diagnostic and research applications. The ongoing outcome is to make these adaptable for different resource level settings to ensure biosafety and biosecurity globally.

4. Virus-Derived DNA Forms Mediate the Persistent Infection of Tick Cells by Orthonairoviru

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Crimean-Congo hemorrhagic fever (CCHF) is a severe disease of humans caused by CCHF virus (CCHFV), a biosafety level (BSL)-4 pathogen. Ticks of the genus *Hyalomma* are the viral reservoir, and they represent the main vector transmitting the virus to its hosts during blood feeding. We have previously shown that CCHFV can persistently infect *Hyalomma*-derived tick cell lines. However, the mechanism allowing the establishment of persistent viral infections in ticks is still unknown. Hazara virus (HAZV) can be used as a BSL-2 model virus instead of CCHFV to study virus/vector interactions. To investigate the mechanism behind the establishment of a persistent infection, we developed an *in vitro* model with *Hyalomma*-derived tick cell lines and HAZV. As expected, HAZV, like CCHFV, persistently infects tick cells without any sign of cytopathic effect, and the infected cells can be cultured for more than 3 years. Most interestingly, we demonstrated the presence of short viral-derived DNA forms (vDNAs) after HAZV infection. Furthermore, we demonstrated that the antiretroviral drug azidothymine triphosphate could inhibit the production of vDNAs, suggesting that vDNAs are produced by an endogenous retrotranscriptase activity in tick cells. Moreover, we collected evidence that vDNAs are continuously synthesized, thereby downregulating viral replication to promote cell survival. Finally, vDNAs were also detected in CCHFV-infected tick cells. In conclusion, vDNA synthesis might represent a strategy to control the replication of RNA viruses in ticks allowing their persistent infection. **IMPORTANCE** Crimean-Congo hemorrhagic fever (CCHF) is an emerging tick-borne viral disease caused by CCHF virus (CCHFV). Ticks of the genus *Hyalomma* can be persistently infected with CCHFV representing the viral reservoir, and the main vector for viral transmission. Here we showed that tick cells infected with Hazara virus, a nonpathogenic model virus closely related to CCHFV, contained short viral-derived DNA forms (vDNAs) produced by endogenous retrotranscriptase activity. vDNAs are transitory molecules requiring viral RNA replication for their continuous synthesis. Interestingly, vDNA synthesis seemed to be correlated with downregulation of viral replication and promotion of tick cell viability. We also detected vDNAs in CCHFV-infected tick cells suggesting that they could represent a key element in the cell response to nairovirus infection and might represent a more general mechanism of innate immunity against RNA viral infection.

5. One to beat them all? Efficacy of a multi-component drug containing GS-441524 against feline coronavirus and SARS-CoV-2

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The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China at the end of 2019 caused the COVID-19 pandemic and is a major public health concern despite available vaccines. A small subset of pediatric patients develop a rare severe clinical condition known as "paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2" (PIMS-TS). A fatal infection in cats called feline infectious peritonitis (FIP) is also caused by a coronavirus, the feline coronavirus (FCoV). About 7–14% of cats infected with FCoV develop FIP and no effective treatment is currently legally available.

A comparison of the clinical symptoms of PIMS-TS and FIP exhibit major similarities in terms of clinical manifestation, age spectrum, and immune reactions. Interestingly, Remdesivir, conditionally approved for COVID-19 treatment, and its metabolite GS-441524, which has been effectively tested against SARS-CoV-2 in a mouse model, have also been successfully used in the treatment of FIP (Jones et. al, 2021).

In a recently published study by our research group, our colleagues demonstrated that the multi-component drug Xraphconn® containing GS-441524 is extremely effective against FIP. Cats with FIP were treated orally once a day over 84 days with Xraphconn®. All cats completely recovered clinically and no relapse occurred up to date. Clinical and laboratory diagnostic parameters improved within a few days and no serious adverse effects occurred (Krentz et. al, 2021).

The aim of a subsequent study was to evaluate the antiviral efficacy and toxicity of Xraphconn® compared to pure GS-441524 in vitro against both FCoV and SARS-CoV-2 (strain Muc-IMB-1) as well as in vivo against SARS- CoV-2. Xraphconn® inhibited both the viral replication of SARS-CoV-2 on Vero E6 cells as well as of FCoV on Crandell-Rees Feline Kidney cells (CRFK) at non-cytotoxic concentrations. Interestingly, Xraphconn® inhibited virus growth of SARS-CoV-2 already at lower concentrations when compared to GS-441254. The efficacy of Xraphconn® was further assessed in K18-hACE-2 mouse model after oral or intraperitoneal administration using different concentrations. Unfortunately, all SARS-CoV-2-infected mice developed severe and lethal disease manifested by weight loss up to 20%,

distinct central nervous signs, and viral RNA was detected in oral swabs as well as all taken organ samples, and thus, no in vivo antiviral effects could be demonstrated in this model.

Therefore, further studies in more suitable SARS-CoV-2 infection models are needed to evaluate the mode of action of GS-441524 and Xraphconn®, the impact of administration-route, the relationship between efficacy and inistration, and the role of additional agents besides GS-441524 in the multicomponent compound Xraphconn®.

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6. Immunohistochemical characterisation of ACE2 receptor distribution in tissues of wild animals

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Natural cases of reverse zoonotic transmission of SARS-CoV-2 into animals have been reported during the COVID-19 pandemic. The transmission of SARS-CoV-2 in particular among free-ranging white-tailed deer in North America has raised concerns for disease spread and control, and potentially implications for virus evolution and reservoir status. To understand the potential tropism of ACE2-dependent virus in wildlife, respiratory and gastrointestinal tissues from artiodactylids, mustelids, non-human primates, and macropods were evaluated for ACE2 receptor distribution *in situ* using immunohistochemistry. ACE2 is expressed on the bronchiolar epithelium of several deer species, badger, and otter. Further characterisation on a subset of species revealed the presence of ACE2 in the mucosal epithelium and occasionally the submucosal glandular epithelium of the nasal turbinates and trachea of roe deer (*Capreolus capreolus*), moose (*Alces alces*) and Asiatic lion (*Panthera leo leo*). The expression of ACE2 in the enterocytes of the small intestines was ubiquitous amongst the species examined. Our results demonstrate the potential sites of ACE2-mediated viral infection in wild animals but also highlight differences between species which could influence host susceptibility and transmission.

7. Genetic characterization of peste des petits ruminants' virus (PPRV).from Sulaimani/ Iraq

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Abstract: An outbreak of peste des petitis ruminants (PPR) in sheep was studied in Sulaimani/ Iraqi Kurdistan Region. The study provided the first molecular characterization of the PPRV lineage associated with fatal PPR infections in small ruminants. RT-PCR was used for the diagnosis of PPRV outbreaks during 2012-2013 and the phylogenetic analysis results were based on F and N genes. It was observed that three tested samples were positive for PPR, from which the two F genes (ppr/Kurdistan/2012, ppr2/Kurdistan/2012) were genetically close to the (KF478924 & JF274480) strain (turkey and Egypt) of lineage IV, respectively with 99% nucleotide sequence homogeneity.

Regarding the N gene, it was noticed that one sample (ppr3/Kurdistan/2013) was genetically close to (FJ795511, DQ840197 & DQ840190) strain (Emirate, Saudi Arabia & Israel) %99, %98, and %97 respectively. The current study suggested that there are at least two sources of PPRV in Iraq. Vaccination is performed as a part of control action in certain endemic areas. However the used PPR vaccine is based on Nig75/1 which is in lineage II, and the circulating field isolates in Iraq according to the phylogenetic tree and sequence analysis are grouped in lineage IV. It may be better to use a domestic strain for vaccination as has been practiced in another country.

Aim of the study: This study aimed to determine the phylogenic relationship and molecular characterization of PPRV circulating in Sulaimani province, Kurdistan region of Iraq, based on a partial sequence of the F and N gene in clinical samples.

Sampling: Mouth epithelial tissue samples from sick sheep were collected by the local veterinary department based on clinical signs.

Sequencing the PCR products:Twenty ul of PCR product of F and N gene was sequenced from both primer in, (Macrogen sequencing crevice, Korea) and (Bioneer sequencing crevice, Korea). Both sequences were aligned and trimmed by using NCBI (bl2seq) then published in Genbank as ppr/Kurdistan/2012, accession number (KC292209), ppr2/Kurdistan/2012 accession number KC252611 for F gene and ppr3/Kurdistan/2013 accession number (KF992797) for N gene.

Sequence and phylogenic tree analysis:The partial nucleotide sequences of F and N genes were aligned with corresponding sequences available in GenBank, The sequence homology, and multiple sequence alignment at the nucleotide and the amino acid level were performed by the CLUSTAL W program. Phylogenic trees were constructed among Kurdistan/ppr isolates and isolates from different lineage around the world, based on the neighbor-joining method using the Kimura2-parameter model in Mega5.2. **Result**: Detection of PPRV by F and N gene-based RT-PCR All the suspected samples were showed a positive result for PPRV, based on agarose gel electrophoresis, which demonstrated the expected amplicon about 372 bp and 463 bp for F and N gene, respectively. The results were confirmed by sequencing of PCR product. **Sequence Homology and phylogenetic analysis**: The analysis of PPRV of Iraqi isolates which was published in NCBI Genbank indicated that the 372 bp F gene sequences of two Sulaimani isolates (Kurdistan/ppr & Kurdistan/ppr2) accession number (KC252611 & KC292209) have 99% homology. The analysis of both Sulaimani isolates with a recent outbreak in Erbil (Kurdistan/2011) was also showed 99% homology whereas similarity with previous outbreaks Iraqi

isolate Iraq/2002 ranged from 97-98%. The F gene sequence of Sulaimani isolates was also aligned with the PPRV isolates outbreak in the neighboring countries and it showed 92-99% homology.

8. Oral Inoculation Model for Hepatitis E Virus in Pigs

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Keywords: HEV, Pigs, Inoculation, Infection

Hepatitis E is a worldwide liver disease of humans, caused by the hepatitis E virus (HEV). Pigs are considered a reservoir of HEV and recognised as a source of zoonotic transmission of the virus through the consumption of undercooked, contaminated pork products. The incidence of non-travel related cases of HEV has been increasing in Europe, including the UK, highlighting the importance of the zoonotic disease. In order to understand the reason behind the increase of autochthonous cases in developed countries, and its association with zoonotic transmission, several *in vivo* studies have been performed. These studies aimed to expand the knowledge of HEV infection in pigs, and thus its progression into the pork food chain. However, many of these studies used intravenous inoculation or oesophageal catheters to infect the pigs, which is not how they acquire the virus in nature (faecal- oral route). Hence, the aim of these *in vivo* studies was to establish a natural route of oral inoculation for HEV in pigs.

These experiments were approved by the Animal Welfare and Ethical Review Body at the Animal and Plant Health Agency (APHA). In the first experiment twelve 10-week old pigs, divided into three groups of four, were inoculated with either 10^7, 10^8 or 10^9 GE of the NLSWIE3 HEV strain. The pigs were monitored for 27 days. In the second experiment, twelve 10-week old pigs were divided into three equal groups: two groups were inoculated with ENG/2013/022, one of these groups received 10^7 GE and the other group 10^8 GE. The third group was inoculated with one dose of NLSWIE3 at 10^8 GE. The pigs were monitored for 35 days. For both experiments, faeces and serum samples were collected at regular intervals, and a selection of tissues were collected at post mortem. HEV RNA was detected using RT-qPCR, and antibodies were detected using two commercial ELISA kits.

In experiment one, 67% of the pigs shed HEV RNA in the faeces, 25% exhibited viraemia, 58% were positive in the bile, and 25% were positive in the liver. This experiment deduced that dose 10^7 GE was optimal for causing an infection in pigs, as this group contained the most positive results. However, further investigation found that the pigs which received doses 10^8 and 10^9 GE possessed higher antibody titres than those which received 10^7 GE. This may have skewed the data, leading to fewer positive detections of HEV RNA in pigs inoculated with 10^8 and 10^9 GE. For this reason, the experiment was refined to obtain more reproducible results.

In experiment two, 100% of the pigs shed HEV RNA in the faeces, and 100% of pigs in the 10^8 GE (ENG/2013/022) group exhibited viraemia. Regarding the post mortem samples, 75% of pigs were positive in the bile, and 42% were positive in the liver. Moreover, one pig in the 10^8 GE (ENG/2013/022) group was HEV positive in the diaphragm.

9. Methicillin-resistance Staphylococcus aureus in beef products

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Abstract

Methicillin-resistance *Staphylococcus aureus* (MRSA) is widely distributed in hospitals around the world and is a pathogen frequently transmitted through animal products to humans causing a variety of diseases. There is a strong relationship between disease developments such as Staphylococcus food poisoning and MRSA in animals, especially beef products. As PVL (Panton-valentine Leukocidn) is the most well-known virulence factor of MRSA with the *Mec* A gene. PVL gene is responsible for the secretion of leukocidin that may play a role in the increased severity of infection with PVL- carrier isolates. Mec A gene is an encoded methicillin binding protein that is responsible for methicillin resistance in this bacteria. MRSA and isolates from clinical samples will be studied through using PCR (polymerase chain reaction) and pulsed-field gel electrophoresis(PFGE) methods. Therefore, the objective of this current ongoing study is to evaluate the prevalence and epidemiology of MRSA in beef products and to investigate the possible connection between food resource contamination and human cases of MRSA.

Material and Methods

Samples of beef products are collected from slaughterhouses. Through using mannitol salt agar for bacterial growth, MRSA will isolate. During using coagulase test MRSA will be characterized, and through an antimicrobial test using MRSA isolates will be sensitive to Vancomycin. The prevalence of the PVL gene and mecA gene will also analyze through using PCR and PFGE methods. DNA will extract from *S. aureus* isolates using the Qiagen DNA purification kit as per manufacturer's instructions (DNAEASY, Qiagen) and all MRSA isolates test for the presence of the *Meca* gene and PVL using PCR as previously described. PCR amplification of *mecA* used previously primers(MRI: GTG GAA TTG GCC AAT ACA GG and MRTGA GTT CTG CAGTAC CGG AT primers), which can amplify a 1399 base pair fragment specific for 2: *Meca*. The P*vl* gene is amplified using standard protocols with the following thermal cycling conditions: 35 cycles, each consisting of denaturation at 95 ° *C* for 30 s, annealing at 55 ° *C*for 1 min, and elongation at 72 ° *C* for 2 min. Primers Luk-PVL(ATCATTAGGTAAAATGTCTGGACATGATCCA) and used . Amplification for both *mecA* and Pvl genes started with an initial denaturation at 95c for the 60s and a final elongation step of 5 min at 72 ° *C*. PCR reactions is carried out in 25 ?L of DNA, 1 ?L primer, 0.5 ?L of Taq DNA polymerase, 0.5 ?L dNTPs, and 2.5 10* PCR buffer. The PCR is run under the following conditions: DNA denaturing was carried out for 5 min at 94 ° *C*. This followed by 35 cycles of 94 ° *C*. This was followed by 35 cycles of 94c for 45 seconds. Annealing for 45 sec at 65c and elongation step for 90 sec at 72 ° *C*. PCR reaction is performed with an ending final extension step at 72 ° *C* for 10 min. The PCR products are electrophoresis using a UV lightbox on a 2% wt/vol agarose gel containing 0.5 mg/ ?L ethidium bromide. The products are run for 40 minutes at 80 V in 1* TAE buffer and 100bp DNA ladder is used as a size marker (M) in all gels. Further, all isolates of *S. aureus* strains are genotyped by amplified PFGE.

Discussion

Previously, MRSA was almost exclusively a problem in hospitals. However, contamination of food products may be a potential threat for the acquisition of MRSA by those who handle the food. In addition, a large hospital outbreak with MRSA due to contamination of food products has been

described. Thus, investigating MRSA colonization in beef by PCR. Moreover, it suggests a possible human source of contamination during meat processing. The results are expected in the future. Further research is needed to examine the evolution of MRSA over time that leads to different clinical signs of infection in humans.

10. Genetic Plasticity of Highly Pathogenic Avian Influenza Viruses of H5 Clade 2.3.4.4b

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Highly pathogenic avian influenza viruses (HPAIV) of subtype H5 belonging to the goose/Guangdong lineage have evolved into numerous genotypes, subtypes and various phylogenetic clades. HPAI H5 viruses of clade 2.3.4.4b have been present in Europe since 2016 and show multiple incursions with dispersion all over the continent, subsequently causing annual seasonal outbreaks in wild and domestic birds with high mortality.

Since 2020, Europe has experienced repeated severe HPAIV epizootics. Incursion and spread of these H5 viruses were tracked via analysis of full genomes to gain insights into phylogenetic relationships, segment reassortments, geographic spread and potential incursion routes. We analyzed whole H5 genomes of different neuraminidase subtypes, mostly H5N8 and H5N1, but also H5N2, H5N3, H5N4 and H5N5 subtypes from HPAI viruses collected in Europe from October 2020 to February 2022. The viruses show a mix of different segments originating from a wide variety of Eurasian LPAI viruses. Dominating reassortants found all over Europe were identified alongside regionally occurring reassortants, pointing towards local reassortment events. In addition, HPAIV H5N8 infections of clade 2.3.4.4b were reported in rare human cases and several genotypes were found in mammals presenting, in part, typical genetic mutations known for host-adaption, thus highlighting the zoonotic potential of these viruses.

Spatio-temporal analysis of strains reveals the continuous presence of clade 2.3.4.4b viruses in Europe from summer 2020 onwards with a Eurasian H5N8 as a common precursor. The respective H5N8 virus and reassortants thereof were reported in Europe from fall 2020 to summer 2021. In particular, H5N1 viruses were detected in Northern Europe throughout the whole year of 2021, proving the danger and capability of an enzootic HPAIV situation in Europe. Spatio-temporal analysis of H5N1 genomes support this finding by showing one genotype present also during summer 2021, subsequently emerging into a severe European HPAIV epizootic in the fall and winter season 2021/2022, additionally enhanced by independent incursions of further H5N1 genotypes and local diversification into multiple reassortants. Emergence and spread of these viruses were tracked va phylogeographic analysis and network-based detection of reassortment events.

The trend of a genetically flexible HPAI strain evolving into multiple genotypes is ongoing, indicating the risk of an enzootic HPAIV situation with severe consequences for avian wildlife, enormous economic losses, and the risk of zoonotic virus emergence.

11. Gross pathology of HPAIV H5N1 2021-2022 epizootic in naturally infected birds in the United Kingdom

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Highly pathogenic avian influenza virus (HPAIV) clade 2.3.4.4b virus has re-emerged in the United Kingdom in 2021-2022 winter season, with over 90 cases of HPAIV detected among poultry and captive birds in England, Scotland, Wales, and Northern Ireland. Globally, HPAIV H5N1 has also had a wide geographical dispersion, causing outbreaks in Europe, North America, Asia, and Africa. It is important to raise awareness of the gross pathological features of HPAIV and subsequently aid disease investigation through definition of pathological indicators following natural infection. As such, here we report on the gross pathology of HPAI H5N1 in poultry species (chicken, turkey, pheasant, guineafowl, duck, goose), and captive or free-ranging birds (mute swan, tufted duck, jackdaw, peahen, white-tailed eagle) that tested positive between October 2021 and March 2022. Pancreatic and splenic necrosis were the common pathological findings in both Galliformes and Anseriformes. In addition to the more severe lesions documented in Galliformes, we also noted increased detection of pathological changes in a broader number of Anseriformes particularly in domestic ducks, in contrast to those reported in previous seasons with other H5Nx HPAIV subtypes. The findings from the 2021-2022 outbreak will also be discussed in conjunction with the observations from the 2020-2021 outbreak. A continual effort to characterise the pathological impact of the disease is necessary to update on the presentation of HPAIV to both captive and free-ranging birds.

12. Study on presence of SARS-CoV-2 in pet dogs and cats - results from Serbia

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The SARS-CoV-2 virus is the human coronavirus causing the COVID-19 pandemic. As the virus spread in the human population, first as sporadic, and then more and more frequent findings of SARS-CoV-2 virus were reported firstly in pets - cats and dogs, and then in large cats in zoos, and in some other animal species. The aim of this small study was to determine whether during the COVID-19 pandemic in Serbia SARS-CoV-2 transmitted from humans to pets - dogs and cats in natural conditions, how frequent the infection was, what is the clinical picture of SARS-CoV-2 infection in dogs and cats, the possible epidemic significance of this infection for humans etc.

A total of 31 dogs and 16 cats were examined, namely swabs (nasal, pharyngeal and rectal swabs) for the presence of SARS-CoV-2 virus and blood sera for the presence of specific antibodies against the mentioned virus. In some of tested animals, sampling of swabs and blood were done in multiple occasions (swabs during the few consecutive days, and blood sera during few months). The sampling was dependent on the animals' availability, in some cases only one sample (samples) was available in one occasion, and in some occasions animals were monthly sampled during prolonged period of time. All tested animals were in close contact with COVID-19 patients (mainly owners) for some period of time, and were sampled in the period from October 2020 to February 2022. The presence of the virus was detected by the RT-qPCR method (Charité-Berlin protocol) recommended by the WHO, and the presence of specific antibodies against SARS-CoV-2 was tested by a commercial ELISA test (ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA).

The presence of SARS-CoV-2 virus was detected in the nasal or pharyngeal swabs of 2 out of 22 tested dogs and in 3 out of 15 tested cats, while the presence of specific antibodies was detected in 4 out of 28 examined dogs and in 3 out of 13 tested cats. The first SARS-CoV-2 positive dog was available only for one swabs sampling and another positive dog was sampled during 4 consecutive days but only the first nasal swabs were found positive for virus presence. That dog was also positive for SARS-CoV-2 antibody presence for 2 months. One out of three serologicaly positive dogs was positive for antibody presence for 8 months (9 sampling occasions). In one out of three SARS-CoV-2 positive cats, the virus presence was detected in nasal or pharyngeal swabs for 5 consecutive days. One cat was positive on SARS-CoV-2 antibody presence during 9 months (11 sampling occasions) after infection, one were positive for 2, and the third one 3 months (study is still ongoing). All positive RT-qPCR findings in animals were determined within the first 14 days from the contact, and specific antibodies 10 or more days after the contact of the animals with SARS-CoV-2 positive persons.

It could be concluded that SARS-CoV-2 was transmitted from humans to their dogs and cats in natural conditions, as well as that these animals can be naturally infected with SARS-CoV-2 virus. This is just the preliminary study that is still in progress and further studies will be necessary to give an answer on questions - to what extent is SARS-CoV-2 infection a health problem of dogs and cats, including the "post-COVID" period after infection, as well as is there any epidemiological significance of the infection of these pets for human population.

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13. Characterization of SARS-CoV-2 in mink cadavers after burial for six month

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Control of infectious disease outbreaks in livestock can require millions of animals to be killed during stamping-out strategies to contain and eradicate the disease. In 2020, during the COVID-19 pandemic, SARS-CoV-2 virus swept through the Danish mink population and the national government decided to cull the entire mink population to ensure human health and safety (Larsen et al. 2021). Thus, in November 2020, about 4 million mink were buried at two sites in Jutland. Due to concerns about contamination of the environment, including ground water reservoirs, the mink carcasses were exhumed after six month for final disposal by incineration. In this context, it was decided to obtain more knowledge regarding the impact of disposal by burial of large numbers of infected animals, including the level of decomposition and microbiological status of the mink cadavers.

From each burial pit (dimensions 100 x 3 x 2.5 m), samples were collected from the top, middle, and bottom layers. Swab samples from three mink cadavers were pooled on location, consisting of either 3 throat swabs (54 pools) or 3 rectal swabs (53 pools). Preliminary testing of the sample pools for SARS-CoV-2 showed PCR positive results in 11% of the pools. Therefore, follow-up samples from exhumed mink were subjected to extended analysis in order to characterize the condition of the virus remaining in the samples, including degradation and potential health risk of the excavated material in relation to dispersion of infectious virus. When possible, throat swabs, rectal swabs and lung tissue from individual cadavers were collected.

Detailed analysis on selected samples included: 1) retesting samples in diagnostic PCRs (E-gene PCR) and confirmatory PCR (N-gene PCR); 2) Attempts to amplify large fragments of the virus genome to evaluate the extent of degradation; 3) further characterization of virus variants in positive samples by next generation sequencing; and 4) virus isolation in cells to recover any infectious virus.

Analyses on the selected samples revealed that virus genome fragments up to ca. 1 kb long could be amplified by RT-PCR. Sequence analysis detected the changes encoding the Y453F substitution in the spike protein, related to the SARS-CoV-2 'mink variant' (pangolin Lineage B.1.1.298), which circulated on most of the infected Danish mink farms during the outbreak period. Original material in the form of throat swabs, rectal swabs and lung tissue, subjected to extensive virological examinations in cell-based assays could not recover infectious virus following up to five cell passages. Thus, even though SARS-CoV-2 RNA could be detected in exhumed mink cadavers 6 months after burial, the residual virus was found to be non-infectious.

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14. SARS-CoV-2 variants observed in farmed mink during the epidemic in Denmark, June-November 2020

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In June 2020, SARS-CoV-2 infection in farmed mink (*Neovison vison*) occurred in the first three farms in Northern Jutland, Denmark. A mink variant SARS-CoV-2 strain (lineage B.1.1.298), which had the spike protein change Y453F, appeared among some of the mink in the first infected farm. This change was located in the receptor- binding domain and the same variant was also found in all the tested mink at the two other farms infected in June and among people in the area (Hammer et al. 2021).

After a silent period in July, the fourth case of infected mink was detected in mid-August 2020 (Rasmussen et al. 2021). The virus variant at this new farm, farm 4, was closely related to the variant that was previously identified on the first farms and appeared part of the same transmission chain. In addition to Y453F, the virus from farm 4 had lost two amino acids in the N-terminal domain of the spike protein (?H69/V70) and acquired some additional changes in other parts of the genome.

In the following weeks of August and September, more farms tested positive for SARS-CoV-2 and was coincident with extensive community spread (Larsen et al. 2021). The variants found in mink in this period all belonged to the

B.1.1.298 lineage and were likely descendants from the virus introduced in the mink population in June. Additional spike substitutions emerged and genome phylogenies showed a segregation of the viruses in up to five clusters, including “cluster 5”, indicating multiple transmissions clusters. The Cluster 5 variant was only observed in a limited number of farms in the first part of September, whereas the other clusters persisted until the cull of all mink in Denmark in November. In total, about 93% of the 290 infected farms that were tested positive for SARS-CoV-2 by the end of November were infected with B.1.1.298 variants.

In October, mink infected with other lineage variants were found on two other farms. These farms were located in the Southern part of Jutland indicating independent introductions of SARS-CoV-2 into these mink. During the last phase of the mink epidemic in October and November, additional lineages of SARS-CoV-2 were detected in mink, of which some were found on more farms. In total, mink in eighteen infected farms were infected with other lineage variants than B.1.1.298.

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15. SARS-CoV-2 infections in wildlife captive animals: 2 females Hippopotamuses tested positive in Belgium

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The novel severe acute respiratory syndrome coronavirus SARS-CoV-2 first described in 2019 causes the infectious disease COVID-19. This highly contagious virus has spread worldwide within few months resulting in the current pandemic. Next to human infections, cases of SARS-CoV-2 infections have also been reported in pets (cat, dogs) and wildlife captive animals (mink, tiger, lion and primates).

We present the case of two female hippopotamuses from Antwerp Zoo (Belgium) who tested positive for Sars- CoV-2 last December. These animals showed very mild clinical symptoms (nasal discharge).

To characterize the course of the infection, the presence of the virus was monitored over time using qPCR in nasal swabs and animal feces. Saliva and blood samples were collected to monitor the seroconversion of the animals.

Whole genome characterization was performed using the Midnight protocol for nCoV-2019 MinION sequencing. In addition, as hippopotamuses are aquatic mammals, the presence of the virus was also assessed in the pond water. The detection of these two SARS-CoV-2 positive hippos, allowed us to follow the course of infection in an animal species that had never before been described as susceptible to this new betacoronavirus and to study the potential adaptation of the virus to its host.

16. An objective clustering workflow for West Nile virus sequences based on affinity propagation and hierarchal clustering

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West Nile virus (WNV) is the most widespread arthropod-borne virus and the primary cause of arboviral encephalitis in the world. Subsequent to its widespread distribution and further geographic expansion, member of the West Nile virus species genetically diverged and are currently classified into different subspecies groups.

However, the demarcation criteria for allocating WNV sequences into different subspecies levels, as well as their nomenclature, remain inconsistent and unstructured.

Therefore, we developed a clustering workflow that utilizes an unsupervised machine learning algorithm called “affinity propagation” clustering and agglomerative hierarchal clustering for the objective and systematic allocation of WNV sequences into different subspecies groups. Our workflow only requires pairwise sequence alignment as input and important clustering criteria (e.g., the number of sequences per cluster, number of clusters etc.) are entirely decided by the mathematical algorithm. Alongside this objective clustering approach, we also proposed to employ a hierarchal decimal numbering system to represent each subspecies group.

