International Coordination of Research on Infectious Animal Diseases (ICRAD) First Call- 2023

Editorial and Abstracts

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International Coordination of Research on Infectious Animal Diseases (ICRAD) First Call: Editorial and Abstracts

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ICRAD First Call 2023

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Abstract

The ICRAD ERA-NET launched its first call in 2020, with funding partners from twenty European countries. The aim was to foster a collaborative and interdisciplinary approach towards developing innovative instruments for the control and prevention of animal infectious diseases. Particularly, with a focus on creating vaccine and diagnostic technology platforms to enhance preparedness for major epidemic threats such as African swine fever and animal influenza. The first ICRAD call received significant support, totaling €17.4 million from partner countries alongside an additional €6.7 million top-up from the European Commission (EC). These funds were allocated to nineteen research project consortiums, consisting of researchers from across Europe. Through their collective efforts, these consortia have made remarkable progress in addressing various infectious disease risks faced by European animal health and the livestock industry. The following text encapsulates abstracts from eleven out of the nineteen projects that received funding through the first ICRAD research call. It's noteworthy to observe that several of these projects are addressing pressing issues related to ongoing animal health outbreaks. For instance, three of these funded projects are dedicated to enhancing our comprehension of the African swine fever virus (ASFV) and fostering the development of a potential vaccine. Another project is honing its focus on avian influenza, with the objective of deciphering the factors contributing to increased virulence in low pathogenic avian influenza (LPAI) viruses. This could potentially enable us to better predict the severity of emerging avian influenza virus (AIV) strains. In addition, there are several other projects that align seamlessly with the overarching goals of ICRAD. These include but are not limited to, the development of innovative DNA vaccine technologies and the creation of diagnostic tools for a spectrum of pig diseases. Overall, we have funded a range of projects that align excellently with the overall goals of ICRAD, supporting innovative, cross-cutting research on animal health and welfare, with associated benefits for the environment and the economy. These projects represent significant strides in improving animal health, welfare, and food production throughout Europe. By fostering collaboration between partner institutes and bringing together experts from various countries and disciplines, these joint efforts can effectively combine knowledge, resources, and expertise to address complex challenges and threats. This coordination is crucial for identifying, preventing, and responding to emerging animal diseases that could not only endanger animal health but also have direct or indirect implications for human well-being. Moreover, this cross-border cooperation can yield more comprehensive research outcomes while developing innovative solutions and strategies for disease prevention and control. Ultimately, international scientific coordination on animal health showcased in the following abstracts demonstrates a strong commitment to cooperation while providing robust evidence to safeguard the health of animals, humans, and the environment. ICRAD, which organized this call and allocated grants to these projects is an ERA-NET, a co-funded project under the European Union's Horizon 2020 Research and Innovation program.

Keywords: ICRAD, ERA-NET, African swine fever virus, Avian influenza, DNA vaccine, Food production, Prevention and control, One-Health

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Characterization of virus- and host-specific modulation of type-I IFN in African swine fever virus virulence or attenuation

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Abstract

African swine fever virus (ASFV) is a dsDNA virus that infects domestic pigs and wild boar, currently causing the largest animal epidemic a ecting numerous countries in four continents and for which there is no commercial vaccine, representing the greatest threat to the pig industry worldwide. The virulence of ASFV varies between strains, with virulent strains producing up to 100% mortality, while attenuated strains produce only chronic disease. One of the key mechanisms determining ASFV virulence is its ability to control the innate immune response, particularly the production of type I IFN through the cGAS/STING pathway. Identifying and characterizing the viral genes involved in IFN control is thus key to understanding the molecular mechanisms of ASFV virulence and vaccine development. The aim of the IFNASF project is to coordinate the e orts of four European laboratories (CBMSO in Spain, LMU in Germany, NVRI in Poland, and SVA in Sweden) in order to answer these questions and to approach a better understanding of the molecular mechanisms of ASFV virulence. To this end, we have carried out di erent approaches combining *in-vivo* and *in-vitro* studies in order to identify and characterize new ASFV genes involved in virulence. On the one hand, we have selected a series of genes by sequence comparison between virulent and attenuated isolates, together with identifying genes absent in spontaneous attenuated mutants (CBMSO). On the other hand, following an *in-vivo* experiment with the virulent Arm/07/CBM/c2 strain (NVRI), a scRNAseq was performed in which viral genes present in cells that did not express genes related to type I IFN were identi ed (SVA). Some of these genes were cloned into appropriate expression vectors, and di erent screenings based on luciferase assays and/or qPCR were performed to analyze the interference of these genes in the production of type I IFN in di erent cell models (CBMSO). After these screenings, genes potentially involved in the control of type I IFN production during ASFV infection were selected and cloned for the generation of recombinant MVA to further study their mechanism of action by taking advantage of the strong heterologous expression system (LMU) and based on this information, ASFV speci c deletion viruses will be generated (CBMSO). Finally, the ASFV deletion mutants will be used for animal trials to analyze the correlation between the presence/absence of these gene(s), their control over IFN-I production, and their virulence (NVRI). Both recombinant MVAs and ASFVs viruses will be, in addition to tools to advance the knowledge of ASFV virulence and its control over the innate immune response, prototypes that could be used as future vaccines or vaccine components against ASFV in the near future.

Keywords: African swine fever, Modulation, Virulence, Immune response

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From proteogenomic host response signatures of persistent Foot-and-Mouth Disease Virus (FMDV) infection to diagnostic markers and therapeutic control (FMDV_PersIstOmics)

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Abstract

Foot-and-Mouth Disease (FMD) is one of the most important and devastating contagious viral diseases of cloven-hoofed livestock with severe global socio-economic impact. More than 50% of ruminants that had been exposed to FMDV, even those in which prior vaccination has prevented clinical disease, will carry the virus in the nasopharynx for a prolonged period, which is referred to as persistent infection or the \carrier state". Carriers are often considered a potential source of infectious viruses and represent an impediment to international trade. Even after decades of research, the mechanisms underlying FMDV persistence remain largely unknown. Investigations performed so far suggest that the maintenance of persistent infection is mainly related to the host's immune responses. Filling the knowledge gap regarding the carrier state is critical to predicting, preventing, detecting, or curing FMDV persistence and ending the mass culling of exposed animal populations during disease outbreaks in free areas. This project aims to (i/WP1) uncover alterations of the host response during persistent FMDV infection of cattle, (ii/WP2) evaluate genes highly regulated during FMDV persistence as candidate host markers of persistent infection, and (iii/WP3) identify pathways that could be targeted to prevent the establishment of FMDV persistence or terminate the infection. In WP1, a work ow of transcriptomic analyses was optimized to investigate 52 selected nasopharynx samples from 16 carrier cattle. A di erential gene expression analysis then identi ed relevant genes as candidate host markers of FMDV persistence. In parallel, viral genomes collected during acute and persistent phases were full-length sequenced and compared, and some variations have been highlighted. We have also started to assess, *in-vitro* and *in-vivo*, the role of FMDV immune evasion in persistence. In WP2, a bank of 78 clinical samples collected from 39 bovines during 6 FMDV outbreaks occurring in Turkey in 2021 was created. Of these 39 bovines, 64% were persistently infected by the FMD virus. A multiplex rt-RT-PCR was developed on two candidate host markers of FMDV persistence and one epithelial marker as quality control. This rt-RT-PCR will be used to validate the potential of these candidate genes as persistence markers by evaluating their kinetic expression during the infection. This prototype of rt-RT-PCR will then be tested on the bank of clinical samples from Turkey (non-persistently and persistently infected animals) to determine its diagnostic and predictive values of FMDV persistence in bovine. In WP3, we have started to decipher the mechanisms of the modulation of FMDV persistence by interference in the type I interferon response, and the work is still ongoing. In parallel, we work to establish a guinea pig *in-vivo* model of FMDV persistence as a small-scale alternative for cumbersome infection experiments in cattle. Adapting the FMDV strain of interest and the inoculation method are in progress. Overall, the outcomes of this collaborative project will contribute to determining mechanisms and factors that may help to prevent or control persistent infection and improve diagnostics.

Keywords: Foot-and-Mouth Disease, FMDV, Proteogenomic, Host response, Diagnostic markers

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A comprehensive proteogenomic analysis of *Brucella* to understand the epidemiology, biology, virulence mechanisms, and host-pathogen interaction