As a proof-of-concept, we applied this workflow to WNV sequences that were designated into specific lineages and/or clusters by previous studies. Our workflow allocated these WNV sequences in agreement with their respective lineages and clades; however, it regrouped some WNV clade 1a sequences into a few clusters. Using this workflow, we classified all available WNV lineage 2 complete coding sequences found in the database and designate them into hierarchically ordered subspecies groups: lineage (L2), clades (L2.1 - L2.5), subclades (e.g., L2.5.1 - L2.5.12), clusters (e.g., L2.5.12.1 - L2.5.12.4), and subclusters (e.g., L2.5.12.1.1 - L2.5.12.1.3; L2.5.12.1.1.1 - L2.5.12.1.1.3 and so on). We also employed this approach to characterize the sequences from the 2020 WNV lineage 2 circulation in Germany, primarily from WNV-infected birds and horses. These WNVs sequences were allocated in at least four subclusters, in which the subcluster previously referred to as the “Eastern German clade” was still the predominant subcluster circulating in Germany in 2020. The presence of minor WNV subclusters and cluster in Germany may reflect the continuous incursion of WNV in Germany from neighboring countries.

Although our study primarily aimed at a more detailed WNV classification, the here presented workflow can be also be applied to the objective genotyping of other virus species.

17. MERS-coronavirus clade C Egypt/2013 isolate exhibits poor transmission potential in camelid reservoir hosts

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Zoonotic spillover events from Middle East respiratory syndrome coronavirus (MERS-CoV) circulating in camelid reservoirs continue ongoing. Despite more than 80% of the camel population being found in Africa, animal-to- human transmission seems restricted to the Arabian Peninsula. There is only minimal evidence of MERS-CoV exposure to camel handlers, but no zoonotic MERS has been reported across Africa. Nowadays, MERS-CoV clade B strains are dominant in the Arabian Peninsula and are responsible for human outbreaks. Nonetheless, differential replication and transmission competence of Arabian and African viruses in camelid reservoir species remain unknown.

To investigate the transmission potential of African MERS-CoV strains, we used a direct-contact llama transmission model. Two llamas were intranasally inoculated with a MERS-CoV clade C isolate (MERS-CoV/Egypt2013) and placed in direct contact with three sentinels at two days-post inoculation. To assess differential transmission patterns between strains, we retrieved experimental data from a previous transmission study (*1*) using llamas inoculated with the MERS-CoV Qatar15/2015 (clade B) strain and in-contact animals.

Animals experimentally inoculated with either MERS-CoV Egypt/2013 or Qatar15/2015 had similar levels of genomic and subgenomic viral RNA in nasal swabs for 2 weeks and shed high titers of infectious virus during the first week post-inoculation. The Arabian isolate was transmitted and caused productive infection to all direct-contact llamas. By opposition, genomic and subgenomic Egypt/2013 RNA were detected at lower levels and cleared faster in two out of three direct-contact llamas, which excreted minimal amounts of infectious virus. Although one sentinel was not productively infected with the African isolate, all three contact animals developed similar levels of serum neutralizing antibodies to MERS-CoV. Statistical analyses in sentinel animals showed a significant reduction in MERS-CoV Egypt/2013 replication and shedding compared to those observed in direct-contact llamas exposed to the MERS-CoV Qatar15/2015 strain.

Altogether, our work provides experimental evidence that the MERS-CoV Egypt/2013 strain displays a lower transmission potential than the Qatar15/2015 strain in a camelid model, which might in turn result in a reduced spillover to humans. In addition, since there is a constant flow of dromedary camels from Africa to the Arabian Peninsula, our results support the hypothesis of MERS-CoV clade B strains outcompeting clade C strains in the Middle East.

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18. Serological survey of SARS-CoV-2 in wild and domestic animals in Italy

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Coronaviruses (CoVs) are reported to cause diseases in humans and a wide range of animal hosts. Their capacity to jump species barriers makes them particularly interesting, especially considering the recent SARS-CoV-2 pandemic, which is suspected to be originated from a live-animal market in Wuhan, China. Currently some animal species are reported as susceptible to the infection, in particular mustelids, felines and some rodents, posing a potential reverse-zoonotic transmission risk and zoonotic implications. In order to widen the knowledge about natural infection in animals, a total of 608 sera collected in 2020-2021, were screened for the presence of antibodies against SARS-CoV-2. In particular, 109 wild boars, 85 roe deer, 22 red deer, 76 chamois, 92 hares, 85 rabbits, 131 bovines and 8 buffaloes were analysed. Animals were from different Italian regions, with the exception of rabbits which were both from Italy and from European countries (Germany, Belgium and France). Sera were tested through a multispecies double-antigen sandwich ELISA-IZSLER kit (Fernández-Bellon et al, 2021) to detect antibodies against nucleocapsid proteins. The 20 positive samples identified (18 from ungulates and 2 from lagomorphs) were further investigated with the additional commercial serological test GenScript SARS-CoV-2 Surrogate Virus Neutralization and the SARS-CoV-2 virus neutralization test (VNT) considered the gold standard test for serology. One chamois resulted positive only to GenScript SARS-CoV-2, whereas all other sera resulted negative both to the additional test and to the VNT. The obtained results highlight the absence of antibodies against SARS-CoV-2 in all tested animals. Concerning cattle and rabbits, a variable susceptibility in experimental infection was demonstrated in previous studies. The negative findings are probably due to the absence of virus circulation among farm personnel in the period of sampling. Wild animals, at least the species here investigated, appear not to play an epidemiological role in virus transmission and spread. However due to the susceptibility recorded in white-tailed deer, it could be interesting to widen the sampling of deer, both considering the high- density populations in Italian Alps, and their hunting importance with related zoonotic implication. At the same time, it would be worth investigating other synanthropic wild species more frequently detected in urban areas, such as rodents, foxes, hedgehogs. Despite the negative molecular results on viscera from carcasses, data on serological status are very difficult to obtain, even if they would be more useful to better understand the role of wild species in virus spread.

19. Experimental West Nile and Bagaza virus infections in quails

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Common quail (*Coturnix coturnix*) is a species of conservation concerns at European and national scales and perform seasonal migrations. Host competence and potential role of wild quails in flaviviruses dissemination was estimated by analyzing the effect of West Nile (WNV) and Bagaza virus (BAGV) in farm raised common quails (*Coturnix coturnix*) hybridized with Japanese quails (*Coturnix japonica*).

Two groups of 10 (WNV-inoculated group) and 12 (WNV-necropsy group) quails were inoculated with Italy/08 WNV strain. Two other groups of 10 (BAGV-inoculated group) and 9 (BAGV-necropsy group) birds were inoculated with Spain/10 BAGV strain. All inoculations were performed subcutaneously in the neck (10,000 pfu/individual). As control group, 10 animals were sham-inoculated, and 4 quails (BAGV-contact group) were maintained in the same cage as the BAGV-inoculated group. Birds were observed daily for clinical signs and a follow-up of viral load in blood, feathers, oral swabs and neutralising antibody titres was made up. Additionally, viral load in organs were also determined after sample collection during necropsy.

All WNV-inoculated quails developed serum neutralizing antibodies indicating that all birds were infected. Nevertheless, the infection did not result in significant mortality or morbidity. WNV genome was undetectable in blood and at very low rates in organs of inoculated birds.

A high percentage of BAGV inoculated quails (80%) became infected developing neutralizing antibodies and viremia. The infection caused morbidity (relevant weight loss) in 25% of the animals. A correlation between morbidity and viral load in brain was observed. Contact transmission did not occur in quails. Low viremia detected in quails when compared to other BAGV-infected phasianid birds as red-legged partridges (Llorente et al., 2015) and grey partridges (Cano-Gómez et al., 2018) suggests a lower host competence of this species for BAGV transmission to mosquitoes.

In summary, neither mortality outbreaks after infection nor a relevant role of the common quail in the dissemination of WNV and BAGV are expected.

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20. Susceptibility of domestic goat to SARS-CoV-2 Beta variant infection

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SARS-CoV-2 is the causative agent of the COVID-19 global pandemic, which was first described in humans in Wuhan, China (December 2019). COVID-19 has a zoonotic origin and a broad number of domestic and wild animal species have been shown to be naturally and/or experimentally susceptible to infection. Livestock animals are constantly in close contact with human population, which could facilitate the virus reverse transmission and therefore, the appearance of SARS-CoV-2 reservoir hosts. Domestic goat (*Capra aegagrus hircus*) is a livestock ruminant which has been already suggested to be minimally permissive to SARS-CoV-2 infection. For this reason, this study aimed at elucidating the susceptibility of goats to SARS-CoV-2.

In silico studies using FoldX and pyDock were used to compare the affinity of Classical, Beta, Gamma and Delta SARS-CoV-2 variants in human, mouse or goat ACE2 receptors. Regarding the experimental study, a total of 18 male goats (*Capra aegagrus hircus*) of 2-3 months of age were used. Three animals were included as non- inoculated, negative controls and the remaining 15 were intranasally inoculated ($2x10^6$ $TCID_{50}$/animal) with an isolate of SARS-CoV-2 (Beta-variant). On days 2, 4, 7, 10 and 18 post inoculation (dpi), three animals per day were necropsied and nasal and rectal swabs (NS/RS), blood (sera) and different tissues (nasal turbinate, olfactory bulb, parotid gland, tonsil, lymph nodes, trachea, lung, spleen, liver, kidney and intestine) were collected for the detection of SARS-CoV-2 by RT-qPCR and/or IHC. Besides, neutralizing antibodies (nAbs) targeting the SARS-CoV-2 RBD were measured by a receptor binding inhibiton assay).

The *in silico* study showed that the SARS-CoV-2 Beta variant had a higher affinity to the ACE2 goat receptor compared to the Delta and Gamma variants. During the whole study period, no animal had clinical signs neither developed fever related to SARS-CoV-2 infection. Low viral loads were detected in NS at 2, 4 and 7 dpi. Low viral loads were also detected in the nasal turbinate (NT) (2, 4, 7 and 10 dpi), trachea (2 and 4 dpi), lung (4 and 18 dpi),

submandibular lymph nodes (2, 4 and 18 dpi) and tonsil (2, 4, 7, 10 and 18 dpi). Since the highest viral loads (Ct: 23-25) were found in tonsil and NT, IHC was only assayed in these tissues; viral antigen was only observed in the tonsil, apparently in dendritic-like cells located in areas surrounding tonsillar crypts. Regarding the serological analysis, low titres of nAbs were detected in goats necropsied at 10 and 18 dpi.

In silico studies were not conclusive about a potential effective binding between goat ACE2 and SARS-CoV-2 Beta variant. However, results obtained from the experimental infection showed a low susceptibility of goats to the virus without displaying any clinical signs related to the infection. The existence of nAbs at the latter times of the experiment suggests that, to a low degree, viral replication could take place in inoculated animals, probably in NT and/or lymphoid tissues. The present investigation emphasises the importance of searching for potential susceptible host species, especially

those in close contact with human population, to confirm or rule out their potential as animal reservoirs for SARS-CoV-2. Furthermore, this study confirms that goats do not play an important role as reservoirs and are not a suitable model for COVID-19 neither for infectivity studies.

21. UK International Coronavirus Network

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The UK International Coronavirus Network (UK-ICN) is a four-year research and innovation network (2021-2025) launched to bring together researchers from animal and human coronavirus communities together, funded by the BBSRC and DEFRA, UK. Its main aim is to provide and support global coordination for the delivery of collaborative scientific research and a sustained long term One Health approach. It brings together partners form all over the world, currently including Europe, China, India, North America, Southeast Asia, Middle East, Africa, Oceania with members from 14 different counties with options for other to join. UK-ICN@liverpool.ac.uk, https://www.liverpool.ac.uk/health-and-life-sciences/research/uk-international-coronavirus-network/

UK-ICN is led by Prof. Julian Hiscox at the University of Liverpool with co-directors from UK Universities and Institutes (authors). Our themes include: One Health and Zoonoses. Surveillance, Detection and Characterisation, Countermeasures and Interventions, Behaviour and Social Policy and SARS-CoV3 and the Future.

The aims of UK-ICN: To provide a community gateway, including a network of networks and international bodies (FAO/OIE/WHO), to facilitate and co-ordinate interactions between members, focusing on animal-human- environment interface research, to foster cross-fertilisation of ideas, provide expert perspectives to identify knowledge gaps, create research opportunities and build an evidence-based road map for one-health, ensure the sustainability of coronavirus research after the impetus of SARS-CoV2 has reduced and to disseminate and preserve knowledge to better combat future emerging coronaviruses.

Outcomes: Better understanding of ecologies at the animal-human-environment interface globally, enhanced understanding of coronaviruses and intervention tools, increased integration of human-veterinary coronaviruses research and innovation capacity and capability, focus on early career scientist (ECS) development, identification of research gaps and future priorities, and a roadmap (STAR-IDAZ; https://www.star-idaz.net/) for future priorities and actionable intelligence for policy makers.

The UK-ICN directorate is supported by a global multi-disciplinary management board. To date, including representatives (n=31) from 14 countries. The expertise includes clinicians, veterinarians, academics, government agencies and industry partners. Our membership includes 84 people and continues to grow with options to join us.

https://www.liverpool.ac.uk/health-and-life-sciences/research/uk-international-coronavirus-network/member/

Annual meetings of the network will be hosted in the UK, focused specific topics workshops in the UK and partner countries. Sponsorship of sessions at major international meetings is available including provision of travel bursaries for early career researchers and training/knowledge transfer within the network.

Upcoming meetings:

April 28-29th, 2022: UK-ICN South Africa Regional Meeting (University of Pretoria, South Africa) May 10-11th, 2022 (DATE TBC): UK-ICN Indian Regional Meeting (Kashmir, India)

Summer 2022: UK-ICN Focused workshop MERS (King Fahad Medical City, Saudi Arabia)

Sept 28–30th, 2022: Cutting Edge Virtual Symposium on Coronaviruses with Disease-X Potential in collaboration with CSIRO and US FDA (Geelong, Australia)

2023 UK-ICN Focused workshop Human and Animal Interface sponsored by Ceva Animal Health Ltd

2023 UK-ICN Focused workshop Livestock and Companion animal Coronaviruses

22. Revisiting rustrela virus – new cases of encephalitis and the capsid enigma

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Rustrela virus (RusV) has been associated to cases of fatal encephalitis in a wide spectrum of mammals, including placental and marsupial animals. The virus has only been recently discovered and is, besides ruhugu virus, one of the only currently known relatives of rubella virus. Wild yellow-necked field mice (*Apodemus flavicollis*) may represent the natural reservoir of RusV, as viral genome could be detected in their central nervous system in absence of apparent clinical manifestation. Unfortunately, the clinical and pathological data for the RusV associated encephalitis as well as RusV genome sequences were so far limited to the first description of the virus [1].

Here we present two new cases of fatal RusV-associated meningoencephalitis in a South American coati (*Nasua nasua*) and a Eurasian otter (*Lutra lutra*) [2]. While the South American coati was housed in a zoological garden with prior history of RusV infections, the wild Eurasian otter had no reported link to the zoo areal, but was found in close proximity. Both animals presented with abnormal movement or unusual behaviour such as convulsion and tremor and ultimately succumbed to the disease. Histopathology of the RusV-infected South American coati and Eurasian otter indicated a non-suppurative meningoencephalitis, that was similar to that of the previously reported RusV-infected donkey, capybara and wallaby from the same zoo. High RusV genome loads were detected using specific RT-qPCR and RNA *in situ* hybridization in the brain tissue of both animals.

As sequencing of the RusV genome proved to be very challenging (mean G+C content = 70.6 mol%), we tested poly(A)+ enrichment, rRNA depletion and post-library bait capturing in order to increase sequencing efficiency. Especially the use of target-specific capture enrichment with specifically designed RNA baits (panRubi myBaits panel) markedly improved virus-to-background sequence ratios, particularly in regions of exceptionally high G+C content ratios. Using this technique, we generated complete RusV genome sequences from both animals and also reanalysed two previously published encephalitic zoo animals and ten RusV-positive wild yellow-necked field mice. *De novo* assembly of the RusV enriched sequencing libraries identified a previously undetected stretch of 309 nucleotides of the intergenic region and the 5' end of the capsid protein coding sequence, indicating that the initial RusV sequence was likely incomplete. This was likely caused by sequence underrepresentation at a low complexity region with an exceptionally high G+C content of >80 mol%.

The initial RusV capsid protein sequence was considered enigmatic, as it was exceptionally short and lacked the RNA-binding domain. With the now revised RusV genome, the predicted capsid protein resembles those of RuV and ruhugu virus in size and includes the potential RNA-binding domain.

In summary, we present new clinical cases from RusV spillover infections that extends the detected RusV host spectrum to carnivore mammals. The clinical and histopathological data will help to expand

the knowledge about this encephalitic rubivirus. Using a sophisticated enrichment strategy, we were able to solve the RusV capsid enigma and provide an updated RusV genome sequence.

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23. Characterization of HPAIVs detected in Denmark in the seasons 2020/2021 and 2021/2022

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In recent years, European countries have experienced the to date largest reported epizootic of highly pathogenic avian influenza viruses (HPAIVs) affecting wild birds, commercial farms as well as captive birds. The avian influenza viruses (AIV) detected during the 2020/2021 season were predominantly clade 2.3.4.4b H5N8 HPAIVs, whereas the current 2021/2022 season has been dominated by H5N1 viruses belonging to the same clade. In the past two seasons, clade 2.3.4.4b viruses of several different subtypes (H5N1, H5N3, H5N5, and H5N8) have been identified in Denmark. While Denmark is geographically a small country, its coastlines and wetlands make it an ideal habitat for migratory waterfowl, resulting in numerous incursions and circulation of HPAIVs.

Ultimo 2020, Denmark experienced its first HPAIV outbreak in a commercial poultry farm ever. During the 2020/2021 season, a total of 16 outbreaks were detected in commercial poultry farms or backyard holdings and as of 11 February 2022, ten cases have been identified in the 2021/2022 season. Especially, Danish turkey farms have suffered from HPAIV outbreaks. During the two seasons, more than 430,000 birds have been culled in relation to HPAIV outbreaks and more than 400 wild birds found dead with HPAIV in Denmark. Additionally, as part of the Danish active surveillance, HPAIVs have been identified in hunted live wild birds and in droppings collected from the Wadden Sea. In Denmark, HPAIV epizootics have never before reached such levels of severity. To elucidate the diversity of the Danish HPAIVs found in the two seasons, whole genome genetic analyses were performed. Our data revealed that the viruses were highly similar to contemporary HPAIVs in other European countries in both seasons, although some viruses contained unique gene constellations. A number of viruses identified in 2021/2022 were found to be highly similar to those found in the previous season, such as the H5N8 viruses detected in two wild birds and a commercial chicken farm, whereas others were identified as novel introductions. The genetic data combined with epidemiological information were used to explore the most likely route of introduction into each infected poultry farm. In a few cases, the data supported that the outbreaks were caused by inter-farm HPAIV transmission. However, the majority of outbreaks in poultry are believed to have been a result of direct or indirect contact with HPAIV-infected wild birds.

The continued circulation of HPAIVs in non-migratory periods, as observed between the 2020/2021 and 2021/2022 AIV seasons, combined with novel incursions could potentially create an enzootic state of HPAIVs in Europe.

Further actions to elucidate where and how these viruses have been maintained need to be established. These growing concerns prompt reconsideration of AIV preventative measures limiting the HPAIV incursions, not only at the wild bird-poultry interface, but also the secondary transmissions between poultry farms.

TOPIC 2 - Pathogen evolution

24. Modeling porcine hemagglutinating encephalomyelitis virus infection in vivo and ex vivo

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

25. Virus shedding in poultry after experimental infection with wild bird-adapted H7N7 LPAIV

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Introduction: Avian influenza viruses (AIV) are a heterogeneous group of pathogens that are classified into two categories, low pathogenic (LP) and highly pathogenic (HP) strains, based on their virulence in chickens. Wild aquatic birds are natural reservoirs of AIVs enabling the virus spillover to many poultry species with varying susceptibility to AIV infection. The occurrence of different virus shedding patterns in poultry indicates differences in host-pathogen interactions which could result in further virus evolution. To investigate the clinical outcome and virus shedding levels in poultry, an experimental infection with LPAIV of H7N7 subtype originating from a wild mallard was performed. The study included three gallinaceous species (chicken, quail, turkey) and one representative of waterfowl (i.e. duck).

Materials and methods: For each species, a group of ten birds were inoculated via intranasal, conjunctival and oral route with a viral dose of 10^6 EID_{50}. The control group included ten animals of corresponding species. In addition to daily clinical observation, oropharyngeal and cloacal swabs were collected at 2, 4 and 7 day post infection and further examined by real time RT-PCR. The assessment of exact virus shedding levels for individual birds was carried out by using the standard curve generated by ten-fold dilutions of inoculum. Analysis of the results included comparison of latency period and duration of shedding as well as overall shedding levels (mean and peak shedding).

Results: Apart from one turkey with moderate diarrhea, animals did not show any clinical signs of viral infection. No statistically significant differences either in the latency period or in the duration of oropharyngeal shedding between all tested species were observed. However, ducks showed the shortest latency period of cloacal shedding and its interspecies comparison disclosed a statistical difference to turkeys ($p < 0.04$). The highest duration of cloacal shedding was demonstrated also in ducks and it was significantly longer than in chickens and turkeys ($p < 0.0002$ and $p < 0.03$, respectively). Quails shed the largest amounts of virus via respiratory route in comparison to all animal groups (p ? 0.01), while the highest cloacal shedding was observed in ducks and differed significantly from shedding levels of galliform species (p ? 0.02).

Conclusions: Our research confirms the possible asymptomatic course of viral infection with LPAIVs even if introduced into poultry. Moreover, inoculation and efficient replication of a wild-type virus, without prior adaptation to poultry, was demonstrated in four domestic bird species and interspecies differences in virus shedding levels were identified.

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26. A panserotype Solid Phase Blocking ELISA for detection of Structural Protein antibodies

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Foot-and-mouth disease (FMD) is one of the most contagious transboundary animal diseases, caused by the FMD virus (FMDv). FMD may be controlled by zoo-sanitary measures and vaccination, but this remains difficult due to the existence of multiple serotypes (O, A, C, Asia 1, SAT 1,2,3) of the causative virus and the lack of cross- protection between them.

As FMD significantly limits trade in animals and animal products, FMD-free countries invest important resources to prevent and prepare for possible incursions. Vaccine banks enable rapid implementation of emergency vaccination in the event of an outbreak. Availability of diagnostics is also crucial for the management of outbreaks, either to monitor virus spread with non-structural protein (NSP) or capsid structural protein (SP)-based tests, or to perform post-vaccination monitoring with SP ELISAs.

Antibody (Ab) titers are influenced by the level of exposure to the specific protein. Seroconversion to SP occurs earlier than to NSPs, and the titers are usually higher; moreover, titers to NSP proteins may be transient and difficult to detect in some subclinically infected animals with low levels of virus replication, regardless of the vaccination status.

Therefore, in a context of an emergency outbreak management in non-vaccinated areas, SP serology may be preferred, using Solid Phase Blocking ELISAs (SPBE) ELISAs related to the serotype causing the outbreak. Even though many research on viral-emergence prediction is carried to improve vaccine and diagnostic selection, the risk of an unexpected serotype cannot be excluded.

In this context, appropriate SP diagnostic kit availability could be an issue: diagnostic banks preparation may be complicated and costly, and inappropriate SP kit stocks could make it ineffective. A SP-*pan*serotype SPBE might solve these problems.

In this study, different SP-*pan*serotype SPBE prototypes were developed, using different *pan*serotype monoclonal antibodies as conjugate. For the best one, the specificity was evaluated with 80 cattle and 80 swine naïve samples, from a non-endemic and unvaccinated area. Measured specificity was 99,4% (IC95% [96,6 – 99,9].

Inclusivity was assessed by testing sera from infected cattle (IAEA, through the Animal Production and Health Sub- program of the Joint FAO/IAEA Division), including sera against the 6 FMDv serotypes (A, O, Asia 1, SAT 1, SAT 2 and SAT 3). All samples were found positive, showing the capacity of the test to detect SP antibodies, regardless of the infective FMDv serotype.

Analytical sensitivity was determined by comparing the last positive dilution for 6 monovalent sample results with the homologous ID Screen® SP-SPBE. Six samples from experimentally vaccinated animals and 15 samples from vaccinated and/or infected animals from endemic areas were tested. Results indicated comparable analytical sensitivity, regardless of the serotype tested.

Even if more validation data is needed to assess the diagnostic sensitivity and the seroconversion detection window, preliminary results indicate the possible use of a *pan*serotype SPBE kit for the specific detection of SP antibodies, regardless of the infective FMDv serotype.

27. Evolutionary-related high and low virulent classical swine fever virus isolates reveal viral determinants of virulence

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Classical swine fever virus (CSFV) has been eradicated from central Europe but remains endemic in different regions of Central and South America, Asia and the Caribbean. In Cuba, CSFV has evolved over the past 30 years as a unique subgenotype 1.4 from an outbreak of the high virulent CSFV/Margarita (CSF0705)/1958 (Margarita) towards viruses with an attenuated phenotype (Perez et al., 2012). Nucleotide sequence analyses of the envelope protein genes of the high and low virulent variants revealed amino acid substitutions located essentially in immunodominant epitopes of the envelope protein E2, which was attributed to a positive selective pressure due to suboptimal vaccine efficacy (Coronado et al., 2019). However, it was unclear, whether attenuation was due to the substitutions in E2 only or whether changes elsewhere in the genome were also involved.

To address this, we applied reverse genetics with a gain-of-function approach using the evolutionary-related low virulent CSFV/Pinar del Rio (CSF1058)/2010 (PdR) and high virulent Margarita strains. Nucleotide sequencing of the complete genome of the two viruses recovered from experimental infections in pigs revealed a total of 40 amino acid differences in the open reading frame and an insertion of 36 uridines in the 3' untranslated region (UTR) of PdR as opposed to the standard 5 uridines found at this position in the Margarita 3'UTR. Interestingly, the amino acid substitutions clustered in the envelope glycoprotein E2 and in the NS5A and NS5B proteins, with 7, 10 and 12 differences, respectively. First, we constructed functional cDNA clones of the PdR and Margarita strains and demonstrated that these recombinant viruses had maintained the phenotype of the parent viruses *in vitro* and *in vivo*. We then generated eight chimeric PdR/Margarita viruses by introducing single or multiple gene fragments from the Margarita strain, with or without the 3'UTR poly-uridine stretch or with the complete 5' and 3´UTRs of Margarita in the PdR backbone. All chimeric viruses had comparable replication characteristics in porcine monocyte-derived macrophages. PdR/Margarita chimera carrying either E2 or NS5A/NS5B of Margarita alone, with 36 or 5 uridines in the 3'UTR remained low virulent in 12-week-old pigs, while the combination of these elements recovered the virulent Margarita phenotype. These results show that CSFV evolution towards attenuated variants under suboptimal vaccination in the field involved mutations in both, E2 and the replicase proteins. They confirm also a synergistic effect of E2 and the replication complex in determining virulence of CSFV, as reported previously (Tamura et al., 2012).

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TOPIC 3 - Vaccine development

28. Dendritic cell responses of live attenuated C-strain and E2 subunit (Porvac) vaccines,against classical swine fever virus

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Classical swine fever virus (CSFV) causes febrile disease in pigs, mostly leading to wasting, haemorrhage and death. Live attenuated *C-strain* vaccine induces protection within 5 days. Unlike previously published subunit vaccines, the vaccine *Porvac*, comprising of CD154 conjugated to CSFV E2, also protects rapidly against CSFV, similar to *C-strain*. To gain insights on mechanisms of action of these vaccines, animals were vaccinated intra- muscularly with: Porvac; a Mock preparation; E2 in the equivalent adjuvant; or *C-strain*. Intranasal challenge infection with a field strain (CSFV:UK2000) was applied 5 days post vaccination. Draining regional lymph nodes (retropharyngeal and pre-scapula) and tonsils were collected either on day of challenge or 1 or 2 weeks thereafter and the associated dendritic cell (DC) populations were examined by analysing both frequency and activation of pDC, cDC1, and cDC2s by flow cytometry.

C-strain induced a substantially different pattern of DC distribution and activation compared to subunit vaccines. Addition of CD40L in *Porvac* did not change the distribution of DCs compared to the E2 only formulation.

Intriguingly, pre-challenge, the DC response in the local lymph nodes was cDC2 dominated in *C-strain* with a trend towards cDC1 in both *Porvac* and E2. MHCII as an activation marker was significantly increased in pDCs and cDC2s by *Porvac* compared to the adjuvanted E2 subunit only, particularly in tonsils on Day 7 post vaccination.

Differences in DC frequency and activation highlight significant differences in the response of DCs to diferent vaccines and the need for more investigations to assess the role of CD154 in conferring protection.

29. Recombinant Rift Valley fever virus expressing peste des petits ruminant antigens: immunogenicity studies in vivo

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Routine vaccination against zoonotic Rift Valley fever (RVF) in endemic countries is not practiced by many farmers due to its sporadic occurrences and shortcoming of the existing vaccines. In fact, RVF vaccination is only established after epizootic outbreaks occur, often too late to contain virus spread among animals and humans. Therefore, other strategies to encourage RVF vaccination and help reduce disease burden should be sought. In a general sense, the choice of multivalent vaccine approaches would facilitate the immunization against both non-prevalent and prevalent livestock diseases.

We and others had previously shown the feasibility of using live attenuated RVFV as a vector platform for vaccine antigen delivery. In this work we describe the rescue of recombinant RVFVs expressing either the Hemagglutinin (H) or Fusion (F) proteins from peste des petit ruminants virus (PPRV) strain Nigeria 75/1. Upon rescue the recombinant viruses showed stability in cell culture and sustained viral growth to high titers. The stability was proved by serial passages in which the expression of both transgenes was shown unaltered. Immunization of mice demonstrated the induction of serum antibodies able to neutralize both RVFV and PPRV infection in vitro. When tested in the ovine species, both neutralizing and cell mediated immune responses specific to PPRV and RVF antigens were elicited.

These results indicate that the use of rationale attenuated recombinant RVFV is a valid strategy for the simultaneous immunization against RVF as well as for other more prevalent ruminant diseases.

30. Generation of SARS-CoV-2 vaccines based on muNS-Mi carrying S, M, and N proteins by IC-tagging methodology

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An unprecedented global effort to develop safe and efficacious vaccines against the pandemic SARS-CoV-2 has been made. Commercialized COVID-19 vaccines are mostly based on the induction of neutralizing antibodies directed to the coronavirus spike (S) protein. Adequate protection against intracellular pathogens depends on the generation of potent adaptive immune responses, which implies the induction of both humoral and cellular arms of the immune system. In this sense, alternative SARS-CoV-2 antigens are being explored to enhance the immunogenicity of new vaccine candidates. Here, we have applied the methodology to produce avian reovirus muNS-Mi microspheres for presentation of different SARS-CoV-2 antigens through their C or N-terminal tagging with the so-called IC-Tag. Microspheres carrying the S protein of SARS-CoV-2, the pre-fusion state of the S protein or the receptor binding domain (RBD), respectively, were generated. The SARS-CoV-2 matrix (M) and nucleocapsid (N) proteins were also incorporated into muNS-Mi microspheres. The appropriate expression and incorporation of these antigens into avian reovirus muNS-Mi microspheres were confirmed. Besides, convalescent patient sera were used to evaluate whether these potential subunit vaccines could be recognized by naturally induced SARS-CoV-2 specific antibodies. muNS-Mi loaded with the pre-fusion S protein were detected by convalescent patient antibodies, while no such result was obtained for the S protein or the RBD. For the N protein, the construction with the IC-Tag in the C-terminal domain (N-IC) was the only one recognized, whereas both construction containing the M protein (IC-M and M-IC) were correctly detected by convalescent serum. Further experiments are being conducted to assess the immunogenicity of these vaccine candidates in BALB/c mice, and safety, immunogenicity and efficacy against COVID-19 disease will be eventually studied in the ACE2 mouse model and rhesus macaques.

31. Optimization of vaccine candidate screening using statistical simulations: an FMD storytelling

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Foot-and-mouth disease (FMD) is an acute viral vesicular disease affecting cloven-hooved animals characterized by painful lesions and a severe discomfort in animals. Due to its potential huge economic and social impact, especially in FMD-free countries, regulatory authorities require high-potency vaccines (containing at least 6 PD50/dose) to control the outbreaks. According to the different regulatory texts, the in-vivo 50% Protecting Dose (PD50) of vaccines is estimated by means of statistical methods after the experimental challenge of animals vaccinated with different fraction doses (full, 1/X, $1/X^2$, ...). Numerous topotypes (among six serotypes) are currently circulating in Africa and Asia with variable cross protections between them. Therefore, the potency of FMD vaccines must be estimated for each strain. At early stages of the screening phase, several vaccine candidates need to be tested to determine their PD50, multiplying the number of challenge studies to be performed. Thereby, the selection of new FMD vaccine candidates have a huge impact in animal ethics (in terms of number and discomfort). In addition, PD50 studies must be performed in BSL3 facilities, which are limited around the world, complicating the screening of new vaccines. In this context, a statistical method based on simulations was developed to optimize the screening of FMD vaccines and to better deal with the 3R rule.

The first step of the method is to collect a sound serological data package by means of injecting fraction doses of the vaccines to determine the fraction dose that would best distinguish the candidates. Then, the candidates are compared in only one challenge study injecting the determined dose to a group of five animals for each vaccine. The dose/protection relationships of theoretical vaccines (6, 7, 8, etc... PD50/dose vaccines) are modelized using log-logistic functions including some hypotheses concerning the steepness and the presence of high/low asymptotes. Finally, simulation of the *in-vivo* variability with binomial laws allows to determine for each vaccine candidate the probability of having a particular theoretical PD50 level according to the observed proportion of animals protected. Based on the highest probabilities, the log-logistic functions also help us to anticipate the best designs of further regulatory PD50 studies to estimate as accurately as possible the PD50 value of a selected candidate (where the Cumulative Distribution Function covers the entire dose range).

Using this method, three FMD vaccine candidates (A, B and C) were tested. Preliminary serological studies suggested that the best fraction dose to distinguish the candidates was 1/9. A challenge study was performed injecting 1/9 dose of each vaccine in pigs (three vaccinated groups of five animals, one unvaccinated control group). Based on the obtained protection pictures (2/5 protected animals with vaccine A, and 5/5 with vaccines B and C), the statistical method allowed to determine that vaccine A had high probabilities to be between 8-9 PD50/dose, and vaccines B and C had high probabilities to be higher than 15 PD50/dose. The usual method to estimate PD50 values (Spearman-Kärber) would probably have given results between 6.47-8.06 PD50/dose for vaccine A, and 15.95 PD50/dose for vaccines B and C (assuming monotone data set). Therefore, similar conclusions were obtained using the statistical method, sufficiently reliable to move forward at the early stages of the R&D process but preventing to challenge 34 extra animals and thus limiting time spent in BSL3 facilities.

32. Duration of immunity: comparison between a live attenuated and an inactivated lumpy skin disease virus vaccine

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Vaccines have proven themselves as an efficient way to control and eradicate Lumpy Skin Disease (LSD) in the field and under standardised experimental settings. In addition to safety and efficacy aspects of vaccines, it is important to know the period during which they confer protective immunity against an infection(duration of immunity), as this impacts the design of an efficient control and eradication program. As data on this topic is currently lacking, it was the purpose of this study to evaluate the duration of immunity induced by a live attenuated (LSDV LAV) and an inactivated (LSDV INAC) LSDV vaccine in a controlled and standardized setting using a previously optimized challenge protocol. Three groups of six animals were vaccinated with a LSDV LAV, according to the manufacturer's instruction, and challenged after 6, 12 or 18 months. In comparison, two groups of six animals were vaccinated (including a booster after 21 days) with an inactivated LSDV vaccine and challenged after 6 or 12 months. Unvaccinated animals were used as controls. In addition to a daily clinical monitoring, serum, EDTA and heparinized blood were collected during the post-vaccination and post-infection periods. These samples were analyzed to study viremia (vaccine related or/and challenge strain), seroconversion and IFNg release upon re- stimulation. After vaccination with the LSDV LAV, a fever spike and local reactions at the site of vaccination could be observed. The side effects of the LSDV INAC were limited to a short fever spike. The onset of seroconversion and the number of antibody positive animals until day 180 post-vaccination, as determined by the IPMA, was very similar for both vaccines. After that point in time, the animals vaccinated with the LSDV INAC demonstrated a strong decrease in antibody levels, resulting in all animals being negative after 1 year post-vaccination. Although some reduction in antibody levels was also seen in the LSDV LAV groups, this was less pronounced with the majority of the animals still being positive after 1 year. When the cellular immune response was evaluated, it could be seen that response was homogenous for the LSDV LAV groups with a medium to strong IFN? responsiveness in all animals up to 12 months. In the LSDV INAC group, however, the results were more heterogeneous between the trials. In the 6-month group all animals reacted strongly after vaccination and remained so until the moment of challenge. In the 1-year group, 2 animals reacted strongly and remained to do so until challenge but the 4 other only reacted weakly or remained negative during the complete duration of the trial. All animals vaccinated with the LSDV LAV were protected against infection after 6, 12 and 18 months as demonstrated by the absence of clinical signs and viremia after challenge. All the LSDV INAC vaccinated animals were protected against infection at 6 months post vaccination, but this was reduced to 67% (4 out of 6) when challenged at 12 months post vaccination. These observations can have important implications for the applicability of these LSDV vaccines in the field. While an annual vaccination campaign with the LSDV live attenuated vaccine seems sufficient, a bi-annual vaccination seems recommendable when using the inactivated LSDV vaccine.

33. Evaluation of the efficacy of live attenuated heterologous vaccines for the control of lumpy skin disease in cattle

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Lumpy skin disease (LSD) is a bovine viral disease with fever and the formation of nodules as main symptoms. It is of socio-economic importance, due to diminished draught power, drop in milk production, infertility and reduction of market value of the animals and hides (Gari et al., 2015). Lumpy skin disease virus belong to the genus of Capripoxviruses, together with sheeppox (SPPV) and goatpox virus (GTPV). The virus is mainly transmitted mechanically via arthropod vectors. (Sohier et al,. 2019). Vaccination has proven to be a very effective tool to prevent and/or control the disease. Previous studies have shown that homologous (LSDV-based) live attenuated vaccines (LAV) provide good protection against LSDV challenge (Haegeman et al, 2021). Since cross-protection is known to occur between viruses of the Capripox genus (OIE terrestrial manual, 2021), several countries however use heterologous (sheep- or goat pox-based vaccines) vaccines. In this study, the efficacy of 4 sheeppox based LAVs (LAV1-4), all based on different strains, and 1 goatpox based LAV (LAV5) to protect against an LSDV challenge were evaluated. The vaccination scheme and dose prescribed by the manufacturer was followed. The dose for vaccination of cattle with an SPPV vaccine is between 3X and 10X higher than the dose for the vaccination of sheep. For each vaccine, 7 cows were vaccinated and infected 21 days post vaccination. The results were compared with 5 non-vaccinated challenge control animals. For the animals vaccinated with the sheeppox LAVs, seroconversion was not detected in 5, 3, 2 and 5 animals of the 4 groups, respectively, in the period after vaccination. Furthermore, those that did seroconverted scored only weakly positive in the immunoperoxidase monolayer assay (IPMA). The induced interferon gamma response was variable between the different vaccines.

Based on the results of the interferon gamma assay, none of the vaccines was able to generate a cell mediated immune response in all of the vaccinated animals. The indications that LAV1-4 might not induce a protective immune response in all animals was confirmed by the LSDV challenge. For LAV 1 to 4, respectively 6, 2, 4 and 3 animals developed clinical signs upon challenge which were comparable to those observed in clinically infected animals in the control group. Besides the typical clinical lesions, also blood viremia, viral secretion and virus spread to different organs was found in the vaccinated animals showing clinical signs. The results with the sheeppox LAVs were in contrast with those obtained with the goatpox vaccine. All goatpox LAV vaccinated animals had seroconverted by the time of challenge and a strong IFN gamma response was detected after vaccination. All animals showed an increase in body temperature and a small local reaction upon challenge. These clinical signs are also seen with homologous LAVs. No nodule formation or enlargement of lymph nodes was observed. The clinical data were supported by the virological analyses. No blood viremia was detected and viral DNA was only found in one organ in two animals, respectively the skin at the inoculation site and the skin of the testis.

None of the SPPV based LAVs were suitable for vaccination to prevent LSDV infection at the vaccination dose used. On the other hand, the protection induced by the GTPV based LAV was comparable to the results obtained by homologues LAV vaccines (Ben-Gera et al., 2015; Haegeman et al., 2021), and could therefore be used to provide protection against LSDV infection. It should however be emphasized that this cannot be extrapolated to other goatpox virus based vaccines without extensive validation.

34. Anti-OVA immune response modulation in sheep by recombinant adenovirus expressing OX40L and CD70

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The tumour necrosis factor (TNF) superfamily OX40L and CD70 and their receptors are costimulating signalling axes critical for adequate T and B cell activation in humans and mice. Characterization of these receptors in ruminants is lacking. In this work we inoculated groups of sheep with these two different immunomodulators expressed from non-replicative human recombinant adenoviruses [described in (Rojas et al., 2020)], to determine whether they could improve the immune response to the model antigen OVA in sheep.

Groups of 7 sheep (21 sheep in total) were inoculated with human recombinant adenovirus expressing OaOX40L or OaCD70 or a control adenoviral vector at the time of immunization with OVA and bled at days 0, 15, 30 and 90 post-inoculation (D0, D15, D30 and 3MD0). Recall responses to OVA were assessed by inoculating the antigen three months (3M) later (3MD0), and blood samples obtained at days 7 and 30 after the second inoculation (3MD7 and 3MD30). PBMCs and serum samples were obtained for analysis of the adaptive immunity induced to OVA.

In the presence of immunomodulators, higher specific IgG titers against OVA were detected, especially in the group inoculated with the AdOaCD70. Moreover, sheep inoculated with AdOaCD70 showed a statistically significant increased production of specific antibodies against OVA at days D30 and 3MD0, and higher anti-OVA-specific IgG- secreting B cell counts detected by ELISPOT when compared to control. Higher IFN-? production was detected in ELISPOT assays in PBMC from the AdOaCD70 group in respect to the control group on days D7, 3MD7 and 3MD30, although this did not reach statistical significance.

Importantly, the immunomodulatory molecules administered (OaOX40L and OaCD70) did not trigger unspecific PBMC stimulation as no statistical differences in the percentage of PBMC populations were detected between recombinant adenovirus administration regimes when compared within the same day. Flow cytometry analysis of PBMC cell population nonetheless showed that monocytes (CD14+ cells) increased at D30 and 3MD7 compared to baseline (D0) in the AdOaOX40L group, and at day 3MD30 in the AdOaCD70 group. NK cells (CD14-CD16+ cells) increased in the AdOaCD70 group at D30 and 3MD30. Phenotypic analysis of T cell activation was also performed by assessing the expression of the activation marker CD62L (which is lost upon activation) and the activation/memory marker CD27 (which is expressed on naïve, memory cells and in early activation, but downregulated on effector T cells). At D15, sheep that received AdOaCD70 showed an increase in effector CD8+ T cells (CD8+ CD62L- CD27-) concurrent with a decrease in early activated cells (CD8+ CD62L- CD27+), which indicated that AdOaCD70 could promote CD8+ T cell effector differentiation.

The findings presented in this study show that the immunomodulators OaOX40L and OaCD70 could promote adaptive immunity in vivo in sheep and thus act as adjuvant. The data presented here is relevant to veterinary vaccine development and the in vivo functional characterization of immune signaling molecules in novel species.

35. Early and Solid Protection Generated by Thiverval Vaccine, a Novel Alternatives Against Classical Swine Fever Virus

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Classical swine fever (CSF) is a highly contagious disease that affects domestic pigs and wild boars. CSF poses a threat for the pig industry from sanitary and economic points of view, and its notification is mandatory to the World Health Organization for animal health (OIE). Despite the implementation of extensive eradication programs, CSF virus (CSFV) remains a challenge for the porcine industry. Inefficient vaccination programs in some endemic areas may have contributed to the emergence of low and moderate virulence CSFV variants, facilitating the virus persistence in the field. The present study aimed to expand and update the information about the safety and efficacy of the CSFV Thiverval-strain vaccine. Two groups of 6-week-old pigs were vaccinated and challenged with a highly virulent CSFV strain at 21- or 5-days post vaccination (dpv). A contact and an infection control group were also included. The vaccine induced rapid and strong IFN-? response, mainly in the 5-day immunized group, and no vaccine virus transmission was detected. Vaccinated pigs developed humoral response against CSFV E2 and E^{rns} glycoproteins, with neutralizing activity, starting at 14dpv. Strong clinical protection was afforded in all the vaccinated pigs as early as 5dpv. The vaccine controlled viral replication after challenge, showing efficient virological protection in the 21-day immunized pigs despite being housed with animals excreting high CSFV titres. These results demonstrate the high efficacy of the Thiverval strain against CSFV replication. Its early protection capacity makes it a useful alternative for emergency vaccination and a consistent tool for CSFV control worldwide.

36. Long-lasting immunity induced upon priming and boosting with HAdV5 and MVA vectors encoding the RVFV proteins Gn and Gc

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Rift valley fever virus (RVFV) causes a severe disease in livestock and humans mainly in Africa and the Arabian Peninsula. The potential risk to emerge in distant geographical regions remains as a veterinary and public health concern. Vaccination is the only measure to control RVF outbreaks in endemic regions but the available vaccines, based in attenuated virus, still pose residual virulence. Currently, there are not licensed vaccines available for human use. Over the past decade, several immunization approaches have been developed, based either in viral vector, modified live virus, subunit, nucleic acid, virus-like particles or virus replicon particle vaccines. In terms of safety, vaccines based in replication-deficient adenovirus vectors are a valid strategy for use in humans.

Replication-deficient adenovirus vectors have been widely used to develop vaccines against human and animal diseases with excellent results in terms of immunogenicity and safety. In previous works, we showed the efficacy and immunogenicity of RVF vaccines based in human adenovirus type 5 (HAdV5) or a replication-deficient chimpanzee adenovirus vector (ChAdOx1), both encoding the same RVFV glycoproteins GnGc cassette. Here, we have studied the long-term immune responses induced by mice immunized with one dose of HAdV5 encoding Gn and Gc and the effect of adding a booster dose with a modified vaccinia Ankara strain (MVA), encoding the same glycoprotein construct. We analysed the antibody response against purified Gn expressed by recombinant baculovirus as well as neutralizing antibody activity at different time points after immunization. We also evaluated the long-term B and T cellular immune response elicited by *ex vivo* T and B-cell interferon-? enzyme-linked immunosorbent spot (ELISPOT) and intracellular cytokine staining (ICS) assays. Both immunization strategies generated a specific antibody response and neutralizing activity that remained detectable during at least 20 months. After MVA boosting, a transient increase in the magnitude of the antibody responses was observed, decreasing afterwards to similar levels of mice primed only with HAdV5-GnGc. Both strategies induced a robust CD8+ IFN?+ T-cell specific response detected up to 20 months after vaccination. In conclusion, these results emphasizes the efficacy of a RVF immunization regime based on the use of HAdV5 and/or MVA viral vectors to elicit strong, long-lasting, humoral and cellular responses and their potential use in different species.

37. Optimization of the modified vaccinia virus Ankara (MVA) as a vaccine vector for sheep

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Nowadays, Modified Vaccinia Ankara (MVA), a poxvirus, is used as a viral vector for the development of new generation vaccines against human diseases and relevant pathogens in animal health such as Bluetongue virus (BTV) or Rift Valley fever virus (RVFV). In this work, we assessed the feasibility of modifying the MVA host range with the aim of obtaining stable avirulent variants with an enhanced replication rate both *in vivo* and *in vitro*. The generation of recombinant MVAs (rMVAs) that are replication competent in target species could provide a novel platform for the generation of rMVAs that express heterologous antigens capable of inducing a higher level of immunogenicity compared to the parental virus. For adaptation, several cell lines of bovine or ovine origin were tested including MDBK (bovine kidney) or GeLo9633 (a spontaneous cell line obtained by subculture of ovine peripheral blood mononuclear cells). In MDBK cells no cytopathic effect (cpe) was observed after 10 consecutive passages. However, in GeLo9633 cells, a slight cpe was observed at the fifth passage suggesting the adaptation of the virus to this cell line. The virus was then serially passaged in these cells for a total of 100 passages (P1 to P100). The time for the onset of detectable cpe was around 72 hours post-infection initially, and was faster at later passages, reaching titers of 10^5pfu/ml. Interestingly, after adaptation the virus was able to grow in other mammalian cell lines such as Vero cells. The P100 viral genome was analyzed by NGS, which allowed us to detect seven point mutations and four large deletions, in various genes that codify for different proteins, enzymes and growth and virulence factors. The adapted virus (MVAp100) was used for the generation of rMVAs expressing either BTV-NS1 protein or RVFV Gn and Gc glycoproteins, showing transgene expression levels comparable to those of a rMVA based on the non-adapted parental virus. The vaccine potential of the generated viruses was assessed *in vivo* both in mice and sheep. Sheep vaccinated with rMVAp100-GnGc showed lower viremia titers and reduced pyrexia upon challenge with virulent RVFV. On the other hand, vaccination with rMVAp100-NS1 induced higher NS1 specific antibodies and higher levels of IFNg in stimulated plasma, with reduced pyrexia levels upon BTV-4 challenge. Additional studies should be done to clarify the role of the genetic changes in the adapted virus.

38. Study of BoHV-1 Seronegative Latent Carrier by IBR marker vaccines in passively immunised calves: preliminary results

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The Bovine alphaherpesvirus 1 (BoHV-1) is responsible for different clinical syndromes, including a respiratory disease named Infectious Bovine Rhinotracheitis (IBR) (1). A peculiar feature of this virus is the possibility to induce latent infections. However, the presence of passive immunity may interfere with an antibody response following traditional vaccination (2) or infection (3) and induce the condition of so-called Seronegative Latent Carrier **(**SNLC). These animals are characterized by: (i) reduced passive immune response; (ii) viral shedding after dexamethasone (DMS) treatment (4). SNLCs are of primary relevance in genetic or artificial insemination centres, control or eradication plans and BoHV-1, as the virus may be present in animals without showing any seropositivity. The aim of this study was to investigate whether calves immunised with IBR live deleted vaccines, in the presence of IBR passive immunity, may become SNLC animals.

Six neonatal calves were vaccinated and divided into two groups of three animals each. Group A was injected with an IBR live gE, tk deleted vaccine by the intramuscular route. Group B was inoculated with an IBR live gE-deleted vaccine by the intranasal route. The neonatal calves were fed with colostrum and milk collected from cattle immunised with an inactivated IBR gE-deleted marker vaccine.

Clinical signs were evaluated throughout the experiment. In addition, serum samples and nasal swabs were collected at different time points from all animals. The serum samples were tested for the specific antibody via competitive gB-ELISA (IDEXX IBR gE), gE-ELISA (IDEXX IBR gB X3) and virus neutralization (VN) test. The competitive ELISA tests were carried out using the protocol provided by the kits, while the VN test was performed according to the protocol described in the O.I.E. Manual (5). Nasal swabs were obtained from each animal and used for virus isolation (5). When the animals became BoHV-1 seronegative, each calf was treated with DMS at 0.1 mg/kg intravenously on 5 consecutive days to demonstrate the BoHV-1 vaccine latent state. Further, nasal swabs and blood samples were collected for the virological and serological investigations.

Vaccine virus shedding and the clinical form of BoHV-1 were not observed during the entire experimental period. The calves became BoHV-1 seronegative to neutralizing antibodies at 180 (Group B) and 270 (Group A) post- vaccination days. Subsequently, at 74 days post-treatment (DPT) with DMS, only the calves in Group A seroconverted for the competitive gB-ELISA test. In addition, these animals showed a low progressive increase in the VN titer (1.38 $\log_{10}$) 120 DPT with DMS. Moreover, in both groups, the gE-ELISA test was consistently negative, excluding virus circulation on the farm. These results differ from those published in the previous study (5,6).

This study would appear to demonstrate that it is possible to induce SNLC status in passively immunized calves inoculated with live double-deletion vaccine. In fact, despite the fact that viral isolation tests provided negative results, these animals showed seroconversion post-DMS treatment: this increase can be taken as an indicator of viral reactivation. The results obtained in this research (IZSUM RC 09-2019) are preliminary, and a second phase involves the same protocol mentioned above, using calves free of passive immunity to IBR.

1)Petrini et al., 2019; 2) Menanteau-Horta et al., 1985; 3) Bradshaw et al., 1996; 4) Lemaire et al., 2000; 5) Manual ests and Vaccines for Terrestrial Animals, 2018; 6) Lemaire et al., 2001.

TOPIC 4 - Vector-borne diseases

39. “One Health" surveillance of West Nile and Usutu viruses suggests endemisation in the South of France

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The number of emerging mosquito-borne viruses has increased in the last decade worldwide. These viruses include West Nile virus (WNV) and Usutu virus (USUV), two closely related flaviviruses. These viruses mainly follow an enzootic cycle involving mosquitoes and birds, but they occasionally infect mammals. Disease in vertebrates can develop ranging from mild febrile illness to severe neurological disorder. Their control mainly relies on surveillance and the adoption of preventive measures in case of detection. However, the epidemiological situation may have recently changed in several European regions, shifting from sporadic epidemics to endemicity. This situation deserves confirmation due to its implications for risk assessment and surveillance strategies. We have evaluated the circulation of WNV and USUV in the South of France, an area particularly exposed to a potential endemisation. An epidemiological survey assessed virus exposure in mosquitoes, birds, horses, dogs and humans between 2016 and 2020. We observed the active circulation of both viruses. Moreover, for both viruses, the same lineages were repeatedly found along years. Overall, these results suggest on-going endemisation and non-negligible exposure for WNV and USUV in the South of France.

40. Rescue and characterization of bluetongue virus (BTV) expressing reporter genes by reverse genetics

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Bluetongue virus (BTV), the prototype member of the genus Orbivirus (family Reovirida e), is the causative agent of an important livestock disease, bluetongue (BT), which is transmitted via biting midges of the genus Culicoides.

This disease causes severe economic losses that are associated with its considerable impact on animal health, both direct such as weight loss, reduced fertility rate, red uced meat and milk production efficiency and death, or indirect like lost revenue and trade restrictions.

The study of BTV requires the use of secondary approaches to detect the presence of v irus in infected cells. By reverse genetics, we have designed and rescued a BTV-1-bas ed virus expressing luciferase (NLuc) or the fluorescent reporter genes iLov or Venus.

To generate these viruses, we custom synthesized a modified segment 5 (encoding NS1 protein) where the NS1 and the reporter proteins were separated by the Porcine tesch ovirus-1 (PTV-1) 2A autoproteolytic cleavage site. As the reporter genes were fused t o NS1, fluorescent signal or luciferase activity were only detected when the virus en ters the cell, replicates, and synthesizes its nonstructural proteins. Fluorescent si gnal was detected in the same BTV-1/iLov or BTV-1/Venus infected cells as those expre ssing NS2 confirming that these reported genes were only expressed in cells with repl icating BTV. Growth curves in Vero cells showed that BTV-1/Nluc, BTV-1/iLov and BTV-1

/Venus showed a replication rate comparable to that of BTV-1 wild-type. In addition, the recombinant BTV expressing the reporter genes were stable at least in the six pas sages in Vero cells analyzed. In contrast, infection assays in IFNAR (-/-) mice showe d an LD50 higher for BTV-1/Nluc, BTV-1/iLov and BTV-1/Venus than for BTV-1 indicating that viruses containing the reporter gene were attenuated in vivo.

By eliminating the need for secondary labeling of infected cells, BTV reporter viruse s provide an ideal tool in the ongoing fight to better characterize the virus and ide ntify new therapies against viral infections of this pathogen.

41. Surveillance and control of WNV and USUV in the European Union/European Economic Area: a cross-sectorial study

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West Nile virus and Usutu virus are arboviruses maintained and amplified in nature in an enzootic cycle between birds and mosquitoes. Under certain conditions, the spillover of both viruses to humans, equines and other wild and domestic mammals may occur. Direct human-to-human transmission may occur through infected substances of human origin (SoHO). Both viruses are becoming endemic in many EU/EEA countries. The results presented are part of a multi-sectoral project launched by the European Centre for Disease Prevention and Control, in collaboration with the European Food Safety Authority and implemented by the Istituto Zooprofilattico Sperimentale delle Venezie and the University of Padua.

Key elements of the project were: an online survey directed to Animal Health, Vectors, Public Health and Blood Safety national authorities, a literature review and an online technical stakeholder meeting. Twenty-nine out of 30 countries provided a reply for at least one sector. Overall, 824 articles were included in the literature review study and 77 and 69 representatives of the three sectors from 13 countries plus the representatives from ECDC funded networks and the EU Commission were present respectively on the first and second meeting day.