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Abstract

Brucellosis is a debilitating and incapacitating bacterial disease a ecting humans, livestock, and wildlife. Several aspects of its biology, host/pathogen interaction, and virulence mechanisms are not understood yet. In the current project, several activities were performed in each work package to explain its epidemiology, virulence mechanisms, and host speci city, as well as to understand the role of wildlife in the transmission and dissemination of the infection. In WP1, an assessment of brucellosis in wildlife in European countries showed that there is a potential threat to people and domestic animals exposed to wildlife. Most of the infections that occurred in wildlife species were due to *B. suis* bv 2, *B. ceti*, and *B. pinnipedialis*, which seems to be not of zoonotic importance. However, *B. suis* bv 3, *B. abortus*, *B. melitensis*, and *B. canis* pose a signi cant zoonotic threat. In WP2, we collected 200 *B. abortus* and *B. melitensis* isolates from di erent hosts in Greece and Turkiye. All isolates were sequenced, and comparative genome analysis using NGS technology showed no signi cant di erences in the distribution of virulence genes among *B. abortus* and *B. melitensis* strains, even for those isolated from di erent hosts. In WP3, 60 selected isolates were cultured every four times independently, and mass spectrometry-based quantitative proteomics analysis was carried out to identify the unique and cross-reactive proteins in isolates from di erent hosts. In WP4, trophoblast cell from bovine tissue was prepared and infected with *Brucella*. Di erent infection times have been evaluated with *B. abortus* and *B. melitensis*; adhesion and invasion evaluations are in progress. In WP5, the culture of four di erent *B. melitensis* was carried out at normal pH, followed by exposure to di erent pH for 1, 2, and 3 hours. This was followed by RNA isolation, cDNA generation, and RT-PCR with speci c primers designed to amplify ve di erent genes. The stability of the genes was assessed using statistical methods, and only one gene showed a more relatively stable status. Primers were designed to amplify the RelE, BrnT, Fic, and cogt genes belonging to the toxin-antitoxin system and the type IV secretion system, as well as OtpR, a response regulator with a signi cant role in *Brucella* metabolism and virulence under acidic stress. In WP6, the preliminary results were published in a review article and two conference abstracts. Additionally, two full articles are under review. To implement knowledge transfer between partners, work protocols were shared among partners, and workshop and training programs were carried out at the FLI, Germany. As outcomes, identi cation and monitoring of the existence of *Brucella* in the environment and wildlife ecosystems is of supreme signi cance in healthcare and community settings. It will help to understand the role of those reservoirs in the epidemiology and transmission of brucellosis. A comprehensive understanding of *Brucella*'s genomic and proteomic contents will facilitate a greater understanding of its biology, improve the development of species-speci c treatment in humans, design better diagnostic tools and vaccines, and clarify several aspects of *Brucella* pathogenesis.

Keywords: Brucellosis, Genomics, Proteomics, Virulence, Host-pathogen interaction

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African swine fever pathogenesis and immune responses in resistant and susceptible hosts

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Abstract

African swine fever (ASF) is one of the most complex viral diseases and has a devastating socio-economic impact. Over the last 15 years, the ASF virus has been transmitted to several territories and is still spreading. The disease originated from sub-Saharan Africa and maintains a sylvatic cycle between warthogs and soft ticks. In warthogs, ASF is not associated with severe clinical signs or mortality. However, introducing the disease into the domestic pig sector or Eurasian wild suids leads to severe multi-systemic disease that can resemble a viral hemorrhagic fever with exceptionally high lethality. Comprehensive knowledge about correlates of protection, severe clinical outcomes, and pathogenesis is scarce. To facilitate our understanding of ASF, the ASF-RASH consortium aimed to identify factors determining host/cellular susceptibility and protection at three levels: the virus, the host species, and their respective immune cells. Within Work Package (WP) 1, we conducted studies into the role of male animals in ASFV transmission. In the rst experiment, domestic and wild boar were inoculated with ASFV isolates of di erent genotypes. Viral antigens, mRNA, and infectious particles were detected in all male reproductive tissues. In a following trial, we demonstrated the suitability of arti cial insemination as an alternative transmission route for ASFV. Viral antigens, viral genomes, and infectious particles were found in male and female reproductive organs and deformed embryos. Furthermore, a trial addressed whether previous infection with a moderately virulent strain (`Estonia2014') can protect pigs from lethal challenges with a highly virulent strain (`Armenia2008'). All nine pigs survived the challenge; however, four individuals were shedding infectious particles upon challenge. Studies into responses upon infection with very low viral doses are ongoing. A study embedded in WP2 demonstrated a striking di erence in the immune cell landscape of SPF and conventionally housed pigs. The latter had higher frequencies of leukocyte populations (identi ed by ow cytometry), and cells had a more proin ammatory phenotype (cytokines). Upon infection with `Estonia2014', SPF pigs were largely protected from excessive in ammation, mortality, and immunopathology, contrary to conventional pigs. Survivors were protected from a lethal challenge (`Armenia2008'), and reactivation of speci c leukocyte subpopulations has since been discussed as a putative correlate of protection. The pathogenesis of ASFV variants originating from four German districts was assessed compared to well-characterized strains Estonia2014' and `Armenia2008' within WP2. Analyses revealed that the pathological ngerprint of all German isolates largely compares to that of `Armenia2008'. All inoculates and organ samples are sequenced to determine whether the virus alters its genome composition during an animal passage (WP5). Since macrophages are the primary target cell type of ASFV, interactions of these cells with representatives of di erent ASFV genotypes (gt I: `E70', gt II: `BEL18') were analyzed within WP4, as legs and ears are often discolored during ASF, explants of veins from ears and legs were utilized. Higher replication rates of gt I were observed in ear vein explants, whereas gt II showed increased replication in leg vein explants.