Results confirm that WNV is endemic in several EU/EEA countries and during the last decade a total of 3,632 human cases of WNV infection were reported in 16 countries (62% of which in Italy and Greece). In the same period, 17 countries reported WNV cases in animals and 13 detected the virus in mosquito vectors. USUV infections are also increasing: eight countries reported a total of 104 cases of USUV infection, most of which occurred in Italy and Austria (54%, 25%), 15 countries reported USUV cases in animals and eight in mosquito vectors.

WNV infection is a notifiable disease in Europe and most of the countries use the EU case definition for WNV infection in humans and the OIE definition for animal cases. Approaches for identifying WNV infections vary among countries: passive surveillance is applied in the majority of countries in both humans and animals. To date, there is no EU case definition for USUV infection in humans and animals, however some countries have developed a national case definition for USUV infection and conduct surveillance nationally. An integrated surveillance system, defined as a surveillance system able to early detect viral circulation through human, animal and vector targeted surveillance, in order to prevent transmission to humans and guarantee blood safety, is present in nine out of 30 EU/EEA countries and in two of them is partially integrated since vectors are not included. In 13 EU/EEA countries specific SoHO safety measures are implemented during the transmission season and in nine they are implemented following a trigger that includes the detection of laboratory-confirmed: i) human case with WNV neuroinvasive disease and/or fever ii) equine case with positive IgM antibodies and/or positive molecular test, iii) bird case and iv) virus detection in mosquitoes.

Besides prevention of human cases through control of SoHO in donors and in returning travellers, 11 countries apply vector control strategies (mosquito control actions and/or citizen education programs)

very differently in terms of mandatory nature, responsibilities for the implementation and application times.

In conclusion, promoting standard operative procedures for all the sectors and well-defined strategies for WNV and e and control could be reinforced at the European level under the “One Health” approach.

TOPIC 5 - Pathogenesis and immunology

42. A quantitative approach to study equine influenza virus pathogenesis

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Equine influenza (EI) is a significant disease of the horse caused by equine influenza virus (EIV). It displays high morbidity and low mortality. Typically, infected animals shed virus for up to 10 days and exhibit conjunctivitis, nasal discharge, anorexia and lethargy. Pathological changes induced by EIV infection include degeneration of the respiratory epithelium (e.g., loss of cilia and goblet cells, necrosis of the epithelial cells) and inflammation of the pulmonary alveoli due to secondary bacterial infection.

To study the spatial and temporal dynamics of pathological changes induced by EIV, we performed image analyses of archived sections derived from respiratory tissues of animals experimentally infected with EIV (A/equine/Ibaraki/1/07) and euthanized at 2, 3, 7 and 14 days post infection (pi). Sections were stained with hematoxylin and eosin to visualise morphological changes. Viral proteins and markers of apoptosis (CC3), mitosis (Ki67) and innate immune activation (MX1) were detected using immunohistochemistry (IHC). IHC was also used to identify B (Pax5) and T (CD3) lymphocytes to characterise inflammation. Viral mRNA was detected *in situ* using RNAscope®. Morphological changes and protein expression were measured using Aperio Imagescope image analysis software.

We observed virus antigen in the nasal mucosa, trachea, bronchi, bronchioles and alveoli at all times post infection. Virus distribution displayed a clear temporal and anatomical pattern, with lower areas exhibiting abundant viral antigen at late times pi. Notably, we detected viral proteins and viral mRNA at day 14 post infection. Innate immunity was strongly and transiently activated at day 3 post infection. Apoptosis and mitosis were observed at all timepoints, albeit at low levels. Epithelial cell density decreased over time and varied according to anatomical location. Pulmonary fibrosis and airway blockage were evident at days 7 and 14 post infection.

Our results show that EIV infects a large proportion of cells in the entire equine respiratory tract, disrupting its normal structure and function, and triggering an early and widespread innate immune response. To our knowledge, the observation of lung fibrosis has not been previously associated with EIV and suggests that the virus induces more severe lesions than previously thought. The presence of viral antigen and mRNA at day 14 post infection is another novel finding that requires further investigation as it suggests that EIV persists in the respiratory tract for longer than expected.

43. Is the trick of CSFV C strain vaccine attenuation just growing slowly?

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Classical swine fever (CSF) remains a global problem to pig production and in Europe is an exotic disease and threat. The C strain of CSFV is an extremely efficacious vaccine, but its modus operandi remains unresolved. Live vaccines, such as C strain, are generally attenuated through multiple passage, often in different hosts. As a consequence, their replication is notably slower than that of pathogenic strains and this applies to C strain both *in vitro* and *in vivo*. Hence it is tempting to speculate that the reduction of replication speed provides the immune system with a head start that allows it to react fast, thereby keeping the vaccine in check and to be prepared for subsequent challenges. In contrast, the immune system cannot hold pace this with the pathogenic, fast replicating strains. This indeed would be a possible conclusion derived from one of our previous studies (doi:10.3389/fimmu.2019.01584), demonstrating a key role for interferon signalling and particularly ISG15 in the action of C strain to limit replication upon challenge and also explain the fast action of C strain to protect within 5 days of vaccination.

We have recently carried out a further study to identify the early events of C strain action on the immune system (doi:10.3390 /ijms22168795). Here, pigs were infected or immunised intranasally with either the pathogenic strain Alfort187 or the C strain vaccine, and the immune reaction in tonsils examined over a period of 90 hours. In this context changes in the dynamics of the myeloid cell compartment were identified, which were largely similar between C Strain and Alfort187 indeed. We were able, however, to identify 2 animals per group that had very similar in viral load in the tonsils - for Alfort187 samples were from animals euthanised a 36hpi whereas for C strain tonsils at 90hpi had an equivalent viral load. These samples were further analysed, and their transcriptomes compared using next generation sequencing of the RNA followed by gene expression analysis using different approaches to determine differentially expressed genes, gene set enrichments and pathway analysis. The results provide clear evidence that C strain initiates a very different host response in comparison to the pathogenic strain, not only driving a head start for the immune system but generating better immune conditions overall. In contrast Alfort187 seems to initiate a rather rogue response, for example in the ISG15 pathway.

44. Chronological brain lesions after SARS-CoV-2 infection in hACE2 transgenic mice

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes respiratory disease, but it can also affect other organs including the central nervous system. Several animal models have been developed to address different key questions related to the disease caused by this agent: the coronavirus infectious disease 2019 (COVID-19). Since wild type mice are minimally susceptible to certain SARS-CoV-2 lineages (Beta variant), hACE2-transgenic mice, previously developed to study SARS-CoV, have been used. These mice succumb to SARS-CoV-2 and develop a fatal neurological disease. Here, we aimed to chronologically characterize the SARS- CoV-2 neuroinvasion and neuropathology. Necropsies were performed at different time points and the brain and olfactory mucosa were processed for histopathological analysis. SARS CoV-2 virological assays, including immunohistochemistry, were performed along with a panel of antibodies to assess neuroinflammation. At 6-7 days post inoculation (dpi), brain lesions were characterized by non-suppurative meningoencephalitis and diffuse astro- and microgliosis. Vasculitis and thrombosis were also seen associated with occasional microhemorrhages and spongiosis. Moreover, neuronal vacuolar degeneration of virus-infected neurons was observed. At 2 dpi SARS CoV-2 immunolabeling was only found in the olfactory mucosa, but at 4 dpi intraneuronal virus immunolabeling had already reached most of the brain. Maximal distribution of the virus was observed throughout the brain at 6-7 dpi except for the cerebellum which was mostly spared. Our results suggest an early entry of the virus through the olfactory mucosa and a rapid inter-neuronal spread of the virus leading to acute encephalitis and neuronal damage in this mouse model.

45. Lumpy skin disease virus encodes an extracellular inhibitor of type 1 IFN signalling

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Lumpy skin disease virus (LSDV) is a poxvirus and the causative agent of lumpy skin disease (LSD), a severe systemic disease of cattle characterised by multifocal necrotic cutaneous lesions. LSD causes substantial economic loss to affected regions through production losses, cost of control strategies and export restrictions. LSD is a rapidly emerging disease, having spread from Africa and the Middle East into Europe, Russia and extensively throughout Asia in the past six years.

Vaccination is the most effective strategy to control and prevent LSD however, reports of adverse reactions and recombination events highlight the need for safer, more effective vaccines. Poxviruses encode a plethora of proteins targeting type 1 IFN to block and evade the activation of the antiviral response. LSDV is a neglected pathogen, consequently little is known about how it evades the antiviral type 1 IFN response. We found that LSDV infection of MDBK cells does not induce a detectable type 1 IFN response suggesting LSDV, like other poxviruses, employs immunomodulatory mechanisms to inhibit activation of the type 1 IFN response. LSDV135 is an orthologue of the vaccinia virus protein B18, a well-characterised type 1 IFN receptor mimic. We hypothesised that LSDV135 encodes a soluble, secreted protein which binds to and inhibits the function of bovine type 1 IFN. A deletion mutant virus (LSDVegfp?135) was generated using homologous recombination in order to study the function of LSDV135. MDBK cells were infected with wildtype LSDV and LSDVegfp?135, and supernatant collected and concentrated. Supernatant from cells infected with wildtype LSDV inhibited the activity of bovine IFN-?, however no inhibition was detected when using supernatant collected from cells infected with LSDVegfp?135. In contrast, supernatant from both wildtype and LSDVegfp?135 infected cells showed inhibitory activity against bovine type 3 IFN (IFN?3). This indicates that LSDV inhibits both type 1 and type 3 IFN via a soluble secreted factor. The activity against type 1 but not type 3 IFN is associated with LSDV135. Therefore, LSDVegfp?135 virus is less able to inhibit the host anti-viral response and could be targeted to improve current live-attenuated vaccine strains to help control and prevent LSD.

46. A new FMDv Antigen ELISA using multiserotype-reactive monoclonal antibodies

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Foot-and-Mouth Disease (FMD) is an international epidemic disease that infects cloven-hoofed animals such as cattle and swine. There are seven FMD viruses (FMDv) serotypes, O, A, Asia1, C, SAT1, SAT2 and SAT3. FMD is one of the most highly contagious viral diseases that causes devastating economic losses. The wide range of hosts, ability of small doses to infect, rapid replication, high levels of viral excretion and multiple forms of transmission make FMD difficult to control and eradicate. As FMD can spread fast, rapid and specific identification of the agent is required, as FMD is clinically indistinguishable from other vesicular diseases such as vesicular stomatitis (VS) and swine vesicular disease (SVD).

The FMDv antigenic diagnostic methods mentioned in the OIE manual are virus isolation, immunological methods

—i.e., sandwich Enzyme-Linked Immunosorbent Assay (ELISA) and nucleic acid recognition methods, such as reverse transcription (RT)-PCR and real-time RT-PCR. The manual mentions the ELISA as “that the preferred procedure for the detection of FMD viral antigen (Ag) and identification of viral serotype”.

In this study, we describe the preliminary performance evaluation of a Double Antibody Sandwich (DAS) ELISA for FMDv detection.

A panel of different monoclonal antibodies (MAb) against each of the FMDv types O, A, and Asia 1 was produced. Most of them could detect only a single-serotype antigen. However, some MAbs recognized different serotypes.

The reactivity profile of each of these *pan*FMD MAbs was investigated by indirect ELISAs, with plates coated with viruses from different serotypes. The MAbs that showed the wider spectrum of recognition were selected and submitted to further testing for their ability to capture and reveal the different virus strains in DAS ELISA format. The *pan*FMD MAbs were labelled to Horseradish Peroxidase (HRP), and various combinations were tested by DAS ELISA using them either as trapping antibody or as HRP conjugate.

For the best couples, the inclusivity was assessed by testing different serotypes including O, A, Asia1, C, SAT1 and SAT2. The analytical sensitivity was evaluated by testing serial dilutions of these viruses. Results obtained with the new *pan*FMDv DAS ELISA were compared to commercially available techniques: a MAb –based ELISA kit produced by reference laboratories and a lateral flow device test based on the well described 1F10 MAb.

The new *pan*FMDv DAS ELISA was able to detect all strains tested. SVD virus was not detected. Interestingly, SAT1 and SAT2 strains were very well detected, whereas a very low signal was observed with the other Ag ELISA. Globally, the new *pan*FMDv DAS ELISA showed an improvement in analytical sensitivity (up to 10-fold) compared to the other techniques and seemed to have a wider spectrum of detection.

Further testing on more strains is ongoing to better characterize the MAbs recognition pattern and the usefulness of their use in viral detection tests. This new *pan*FMDv Ag ELISA, easy to implement, gives results in less than 90 min, allows for rapid and specific FMDv multiserotype detection. It could be a useful tool for detecting and controlling FMDv outbreaks.

47. Presence of seronegative sows after routine vaccination against Porcine Reproductive and Respiratory Syndrome

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Keywords: Pig health, PRRS, vaccination, immune responses

Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS), an infectious disease caused by the PRRS-virus (PRRSv), is responsible for major production and economic losses in the worldwide swine industry. Vaccination against PRRS in sows and/or piglets is the main tool to prevent and control the disease. Unfortunately, the effectiveness is suboptimal and inconsistent, with PRRS outbreaks occurring despite routine vaccination being practiced. This cross-sectional study aimed to identify non-responding sows: sows who remain PRRS-seronegative despite being routinely vaccinated, by measuring the antibody (Ab) response in different serological assays.

Materials and methods

A total of 70 Belgian sow herds, practicing routine PRRS-vaccination, were visited between October 2020 and May 2021. In each herd, 20 breeding sows of different parities were blood sampled, resulting in a total of 1400 samples (parity 1: 19.57%, parity 2: 21.14%, parity 3: 19.07% and parity 4+: 40.21%). IDEXX ELISA was used to detect Abs directed against the Nucleocapsid (N) protein. CIVTEST ELISA was used to detect Abs against a Glycoprotein extract. Negatives in either or both tests were further analyzed on the INgezim 2.0 and IDScreen ELISA kits, both detecting Abs against the N protein. The presence of neutralizing Abs, in a selection of ELISA seronegative and seropositive samples, was assessed by means of SN assay on the PRRSv DV strain.

Results

In total, 49/1400 (3.5%) sows were identified as seronegative in IDEXX ELISA. These 49 sows originated from 28/70 (40%) of the herds, with the within-herd prevalence ranging from 1 to 4 (on 20 sampled). In CIVTEST ELISA, 58/1400 (4.14%) sows were classified as seronegative, originating from 40% of the herds and the within-herd prevalence ranged from 1 to 6. Almost all IDEXX seronegatives were also seronegative in both INgezim (95.9%) and IDScreen ELISA (91.8%). CIVTEST seronegative but IDEXX seropositive samples showed a lower percentage of seronegatives in the additional ELISA tests (85.7% and 77.1% respectively). A discrepancy between ELISA and SN results was shown: 43.21% of ELISA seronegatives (in either or both tests, n=81) tested seropositive in SN assay and 29.83% of ELISA seropositives (in both tests, n=238) were seronegative in SN assay.

Discussion and conclusion

Although a low number of PRRS-vaccinated, seronegative sows was identified, there was at least one seronegative sow in 40% of the herds. The clinical importance of the observed non-responders as well as the underlying immunological mechanisms warrants further investigation, as it can be hypothesized that these sows might be at elevated risk for PRRS. Furthermore, it can be reasoned that piglets born from PRRS seronegative sows might receive less maternal immunity and/or react different to PRRS vaccination, compared to piglets born from PRRS seropositive sows. The influence of the sow PRRS immune status on the progeny will be analyzed in a follow-up study. Finally, the

observed discrepancy between ELISA and SN results suggests that ELISA alone might be insufficient to classify pigs as seronegative for PRRS.

48. Extracellular vesicles in bovine leukaemia virus (BLV) infected cattle.

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Bovine leukaemia virus (BLV), member of the genus *Deltaretrovirus,*family *Retroviridae* is a cattle retrovirus responsible for bovine enzootic leukaemia. Its genetic structure, sequence and pathogenicity present many similarities to human T-cell leukaemia viruses (HTLV-1 and 2). Approximately in one-third of infected animals persistent lymphocytosis with polyclonal expansion of B-lymphocytes is observed, but some animals are frequently asymptomatic at aleukaemic state and they are virus carriers. In only small percentage of infected cattle develops malignant lymphoma after a long latency period. Cell-to-cell transmission is the most efficient route of leukaemia virus infection. BLV is present in circulating peripheral blood lymphocytes of infected animals and both horizontal and vertical transmission occurs often through infected blood. Extracellular vesicles (EVs) such as exosomes and microvesicles are nanosized membranous microvesicles, ranging in size from 30 to 1000 nm in diameter. They are extracellulary released from variety of mammalian cells. Exosomes are lipid bilayer membrane-enclosed extracellular vesicles. They play an important role in intercellular signalling by transporting many biomolecules: several types of RNA, lipids and proteins. They participate in intercellular exchange of DNA, RNA, miRNA, proteins and other components. These microvesicles are present in all body fluids in physiological and pathological conditions and represent actual state of host organism. Under pathological conditions their number, size and content are found to be altered and have been shown to play crucial role in diseases progression. The aim of the study was exosomes isolation, determination of exosomal matkers and infectivity. Blood samples were collected from BLV-infected and BLV free cows. Exosomes were isolated by ultracentrifugation from blood sera, plasma and cell culture supernatant of BLV infected dendritic. Stem cells CD34+ generated from the blood and lymphoid tissues were cultivated *in vitro* with exosomes of BLV infected cows. Real-time PCR and immunofluorescence (IF) were used for determination of exosomal infectivity. Analysis of exosomes in cell cultures was performed with the use of Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). Western blot (WB) analysis was performed for determination of structural BLV proteins: gp51 (Env) and p24 (Gag) and exosomal markers tetraspanins: CD9, CD63 and flotillin-1. The presence of exosomal markers were found in all investigated samples, but viral antigens were detected only in exosomes of BLV infected cows. RT-PCR and IF tests confirmed BLV infection of stem cells CD34+ generated from spleen after cocultivation *in vitro* with leukaemic cow exosomes.

As conclusion: Virus-altered EVs that can be found in all body fluids are increasingly being used as biomarkers for identification the presence and severity of a viral infection. The expression and changes in pathogen-derived factors in the EVs can serve as diagnostic biomarkers and indicators of disease progression. The change in EVs cargoes during disease progression makes them important biomarkers.

49. Novel antibody signatures in Bluetongue virus infected ruminants improve predictions of infectious timelines

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Bluetongue is a high-consequence, non-contagious, haemorrhagic disease of ruminants caused by the arbovirus, Bluetongue virus (BTV). The identification of infectious ruminant hosts in the field remains a key aspect of global BTV control, however this is hampered by the very broad characterisation of infection provided by current diagnostic tests. The infectiousness and transmission risk of PCR-positive ruminants is often uncertain given viral RNA remains in the blood significantly longer than transmissible virus. Furthermore, commercial enzyme linked immunosorbent assays (ELISAs) only detect antibodies raised against the group-specific viral protein 7 (VP7) which only confirms infection or vaccination within an animal's lifetime. As BTV-infected ruminants raise antibodies against all BTV proteins, including the non-structural proteins important for viral replication, these underexplored antibody dynamics might provide new depths and insights into BTV infection timelines which could, in turn, benefit our current diagnostic strategy. Here, we have generated bacterially expressed recombinant non-structural protein 2 (NS2) and have used this alongside commercially available VP7-coated plates (ID-Vet, Grabels, France) to develop novel ELISAs to detect anti-NS2 and anti-VP7 antibodies of varying isotypes (IgM, IgG Fc, IgG1 and IgG2) in BTV antibody positive cattle and sheep sera. Following their development, these novel assays were used to investigate isotype-specific anti-BTV antibody dynamics across a vast collection of archived time-course serum obtained from BTV-infected cattle and sheep. The *in vivo* studies from which these sera originate include animals infected by different inoculation routes and with different BTV strains, clinical outcomes and viremia dynamics. IgM- specific anti-VP7 antibodies were detected first during infection in both cattle and sheep, appearing from around 7-8 days post infection (dpi) and peaking at 10-12 dpi, with IgM levels reaching peak earlier in sheep. The decline in IgM-specific anti-VP7 antibodies thereafter coincided with an increase in IgG Fc-specific antibodies, demonstrating that the presence, absence or potentially specific titres/ratios of IgM/IgG Fc-specific anti-BTV antibodies might allow a better estimation of time since BTV infection. We also present here, for the first time, the dynamics of isotype-specific anti-NS2 antibodies in BTV-infected cattle and sheep and compare these signatures to those of anti-VP7 antibodies to better characterise time since infection and assess potential correlation between viremia, clinical disease and antibody dynamics. These novel isotype-specific anti-BTV antibody ELISAs might play an important role in future diagnostic strategies for BTV during testing for animal movement for importation/export and trade purposes, particularly as an improved prediction of time since infection might allow better analysis of the transmission risk to the *Culicoides* insect vector.

50. Comparative Virus-Host Protein Interactions of the Bluetongue Virus NS4 Virulence Factor

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Bluetongue virus (BTV) is the etiologic agent of a non-contagious arthropod-borne disease transmitted to wild and domestic ruminants. BTV induces a large panel of clinical manifestations ranging from asymptomatic infection to lethal hemorrhagic fever. Despite the fact that BTV has been studied extensively, we still have little understanding of the molecular determinants of BTV virulence. In our report, we have performed a comparative yeast two-hybrid (Y2H) screening approach to search direct cellular targets of the NS4 virulence factor encoded by two different serotypes of BTV: BTV8 and BTV27. This led to identifying Wilms' tumor 1-associated protein (WTAP) as a new interactor of the BTV-NS4. In contrast to BTV8, 1, 4 and 25, NS4 proteins from BTV27 and BTV30 are unable to interact with WTAP. This interaction with WTAP is carried by a peptide of 34 amino acids (NS422?55) within its putative coil-coiled structure. Most importantly, we showed that binding to WTAP is restored with a chimeric protein where BTV27-NS4 is substituted by BTV8-NS4 in the region encompassing residue 22 to 55. We also demonstrated that WTAP silencing reduces viral titers and the expression of viral proteins, suggesting that BTV-NS4 targets a cellular function of WTAP to increase its viral replication.

51. Immunohistochemistry as a useful tool for Rift valley fever virus detection on formalin-fixed paraffin-embedded tissues

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Rift valley fever virus (RVFV) is an arthropod-borne RNA virus which causes disease in livestock, wild ruminants and humans. Epizootics and epidemics have occurred in several countries in Africa and the Arabian Peninsula, with continuing reports of severe human illness and livestock disease. Understanding Rift valley fever (RVF) pathogenesis and host´s immune response is critical to develop new vaccines and antiviral treatments. RVF pathogenesis is different among susceptible species. Along with the pathogenicity of the virus strain, other host factors such as age or breed are determinant for the outcome of the disease. That is why choosing the most suitable animal model has not always been straightforward. Mice are one of the most susceptible animal models to RVFV infection. However, farm animals as sheep may predict vaccine outcome or therapeutic treatments in humans more accurately than rodents, making sheep model very attractive for RVF studies.

The pathogenetic mechanisms of the most frequently lesions observed in RVFV infected mice and sheep such as hepatitis, renal failure, encephalitis, vascular endothelial injury or immunosuppression have been poorly studied. To throw light on these pathogenic mechanisms, “in vivo” studies using a combination of histopathological and immunohistochemical methods on tissue samples from experimentally infected animals are required. Along with other RVFV detection techniques as PCR or virus isolation, immunohistochemistry on formalin-fixed paraffin- embedded (FFPE) tissues is an excellent and complementary tool for RVFV diagnostics and research. Formalin is a cheap fixative, stable within a wide temperature range, easy to transport and store. FFPE tissues are non- infectious, can be handle outside biocontainment facilities and can be stored at room temperature for decades in tissue banks, allowing samples to be compared with retrospective archived materials. However, the availability of commercial antibodies against RVFV antigens is scarce.

In the present study, we have set up an immunohistochemical technique on FFPE tissues from moribund mice euthanized 6 days after intraperitoneal infection with 500 pfu of the virulent isolate rZH548 (Egyptian lineage) and sheep euthanized 3 days after intramuscular infection with 10^7 pfu using the same isolate. Different tissue samples were fixed in 10% buffered formalin solution for 48-72 hours, routinely processed and embedded in paraffin wax. For immunolabelling RVFV, a non-commercial polyclonal rabbit serum against purified RVFV was tested at different dilutions in combination with different antigen retrieval methods. In both mice and sheep, the liver displayed the most distinctive histopathological lesions characterized by multifocal-random to diffuse necrotizing hepatitis. Immunolabelling was most consistent and unequivocal in liver, followed by spleen and kidney. In mice, RVFV antigen-positive cells included hepatocytes, Kupffer cells, circulating monocytes, microvascular endothelial cells, splenic macrophages, fibroblasts of the splenic capsule, renal interstitial fibroblasts, mesangial cells and reticular fibers of the white adipose tissue. In sheep, positive cells included hepatocytes, Kupffer cells, circulating monocytes, and microvascular endothelial cells along with abundant fibroblasts in splenic trabeculae and occasional interstitial fibroblasts in the kidney and tunica muscularis of the rumen.

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107. Role of the Erns RNase and the 3´UTR poly-uridine insertion to prevent the cytokine storm and classical swine fever

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The understanding of attenuation mechanisms behind the low virulence classical swine fever virus (CSFV) strains prevalent in endemic areas remain scarce, despite its potential contribution to vaccine and diagnosis development against CSFV. Our previous study revealed that the unique uninterrupted poly-uridine (poly-U) sequence found in the 3'UTR of the low virulence CSFV field isolate Pinar de Rio (PdR) is a new virulence factor that could reduce CSFV replication and attenuate virulence in piglets. Moreover, we also revealed previously the loss of pathogenicity and replication capacity in pigs when the E^{rns} RNase function was abrogated in this PdR strain. The present study aimed to assess the synergistic role of the E^{rns} RNase activity and the poly-U insertion in the 3'UTR of the PdR strain in the innate and adaptive immunity regulation, in the viral replication and their relationship with the CSF pathogenesis in two age ranges of pigs. We constructed a double mutant vPdR-H_{30}K-5U with an inactivation of the E^{rns} RNase function and with 5 uridines instead of the 36 in the 3'UTR. A group of nineteen 5-day-old piglets and another group of six 3-week-old pigs were infected with vPdR-H_{30}K-5U. The vPdR-H_{30}K-5U induced severe clinical manifestations in the two infected groups, higher mortality rate was shown in 5-day-old piglets (89.5%) than in3-week-old pigs (33.3%), indicating the important role of pig's age for CSFV susceptibility and the pathogenesis modulation by the synergistic effect of the 3´UTR and the lack of the E^{rns} RNase activity. The pigs infected with vPdR-H_{30}K-5U showed high viral replication in tonsils but mild viremia and low viral excretion, likely due to a modulation of viral replication by the 3'UTR and the host innate immunity by the E^{rns} RNase activity. Among nine cytokines quantified, only IFN-? and IL-12 were highly elevated in the two groups. In addition, high IL-8 levels were found in the newborn but not in the older pigs. This points towards a role of these cytokines in the outcome of disease, with age-related differences. In addition, infection with vPdR-H_{30}K-5U resulted in the reduced adaptive immune response from the pigs, which correlated with the viral replication and innate immune response. These results give a better understanding of the relationship between CSFV virulence and viral replication and host immunity, providing new directions for the study of the natural CSFV attenuation molecular determinants.

TOPIC 6 - Diagnostic tools and Disease surveillance

52. Bovine Ephemeral Fever (BEF): monoclonal antibodies as strategic tools for the development of serological assays

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Introduction. Bovine Ephemeral Fever (BEF) is an emerging arthropod-borne viral disease affecting mainly domestic cattle and water buffalo. The etiological agent (BEFV) is a member of the genus Ephemerovirus within the family Rhabdoviridae. BEF is included among the so-called "FAST diseases" (Foot-and-Mouth Disease and similar transboundary animal diseases) by the European Commission for the Control of Foot-and-Mouth Disease (EuFMD) since it represents a growing risk of introduction and spread into Europe from affected neighbours such as Turkey and the Middle East. BEF causes economic losses by a sudden drop in milk production in dairy cattle and loss of condition in beef cattle. The impact of BEF on the cattle industry may be underestimated, however, the introduction of BEFV into European countries is possible, similarly to the past incursion of other arboviral diseases (e.g. bluetongue). The improvement of laboratory skills through the development and standardization of diagnostic tools is essential for constant monitoring, prevention and control of BEF in the affected nearby countries and to increase the capacity of early detection of FAST introductions in Europe. In this study, a panel of monoclonal antibodies (mAbs) directed against BEFV has been characterized and evaluated for the development of a competition-ELISA (c-ELISA) for the detection of anti-BEFV antibodies in bovine sera.

Material and methods. A BEFV field strain kindly provided by Dr. Hagai Yadin (Kimron Veterinary Institute, Israel) was used in the study. MAbs were produced in Balb/c mice according to an internally standardized method and were screened by indirect immunoperoxidase (IPMA) using BEFV infected VERO cells. The ability of mAbs to neutralize virus infectivity was investigated by a virus-neutralization test (VNT). To identify mAbs reactive to immunogenic epitopes, competitive ELISAs were performed with six BEFV naturally infected bovine sera (VNT titre ranging from 1/8 to 1/256). Then two mAbs were selected, cloned and tested in sandwich ELISA to evaluate their capability when used in different combinations either adsorbed or HRP?conjugated to efficiently bind the BEF viral antigen. Further mAbs characterization through western blotting (WB) was performed.

Results. Nineteen mAbs reactive against BEFV were obtained, none of them showed VN activity, however, all showed to compete with BEF positive sera. Among them, two mAbs, namely 3E6 and 3A2, were selected and tested in sandwich ELISA, showing to be able to catch and detect the viral antigen when used in heterologous combination. In particular, the sandwich ELISA with mAb 3E6 used to capture crude BEF viral inactivated antigen, and mAb 3A2, used as tracer, was evaluated as a prototype of a c-ELISA, showing to be able to discriminate between BEF positive and negative sera. Both 3A2 and 3E6 mAbs, when tested in WB with BEF antigen, showed reactivity against a protein of about 50KDa, corresponding to the nucleoprotein N, while the other mAbs did not react in WB.

Discussion and Conclusions. The mAbs produced represent a valid tool for the development of diagnostic assays. All them recognize immunogenic epitopes and two recognize two different linear epitopes on N protein, and were thus suitable to be emplyed in competitive ELISA serological assay. The mAbs characterization is still ongoing to better exploit their properties, and competitive ELISA validation is in progress. Our preliminary resultsare promising for improving the capacity for FAST prevention by enhancing laboratory diagnostic skills.

53. Developing an xMAP multiplex assay for the rapid and simultaneous detection of AHSV serotypes 1-9

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African horse sickness (AHS) is an arthropod-borne disease of equids that is often fatal in naïve horses and mules. AHS is currently endemic in most of sub-Saharan Africa but outbreaks have occurred in India, the Middle East, Spain and Thailand. The causative agent is the African Horse Sickness Virus (AHSV), genus *Orbivirus*, for which there are nine antigenically distinct serotypes. In order to facilitate surveillance programmes and deploy appropriate control strategies where outbreaks occur, rapid, accurate and cost-effective diagnostics are essential. Over the past decade, Real Time-PCR assays have been developed that allow for differentiation of AHSV based on serotype, however when the serotype is unknown these assays can be costly and time consuming. xMAP (Multi- Analyte Profiling) technology allows for simultaneous detection of multiple targets and has previously been used to detect all 24 serotypes of Bluetongue Virus, also part of the *Orbivirus* genus, proving itself to be a useful diagnostic tool when analysing large sample sets. xMAP technology works by detecting fluorescent beads that are hybridised with amplified nucleic acid specific to each AHSV serotype, if it is present in the sample. Due to the multiplexing nature of the xMAP platform, the cost and time involved in the analysis of samples can be greatly reduced. Here we describe the development and optimisation of an xMAP multiplex assay for the simultaneous detection of AHSV serotypes 1-9, using data obtained for sensitivity, specificity and limit of detection when compared to Real Time- PCR.

54. Implementation of air sampling to facilitate AI/ND diagnostics and study their viral dispersion dynamics

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Avian influenza and Newcastle disease are two highly contagious poultry diseases that transmit mainly via oro-fecal route. Viral accumulation within the aerial environment can lead to airborne transmission via droplets or contaminated dust. A non-invasive air sampling approach could be implemented for easy sampling in case of suspicions on farms or markets on the one hand and be used to evaluate contamination and dispersion dynamics within the environment on the other. In addition, the coriolis could be used for its potential to evaluate the cleaning and disinfection procedures implemented after clearing an outbreak.

The aim is to evaluate possible application of Coriolis®µ (Bertin, France) air sampling for its application early, during and after a notifiable HPAI or NDV outbreak in a poultry flock. Initial evaluations focused on establishing the link between viral excretion sampled directly from the bird and viral presence sampled from air on suspected outbreaks sites. This non-invasive detection coupled with classical swabbing provides essential data on its applicability for diagnostic sampling and the evaluation of airborne dispersion. In a laboratory context, the Coriolis air sampling demonstrated its ability to early detect HPAI viral particles accumulated in the aerial environment of BSL-3 isolator during an highly pathogenic H5N8 infection, as well as the prolonged detection after individual viral excretion had ended. Same observations on viral persistence was made within BSL-3 isolators air samples after a live-NDV vaccination. The air samples allowed viral recovery and other downstream analysis from the diagnostic air samples.

At HPAI outbreaks sites the application of the Coriolis was used to assess the risk of air contamination within and around two HPAI outbreaks sites. Viral presence was detected in the air of contaminated barns but also outside around the air expulsion systems. An impact of the density and the type of breeding was observed although requires to be confirmed by further field evaluations. This external contamination of the aerial environment can contribute to the HPAI spill-over back into wild birds.

The Coriolis air sampler showed its potential for an application as diagnostic sample with subsequent viral and molecular biology analyses, which allows a quick status of the biological contamination of the indoor and outdoor air but also allows the study of airborne transmission around HPAI outbreaks. In order to assess more precisely the impact of animal density on viral persistence and contamination in the aerial environment, a similar analysis will be performed in parallel of a live-NDV vaccination on farms selected according to their animal concentration.

55. CWD in Europe: evaluation of diagnostic performances of rapid tests and confirmatory western blot methods

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Chronic Wasting Disease (CWD) is a fatal neurodegenerative disease affecting cervids. It belongs to the group of Transmissible Spongiform Encephalopathies (TSEs) or prion diseases, that affect animals and humans, that include bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, and Creutzfeldt-Jakob disease (CJD) in humans. The infectious agent of TSEs is the misfolded pathogenic form (PrP^{Sc}), also called prion, of the host-encoded cellular prion protein (PrP^{C}), which accumulation leads to neurodegeneration and, eventually, to death. CWD, like classical Scrapie, is highly contagious, shedding through bodily fluids, and can remain infectious in the environment for years. CWD was detected for the first time in mule deer in Colorado in 1967 and is, since then, spreading persistently through North America in different cervid species. CWD has been exported to South Korea, and in 2016, cases have been discovered in Norway in reindeer and moose, followed by identification in moose in Finland and Sweden. To prevent the spread of CWD within the EU, and/or to control the disease where it occurs, the European Commission (Regulation EU 2017/1972) has implemented a CWD surveillance programme in cervids, performed by using diagnostic rapid tests approved by the EC Regulation, in the six countries having reindeer and/or moose (Estonia, Finland, Latvia, Lithuania, Poland, and Sweden). Experimental transmission studies in rodents have proved that the European CWD strains are all different from those from North America. Data on the performances of authorized rapid tests are limited for CWD from North America and, due to the paucity of positive material, is currently minimal for CWD from Europe. The aim of this study was to evaluate the diagnostic performances of the three so-called "rapid" tests (Bio-Rad TeSeE SAP, Bio-Rad TeSeE Sheep/Goats, and Idexx HerdCheck BSE-Scrapie Ag test), all ELISA tests that are commercially available and approved for TSE diagnosis in cattle and small ruminants, to detect the CWD strains circulating in Europe. The performances of these three tests were also compared to two different confirmatory western blot methods, one commercially available (Bio-Rad TeSeE Western Blot) and one (SAF-Immunoblot) developed at the Italian NRL for TSEs. Five moose and two reindeer, detected as positive through the Norwegian surveillance programme, were analyzed in this study, as well as negative pools of both moose and reindeer brain tissue. To evaluate the analytical sensitivity of these methods, dilution series (1:2 to 1:128) of each homogenate were prepared in negative brain material, before being analyzed in parallel with the three rapid tests and the two western blot methods. For SAF-Immunoblot method five monoclonal anti-PrP antibodies were chosen among the most commonly used for the diagnosis of animal TSEs and raised against different regions of the prion protein: SHa31, 9A2, 6H4, L42, and SAF84. The three rapid tests and the two confirmatory western blot methods were able to detect the seven positive samples, while some differences in analytical sensitivity have been observed. Although this study presents a limitation due to the small number of samples analyzed, it is conceivable that the rapid and confirmatory diagnostic systems applied in Northern Europe for the CWD surveillance in the cervids populations are reliable tools.

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56. Machine learning approach for avian influenza virus subtyping using hemagglutinin genomic sequences

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Many disease causing viruses are clustered into subtypes with clinical significance. Most methods for subtype classification require the alignment of the input sequence against predefined subtype reference sequences, which enables algorithms to compare homologous sequence features. This process can be relatively computationally expensive, especially for long sequences. Moreover, highly divergent genome regions may affect the alignment algorithm’s performance. In order to overcome these obstacles, various machine learning (ML) algorithms have been used for the subtype determination of different viruses. In this work, an alignment-free artificial intelligence (AI) approach has been implemented for avian influenza virus (AIV) subtyping by using only hemagglutinin (HA) genomic sequences. Publicly available HA sequences were retrieved from the NCBI GenBank and the Influenza Research Database. Hundred randomly chosen unique both complete and partial coding HA sequences were retrieved for each H subtype, except for H14 and H15 for which 47 and 23 sequences were retrieved, respectively, given that those were the only available sequences at the time of query. The average length of the sequences was 1725 nucleotides, the minimum length being 1516 and the maximum 1781. An algorithm was designed for the conversion of the sequences to *k-mers* of length 6 (hexamers), since according to simulation studies, genetic information is better represented when k equals 5 or 6. A *k-mer* is a subsequence of k nucleotides in a DNA sequence and in order to obtain all *k-mers* from a sequence, the algorithm starts by extracting the first k nucleotides and then continues the extraction by moving just a single nucleotide in the given DNA sequence for the start of the next *k-mer.* The method used in this work is based on the Natural Language Processing, a subfield of AI. Specifically, a bag-of-words model was applied, which is used for simplifying textual data before ML algorithms are applied for its analysis. In this model, a text such as a sentence or in this case a genomic sequence is represented as the bag of its words or in this case, hexamers. A CountVectorizer tool was used to transform the sequences into a matrix of token counts (counts of each hexamer that occurs in the entire sequence). Four ML classification models (Naïve Bayes, k-nearest Neighbors Classifier, Random Forest Classifier and Support Vector Classifier) were applied for the classification of the HA sequences. In order to train the ML models, the data was randomly split into training (80%) and test data (20%). All models were implemented from the python scikit-learn library. The accuracy, F1, precision and recall scores were evaluated for all models by using a confusion matrix.

The empirical results showed that all models performed the classification task with scores >99% which suggests that this approach could be applied for accurate subtyping of AIV sequences. However, there are some limitations that should be considered. The dataset is relatively small, so in order to evaluate these ML models further, more samples should be included (especially for H14 and H15 in order to be certain that the models can classify them appropriately) and sequences of different length should be used. Further improvement of these models can be achieved by incorporating recombinant and mixed subtypes or by using parallel classification of different AIV genomic sequences.

57. FMD serological blocking ELISA based on VHH for post-vaccination monitoring

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Currently, antibodies to Foot-and-Mouth Disease Virus (FMDV) are detected in animals using the virus neutralization test (VNT). While VNT is recognized as correlated to protection, the method is laborious, time consuming and reliant on live virus (to be handled in BSL3 facilities) and cell cultures. Due to these constraints, alternative methods are being investigated by different labs. We have chosen an approach using VHH specific of 146S particles (or nanobodies) to set up a blocking enzyme-linked immunosorbent assays (ELISA) for the detection of specific antibodies against O1 Manisa in swine and cattle sera.

First, dilutions of serum are mixed with a defined quantity of FMD antigen (concentrated and inactivated) and then added on a plate coated with FMDV serotype specific VHH. The non-bound antigen present in the mix is captured by the coated VHH and detected by a homologous biotinylated-VHH. Titer of 146S specific antibodies is expressed as log10 OD50.

Preliminary results with O1 Manisa showed low variability (standard deviation below 0.1). Serotype specificity was also confirmed. Using 1500 sera from pigs and cattle vaccinated with either a monovalent or a multivalent vaccine (commercial or experimental vaccines), we demonstrated a good correlation of the ELISA titers with the VNT titers (with $R^2 = 0.72$ in pigs and $R^2 = 0.77$ in cattle).

ELISA provides more reproducible results compared to VNT and has the advantage to be performed in non –BSL3 environment. Next step will be to confirm those results on other serotypes. In conclusion, the developed serological blocking ELISAs, using serotype specific VHH targeting 146S FMDV particles, can be considered, based on these teresting alternative to VNT to evaluate vaccine immunogenicity and performance.

58. A multi-species Double antigen ELISA for the detection of SARS-CoV-2 N antibodies in animals

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Introduction

The COVID-19 pandemic in humans has led to increased interest in determining the prevalence of SARS-CoV-2 in animal species. The virus has already been detected in cats and in mink farms. The OIE made a review of the susceptibility of 10 animal species to SARS-CoV-2 and their possibility to transmit the virus.

Scientists are proceeding to epidemiological surveys in a variety of species and require an accurate and easy-to- use serological test.

Innovative Diagnostics launched a double antigen ELISA based on a truncated nucleocapsid to detect anti-SARS- CoV-2 antibodies in multiple species.

Materials and methods

Diagnostic specificity was evaluated on 788 samples collected in the pre-epidemic period, including 314 dog sera, 92 cat sera, 88 cattle sera, 86 horse sera, 83 goat sera, 86 sheep sera and 39 wild animals sera.

Sensitivity was evaluated with:

- A cat sample found positive by RT-PCR.
- A sample from an experimentally infected ferret, collected at 21 days post-infection, and found highly positive by both the indirect immunofluorescence antibody test (IFAT) and the Sero Neutralisation Test (SNT).
- 8 minks with clinical cases, from one infected farm.

Cross-reactions with other coronavirus from animal species were measured using 38 samples collected in the pre- epidemic period, including 30 sera from chickens infected with the Infectious Bronchitis Virus (IBV) and 8 sera from pigs infected with the Porcine Epidemic Diarrhea virus (PEDV).

Results/Discussion

Out of 788 sera, 781 sera were found negative. The measured specificity was 99.1 % (CI95%: 98.2 % - 99.6%). The cat serum was efficiently detected using the ID Screen® SARS-CoV-2 Double Antigen Multi-Species (COVIDA) ELISA test.

The ferret sample gave a high positive result with a S/P% of 95%, confirming susceptibility of ferrets to infection by SARS-CoV-2.

The 8 mink samples tested were found positive, with a S/P value of 112 to 316% (median value: 195%).

Out of 38 samples used to assess the exclusivity, 37 were found negative and only 1 was found doubtful with the ID Screen® ELISA. This test does not cross-react with other coronavirus such as IBV and PEDV, which means that the ID Screen® ELISA has a very good exclusivity.

Conclusion

The ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA shows high specificity in a number of animal species, and successfully detected a RT-PCR positive cat serum (1), an IFAT/SNT positive ferret serum and whole blood and plasma samples collected on positive minks.

The ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA demonstrates high exclusivity against avian and porcine coronaviruses. No cross-reactivity with other coronaviruses (such as feline or canine coronaviruses) haven't been tested yet, due to the absence of characterized samples. However, due to the use of a truncated protein N sequence, no or very limited cross-reaction should be expected in other animal coronaviruses.

59. Proven performances for FMDv NSP antibody detection with the ID SCREEN® FMD NSP Competitive ELISA

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Foot and mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals, caused by the FMD virus (FMDv). The differentiation of herds which have been infected from those which have been vaccinated is a critically important follow-up activity to protective emergency vaccination. Both infection and vaccination elicit antibodies against structural antigens. Only assays that measure levels of antibodies against non-structural protein (NSP) can differentiate infected and vaccinated animals (DIVA).

Here, we make a review of the performances and the advantages of a blocking ELISA kit designed to detect anti FMDv NSP specific antibodies. This test offers an overnight protocol as well as a short protocol for same-day results.

Test specificity was assessed for both protocols by testing 2009 sera from non-endemic and non-vaccinated areas (France). The global specificity was high 99.7% $CI_{95\%}$ [99.4 % - 99.9 %], regardless of the species tested: Cattle (n=1091): 99.7 [99.2-99.9]; Swine (n=538) 99.6 [98.7-100]; Ovine (n=183) 99.5 [97.0 – 100]; Caprine (n=197) 100 [99.4 – 99.9].

Sensitivity was evaluated using the Pirbright Institute International reference panel of NSP sera, composed of 36 sera derived from vaccinated / challenged or unvaccinated / infected animals (Parida *et al.*, 2007). The ID Screen® sensitivity was equivalent or highly superior to the best ELISAs evaluated in the study, which included several commercial ELISAs.

The ability of the ID Screen® to be a DIVA was evaluated by testing 28 animals vaccinated with O monovalent highly purified vaccine at 0 day post vaccination (dpv) and 50 dpv. Whereas the animals seroconverted to FMDv (they were positive on a type O serotype-specific Solid Phase Blocking ELISA), all vaccinated samples were found negative at 0 dpv and 50 dpv.

A real-time stability study showed that the kit was still working with satisfactory results after storage for 18 months at 21°C, indicating a very high stability of the kit.

The Pirbright Institute validated the ID Screen® ELISA and “shown that both the formats of this test have equivalent specificity and sensitivity with the established Prionics PrioCHECK® FMDv NS 3ABC test” (EVIDENCE Project final report, available online).

In an “Inter Laboratory exercice to evaluate NSP kits”, comparing the Priocheck® NSP ELISA with the IDvet NSP ELISA, the World Reference Laboratory concluded that both kits “have equivalent performance for the detection of FMDv NSP specific antibodies” (euFMD Opensession, Italy, 2018).

The ID Screen® FMD NSP ELISA was included in different ringtrials (VETQAS and FLI, Germany where the kit is registered). Results from the German ringtrial (Inter-laboratory validation of foot-and-mouth disease diagnostic capability in Germany, Hoffman et al., 2017) indicated an excellent reproducibility between all the laboratories as well as a higher specificity compared to other techniques.

The ID Screen® FMD NSP Competition ELISA demonstrates high specificity and excellent performance on reference panels. The ELISA correctly identified all strains tested and efficiently detected carrier animals. The kit offers both short and overnight protocols, that give similar results, meaning that laboratories have the possibility of offering same-day results.

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

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Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by the Crimean-Congo hemorrhagic fever virus (CCHFV), which is a Bunyavirus belonging to the family Nairoviridae, genus Orthonairovirus. CCHF was first described in the mid-1940s on the Crimean peninsula. The disease occurs in humans sporadically throughout much of Africa, South Asia, and Southern Europe. CCHFV is transmitted to humans by tick bites, crushing of ticks and exposure to blood, tissues or bodily fluids of viremic livestock or infected patients. The virus causes an often fatal hemorrhagic illness in humans, with up to 50% case fatality rate. On the contrary, animals do not develop clinical symptoms upon infection but viremia and CCHFV-specific antibodies can be observed. The detection of anti- CCHFV antibodies in livestock is therefore used to reveal CCHFV infection risk areas. CCHFV-specific antibodies can be detected either by viral neutralization, ELISAs or indirect immunofluorescence assays.

Materials and methods

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

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

Results and discussion

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

Part of this work was done within the EU-funded grant FP7 (EDENext). The contents of this publication are the sole responsibility of the authors and don't necessarily reflect the views of the European Commission.

61. A new high-performance ELISA assay as an alternative to CFT for Glanders diagnosis

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Introduction

Glanders is a zoonotic disease caused by Burkholderia mallei, a gram-negative bacterium. The complement fixation test (CFT) for testing of equine sera is a fastidious method which may not be used for high-throughput testing. Moreover, its standardization is not facilitated by the diversity of antigens used and protocols, which lead to differences in diagnostic sensitivity. Anti-complementary sera cannot be interpreted correctly, and the specificity of the CFT is sometimes questioned. Finally, false positive results pose a problem for diagnosticians, animal health authorities and owners, while false-negative results can turn a risk into a possible threat.

Materials and methods

The ID Screen® Glanders Double Antigen Multispecies ELISA is a double antigen enzyme-linked immunosorbent assay (ELISA) based on a recombinant protein.

Specificity was evaluated with equid sera from disease-free and non-vaccinated regions (n=680), and with sera from human blood donors from France (n=100).

Sensitivity was evaluated with samples from infected horses, donkeys and mules from endemic areas (India and Pakistan; n=86). These sera were collected from animals with clinical signs and all samples were positive by the OIE prescribed test, the Complement Fixation Test (CFT).

Results and discussion

Measured specificity was found to be 100 % in equids (CI95%: 99.4 – 100, n=680) and 100 % in humans (CI 95% : 97.9 – 100, n=100).

Measured sensitivity on CFT positive samples was 100 % (CI95%: 95.7 – 100, n=86), regardless of the species tested.

Conclusion

The data presented in this study indicates that the ID Screen® Glanders Double Antigen Multispecies ELISA test offers excellent diagnostic performance.

Measured specificity, n=400	
ID Screen® ELISA	99.8 % (CI $_{95\%}$: 98.6- 100)
CFT	97.0 % (CI $_{95\%}$: 94.8 - 98.4)
Western Blot	99.2 % (CI $_{95\%}$: 97.8 -99.8)

These results were confirmed by a published independent study (Mandy C. Elschner *et al.* 2021, Germany, FLI), with an excellent specificity and sensitivity for the ID Screen® ELISA, in comparison to CFT and Western blot.

Measured sensitivity, n=370	
ID Screen® ELISA	98.1 % (CI $_{95\%}$: 96.1– 99.2)
CFT	96.5 % (CI $_{95\%}$: 94.1– 98.1)
Western Blot	97.3 % (CI $_{95\%}$: 95.1-98.7)

This paper concludes that *« this new [..] ELISA test appears a suitable confirmatory test and a realistic alternative for serological testing of horses for trade or movement. »*

62. Results of avian influenza surveillance in Vojvodina Province of Serbia in 2021

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Avian influenza (AI) is highly contagious infectious disease of birds that cause high health and economic loses to poultry industry, and in the same time, as zoonotic disease, represents the constant threat to human health. During the season 2020/2021, and 2021/2022, a large epizootic of AI occurred. The whole Europe was affected by a large number of 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 H5 virus subtype.

In Serbia, according to the Program of Measures of Animal Health, passive surveillance of the occurrence of AI is carried out, which includes examination of the presence of AIV in cases of found dead wild birds, as well as examination of causes of death in flocks of domestic poultry with an increased number of deaths. From season 2021/2022 an active surveillance program for AI (based on testing the cloacal swabs of domestic poultry from backyards and small village open markets, and on intensive wild birds surveillance (dead animals, cloacal swabs or fresh feces)) were implemented on the whole territory of Serbia. In addition, an intensive surveillance of AIV in wild birds in the frame of cross-border IPA project "BirdPROTECT" have been done in northern part of Serbia – Vojvodina Province. The detection of AIV presence was done by real-time RT-PCR method with primers specific for the matrix gene of all influenza viruses, and after the confirmation of virus presence, additional testing's were done with primers specific for different virus subtypes.

In Vojvodina Province, northern part of Serbia, during the implementation of passive and active surveillance in seasons 2020/2021 and 2021/2022, a total of 590 wild bird samples and 609 backyard poultry samples from 190 backyards and one small private zoo, as well as 60 samples from 6 open village markets were examined respectively. The first positive case of AIV in Serbia in the 2020/2021 season was determined on March 2, 2021 in dead swans in the settlement of Kula on the Great Ba?ka Canal, where the presence of HPAI H5N8 virus subtype was determined. Out of all detected HPAI of H5 virus subtype only the H5N8 subtype in swans in multiply occasions and H5N2 in one mallard in one occasion were detected until November 2021. The first HPAI H5N1 was detected in dead swan on Canal DTD in Novi Sad in November 2021, and after that in domestic poultry in one small zoo in town Temerin and in one backyard in town Srbobran in South Ba?ka District, as well as in one backyard in village Lipar near town Kula in West Ba?ka District. In total, the presence of AIV was detected in 14 examined wild birds (8 swans and 6 mallards) from 9 locations (in 5 districts) and in domestic poultry (chicken turkey, ducks and geese) from one small zoo and 2 backyards on 2 location in South Ba?ka and one location in West Ba?ka District. During this time no large poultry farm was infected, and so far there were no significant economic losses.

Considering to the obtained results, as well as the still unfavourable epizootic situation of AI in the whole Europe, it is necessary to continuously implement a program based surveillance of influenza virus in the territory of the Republic of Serbia in the future.

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63. Improvement of a PRRSV diagnostic PCR method

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is an endemic disease in the UK and worldwide with huge economic consequences. For diagnosis and surveillance, the presence of PRRS virus (PRRSV) is detected by real-time RT-PCR. The current in-house PCR assay was less sensitive to currently circulating PRRSV-1 strains than desired, so the aims of this project were to improve the sensitivity of the PCR while maintaining its ability to detect a wide range of PRRSV-1 and PRRSV-2 strains, to compare it to a commercial PRRSV PCR kit (VetMAX™ PRRSV 2.0 PCR kit), and to develop a control to ensure proof of extraction and demonstrate sample suitability.

Methods and Materials

Extraction – as previously optimised, all extractions were carried out using the MagMAX™ CORE extraction kit as per the manufacturer's instructions. All tissues were first homogenised in PBS using the GentleMACS™ homogeniser.

Primers – Proposed primers and probes for PRRSV-1 were compared against 324 sequences for ORF7. New primers were designed to match these sequences. Existing primers and probes for PRRSV-2 were retained.

PCR - carried out with manufacturer's recommended conditions or the touchdown PCR conditions previously used in the in-house PCR. Commercially available assays were also evaluated.

Extraction/Sample Controls – Identified from a literature review or designed using Primer3. Tested on conventional PCR and visualised on an agarose gel. For the successful control chosen a probe was designed using Primer3.

Results

A new kit and conditions were identified for the improved in-house assay. On validation between the new in-house protocol and the VetMAX™ PRRSV 2.0 kit, the commercial kit showed consistently better sensitivity across a wide range of sample types. No issues were found in detecting a range of diverse PRRSV-1 strains. In the in-house protocol, multiplexing of the PRRSV detection and a ?-actin internal control could not be validated without a loss of PRRSV sensitivity. Additional sample types were tested with the new VetMAX™ PRRSV 2.0 PCR and found to be suitable for use in detection of PRRSV.

Conclusion

The in-house PCR sensitivity was not sufficiently improved compared to the VetMAX™ commercial kit, which proved to be far more sensitive and this was selected as the new standard to be validated for use with an expanded range of sample types. An independent PCR was established for testing using a ?-actin internal control to determine extraction success and sample suitability if required.

64. Development of a sensitive and specific Leishmania Lineblot, able to differentiate between vaccination and disease

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Introduction: Canine leishmaniasis is a zoonotic disease by the protozoan parasite Leishmania transmitted by the bite of an infected phlebotomine sandfly. *Leishmania infantum* is the most common and important cause of canine leishmaniasis worldwide. Other Leishmania spp. reported from dogs include *L. mexicana*, *L. donovani*, and *L. braziliensis.* Leishmaniasis can be categorized by two types of diseases in dogs: a cutaneous reaction and a visceral reaction, the most severe form of leishmaniasis.

Infection does not invariably lead to illness. In fact, most infected dogs remain asymptomatic and may never develop clinical manifestations.

Diagnosis of canine leishmaniasis is based on the presence of clinical signs together with positive specific antibody assay. Due to the close contact of dogs and humans it is important to monitor the presents of Leishmania in the animal population in order to secure safety for humans. In the human field you find 300 000 estimated cases of visceral leishmaniasis (VL) and over 20 000 deaths annually. 1 million cases of cutaneous leishmaniasis (CL) have been reported in the last 5 years. 310 million people are at risk of infection in six countries reporting over 90% VL cases worldwide.

Aim: The aim of this work was to develop a serological Lineblot assay to detect IgG and IgM antibodies against Leishmania in serum or plasma samples derived from all mammals, including dogs and humans.

Methods: For this approach a set of native antigens and different recombinant antigens (developed by the Philipps-

University Marburg) have been used to print Lineblot membranes. The recombinant antigen from Marburg has been designed for high sensitivity and specificity, and it can be used for the differentiation between vaccination and disease state. For confirmation and screening purpose a ELISA system has been developed. To have a point-of- care (POC) device for resource limited setting also lateral flow rapid tests are in development. Both assays are utilizing the recombinant antigen from Marburg.

Results: In house sample collection was used for development and evaluation of the assay. In addition, samples and external validations from the University of Marburg have been used. The new Lineblot assay shows a high specificity and sensitivity and is able to differentiate between vaccination and disease. Cross-reactions can be identified more easily. Currently different studies are running in different endemic regions like Brazil, Sudan or India with human and veterinary samples. Preliminary results of these studies will be shown.