Keywords: African swine fever, Pathogenesis, Immune responses

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Channel-based biosensors to support improved bovine mastitis management

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Abstract

Bovine mastitis a ects animal health and welfare, reducing milk yields and quality. Therefore, prompt diagnosis at the early stages of disease when intervention can be most e ective is key. To that purpose, Biosens4PrecisionMastitis aims to deliver diagnostic tools to detect biomarkers of the early immune response of cows: miRNAs, cytokines, and antimicrobial peptides. The project was set to work with naturally infected cows to identify bovine mastitis biomarkers in milk initially. Representative samples from the herd of 35 Holstein-Friesian dairy cows were collected in four rounds. Based on SCC values, ve healthy cows with clinical mastitis and ve with subclinical mastitis were identi ed to collect their milk on consecutive days (from day three to 14). Samples were initially analyzed by microbial culturing to determine the total number of bacteria and quantify the most common microorganisms causing mastitis (i.e., S. agalactiae, S. uberis, S. aureus, etc). Results did not re ect the infection's severity but could be used to guide the antibiotic therapy of cows with mastitis at an advanced stage. The total RNA isolated from the selected milk samples was sequenced, providing unique miRNA patterns. Speci c miRNAs showing increased expression levels in the clinical and subclinical samples were selected as potential biomarkers, some agreeing with previously identi ed miRNAs for bovine mastitis (e.g., miRNA221, miRNA223, miRNA146b, miRNA29a, miRNA29c), while others were identi ed for the rst time (e.g., miRNA146a, miRNA142-5p, miRNA15a, miRNA-155, miRNA let-7i). In addition, milk smears of 30 cows (10 healthy, 10 with subclinical mastitis, 10 with clinical mastitis) were prepared and immunohistochemically stained with 16 factors (Hdef2, Hdef 3, IL-10, IFNg, IL-2, LL-37, IL-1a, IL-4, IL-6, IL-12, IL-13, IL-17A, TNFα, TGFβ1, NFκB, IL-8) at six di erent days. Milk of healthy cows showed high numbers of immunoreactive cells, indicating the readiness of host immunity under continuous pathogen exposure. Consequently, it was con rmed that local cellular and humoral immune responses play a role in defense responses. Data from mastitic cows showed that in ammatory responses to intramammary infection were driven by IL-1α, IL-4, IL-12, IL-17A, and IFN-γ in subclinical mastitis and by IL-1α, IL-4, IL-6, and IL-17A in clinical mastitis. Two factors were identi ed as potential biomarkers of bovine mastitis: IL-10, which provides anti-in ammatory defense, and β-defensin 3, with microbicidal activity. In parallel, novel sensing platforms were developed to Provide the highest sensitivity, selectivity, and accuracy for detecting the previously identi ed biomarkers. A porous silicon nanostructured electrochemical transducer prepared by the polymerization and carbonization of furfuryl alcohol was fabricated and characterized, showing outstanding electrochemical performance with fast electron transfer kinetics, large e ective surface area with low background current, wide potential window, and high reproducibility and stability. To demonstrate its biosensing capabilities towards detecting miRNAs, miRNA223 was initially used as a target. Thorough optimization (e.g., pore size, capture probe concentration, incubation time) was performed, delivering a biosensor to detect low pM concentrations of miRNA223 in diluted milk accurately. Current work focuses on demonstrating the on-farm performance of a multiplexed biosensor.

Keywords: Bovine mastitis, Biomarkers, Management

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Assessing swine as potential hosts for emerging Coronaviruses