Conclusion: Here we show the performance characteristic of a newly developed assays. Due to the improved antigen design and test setup a superior assay performance was achieved. This new Leishmania Lineblot can be used to diagnose disease in symptomatic as well as in asymptomatic dogs (and humans) to be able to administer proper treatment. It can also be used for confirmatory testing as well as for the differentiation between disease and vaccination. In areas where Leishmania is endemic and transmitted by insect vectors, it is an important agent of human disease and dogs are

considered the most important peridomestic reservoir host. The new Lineblot can be used to monitor seroprevalence and vaccination status in the animal population to help to control and manage the disease in these regions. In addition, this Lineblot will be a tool for screening imported or returning animals from endemic countries. This development is assisted by the other 2 newly developed assays, lateral-flow rapid tests and ELISA.

66. Epidemiology of Rotavirus A and non-A in swine farms in Northern Italy

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Rotavirus (RV) represents one of the major cause of pig enteritis that induces economic losses to swine industry. Four groups (A, B, C, H) have been identified in pigs, with RVA the most characterized. Currently, non-A groups have been detected worldwide, suggesting their epidemiological importance. However, very little is known about their prevalence and genetic variability in Italy. The aim of this study is to provide information on the distribution and genetic diversity of RV groups in different aged pigs suffering enteritis in Northern Italy.

Two multiplex porcine-vp6 RVA/B and RVC/H Real time PCR (RT-qPCR) assays were developed. Nine hundred and sixty-two diarrheic samples, belonging to young (318 suckling and 239 weaning) and adult (405 fattening) pigs, were tested in RT-qPCR, over a period of three years (October 2016-December 2019). A group of samples positive to RVB (29), RVC (25) and RVH (25), representative for year and geographical origin, were sequenced for VP6, VP4 and VP7 and aligned by CLUSTAL W algorithm. Phylogenetic trees were constructed using Maximum Likelihood methods.

RV groups were detected in 72% of suckling, 84,5% of weaning and 78,8% of fattening pigs with enteritis. RVA was predominant in young pigs (58% of suckling and 64% of weaning pigs) while, in adults, RVA, B, C groups are equally distributed with percentages of 51%, 52% and 47%, respectively. Whereas RVA was present as single infection in a higher percentage of piglets (25% vs 7% of adults), RVB, C and H were mostly present in mixed infections in adults. Phylogenetic analysis of VP4, VP7 and VP6 genes of non-A groups revealed the presence of different spatio-temporal clusters suggesting a high genetic divergence. Moreover, we detected new genotypes for all the gene and groups analysed.

This study provides novel data on RV prevalence in porcine livestocks in the North of Italy, showing high diffusion of Rotavirus in enteric clinical cases and especially, a high presence of non-A RV groups associated to a great genetic variability. Moreover, the different distribution of RV groups in the age classes suggests a different epidemiological impact.

67. Health monitoring of laboratory animals: preliminary investigation of pathogens in environmental samples

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Introduction: The development of molecular methodologies, as a tool capable of providing information on the health status of housed animals, has so far taken on a key role in the perspective of the 3Rs principle (1). The FELASA guidelines (2) and the legislative decree 26/2014 (3) sanction the implementation of a health surveillance plan within the experimental farm. The presence of pathogens in the environment is the main source of diseases and epidemics for laboratory animals; these can influence their state of health and the validity and reproducibility of the experimental data. The health screening of pathogens, also performed on environmental samples, guarantees an improvement in the health and environmental conditions of the animals with the aim of reducing the number of sentinel animals (4). The aim of the work was to refine the techniques for the extraction of nucleic acids from environmental matrices for the subsequent detection of any circulating pathogens.

Methods: Samples of fecies, enrichment material, fur and sawdust were provided by the IZSLER animal enclosure. DNA and RNA extraction was performed with extraction kits provided by the QIAGEN company (Milan, Italy). The extracted and quantified nucleic acids were amplified for *Minute Virus of Mice* (MVM), *Ectromelia Virus* (ECTV), *Murine Adenovirus*, *Murine Polyomavirus*, *Murine Hepatitis Virus* (MHV), *Mouse Pneumonia Virus* (PVM), *Reovirus-3* (REO3), and *Theiler's Murine Encephalomyelitis Virus* (TMEV). To ascertain the systemic presence of the virus, blood samples were collected from eight different mice and five mice were sacrificed from which lungs, heart, stomach, intestine, liver, kidneys, spleen and brain were removed.

Results and conclusions: From the first raw environmental samples, it was not possible to obtain a sufficient amount of nucleic acids useful for subsequent investigations. The poor yield obtained may depend on a low presence of viral agents in the environment, their different stability or the specificity of the commercial kits used. New samples were collected from other cages prior to their weekly cleaning. This time it was possible to extract both nucleic acids; this may be due to the greater sensitivity of the kits used or to the collection of particularly dirty material. The search for viral contaminants, using Real-Tima PCR, gave a positive result only for MHV. The blood samples taken from 8 different mice from the cages from which the environmental samples were collected, were all negative for the MHV virus while three of the five sacrificed mice were positive. This preliminary work underlines how the use of environmental matrices can be effective in ensuring controlled environmental conditions and ensuring the surveillance of the health of animals for the entire duration of their housing as well as reducing the use of sentinel animals.

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68. Genetic variability of the N gene of bovine Coronavirus isolates circulating in Northern Italy

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Bovine coronavirus (BCoV), now classified as *Betacoronavirus* 1, is a member of the family *Coronaviridae*, genus *Betacoronavirus* and causes respiratory and enteric diseases in cattle and wild ruminants worldwide. BCoV is responsible for three distinct clinical syndromes: calf diarrhea, winter dysentery in adults and respiratory infections in cattle of various ages, including the bovine respiratory disease complex (BRDC), with shedding detected in nasal secretions, lungs and feces.

The genome is a single-stranded, positive-sense RNA of 27 to 32 kb encoding five major structural proteins. Among these, the 50-kDa nucleocapsid (N) protein is highly conserved among strains and plays an essential role in viral replication. Although most molecular phylogenetic analyses are based on the more variable Spike (S) protein gene, genetic variability has also been found in the N gene, with potential variations affecting the viral life cycle.

In this preliminary study, 31 nasal swabs resulted positive for BCoV by PCR and collected between 2020 and 2022 from 15 cattle farms in Northern Italy were used to study partial sequences of the N gene.

All cattle showed respiratory signs and were recently acquired from Italy, France or Hungary.

A 454-bp fragment from the N gene was amplified using the primer pairs reported by Takiuchi et al. (2006). The resulting amplicons were purified and Sanger sequenced in both directions (BMR Genomics, Padua, Italy).

Nucleotide BLAST analysis was first used to verify the identity of each fragment, then sequences were aligned with all N gene sequences available and phylogenetic analysis was performed using the Maximum Likelihood method with Kimura 2-parameter model with gamma-distributed rates among sites as nucleotide substitution model in the MEGA X software.

Comparing our strains to nucleotide data collection, overall we observed a closer similarity to sequences isolated in cows in France, although they were clustered into different groups. Indeed, 18 samples showed the highest percentage of identity with a French strain isolated in 2012 (GenBank ID KT318089.1) and 3 samples were clustered with other 15 French strains. Nine samples were clustered with a strain isolated in Southern Italy in 2020 (GenBank ID MW074864.1). Interestingly, one sequence from an animal acquired from Hungary showed the highest percentage of identity with the Mebus strain.

These preliminary results confirmed that the N gene sequence is well conserved in all the strains collected, although some relevant point mutations have been observed. Indeed, the sequences obtained showed several synonymous mutations, while 8 non-synonymous mutations were found, that are worthy of interest in future investigations.

Our findings provided new Italian sequences of BCoV strains, which are quite limited in public genetic databases nowadays. Further analysis on more variable genes, such as the S gene, will be carried

out to investigate BCoV variability. It will be also of great interest to deepen the study of simultaneous circulation of different BCoV strains in the same farm.

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69. The oie virtual biobank project

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The OIE launched the international OIE Virtual Biobank project in 2019 to promote the preparation and distribution of standard reagents for diagnostic testing among Reference Centres. The OIE Virtual Biobank project consists of a web-based catalogue of the biological resources that are held in biobanks hosted by OIE Reference Centres and National Reference Centres. This catalogue represents a source of information to search, locate and retrieve samples, especially diagnostic reagents and reference reagents, along with associated metadata.

The OIE virtual biobank project will be implemented and managed by the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna-IZSLER (hereinafter referred to as the IZSLER institute), OIE Collaborating Centre for Veterinary Biologicals Biobank (Italy), under the guidance of the OIE.

The IZSLER institute, which has already developed a data management tool for the national network of biobanks located at Istituti Zooprofilattici Sperimentali, undertook the technical and financial commitment to implement and maintain the OIE Virtual Biobank network.

The project includes the development of a two-level distributed system based on three components: 1) The OIE Virtual Biobank (OIE-VB) is the first level of the system, which is a web-based centralized system. The OIE-VB includes a web-portal through which users can search biobank materials (as well as access public domain information related to those materials) and authenticated user can submit requests for biobank materials; 2) The Biobank Protocol provides interconnectivity capabilities, communication rules and standards between the OIE-VB and the connected Biobanks; 3) The Biobank Management System is the second level of the system, which is a software solution for the local management of biobank materials

The OIE Virtual Biobank project will be implemented in two phases, a pilot phase to test the information technology options which will be adopted, followed by the full implementation of the system. A core group of OIE Reference Centres willing to participate in a pilot phase will be selected from different geographic regions and on the base of different organization levels of their biological collections.

The primary goal of the *OIE Virtual Biobank* project is to enable the identification and localisation of biological materials available globally, especially international reference standard reagents and diagnostic reagents. The *OIE- VB* will therefore support, build upon and expand the already initiated *OIE*'s Standardisation Programme for the production, validation, distribution and adoption of *OIE*-approved international reference standard reagents for diagnostic testing. The use of these standards would also foster the mutual recognition of test results in the context of international trade.

70. Isolation and maintenance of primary mosquito cells for diagnostic purposes: development of an experimental protocol

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Introduction: Recently, the European climatic tropicalization has caused a drastic increase in diseases transmitted by *Arboviruses*. In literature, there are many applications of insect cell lines [1] used for study relating to virus biology, epidemiology and for the evaluation of the *in vitro* efficacy of new antiviral compounds [2]. Isolation of *Arboviruses* requires the use of primary cells of arthropods; embryonic and larval tissues are the main sources used. Differentiated tissues are used less frequently, despite the fact that they are the most susceptible to infection and the presence of stem cells within them has been observed [3]. In accordance with the 3R principle, the aim of this work concerns the development of a technique of isolation of primary cells from differentiated mosquito tissues to achieve multiple diagnostic and research purposes, such as the in vitro evaluation of drugs, antiviral compounds and insecticides, reducing the use of animal experiments [4].

Methods: *Cx. pipiens* and *Ae. albopictus* were reared under controlled conditions (70% RH, 14:10 L/D Photoperiod and 25°C temperature). Eggs, larvae and adult mosquitoes have been used. They were washed with 5% bleach, 70% ethanol and purified H2O. Samples were collected in EDTA-trypsin. Intestines and ovaries were extracted from adults, larvae were mechanically shredded and eggs were treated with 1% pronase for shell elimination. The collected material was placed in flasks with two different culture media:

Mitsuhashi and Maramorosch Insect medium + Leibovitz's L15 (1:1) + 0.05% BSA + 1% NEAA + 2 mM L- glutamine + 10% inactivated FBS;

Grace's Insect medium + 20% FBS + 1X antibiotics.

The flasks were incubated at 27 °C without CO_2 and supplemented with 1ml of the specific media every 10-12 days.

Results and conclusions: Tissues incubated with medium 1 showed contaminations since the first microscopic observation while those treated with medium 2 showed divergent results. At 24 hours, the larvae exhibited fungal contaminations; on the contrary, the flasks containing eggs and adult tissues were sterile and free of external contaminations. At 24 hours, intestines and ovaries showed contractile activity. In the flask containing the eggs, however, a cellular arrest and the presence of some crystals have been observed, probably due to the precipitation of a component of the media or to a waste product. At 50 days post explantation, some samples of intestine and ovaries showed signs of distress: granulated cells and no replicative activity. At 80 days after seeding, crystallization have been observed in all the flasks and no cell growth have been occurred.

The knowledge currently available on mosquito cultures is poor and the choice of the most suitable culture medium is the first step to set up. The preliminary investigations have revealed that the use of

antibiotics it is of fundamental importance for these cells, due to the dirty environment in which mosquitos live. The results obtained are encouraging and the procedure will be optimized.

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71. Intra-farm circulation of swine influenza virus in North-east Italy between 2013 and 2020

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The endemic strains of swine influenza virus (SIV) circulating in Europe are classified as human-like H1N2 (huH1N2), avian-like H1N1 (avH1N1) and H3N2, with avH1N1 lineage being the dominant subtype. In addition, since the first identification of the human pandemic H1N1 ($H1_{pdm}N1_{pdm}$) in feral swine in 2009, this viral strain has been repeatedly detected into swine population, in several countries worldwide. To describe accurately the circulating strains in Europe, in 2015, Watson et al. proposed the classification in 23 different genotypes (A-W) according to their gene constellation. Afterwards, other genotypes were described in Europe.

The segmented genome of SIV favors reassortment and in addition, swine may act as a mixing vessel promoting reassortment events between human and avian strains and the generation of strains with pandemic potential. Therefore, surveillance of SIV is of crucial importance both for animal and human health perspectives.

Between 2013 and 2020, the passive surveillance in pig farms with respiratory signs conducted in Veneto and Friuli Venezia Giulia, in North East Italy, resulted in the collection of 3,522 samples, nasal swabs or lungs, processed at Istituto Zooprofilattico Sperimentale delle Venezie. Samples were screened by a Real time RT PCR targeting the M gene, and the HA and NA genes of all M gene positive samples were characterized by multiplex Real time RT PCRs.

Subsequently a selection of 42 characterized SIV positive samples, were submitted to virus isolation and cell cultured SIVs strains fully sequenced through NGS. The selected samples for NGS, were collected from ten swine farms where SIV circulation was reported at least twice over a six months period.

The sequenced SIVs belonged to the H1N1 and H1N2 and H3N2 subtypes and through the complete genome analysis it was possible to identify nine different genotypes (AH, C, D, H3[84], M, Novel, P, T, U) circulating in ten pig farms. Six out of ten farms displayed multiple introductions of different genotypes, probably resulting from distinct events. Two of these farms showed the circulation of multiple genotypes in the same year and one of them hosted three different genotypes between 2013 and 2019.

Five farms showed evidence of circulation of the same genotype over a time that ranged from two to seven years, with one farm displaying the circulation of exactly the same virus between 2013 and 2019. In one farm, we identified two samples belonging to the P genotype ($H1_{pdm}N1_{pdm}$) that could represent a recent spill over event by humans.

Our results highlight the importance of monitoring swine farms constantly in order to reveal persistence and/or introductions of different genotypes into pig farms. Such intra-farm longitudinal monitoring approach coupled with proper collection of epidemiological data, may aid in identifying risk factors linked to new virus introductions or persistence of the virus within each farm. Ultimately, data generated may help improving farm management to reduce the risk of multiples introductions and, consequently, reassortment events enabling emergence of new viruses with zoonotic potential.

72. Raptors in the focus of monitoring approaches for highly pathogenic avian influenza viruses

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Over the past years, outbreaks of highly pathogenic avian influenza virus (HPAIV) repeatedly affected wild bird populations in Germany. Additionally, HPAIV detection in poultry farms caused enormous economic losses and raises the ever-present risk of zoonotic spillover to humans. Fulfilling the paradigm of "One-health", wild bird populations have become a focus of viral pathogen surveillance in several countries. While previous HPAIV epidemics correlated with bird migration in autumn and winter months, changing climatic conditions may alter the seasonal pattern of HPAIV activity.

HPAIVs have been detected in a broad range of wild bird species, with waterfowl species considered as the major reservoir. Predatory species are intensively discussed as sentinel species in surveillance approaches for zoonotic avian viruses. Their position at the end of the food chain suggests improved hunting success on infected and therefore weakened prey. Scavenging bird species that feed on deceased birds and carcasses are also at high risk of infection.

Therefore, sampling raptors and scavengers could help to set up an effective wild bird monitoring on AIV. While adult individuals might show seroconversion upon prior and survived infection, sampling of nestlings may reveal the presence of maternal antibodies. For resident species, the detection of maternal antibodies may indicate recent circulation of these viruses in the monitoring region.

In our study, we chose a monitoring region in the northeast of Germany, Mecklenburg-Western Pomerania, highly affected during the HPAIV outbreak season 2020/21. We took advantage of established bird ringing projects for our sampling activities, to keep the associated disturbance to the animals at a minimum. Wild breeding raptors are usually ringed by the end of the first month after hatching. Hence, the majority of our samples originate from nestlings. However, we also included samples collected from injured and recovered birds from a wild bird rescue center. All individuals were checked for any clinical signs indicating a current infection. Swabs and serum samples were collected to screen for viral RNA and AIV-reactive antibodies, respectively. The majority of sampled individuals belonged to the taxonomic orders Accipitriformes (n= 124) and Charadriiformes (n= 66). While there was no evidence for a current infection of any individual, we detected seroreactivity against the AIV nucleoprotein in 5 out of 19 (26,3%) Herring gull (*Larus argenatus*) fledglings in a commercial ELISA. Furthermore, for the first time in Germany, our analyses suggest AIV antibodies in nestlings of white-tailed sea eagles (*Haliaeetus albicilla*), assumed to represent maternal antibodies.

Facing the next HPAIV epidemic in Europe during winter 2021/22, we suggest to continue monitoring of raptors and sampling of nestlings, to gain more comprehensive knowledge on virus- and seroprevalences within their populations. This will help to set up risk assessments for raptor species, especially in the age of nestlings, given the current trends towards a possibly endemic HPAIV activity. Moreover, our sampling strategy enables the combination of different monitoring approaches, as predatory bird species are often sampled within environmental toxicology studies. In terms of animal welfare, we particularly suggest interdisciplinary collaboration to keep the level of disturbance for wild birds to a minimum, while gaining as much information as possible.

73. INgezim COVID 19 S VET: a multi-species ELISA for detection of specific antibodies against S protein of the SARS-CoV-2 virus

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Coronavirus disease (COVID-19) is a zoonotic disease caused by the SARS-CoV-2 coronavirus. It was declared a public health emergency outbreak by WHO in January 2020. Since then, cases of natural infection have been reported in companion (cats, dogs and hamsters), production (mink), wildlife (white-tailed deer) and zoo (monkeys, gorillas, tigers and lions) animals, showing different degree of susceptibility to infection and a wide range of clinical signs, from none to very severe signs. To date, some cases of human-to-animal, animal-to-animal and animal-to- human transmission have been reported. Although animals do not seem to play an important role in the spread of the virus among humans, all these facts have led the OIE to promote studies on the prevalence of infection in animals, and the European Food Safety Authority (EFSA) to issue instructions for surveillance of mink farms.

In this work, we have evaluated the performance of the nucleoprotein (N) and the receptor binding domain (RBD) of the S protein of SARS-CoV-2, as antigenic markers for veterinary serological diagnosis, using mink sera as proof of concept. To achieve this goal, we developed two ELISAs and a duplex immunoassay microarray all in double recognition (DR) format, to detect N-specific and RBD-specific antibodies in mink serum. Both RBD-based ELISA and MI had a sensitivity and specificity of 100%, discriminating accurately between farmed mink exposed (n=101) and unexposed (n=163) to SARS-CoV2. In contrast, we found a worse performance of N in DR-ELISA, not only for mink, but also, for feline and canine sera.

Based on these results, we developed and commercialized INgezim COVID 19 S VET, a multispecies indirect ELISA kit for detection of S-specific antibodies in plasma and serum samples. Our test showed a sensitivity and specificity higher than 98.1% in mustelid (n=454), feline (n=234) and canine (n=362) sera, showing no cross- reactivity with dog and cat sera positive for antibodies to canine coronavirus and feline coronavirus, respectively. The assay was able to detect specific antibodies in two sera of experimentally infected cats from day 10 post- infection. INgezim COVID 19 S VET also showed an optimal concordance with seroneutralization assays for 21 canine (95% concordance) and 17 feline (99.9%) samples.

TOPIC 7 – African Swine Fever

83. Analysis of ASFV and host gene transcription within PBMCs of ASFV-infected pigs

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As part of a study on the transmission of African swine fever virus (ASFV), a group of 4 male pigs were inoculated intranasally with 4 $\log_{10}$ $TCID_{50}$ of the ASFV POL/2015/Podlaskie strain of the genotype II virus which has spread widely across Europe and Asia. The animals were monitored on a daily basis. Fever (>41°C) became apparent at 4 days post inoculation (dpi) and clinical signs from 5 dpi. At 6 dpi, all the animals were euthanized, with 3 of them showing marked clinical disease. All 4 pigs had high levels (>10^8 genome copies/ml) of ASFV DNA in their blood at

this time. Peripheral blood mononuclear cells (PBMCs) were isolated from the pigs prior to inoculation on day 0 and at 3 and 6 dpi. From these cells, total RNA was isolated and then, following DNAse treatment and poly(A+) selection, the RNA transcripts were sequenced (by RNA-seq, about 25-40 million sequence reads were obtained per sample). About 90% of these sequence reads mapped to the pig genome while up to 4% (almost 2 million reads) mapped to the ASFV genome at 6 dpi. Only very few virus derived mRNA reads (<1000 in each sample) were detected at 3 dpi. Thus, there was a very major change in viral mRNA expression between 3 and 6 dpi. The pattern of ASFV gene expression in the PBMCs was very consistent between the 4 different pigs. However, there were very marked differences in the level of expression of individual virus genes (this has been published by Olesen et al., 2021). There are almost 200 different ASFV genes that have been annotated, however the function of many of these genes is unknown. Within the PBMCs, marked changes in the expression of host mRNAs were also apparent. About 1300 different host genes were up or down regulated at 6 dpi. These changes were also consistent between the pigs and showed the nature of the host response to infection resulting from ASFV infection.

Changes in expression of selected host genes, which were initially detected by RNA-seq, are being confirmed by targeted analyses using RT-qPCR assays.

84. African swine fever virus MGF505-2R negatively regulates IFN-? production by targeting cGAS-STING pathway

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African swine fever virus (ASFV) is a complex cytoplasmic dsDNA virus that causes a serious hemorrhagic fever in domestic pigs and wild boars and is currently expanding throughout the world. Differences in virulence among the different ASFV strains vary from highly virulent strains with 100% of mortality, to attenuated strains inducing low or no mortality. Our group recently described that one of the main features differencing virulent vs. attenuated strains relies on their ability to control IFN-I pathways. In particular, virulent strain Arm/07 controls IFN-? production through the modulation of the cellular cGAS-STING pathway, an important signaling pathway involved in the activation of the innate immune system during dsDNA virus infection. However, attenuated NH/P68 strain does not inhibit cGAS-STING pathway. The viral molecular mechanisms involved in this phenomenon are not fully understood.

ASFV genome encodes multiple structural and non-structural proteins some of them contributing to evasion of the host immune system, which are mainly grouped into the multigene families, located at both 5´and 3´ends and including MGF505, MGF360, MGF110 and MGF100. The genome of NH/P68 strain lacks many MGFs genes compared to the ASFV virulent strains, this event being apparently related to the NH/P68 attenuated phenotype.

MGF505-2R is a non-essential gene missing in the NH/P68 genome, which contains an “LxCxE” motif, previously reported as a STING-binding motif and that may putatively play a role in inhibition of the DNA-sensing pathway.

In this study, we determined that viral ASFV protein MGF505-2R is involved in negative modulation of IFN-? production. Ectopic expression of MGF505-2R impairs IRF3 and TBK1 phosphorylation stimulated by MVA-T7 infection. ASFV MGF505-2R also inhibits the IFN-b levels induced by IRF3 or TBK1 overexpression. To further confirm the results from the ectopic experiments, we have generated an ASFV MGF505-2R deletion mutant (ArmC2DMGF505-2R) which we have found to induce phosphorylation of STING, TBK1 and IRF3 and to produce similar levels of IFN-b mRNA as compared to NH/P68, in contrast to the undetectable amounts of IFN-b found during virulent Arm/07/CBM/c2 infection. Altogether, these results point out to MGF505-2R as an inhibitor of cGAS-

STING pathway that might have a role in ASFV virulence. Further experiments exploring the specific molecular mechanism (s) displayed by MGF505-2R are currently ongoing.

85. Adherence to and impact of African Swine Fever quarantine measures on value chain actors in Uganda

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Pig production in Uganda has been constrained by African Swine Fever (ASF). With no effective vaccine for ASF disease, biosecurity and movement control are the only available control measures in Uganda. To effectively implement ASF biosecurity measures, there is need for knowledge on implementation of movement control measures, and perceived economic impact of imposed quarantine measures on value chain actors. A study conducted in districts of Kisoro and Moyo, involved pig value chain actors (PVCAs) selected using predetermined inclusion and exclusion criteria. Ten focus group discussions were conducted in a participatory epidemiology approach and data analyzed using matrix-table. Results indicated PVCAs have knowledge of imposed quarantine control measures such as ban on trade and movement of pigs and pig products. PVCAs complied with measures associated with trade activities in; livestock markets, designated slaughter slabs, and pork joints, in addition to confinement of free-range pigs, suspension of issuing movement-permit and pork inspection. However, measures implemented against quarantine regulations were the illegal trade in pigs and pork within the district and across national and international borders, illegal slaughter of pigs at farmers' homes and in the bush, and illegal movement of pigs and pork. The most economically affected stakeholders were farmers and traders as ranked by participants. Sale of pigs and pork forgone by compliant farmers and traders, cost incurred in implementing biosecurity and buying feeds were perceived as the largest contributors to economic loss. In conclusion, absence of live pigs in livestock markets and designated slaughter slabs and pork in pork-joints gave hope in controlling movement of pigs and pig products. Pigs' purchase and slaughter at farmers' home motivated value chain actors to sustain movement of pigs and pork contrary to quarantine control measures. Perception of incurred cost of production during quarantine impacted economically on compliant farmers. We recommend future an in-depth quantitative research on economic impact of quarantine imposition on value chain actors.

87. Novel p22-Monoclonal Antibody Based Blocking ELISA for the Detection of African Swine Fever Virus Antibodies in Serum

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African swine fever (ASF) is a highly infectious viral disease of pigs, resulting in significant economic loss worldwide. There is no approved vaccines and treatments, the control of ASF entirely depends on early diagnosis and culling of infected pigs. Thus, highly specific and sensitive diagnostic assays are required for accurate and early diagnosis of ASF virus (ASFV).

Currently, only a few recombinant proteins with high antigenic properties have been tested and validated for use as reagents in ASF diagnostic assays. The most promising ones for ASFV antibody detection were p72, p30, p54, and pp62. So far, three ELISA kits with high sensitivity and specificity have been commercialized, which are based on recombinant proteins p72 (INGEZIM PPA COMPAC K3, Ingenasa, Spain), p30 (SVANOVIR® ASFV-Ab, Boehringer Ingelheim Svanova, Sweden), and a combination of p72, p30 and pp62 (ID Screen® ASF, IDVet, France).

ASFV p22 protein, encoded by KP177R gene, is located in the inner membrane of viral particle and appeared transiently in the plasma membrane early after virus infection. The p22 interacts with numerous cellular proteins, involved in processes of phagocytosis and endocytosis through different cellular pathways. However, p22 does not appear to be involved in virus replication or swine pathogenicity. We proved that p22 is highly immunogenic in ASFV infected sera (unpublished).

In this study, we expressed recombinant p22 protein (rP22) in *E.coli*, and developed seven monoclonal antibodies (mAb) against rP22, all of which showed high inhibition capacity against the binding of ASFV infected sera with rP22. One of those mAbs, 3B7 was chosen to develop the mAb-based blocking ELISA (bELISA). Totally 806 pig serum samples were tested to evaluate the bELISA. To determine the percent inhibition (PI) cut-off value, receiver- operating characteristic (ROC) analysis was applied. 98.10% of specificity and 100% sensitivity were recorded when the threshold PI cut-off value was established at 47%.

In summary, a highly sensitive, specific, rapid, and repeatable p22-mAb based bELISA assay was developed, and optimized for detection of antibodies against genotype I and II ASFVs. It is an alternative to other serological diagnostic methods, and will play a valuable role in the containment and prevention of ASF. Also, this study will assist in further investigation of the immunogenic importance of p22 protein in ASFV infection.

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88. Superficial Inguinal Lymph Nodes for Screening Dead Pigs for African Swine Fever

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African swine fever (ASF) continues to spread across the globe and has reached closer to North America. As a result, surveillance measures have been heightened and the utility of alternative samples for herd-level monitoring and dead pig sampling have been investigated. Passive surveillance based on the investigation of dead pigs, both domestic and wild, plays a pivotal role in the early detection of an ASF incursion. The World Organization for Animal Health (OIE)-recommended samples for dead pigs are spleen, lymph nodes, bone marrow, lung, tonsil and kidney. However, obtaining these samples requires opening up the carcasses, which is time-consuming, requires skilled labour and often leads to contamination of the premises. Therefore, we investigated the suitability of superficial inguinal lymph nodes (SILNs) for surveillance of dead animals. SILNs can be collected with no to minimum environmental contamination in minutes. In this study, we demonstrated that the ASF virus (ASFV) genome copy numbers in SILNs highly correlate with those in the spleen and, by sampling SILN, we can detect all pigs that succumb to highly virulent and moderately virulent ASFV strains (100% sensitivity). In conclusion, sampling SILNs could be useful for routine surveillance of dead pigs on commercial and backyard farms, holding pens and dead on arrival at slaughter houses, as well as during massive die-offs of pigs due to unknown causes.

89. Dose-dependent disease dynamics in virulent- and attenuated African swine fever virus infection.

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Introduction: African swine fever virus (ASFV) causes one of the most dangerous disease of pigs and wild boar – African swine fever (ASF). Till now, nor safe vaccine, neither effective treatment is available against ASF. Accurate diagnosis and knowledge of disease dynamics may play a crucial role in early recognition of the disease and contribute significantly to minimize its spread. Here, we present the low-dose-effect on infection caused by recently circulating in Europe – virulent genotype II of ASFV in comparison to high doses of virulent or attenuated African swine fever strains.

Materials and methods: A total of twenty-four pigs, aged six weeks, were divided into four experimental groups, infected intramuscularly with virulent Armenia 07 ASFV strain Arm/07/CBM/c2: Group I – Arm07 LD (low dose, n=6, 10^1 TCID50/mL), Group II - Arm07 HD (high dose, n=6, 10^3 TCID50/mL) or attenuated NH/P68 ASFV strain: Group III – NH/P68 (n=6, 10^3 TCID50/mL). Group IV– control group (n=6, non-infected). Evaluation of clinical signs was assessed on the daily basis and scored accordingly to the clinical score scale. To assess the beginning of viremia in the groups, each pig was sampled every two days interchangeably from 1 to 14 days post infection (dpi). Then blood was collected 21 and 28 dpi and in case of any clinical signs (i.e. fever). Samples of blood (1:10 v/v PBS) were used for DNA extraction using Qiagen DNA Mini Kit protocol (Qiagen, Germany) and subjected for real-time PCR (VIROTYPE®, Qiagen, Germany) according to manufacturer's instructions.

Results: Fever, apathy and reduced feed intake, were found in all animals infected with virulent ASFV strain. One pig (Arm07 HD) presented dyspnoea, recumbence and bloody diarrhoea (euthanasia, 7th dpi). Animals infected with attenuated virus presented only moderate fever. Maximum clinical score was found in Group Arm07 HD. Statistically significant difference in severity of the disease (maximum clinical score) was identified between animals infected with virulent and attenuated strain, but not between high and low dose of animals infected with virulent strain. The minimum incubation period was 7 days in Arm07 LD group vs. 2 days in Arm07 HD group and 1 day in NH/P68 group. Mean incubation period was significantly ($p< 0.001$) shorter in Arm07 HD and NH/P68 groups than in Arm07 LD group (average 3.3(±0,74) and 3.0 (±2.3) vs. 14(±4.2), respectively). However, mean time from first clinical signs to death in groups infected with virulent ASFV were almost identical for high dose (2.8 days (±0.74)) than low dose group (3 days (±0.8)). Mortality reached 100% in Arm07 HD group, 83% in Arm07 LD group (one animal was not infected), while in NH/P68 group all animals survived till the end of experiment (euthanasia, 28 dpi). Viral DNA was detected in blood of 5/6 pigs infected with virulent strain of ASFV, while only sporadically in 4/6 animals in animals infected with attenuated strain, at high Ct value (~38 Ct).

Conclusions: The evolution of the disease was more dynamic in group inoculated with high dose of virulent ASFV (Arm07 HD). Despite delayed incubation period, and in contrast to attenuated strain, a low dose of virulent strain (Arm07 LD) did not prolong the period between first clinical signs and death. Significantly delayed incubation period in animals infected with low dose of virulent ASFV may pose a risk for accurate diagnosis of the disease within pig farm, creating additional threat of uncontrolled ASF spread. It is noteworthy that despite the similar incubation period between virulent and attenuated strains, the latter leads to 100% survival rate.

Acknowledgments: This study was funded by ICRAD ERA-NET, project IFNASF: "Characterization of virus- and host-specific modulation of type I IFN in African Swine Fever Virus virulence or attenuation", the global network of animal health STAR-IDAZ under Framework Programme 7 (FP7) in Poland, coordinated by The National Centre for Research and Development No. ICRAD/I/IFNASF/03/2021. We thank Dr. Carmina Gallardo and ERL (CISA- Valdeolmos), for valuable help.

90. Peppermint extract – a natural-derived disinfectant, effective against African swine fever virus

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Introduction: 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 is 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 based mainly on proper biosecurity. Due to growing awareness of the potential ASF threat among pig producers, disinfection processes are considered as one of the most important preventive measures. Currently, a variety of chemical compounds are applied for the disinfection of pig farms. Meanwhile, these chemicals may pose a potential risk, due to their toxic, irritant or corrosive effect. Environmental degradation, among others, is caused by overexploitation of its resources, overpopulation and contamination by detergents and chemicals. Ideally, natural virucidal compounds should be safe for both humans and animals, biodegradable, easily available and inexpensive. Therefore the aim of the study was to determine whether any plant-based natural compounds may show a virucidal effect against African swine fever virus (ASFV), and simultaneously be depleted of some of the side effects typical for chemical compounds.

Material and methods: Fourteen plant extracts (pure extracts n=5, hydroglycerin-based n=6, hydroglycolic-based n=3, namely: black currant, black chokeberry, strawberry, raspberry, thyme, field horsetail, peppermint, aloe vera, asiatic pennywort, lime Lemon balm, cucumber, common nettle, fenugreek) were selected and screened for their virucidal effect against ASFV. The examination was carried out using the suspension test inspired by the PN-EN 14675: 2015 European Standard procedure. All tested plant extracts were analysed in triplicate, diluted with water of standardized hardness, to obtain three dilutions of each extracts. The tested mixture contained 1 part of virus stock, 1 part of interfering substance at low or high soiling level (BSA - bovine albumin 3.0g/l or BSA + YE - bovine albumin 10g/l, plus a yeast extract 10g/l, respectively) and 8 parts of selected plant-extract. The obtained mixture was incubated at 10 ± 1°C for 30 min ± 10 s. Determination of 50% endpoint titer was assumed using Spearman–Kärber method. A 10-fold serial dilution was done in 96-well plates (in quadruplicates) containing Vero cell culture. The presence of cytopathic effect was observed after 7 day-incubation (37 °C ± 2 °C, 5% CO_2). If the difference between tested extract and virus control was ? 4 log (TCID50/ml), the extract was considered as virucidal against ASFV.

Results: Our research showed that peppermint extract (1.05%) shows virucidal activity against ASFV, with the mean log reduction (±SD) at 4.41 (±0.23) – BSA and 4.17 (±0.11) – BSA+YE. The remaining thirteen plant extracts showed low or moderate virucidal activity against ASFV, reducing the virus titre from 0,08 to 2,58 log10. In case of extracts suspended in a hydroglycolic medium, the effect of high virus titre reduction resulted only from medium composition. High soiling was shown to have a significantly negative impact on disinfection effectiveness, which confirms the crucial role of pre-cleaning prior to proper disinfection.

Conclusion: Our research has proven the existence of a naturally-derived disinfectant, effective against the ASF virus, which may be safe for animals, humans and the environment, which is additionally ecological, biodegradable, inexpensive and easily available.

91. Molecular determinants of ASFV hemadsorption

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African swine fever virus (ASFV) is a dsDNA virus causing African swine fever (ASF), a devastating disease affecting domestic pigs and wild boards. ASFV is currently spreading through Asia, Oceania, Europe and Dominican Republic threatening the pig industry worldwide since no fully safe commercial vaccine is available. Differences in virulence among ASFV strains vary from 100% of mortality in acute infection, to low or no mortality induced by attenuated strains. One of the main features associated with virulence is the hemadsorption (HAD) which induces “rosette” formation, representing erythrocytes bound to infected cells. However, the molecular mechanisms connecting HAD and virulence are still unknown. Regarding to the viral factors involved, ASFV EP153R and CD2v proteins (EP402R) were reported to be responsible of HAD.

Here, we demonstrate by ectopic expression and ASFV mutants that HAD relies exclusively on CD2v, and more specifically, on its Nt domain, whereas no role for EP153R has been found. We show here that within the CD2-Nt domain, the predicted signal peptide and transmembrane domains are essential for HAD function. On the other hand, CD2v-Nt is highly glycosylated, and inhibition of glycosylation prevented HAD. In addition, we identified several key residues within the multiple predicted glycosylation sites within CD2v-Nt. Interestingly, CD2v-Nt mutants lacking these residues did not hemadsorb, although the molecule still presented a highly glycosylation pattern. Finally, we investigated the CD2v-Nt status of the attenuated, non-HAD NH/P68 strain. The NH/P68 CD2v- Nt was expressed and glycosylated and key residues for HAD were identified. However, no HAD was observed, in agreement to what we observed during NH/P68 infection. A role of the signal peptide, which is present in CD2v from virulent strains, but absent in NH/P68, is currently under study.

92. Salt inactivation of swine fever viruses in pig intestines: in vitro model validation

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An in vitro 3D collagen matrix model was developed to determine the efficacy of salt to inactivate animal viruses. This model simulates a natural casing which is the edible sausage container derived from animal intestines, actually from the submucosa layer of the small intestines of pigs, cattle and small ruminants. To preserve natural casings from decay by micro-organisms, a standard salt incubation of 30 days is applied during the final stage of processing. Natural casings are traded worldwide and may come from areas where contagious animal viruses are prevalent. In order to validate the above described in vitro model, first a pilot in vivo experiment investigating the intestines of classical swine fever infected pigs (CSFV) was performed. Sufficient viral loads (mean/SD: 4.5/0.3 $\log_{10}$ $TCID_{50}$/gram) were found in a homogeneous distribution throughout the entire intestinal tract, which is prerequisite for the performance of an inactivation study. During the actual validation study, intestine samples of pigs experimentally infected with CSFV and African swine fever virus (ASFV) were salt treated. Decimal reduction (D) values were determined at 4 °C, 12 °C, 20 °C and 25 °C. Standard salt procedure showed an efficient inactivation of ASFV and CSFV over time in a temperature dependent way. D-intestine values of both viruses, treated with standard salt treatment, were in line with D-collagen values. This validation confirmed the suitability of the 3D collagen matrix model for assessment of virus inactivation. This model can replace animal experiments focusing on the intestinal tract as a vehicle for disease transmission.

93. Performant competitive and indirect ELISAs African Swine Fever diagnosis on domestic swine and wild boar

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Introduction

African Swine Fever Virus (ASFV) control programs require reliable diagnostic tests. IDvet offers an indirect ELISA (code product: ASFS) and a competitive ELISA (code product: ASFC) for the detection of antibodies. This work presents validation data obtained on these ELISA.

Materials and methods

The ID Screen® ASF Indirect ELISA kit includes plates coated with three recombinant ASFV antigens (P32, P62, and P72).

The ID Screen® African Swine Fever Competition ELISA allows the detection of P32 antibodies. The test was performed as per manufacturer's instructions.

Panels of known positive and negative samples, as describe below, were tested.

Results

Indirect ELISA ASFS

Specificity:

- 763 disease-free sera from domestic pigs were tested, wild boars, and Iberian pigs, measured specificity was 99.61% (CI 95%: 98.96% - 99.90%).
- 90 negative sera tested by both the serum and filter paper protocols were correctly identified by both protocols.

Sensitivity:

- 3 sera from vaccinated and challenged pigs gave positive results.
- 8 reference sera from the ASF EURL were correctly identified as positive.
- 3 positive sera, titrated and tested by both the serum and filter paper protocols, the analytical sensitivity was similar regardless of the sample type tested.
- All spiked meat juice samples were correctly identified as positive.

Competitive ELISA ASFC

Specificity:

- It was evaluated through the analysis of 280 disease-free sera from domestic and Iberian pigs. Measured specificity was 100.0% (CI 95%: 98.7% - 100.0%).

Sensitivity:

- 8 positive reference sera from the ASF European Reference Laboratory (EURL-ASF, Madrid, Spain) were correctly identified. Seroconversion was detected between 6 and 13 dpi.
- The test correctly identified genotypes tested, including genotype II.

The test was also evaluated by the EURL.

Results indicate a specificity of 99.4% (n=177) and a sensitivity of 95.8% (n=213).

Perfect agreement (k=0,95) with the immunoperoxidase test was obtained.

Conclusion

The ID Screen® African Swine Fever Indirect ELISA is the only test presenting no false positive on wild boars.

The use of filter papers makes sampling easier, especially for wild boars.

It shows excellent specificity and sensitivity, correctly detected reference sera from the EURL for ASF (INIA-CISA, Madrid, Spain). ID ELISAs offer a very good performance, with the highest analytic sensitivity on domestic pigs.

Both kits are currently massively used in Europe for the control of the current outbreak.

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

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Introduction

African Swine Fever (ASF) is a highly contagious hemorrhagic disease and one of the most important infectious viral diseases of swine pigs and warthogs, caused by a double-stranded DNA virus belonging to the family Asfarviridae, genus *Asfivirus.* Control and eradication programs require accurate and reliable diagnostic tests. IDvet developed a new triplex qPCR kit for ASF diagnosis, **ID Gene™ African Swine Fever Triplex (cat.**

IDASFTRI), which offers **ultra-rapid (35 min)** or **rapid (55 min) protocols**, and both exogenous and endogenous internal controls to **ensure accurate results**. The kit is **ready-to-use**, and can be **shipped** at **ambient temperature worldwide**, **reducing shipping costs** and the **environmental footprint**.

Materials and methods

The ID Gene™ African Swine fever Triplex (IDASFTRI) qPCR simultaneously amplifies VP72 gene target DNA as well as endogenous and exogenous non-target positive controls.

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

Analytical sensitivity was evaluated with a synthetic nucleic acid and the limit of detection of the PCR (LDPCR) was determined. The Method Detection Limit (MDL) was determined by using negative swine blood, bone marrow, and oropharyngeal fluids samples spiked with the genotype I ASFV Georgia 2007/1 strain at $10^{7,8}$ HAU/mL (NRL laboratory for ASF, ANSES Ploufragan Laboratory). The spiked samples were extracted with IDvet's nucleic acid purification columns (SPIN) and magnetic beads (MAGFAST, 20 min).

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

Results and discussion

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

The LDPCR (95%) was established around 5 copies/PCR. The MDL obtained with IDvet's extraction methods for spiked swine oropharyngeal fluids samples was $10^{2.8}$ HAU/ml, and $10^{3.8}$ HAU/mL for spiked swine blood and bone marrow samples.

The ultra-rapid and rapid amplification programs show excellent agreement (Kappa coefficient: 1 and correlation coefficient: 1,75 %) (mean difference ?Cq = 1).

Conclusion

The ID Gene™ African Swine Fever Triplex kit offers:

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

The kit is currently under evaluation and validation by the EURL and FLI.

95. Multihost epidemic modelling of African Swine Fever: Disease control insights at the domestic-wildlife interface

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The global spread of African swine fever (ASF) poses an unprecedented threat to the swine industry. Existing mathematical models have proven useful for quantifying disease transmission and informing control strategies among domestic pigs and wild boars separately. Our research seeks to bridge the modelling gap between the domestic and wildlife compartments, where we mechanistically explain the infection dynamics of the current ASF epidemic in Romania, a country with a preponderance of low-biosecurity backyard pig farms susceptible to spillover infection from wild boar hosts.

A multihost spatiotemporal simulation model was designed and fitted to current outbreak data. Backyard domestic pig farms were represented at the village scale due to the ubiquity of backyard farming in villages and commercial farms were represented by their point coordinates retrieved from national veterinary registries. Wild boar presence was simulated through a hexagonal raster of landscape coverage. CORINE Land Cover data was rasterized into 25km² cells sized to estimated wild boar home-ranges, with wild boar presence modelled as a function of raster cell forest density. Domestic pig herds iterated through susceptible (S), infectious-undetected (Iu), infectious-detected (Id), removed-by-culling (R), and re-susceptible (S) states, while wild boar cells were considered to be perpetually infected following detection of infection, allowing for S-Iu-Id states. The simulation was initiated through introducing the virus into 3 wild boar raster cells near where the first wild boar cases were detected.

Using approximate Bayesian computation sequential Monte Carlo (ABC-SMC) methodology, transmission rate parameters were estimated for ASF transmission between domestic pig herds, from domestic pig herds to wild boar, from wild boar to domestic pig herds, and between wild boar, as well as for re-infection among domestic pig herds related to prior infection (such as from stored but infected meat), infection from the edge effect at international borders, and an external force of infection to account for unaccounted sources of transmission.

Following parameterization of our model, the relative contribution of individual epidemiological units to epidemic propagation was determined, and hypothetical control strategies were explored. Our results show that wild boar play a critical role in infection propagation among backyard herds, but only marginally contribute to infection of commercial farms. Additionally, our results suggest that culling all herds in a village within two weeks of detection would have shortened the mean epidemic duration by 8 weeks (95% credible interval: 4-15).

Unravelling transmission processes of ASF in a multi-host environment is paramount to design integrated and efficient surveillance and intervention strategies. Model outputs will continue to be used to assess the effectiveness of additional control strategies during the initial phase of the epidemic.

96. Development of African swine fever virus recombinant Volgograd/D(1L-5-6L)MGF110

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African swine fever is a contagious viral disease of wild and domestic pigs with high mortality rate (Blome S., et.al.,2020). In recent years ASF has wide spread to European and Asian countries and became one of the most difficult problems to pig industry all over the world (Wang, F., et.al.,2021). Nowadays, design of recombinant ASF viruses, with one or several gene deletions, is considered as one of the most promising approaches to vaccine development. Recent reports elucidate the important role of multigenic families, which are situated on the ends of the genome (Zhu, Z et al., 2021). Interesting information about potential function of MGF 110 in viral pathogenicity was presented in study ofthe“Estonia-2017”strain(Zani L., et.al.,2017).Another possible function of MGF members is apoptosis process regulation, which can be one of the mechanisms of immune evasion (Fr?czyk M., et.al.,2016; Golding J., et.al.,2016). Based on these data, the main goal of our work is to study MGF 110 functions and define their role in pathogenicity and host immune response evasion.

The aim of this study was to construct an African swine fever virus recombinant Volgograd/D(1L-5-6L) MGF110 with a deletion of 1L-5-6L genes MGF110 multigene family by homologous recombination.

“Volgograd/14c” was chosen as parental strain because it belongs to the widest spread genotype II and adapted to COS-1 cell culture. “Volgograd/D(1L-5-6L) MGF110” virus with a deletion of six genes (1L-5-6L) of MGF110 multigene family (7004-10107) was generated by homologous recombination method using the Lipofectamine 3000 kit (Invitrogen, USA).As a result, the appearance of fluorescence of the green fluorescent protein EGFP, built into the construct instead of part of the genes MGF110, indicated the presence of a recombinant ASF virus. MGF 110 1L-5-6L members 3103 b.p. size was deleted. Then, a series of six passages was carried out in the transplanted COS-1 cell culture using the limiting dilution method. After that, the recombinant Volgograd/D(1L-5-6L) MGF110 was selected by the plaque formation method and accumulated for further research.The titration results showed that the infectious activity of Volgograd/D(1L-5-6L) MGF110 in COS-1 cells on the 3rd day after infection was 6.08±0.15lg TCID50/cm^3 and 7.00±0.21 lg TCID50/cm^3 on the 6th day after infection.Thus, it can be concluded that deletion of the 1L-5-6L genes of the multigenic family 110 does not affect virus replication *in vitro.*

The obtained PCR results with electrophoretic detection demonstrated the absence of the original parental type of the Volgograd/14c virus in the Volgograd/D(1L-5-6L) MGF110 recombinant.

Conclusion: In this study, we demonstrated recombinant virus with deletion of 1L-5-6Lgenes of multigenic family 110 from ASFV strain (genotype II, serogroup 8). In future work we planned to study the characteristics of the deletion mutant in *vitro* and *in vivo*, and to check protective properties of developed mutant strain against parental challenge.

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97. Comparison of commercially available ELISA kits for accurate detection of antibodies against African swine fever virus

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Rapid spread of African swine fever virus (ASFV), an enveloped, double-stranded DNA virus causing severe disease with often high fatality rates in Eurasian suids, remains a threat for local pig populations and economies. In Germany, seroprevalence rates are continuously increasing after 1.5 years of virus circulation in the affected Federal States of Brandenburg and Saxony. Moreover, some recently emerged ASFV variants might result in a lower lethality in infected individuals and thus a higher number of recovered, sero-positive and virus negative pigs. To accurately track the evolution of seroprevalence and aid the understanding of disease dynamics reliable serological data and accurate assessment of antibody titers is key. However, samples with antibody titers at the lower detection limit and low-quality field sera obtained from wild boar might hamper data interpretation. To ensure serological testing with precise and resilient results, 4 commercially available ELISA kits were experimentally compared. This study provides comprehensible insight into the performance of indirect and competitive ELISA kits. Competitive ELISA kits included in the study detect antibodies against ASFV proteins p32 (ID.vet ASF Competition test) and p72 (Ingenasa Ingezim PPA COMPAC). Indirect screening assays cover detection of antibodies against proteins p32, p62, and p72 (ID.vet ASF indirect screening test), as well as cd312 and p30 (Ingenasa Ingezim ASFV-R). We assessed performance of these kits by employing sera derived from experimental ASFV infection, as well as various wild boar field sera. In conclusion, we determined the screening pipeline with the highest precision, enabling monitoring of seropositive suids and disease progression dynamics upon infection with different ASFV variants in the future.

98. Inactivation protocols for African Swine Fever Virus in serum and saliva samples

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African swine fever virus (ASFV), an enveloped, double-stranded DNA virus which belongs to the *Asfarviridae* family, is the causative agent of African swine fever (ASF), a highly lethal disease in domestic and Eurasian wild suids. Analysis of various biomarkers of the immune system, inflammation, among others, can be studied from biofluids. Nonetheless, these samples present a serious biohazard to animal health and, precautionary measures must be taken in order to reduce or eliminate the potential risk of accidental spread. However, the effects of these actions on the biomarkers are not known. The objective of this study was to analyse the effect of three different inactivation protocols using Tergitol-type NP-40 (NP-40), polyoxyethylene-p-t-octylphenol (Triton X-100), or 95ºC heating, on ASFV inactivation and on a panel of biomarkers in serum and saliva of pigs. ASFV "Armenia 2008" (genotype II), a highly virulent isolate, was applied for validation of virus inactivation. ASFV in serum and saliva samples was exposed for 60 min to dilutions ranging from 0.5% (v/v) to 0.01% (v/v) of each detergent. Samples treated with 0.5% (v/v) concentration of both detergents showed absence of virus infectivity (minimum 99.99% of infectivity reduction). In serum, TX-100 affected significantly amylase, Adenosine deaminase (ADA), total protein, cupric reducing antioxidant capacity (CUPRAC), trolox equivalent antioxidant capacity (TEACH), gamma-glutamyl transferase (GGT) and haptoglobin (Hp); while NP-40 affected ADA, ferric reducing ability of serum/saliva (FRAP) and lactate dehydrogenase (LDH). In saliva, TX-100 affected FRAP, GGT and LDH; NP-40 affected protein content and FRAP; and 95ºC heating changed significantly the levels of salivary alpha-amylase (sAA), ADA, TEACH, and LDH. The inactivation protocols presented here agree with previously related data on detergent-treatment.

However, NP-40 would be the desired detergent for more accurate measurements on salivary and serum biomarkers while achieving a high degree of virus inactivation.

99. Decoding a virus Achilles heel: the African swine fever virus interactome

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African swine fever virus (ASFV) is considered the major re-emerging threat in livestock populations. It has increased in its incidence and spread over the last ten years in Europe, Asia and Africa, causing a high socio- economic burden and impact on animal health. Therefore, a significant challenge is the development of strategies to prevent and control ASFV since efficient vaccines or antiviral treatments are not yet available. However, knowing the functions of viral proteins and their interactions with host proteins is a prerequisite for the rational development of new antiviral strategies or vaccines. Functional characterisation of virus-host protein-protein interactions will be critical to understanding how viral proteins target cellular functions to allow viral replication and spread.

To achieve this goal, six partners from leading research institutes in animal health (ANSES, FLI, CSIC, INRAE, and TPI) and bioinformatics (UTARTU) have combined their expertise in ASFV and protein-protein interactions to define and validate the first ASFV-host interactome. The project, coordinated by ANSES, has received funding from ICRAD, an ERA-NET co-funded under European Union's Horizon 2020 research and innovation programme (https://ec.europa.eu/programmes/horizon2020/en), under Grant Agreement n°862605. The African Swine Fever Virus Interactome project (ASFVInt) aims to identify cellular signalling pathways, functional modules, and machinery manipulated by the virus to its benefit or even are essential for ASFV replication. Knowledge of such pathways would represent a valuable resource for developing antiviral strategies. Collectively, deciphering virus- host molecular interactions opens new perspectives to predict/simulate future emergencies and develop effective countermeasures for disease control, such as novels spectrum anti-infectious compounds and rationally designed ASFV vaccines.

100. Protective immunity conferred by attenuated African swine fever virus infection is strongly dependent on host factors

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African swine fever virus (ASFV) causes a lethal hemorrhagic disease in domestic pigs and wild boars. Since 2007, a highly virulent genotype II strain has emerged in Georgia and spread throughout Eastern Europe and Asia, killing millions of swine in the last 3 years. There is no vaccine commercially available, and attenuated experimental vaccines have shown moderate success. We have established that disease severity with the ASFV attenuated field isolate Estonia 14 is more severe in farm-raised than in specific pathogen-free (SPF) pigs, associated with profound differences in microbiota and baseline immune status. In contrast, with the virulent ASFV Armenia 08 strain the disease was lethal for both SPF and farm pigs. Here, SPF and farm pigs were first inoculated oronasally with the attenuated Estonia 14 strain. All animals survived the infection and SPF pigs developed a milder disease course compared to farm pigs as we previously observed after intramuscular injection. All animals seroconverted by day 28 post-infection with the attenuated virus, yet ASFV was detected in whole blood up to 4 months post-infection. While viremia became undetectable, both groups were re-challenged with the virulent Armenia 08 strain. Farm pigs developed a severe clinical disease with high fever, high viremia and a strong thrombocytopenia and leukopenia. Surprisingly, SPF pigs showed complete protection from disease with no clinical nor hematological symptoms and only mild viremia. These findings show that SPF pigs are a promising model to identify innate immune and microbial factors associated with resilience to ASFV infection and will help identify protective adaptive immune responses. Our data indicate that the immunological and hygienic background have a major influence on the severity protection of ASF with consequences on vaccine development and disease control.

101. Inactivation of ASFV inoculated in liquid plasma by spray drying and storage for 14 days at 4?C or 20?C

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Introduction. African swine fever virus (ASFV) is a dsDNA virus that can cause high mortality in pigs of all ages. Spray-dried porcine plasma (SDPP) is a highly digestible ingredient used in feed because it benefits growth, gut function and immunity[1]. The spray-drying process for SDPP inactivates a diversity of viruses of interest to the swine industry[2]. The current study objectives were to test if spray-drying conditions along with post-drying storage of product for 14 days can inactivate ASFV inoculated in liquid plasma.

Material and Methods. Fresh liquid porcine plasma was inoculated with ASFV (Badajoz 1971 – BA71) to a final $TCID_{50}$ (median tissue culture infective dose) concentration of $10^{5.18}$ per g of liquid plasma. Triplicate 1 kg samples of spiked plasma were spray-dried (SD) using a Büchi 290 Mini Spray Dryer set at an inlet temperature of 200°C and outlet temperature of 80°C. Because the retention time (RT) in the lab dryer is < 1sec, the dried plasma samples were held at 80?C for 30 or 60 sec to simulate RT in commercial dryers. The final dried samples were stored at 4?C or 20?C for 14d. Liquid and SD samples were analyzed for ASFV infectivity in two mirror 24-well plaques containing VERO cells monolayers. The wells were inoculated at different dilutions of liquid plasma or SDPP dissolved 1:9 in PBS. One plaque was immediately frozen at -80?C and the other was incubated at 37?C for 3 d. Each dilution was replicated 9 times. After incubation both plaques were analyzed for ASFV by qPCR. Virus replications were recognized when an increase of Ct values was detected in incubated wells regarding control frozen wells not incubated. The amount of virus in samples were calculated by the Reed and Muench method. The log_{10} reduction values (LRV) for the various processing or storage time factors were calculated.

Results and discussion: We used a different method to differentiate by qPCR the amount of viable virus in samples able to infect VERO cells after 3 d incubation. SD at 80?C with 0, 30 or 60sec RT inactivated 3.2 to 4.2 Log $TCID_{50}$/g ASFV (Table 1). Under commercial drying conditions with RT at 60 sec, the 4.2 LRV of ASFV was very similar to that observed in a previous study[2] using immune peroxidase monolayer assays (IPMA) against ASFV antigens as an analytical method to detect infectivity in liquid or dried porcine plasma, thus providing confidence in the new method used for this study. All SD samples when stored at 4?C or 20?C for 14 d were absent of infectious ASFV (Table 1). Other research[3] proved that SDPP experimentally contaminated with ASFV post drying and stored for 14d at 21?C showed complete inactivation (>5.7 LRV ASFV); however limited inactivation was observed when contaminated SDPP was stored at 4?C for 14 d. Our results suggest that post-drying storage at 4?C or 20?C for 14d further inactivates at least 1.95 Log ASFV.

Conditions[1]	SDPP ($TCID_{50}$/g)	LRV during SD ($TCID_{50}$/g)	Storage (4?C or 20?C) for 14d ($TCID_{50}$/g)	LRV (SD + Storage 14d) Log $TCID_{50}$/g
SD at 80?C; RT none	1.94±0.07	3.23	Negative	>5.18
SD at 80?C; RT 30 sec at 80?C	1.35±0.06	3.82	Negative	>5.18
SD at 80?C; RT 60 sec at 80?C	0.97±0.07	4.20	Negative	>5.18

SD = spray-drying; RT = retention time; SDPP = spray-dried porcine plasma; LRV = log_{10} reduction values.

[1]Intial ASFV inoculation in liquid plasma was $10^{5.18}$ $TCID_{50}$/g

Conclusions: Under these study conditions, the combination of SD and post drying storage for 14 d at 4?C or 20?C was able to inactive >5.18 log ASFV inoculated in liquid porcine plasma, demonstrating that the manufacturing process for SDPP can be considered safe regarding ASFV.

Funding: Partial funding for this study was obtained by European Association of Animal Proteins (EAPA).

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102. Performance characteristics of real-time PCRs for African swine fever virus genome detection

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African swine fever (ASF) became a major threat to the global pork industry. Eradication efforts depend on reliable pathogen detection and real-time PCR (qPCR) is an integral component of routine laboratory diagnosis. A number of commercial kits have arisen on the market during the pandemic spread of the disease. In Germany, general validation is assured through federal approval. However, kits were never systematically compared to each other. Therefore, we compared 12 commercial qPCR kits to an OIE recommended protocol. Samples were chosen to represent different matrices, genome loads and genotypes. Moreover, user-friendliness, internal controls and the time requirements were evaluated. All qPCRs were able to detect ASFV genomes in different matrices across all genotypes and disease courses. Overall specificity was 100 % [95 % CI 87.66 - 100], and the sensitivity was between 95 % and 100 % [95 % CI 91.11 - 100]. As one would expect, variability occurred in samples with low genome loads. In conclusion, all kits are fit for purpose and any of the tested systems can be chosen for ASFV diagnosis based on technical compatibility and preference of the internal control systems.

103. African swine fever virus adhesion proteins CD2v and EP153R

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The ASFV CD2v transmembrane protein is expressed on the surface of infected cells and extracellular virions causing binding of virions to red blood cells (HAD). The EP153R gene codes for a C-type lectin domain containing type II transmembrane protein. The binding of extracellular virions to red blood cells may prolong the period of virus persistence in blood and facilitate virus dissemination. We showed that deletion of the CD2v gene dramatically reduced the period of persistence in blood of a moderately virulent gene deleted ASFV (BeninDDP148R). In contrast deletion of the EP153R gene from the same virus did not reduce the period of virus persistence in blood. Deletion of either of these genes had little effect on reducing the moderate clinical signs caused by the BeninDDP148R virus. Strong immune responses and high levels of protection against challenge were obtained.

Surprisingly deletion of both the CD2v and EP153R genes from BeninDDP148R, dramatically attenuated the virus. Thus, these proteins appear to act synergistically. However immune responses and protection induced following immunisation of pigs with the CD2v/EP153R gene deleted virus were reduced (Petrovan et al., 2021). Since the CD2v and EP153R proteins are known to be important in induction of immune responses, we predicted that reintroducing the gene for a modified CD2v protein that does not bind to red blood cells would improve immunogenicity. To test this, we mapped the residues of the CD2v protein required for binding to red blood cells. A non-HAD CD2v gene was introduced to replace the HAD CD2v gene from a virus also lacking the EP153R gene (BeninDCD2vDEP153RCD2vmut). In pigs immunised with the virus expressing the non-HAD CD2v mutant antibody and cellular responses were boosted, virus remained attenuated and higher levels of protection were achieved compared to the virus with both CD2v and EP153R genes deleted. The results show this is a promising strategy to produce safe and effective ASFV vaccine candidates.

104. Risk Factors of African Swine Fever in the Samara Region, Russian Federation

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African swine fever (ASF) is an incurable viral disease of domestic and wild pigs. The largest epidemic of ASF in the history of Eurasia caused by a highly virulent virus of the II p72 genotype began 15 years ago in South Caucasus and since then has spread without the involvement of natural hosts or biological vectors. In 2020, a massive ASF epidemic emerged in the southeastern region of European Russia in the Samara Oblast and included 41 outbreaks of ASF in domestic pigs and 40 cases in wild boar. The Samara Oblast is characterized by a relatively low density of wild boar (0.04–0.05 head/km2) and domestic pigs (1.1–1.3 head/km2), with a high prevalence of small-scale productions (household farms). We studied the driving forces of the disease and performed a risk assessment for this region using complex epidemiological analyses. The socioeconomic and environmental factors of the ASF outbreak were explored using Generalized Linear Logistic Regression, where ASF infection status of the Samara Oblast districts was treated as a response variable. Presence of the virus in a district was found to be most significantly ($p < 0.05$) associated with the importation of live pigs from ASF-affected regions of Russia (OR = 371.52; 95% CI: 1.58–87290.57), less significantly ($p < 0.1$) associated with the density of smallholder farms (OR = 2.94; 0.82–10.59), volume of pork products' importation from ASF-affected regions of Russia (OR = 1.01; 1.00–1.02), summary pig population (OR = 1.01; 0.99–1.02), and insignificantly ($p > 0.1$) associated with presence of a common border with an ASF-affected region (OR = 89.2; 0.07–11208.64). No associations were found with the densities of pig and wild boar populations. The colocation analysis revealed no significant concentration of outbreaks in domestic pigs near cases in wild boar or vice versa. ASF-infected districts were associated with the transportation of live pigs from ASF-affected regions of Russia, suggesting socioeconomic links as the main factor of disease spread within the region. The results clearly underline the importance of considering animal transportation data as an explanatory factor in further modeling efforts.

105. African Swine Fever Virus: The Multigene Family and Innate Immune Evasion

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African swine fever virus (ASFV), for which there is no available vaccine, is a large DNA virus which encodes for 150 to 170 proteins, many of which are implicated in the evasion of host defences. Many ASFV proteins appear to inhibit interferon (IFN) induction and its response. The deletion of viral genes encoded by the Multigene Family (MGF), particularly MGF360-12L and MGF505-1R, has been shown to attenuate the virulence of ASFV in pigs as well as viral replication in porcine macrophages, yet their molecular mechanism of action remains elusive. Part of the basic research required to develop an effective live-attenuated vaccine against ASFV involves achieving a comprehensive understanding of how the virus evades the IFN response, and the establishment of in vivo and vitro correlates of virus attenuation and immune-induction post-infection. To facilitate the ongoing work for the development of a vaccine against ASFV, we aimed to attain mechanistic data regarding the role of members of the viral MGF family in the subversion of the host IFN response. We used dual-luciferase assays to determine which host pathways may be targeted, using reporters of host interferon stimulated genes (ISGs). We also assessed, in porcine macrophages, the effect of deleting these MGF genes on the IFN response, using quantitative methods, such as qRT-PCR and ELISA, in combination with inhibitors of different host pathways.

106. Prediction of ASF spread in Poland using Machine Learning, Generalised regression and Heuristic computer simulation

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Aim: We study the geographical propagation of African Swine Fever (ASF) through various approaches. Advances in computing power, together with the amount of data obtained from disease surveillance and registries enable machine learning, standard statistical tools as well as computer simulation to be applied to the field of Veterinary Epidemiology. Moreover, numerous mathematical models in the context of the SARS-CoV-2 pandemic show the importance of predictive models in infectious disease.

Methods: We propose a comparison of i) statistical regression (GLM) ii) heuristic approach (quasi-gravity epidemiological SIR model solved with computer simulations) and iii) machine learning approach (XBoost). We utilised 6018 disease notifications in Poland from February 2014 to January 2020 for models training and validation. A spatial representation of notifications with a given area/region in a given time interval was built. We take into consideration 68 sequential months or 17 quartal and hexagons from 100km² to 10000km². Our ground truth is a binary state if there was registered at least one notification in a given polygon in a given time period. We have inferred our models with additional variables with precision to county/poviats (old NUTS-4) as WB (wild boars): representing natural infections between WB; pig: representing agricultural infection between farms; human: representing human mediated virus translocation due to movement of contaminated formitts or products; interactions between each layer.

Results: Notification registry is not only showing disease propagation, but it's also sensitive to surveillance methodology, which vary in time. During the considered period national and European regulations have changed dozens of times. For example surveillance in WB was changed from passive to active (e.g. by searching for carcasses). In results much more cases can be found (with even 12 times higher test positivity rate among wild boars). Thus, five distinguished phases of epizootic were revealed: 1) Feb 2014 to Jun 2016: Sub epidemic; 2) Jul 2016 to Jun 2017: Pre epidemic; 3) Jul 2017 to Mar 2018: Epizootic - early; 4) Apr 2018 to Apr 2019: Epizootic - stable; 5) Mai 2019 to Jan 2020: Epizootic - extended.

We found that predictability of epizootic state (defined binary) one period in advance on the border between disease free and in affected regions reaches over 95% sensitivity and specificity with XBoost, which outperformed all other methods.

Conclusions: We confirmed that ML is powerful in predicting short-term local transmission of ASF knowing simple proxies of pig farming structure, WB habitat and density of human population. Long distance jumps have been much more difficult to predict however, quasi-gravity model seems to give a good qualitative picture of the long and medium term most paths of propagation.

TOPIC 8 – Risk Assessment and Epidemiology

74. Virus exposure bioassays to assess uptake and survival of a coronavirus in insect larvae

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The mass production of edible insects offers the potential for a new, more sustainable, source of nutrients for livestock feed. In order to ensure an increasingly sustainable production, there is a need to explore new substrates for the insects, including those of animal origin, such as slaughter byproducts, meat offal, manure and even communal waste. However, using substrates of animal origin could pose certain risks that need to be assessed, such as the transmission of viruses.

Experimental exposure studies of insects that are used for feed and food with animal viruses can provide more knowledge about such hazards. This can then be used for future risk assessments [1,2].

In a series of experimental studies, we have exposed two insect species, *Tenebrio molitor* and *Hermetia illucens,* to a porcine respiratory coronavirus (PRCV). This virus was used as a model for other coronaviruses. The studies had the aims of establishing virus exposure bioassays for larvae of the two insect species and of providing knowledge of the survival of PRCV within these insect larvae.

For virus exposure of *T. molitor* larvae, a newly developed virus exposure bioassay, using direct oral inoculation of a virus suspension, was established using PRCV. For *H. illucens* larvae, inoculation via feed spiked with PRCV was used. Both species of larvae were sampled immediately following virus exposure and until 8-9 days post inoculation. The presence of viral RNA was determined using quantitative RT-qPCR and infectious virus using virus isolation in cell culture (ST117 cells).

Using RT-qPCRs, PRCV RNA was detected in *T. molitor* on day 0 (directly after feeding) and for up to 3 days later, and in *H. illucens* on day 0 (directly after virus exposure) and for up to 2 days more. The amounts of viral RNA were low in the samples after day 0. Using virus isolation assays in ST117 cells on samples with the highest amount of viral RNA, infectious PRCV was detected in 1 *T. molitor* larva (out of 12) sampled at 0 days post exposure.

It can be concluded that PRCV does not survive well in the fed insect larvae and it appears that the virus is quickly degraded. During the spring of 2022, *T. molitor* and *H. illucens* larvae will be exposed to African swine fever virus using the virus exposure bioassays validated using PRCV.

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75. A new COST Action to improve biosecurity in livestock

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Biosecurity is of paramount importance to prevent the introduction and spread of pathogens and, consequently, to preserve the health of farmed animals. Healthier animals result in better animal welfare, better sustainability of animal production systems and less antimicrobial use. Despite these benefits, biosecurity is limited by different factors: i) lack of knowledge on ways for improvement, especially in extensive systems or settings with low resources; ii) shortage of adequate ways to enhance communication; iii) diversity of methodologies to assess and measure the implementation of biosecurity measures and their cost-effectiveness and iv) low number of trained professionals. To approach these challenges, a COST Action on biosecurity has recently emerged (since November 2021) and during the following four years will evaluate how biosecurity is currently used and will use participative approaches to understand motivators and barriers for biosecurity implementation. Knowledge generated through these activities will act as the baseline upon which adequate communication and training on biosecurity will be developed. The Action will specifically perform a comparison of existing methods used to evaluate biosecurity. Exploiting these tools will promote the development of tailored options in farms based on the evaluation of their risks, on the feasibility of selected biosecurity measures and on their economic benefits.

Moreover, the Action will identify training needs through the evaluation of existing training materials and will develop new courses, increasing therefore the number of trained professionals. Finally, the Action will recommend priority research areas for future biosecurity improvement in livestock production systems. The Action objectives will be achieved from a transdisciplinary group where Early Career Investigators will play a key role in their attainment. At present, the Action is composed of 154 participants from 36 different countries. The Action includes economists, epidemiologists, infectious disease and animal production experts, wildlife experts, sociologists, psychologists, and communication experts, among others. Most of the participants are based in Europe, but not only (e.g. Australia, Argentina or Canada). In addition, stakeholders from government bodies, the industry or international organizations such as FAO and OIE have also joined the Action.

76. Bovine cysticercosis and simplification of meat inspection in Finland

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In Finland, the possibility of cattle to be exposed to *Taenia saginata* was studied, and the prevalence of the infection in carcasses was assessed in order to investigate the consequences, if the incisions of musculus masseter will not be made. At the same time, the goal was to improve the skills of meat inspection professionals in detecting cysticerci through additional training.

Bovine cysticercosis is caused by the human tapeworm *Taenia saginata*. The worm is spread worldwide being the most common tapeworm among humans. In Western Europe, the prevalence of *T. saginata* in humans varies between 0.07-0.20%. Human cases are rare in Finland and mostly travel related. The infection in humans is usually asymptomatic. In Western Europe, the prevalence of cysticercosis found during bovine meat inspection varies between 0-8%. In Finland, only one positive bovine carcass has been reported in 1996, and another carcass in 2002 was highly suspected but not confirmed. Tapeworm is controlled at meat inspection, but the diagnostic sensitivity for the detection of bovine cysticercosis is considered low. The sensitivity increases with the number of cysts in a carcass, but it does not help in low-prevalence countries.

The life cycle of *T. saginata* consists of bovines (intermediate host) and humans (definitive host). Bovines become infected by ingesting *T. saginata* eggs. In bovines the developed larvae migrate into muscle tissue and form cysticerci. Humans get infected by eating raw or undercooked beef containing live cysticerci. An adult tapeworm develops in the human intestine. After maturation, gravid proglottids containing tens of thousands of eggs are released into the environment in human feces.

The detection of the tapeworm larvae (cysticerci) in cattle is based on meat inspection according to (EU)2019/627. According to this new regulation it is not compulsory to incise the masseter muscles of bovines if certain requirements are met. In Finland these requirements will be met after completing a risk assessment and if two years of zero bovine cases is attained. At the moment, incised masseter muscles of all bovines over 8 months old are visually inspected for cysticerci in Finland.

The project was carried out by sampling carcasses and completing a risk assessment. Sampling was carried out by both random and suspicion-based sampling in slaughterhouses 2020-2021. Samples were collected from 784 carcasses of over four years old beef and dairy cattle. Over eight months old Highland cattle were included, because they graze year around and are considered to be exposed to *T. saginata* eggs. The samples were taken from the masseter muscle (min. 1x2x3cm, most often bigger) and the right ventricle of the heart (50-100g). Samples were cut into 5mm slices to search for the cysticerci visually and by palpation.

To conclude, cysticercosis is very rare in Finnish cattle. Three suspicion-based samples were received and analyzed. No cysticerci were detected in any sample in the project. Trainings for meat inspection professionals were held on several occasions to increase detection sensitivity. Predisposing factors for cattle cysticercosis were recognized from literature to be grazing and farm workers with previous exposure to *T. saginata*. Lack of hygiene was identified noteworthy.

The results support the ending of the masseter incisions in meat inspection. In case of infection occurrence, knowledge on predisposing factors is needed, and the findings may also be used for tailored risk management of regional or farm level.

77. Risk factors associated to Streptococcus suis cases in swine farms in Spain

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Streptococcus suis infection is one of the main bacterial disease that affect piglets worldwide, causing a big impact in the industry due to the associated-treatments and mortality. *S. suis* is part of the natural microbiota of the pig respiratory tract, but on certain occasions, it can develop disease, which is characterized by meningitis, arthritis, and sudden death among others less common clinical signs. Furthermore, it is considered a re-emerging zoonotic pathogen, being associated with disease in workers of the swine industry, or people consuming raw or undercooked pork. Despite its importance, little is known about the factors that can trigger an outbreak in a farm.

In the present study, three consecutives batches of piglets from two farms with confirmed *S. suis* disease were sampled at 3, 5, and 7 weeks of age (once in farrowing and twice in weaning). In the visits, presence of animals with *S. suis*-associated disease (arthritis and nervous signs) were recorded, as well as environmental factors (temperature and relative humidity) and sow parity. Nasal and vaginal swabs were collected from sows and nasal swabs and blood from piglets. In addition to *S. suis*, the presence of concomitant pathogens traditionally associated with *S. suis*, such as porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine circovirus 2 (PCV-2), and *Glaesserella parasuis* (virulent and non-virulent strains) were also evaluated. Animal stress was measured using three different parameters (cortisol, POX-Act, and haptoglobin). For both farms, *S. suis* was isolated from lesions of diseased animals, confirming the presence of the disease.

In order to study the effect of all those parameters on *S. suis*-associated disease a mixed effect logistic regression model with mother, batch, and farm as random effects was implemented.

S. suis-associated disease at farm level was conditioned by the age of the animal at farrowing (older animals were more likely to develop disease), the stress at farrowing (reflected in higher levels of cortisol and haptoglobin), the relative humidity at farrowing (a higher mean relative humidity increased the risk), presence of PRRSV at weaning, as well as the parity of the mother (gilts were more likely to have piglets with *S. suis* problems).

These results shed some light on the importance of treating *S. suis* as a multifactorial disease. Also provides some indications to reduce the impact of *S. suis*, such as to maintain a good health status on the farm by keeping theanimals in low-stress conditions and with good air quality.

78. OutCosT-Rum, a new spreadsheet-based tool to evaluate the economic impact of transboundary animal diseases of ruminants

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Transboundary animal diseases (TADs) can affect not only animal productivity and welfare, but also livelihoods and economies, having serious socio-economic consequences. Such consequences highly differ within and across countries depending on the disease and its epidemiology, the structure of the livestock sector, the social context, and the applied control measures. Due to this, it is often challenging to determine the economic impact of disease outbreaks and related control strategies. Our purpose here is to describe a tool for the evaluation of the economic costs associated to TADs affecting ruminants and the related control measures implemented. The *OUTbreak COSting Tool* for ruminants (OutCosT-Rum) is built in an Excel spreadsheet, and computations are based on a deterministic model. The tool allows considering up to eight different types of farms. Farm types are flexible and can be defined in the input worksheet, according to the country's specific definitions and classifications (i.e. by production system or number of animals). The input parameters required by the model include: i) production data; ii) market prices; iii) data related to the ruminant populations in the affected area; iv) data on outbreaks and affected animals; and v) data related to the different control/eradication measures implemented in affected farms, in neighbouring and other at-risk farms, or in the general susceptible population. Finally, specific sections are dedicated to collect data on surveillance and control activities in wildlife, and to other activities, which might be carried out, such as trainings, communication, and coordination tasks. As results, the tool calculates the costs of 93 different items. The cost estimates are presented for the target area, by farm type and by who assumes the cost (whether the veterinary services, farmers, or other stakeholders). Besides, OutCosT-Rum provides a framework for the qualitative assessment of other indirect and non-monetary costs related to the epidemics, such as the impact on human health, environment, animal welfare, socio-economic vulnerability, trade, and political response. The level of concern (i.e. low, moderate, high and extreme) associated to each item is obtained through a semi- quantitative scoring method by combining the importance of the consequence and the likelihood of the occurrence (both scored by the users). As part of the validation process, the developed tool will be tested by calculating the cost of TADs epidemics in the European neighbourhood: the foot-and-mouth disease and Peste des Petites Ruminants outbreaks that occurred in Bulgaria in 2011 and 2018, respectively; and the outbreaks of sheep pox and goat pox that affected Greece in 2015-2018. The OutCosT-Rum can be used to estimate the cost of past outbreaks, but also to assess the potential cost of future epidemics and alternative response strategies (i.e. best- and worst-case scenarios). Therefore, it is envisaged as a tool to support decisions on the control strategy to implement and on the allocation of available resources, and to guide management policies during outbreaks. Besides, it can contribute to raise awareness on the impact of TADs, and to heighten the perceived transparency and public trust in local government.

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79. Network analysis as a tool for the control of foot-and-mouth disease and brucellosis in Paraguay

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Beef exports represent an important part of Paraguay's agricultural sector and cattle movements constitute a high- risk activity due to the possible spread of bovine diseases that can have a significant impact on the country's economy.

Network analysis has been used as an epidemiological tool for the control and prevention of transboundary diseases. Beef exports represent an important part of Paraguay's agricultural sector and cattle movements constitute a high-risk activity due to the possible spread of bovine diseases that can have a significant impact on the country's economy. The objectives of this work were to analyze and compare two cattle trade networks in Paraguay: the general cattle trade network (relevant for foot-and-mouth disease) and the cow trade network (relevant for bovine brucellosis). Both networks were studied during the period 2014-2018. The cattle and cow movement networks in Paraguay had free scale and small world properties. Higher values of the centrality indicators were found for markets than for farms. These results indicate that foot and mouth disease (in case of re- emergence) or brucellosis could spread far away from an outbreak through the markets and also locally. These cattle movement network characteristics should be taken into account when implementing surveillance or control measure for these diseases in the country.

80. Analysis of risk factors associated with bovine brucellosis in Paraguay

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Brucellosis is a zoonosis caused by bacteria of the genus *Brucella*. Bovine brucellosis (BB), caused mainly by *Brucella abortus*, is associated with abortions, reduced fertility and decreased milk production, resulting in significant economic losses in the cattle industry. In Paraguay, whose main economic activity is cattle raising, brucellosis is an endemic disease and one of the main livestock diseases. Several control plans were proposed in the past, but their implementation has not been widespread and the disease is still present in most farms.

Adequate knowledge of the epidemiology of the disease is of great importance for public health, particularly for producers, whose livelihood depends entirely on cattle. Therefore, the objectives of this study were (i) to establish the intra- and inter-herd prevalence of BB in a representative farm sample throughout the country, and (ii) to identify the risk factors associated with bovine brucellosis at the herd level in different districts of Paraguay. A cross- sectional study was conducted using a structured questionnaire in 149 farms and 2,223 cattle were sampled to carry out serological tests. This study suggests the need to raise community awareness about the risk factors associated with brucellosis transmission between animals and farms.

81. Qualitative Risk Assessment for HPAI in Nigeria: implementing a One Health Approach

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Outbreaks of transboundary animal diseases like Highly Pathogenic Avian Influenza A (HPAI, H5) constantly affect the livelihood of the general population, especially in low to middle-income countries that depend on livestock, with an impact on public health and food security. The economic and social implications of the disease remains a challenge in areas where preventive and control measures are difficult to implement, especially in rural areas of Nigeria. However, early warning and effective and sustainable management strategies are the main methods of preventing and reducing the overall effect of the disease outbreak. Therefore, qualitative risk assessment is a tool to help decision-makers identify exposure and risk pathways and implement mitigation measures to help reduce the onset, spread, and impact of HPAI. However, there is a need for a practical approach to conducting a risk assessment involving key in all relevant sectors from the grassroots to the national level in Nigeria. The response to the previous outbreaks revealed a gap in coordination between the human and animal health sectors, alongside other relevant stakeholders. This led to an increased spread of the disease across many states of the country. This study aims to develop risk questions, adopt a suitable risk assessment tool for HPAI, extract available data on HPAI outbreaks for risk mapping and risk pathways, and itemise transdisciplinary approaches for a local risk assessment from the generated results.

The study involves the screening and assessing online repository for risk assessment tools. According to the guidelines by the OIE risk analysis framework, the risk assessment will focus on entry, exposure, consequence assessment, level of likelihood, and uncertainty. A compilation of data on HPAI outbreaks in Nigeria to identify the hotspots for the disease outbreak will be carried out. Further analysis will be done using R-studio, and other visualisation with QGIS.

The recommended local risk assessment will consider the chain of command, risk assessment for high-risk areas and identify the specific risk factors with the proposed mitigation measures. This study will determine the strengths and gaps in conducting the risk assessment. The expected outcome of the research will identify and evaluate key risk questions, hotspots, and possible points of entry and exposure to HPAI in Nigeria. This will support appropriate risk mitigation and communication plans to avoid future outbreaks through a transdisciplinary approach.

82. Can the social tensions (ecologists and animal breeders protests) propagate from East to West of Europe with ASF?

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Background: Animal epizootics show a widely destructive effect on livestock production, but also cause tensions between various groups of interest. Pre-existing social conflicting matters of the animal production conditions has been manifested in reconfiguration of social relations by strengthening the tendency to issue farm animal protection in the general population (mostly urban) and "hijacking" profitability and cultural values of farmers. Such a structural configuration facilitates mobilisation of own groups of interest and ends up with conflict between animal breeders, far-right, agricultural parties, Christian organisations as well as opposing, government, pro-European opposition and ecological organisations. COVID-19 crisis and pressures on farmers related to economic and climate challenges cause more and more farmers to escape from animal production. Thus, changing and ending livestock production as well as deanimalization processes speed up in regions affected by ASF among others. The European Green Deal is projected to affect livestock production by decline between 5% and 15%, with the animal breeders income being the hardest hit among all farmers in the perspective of the next 10 years.

Case study ecologists: Series of national-wide protests started January 2019 with protests against wild boar depopulation. In turn, in several Polish cities animal right defenders organised demonstrations against hunters and government plans of wild boars depopulate in and as a way to slow down the spread of ASF.

Case Study farmers: On the other hand, farmers call for significant reduction of the wild boar population. In September 2020, the governing party (Law and Justice) proposed the so-called "Five for Animals" which attempted to extend animal rights, but at the same time it impacted a number of sectors of Polish agriculture. Moreover, the feeling of abandonment (by the state) and powerlessness (for instance due to ongoing outbreaks of ASF and HPAI) led to protests distributed over 1000 locations across the country in October 2020.

Methodology: We have collected 9 739 tweets between 1-31.10.2020 in Polish language with hashtag #ProtestRolników (farmers protest) and 5285 retweets with #ASF language also in Polish from 19.12.2018 to 18.01.2019. We primarily applied Social Network Analysis (SNA) of the Internet media users connected via their tweets sharing activities. Moreover, our investigation was extended by time series analysis as well as NLP techniques such as sentiment analysis and keyword analysis. We performed multilabel classifications using the deep neural language model Bidirectional Encoder Representations from Transformers (BERT) to mine main topics of discussion

Results and Conclusions: Ecological protests have been massively discussed by mainstream media and the general population constitute a general component linked closely to ecological activists clusters. Animal breeders' protest communication has a highly modular and hierarchical structure with farmers as a general component, but with clear boundaries between internet communities and opinion leaders. There was only a small presence of the general public (i.e. mainstream media) in the discourse. Tweeting activity concentrates around late mornings (the time after post sunrise grooming of animals).

Level of optimism (text sentiment) among farmers protesters than animal right defender protesters was significantly higher than - probably due to a feeling of unity. Level of verbal aggression is opposite, which suggests that language repertoires differ.

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