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Abstract

Spillover of Coronaviruses (CoVs) from wildlife might cause severe human diseases. In this context, the industrialization of farming plays a paramount role as it translates into an increased likelihood of viral ampli cation in bridging hosts, meaning wider opportunities for human infections. Emerging CoVs are also a major threat to swine production worldwide. Among swine emerging CoVs, Swine Acute Diarrhoea Syndrome Coronavirus (SADS-CoV) and Porcine Epidemic Diarrhoea virus (PEDV) show phylogenetic relatedness with bat viruses, suggesting these hosts as major source for novel coronaviruses. In particular, the bat species Rhinolophus sinicus is considered the proximate source for SADS-CoV, which shares 95% identity with batCoV HKU2 over the complete genome. Similarly, partial sequences showing 92.6% identity with PEDV have been found in bats of the genus Murina, also clustering with European Myotis bats. Indeed, bats carry the highest diversity of CoVs among mammals, and therefore, they are speculated to be the evolutionary source of most Covs of the genera Alpha- and Beta-coronavirus, including several human CoVs. ConVErgence project aims to investigate swine's role as potential hosts for emerging CoVs. It initially stemmed from data from a small subset of Italian swine farms, indicating a wide bat biodiversity around the farms, with Pipistrellus kuhlii the most represented species. Preliminary investigations showed the undetected circulation of CoVs in pigs and evidence for viral exchange from pigs to bats. ConVErgence novelty stands on the methodologies applied. Indeed, ConVErgence exploited cutting-edge technologies from di erent elds, including veterinary medicine, ecology, virology, epidemiology, and mathematical modeling. Within the ConVErgence framework, we enlarged the study area, including 18 swine farms in Northern Italy. Samples were collected to rule out a 5% prevalence of CoVs within the population. We used a checklist to describe the main practices related to biosafety and to identify possible contacts with humans. We combined bioacoustics and visual inspections to investigate bat activity within farms and possible interface with pigs. Longitudinal samplings were performed both in farms and in P. kuhlii colonies. Molecular analyses supported the endemic circulation of two swine CoVs but showed no evidence for the circulation of CoVs of bat or human origins. Further molecular and phenotypic characterizations are currently ongoing. Serological investigations and the assessment of swine susceptibility to selected human and bat CoVs are currently in progress. To date, we found no evidence of pig exposure to human-origin CoVs. First, mathematical models were built to describe the intra-year dynamics of viruses in bats, preliminary tted to available data on lyssaviruses. ConVErgence also developed a general framework for infectious disease circulation (non-pathogen speci c) within pig farms. A mathematical model to investigate the transmission/persistence of CoVs in swine will eventually be adjusted to eld data.

Keywords: Coronaviruses, Swine, Wildlife, Emergency

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Analysis of the health status of Belgian 'healthy' pig farms using an integrated, high-tech approach

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Abstract

Pig disease results from complex interactions between infections, environment, and management. To fully understand the conditions that lead to clinical signs, it is important to use highly sensitive technologies that detect pathogens (third-generation sequencing; PathoSense), sense environmental conditions (Healthy Climate Monitor), and determine the biosecurity (Biocheck.UGent digital platform). The present study examined whether `healthy' farms are healthy or if hidden problems exist. Three sows in the same farrowing room were selected within each 5 `healthy' farms. Two days post-partum, three viable piglets of each sow were selected and ear tagged. The pigs were sampled weekly (nasal, tracheobronchial, and fecal swabs) until 12 weeks of age. Samples were transported at 4°C, pooled, and analyzed by a new metagenomics platform (PathoSense b.v.) for identifying all viruses and bacteria based on third-generation sequencing. A Healthy Climate Monitor was used to gather environmental data (T°, relative humidity, [NH3], [CO2], and [dust particles with diameters of 1 µm, 2.5 µm, and 10µm]). Biosecurity levels were assessed through the Biocheck.UGent questionnaire. Sneezing, coughing, and diarrhea were observed on 3, 3, and 5 of the ve farms, respectively. During the observation period, 4 to 7 viral respiratory infections (porcine in uenza virus A (5/5), porcine cytomegalovirus (5/5), porcine hemagglutinating encephalomyelitis virus (5/5), porcine reproductive and respiratory syndrome virus (4/5), porcine parain uenza virus 1 (4/5), porcine respiratory coronavirus (3/5)) and 6 to 7 viral enteric infections (rotavirus A, B, C, and H (5/5), sapovirus (4/5), kobuvirus (3/5), sapelovirus (2/5), teschovirus (2/5), rotavirus F (1/5)) occurred. In addition, several bacterial co-infections occurred. Compared to the reference values, ambient temperature was too high in the farrowing units of farms 2 and 3 and the fattening unit of farm 3. Relative humidity was lower in the farrowing units of farms 3 and 4. Carbon dioxide levels were higher in the nursery and nishing units of farm 2. Ammonia levels were higher in the fattening unit of farm 3. Dust concentrations were elevated (i) for 1µm particles in the farrowing and nishing units of farm 3, (ii) for 2.5µm particles in the farrowing units of farms 1 through 4 and in the nishing units of farms 2 and 3, and (iii) for 10µm particles in the farrowing and nishing units of farms 2 and 3. Overall biosecurity scores were lower on farms 1 (63/100) and 3 (74/100), while the scores of farms 2 (83/100), 4 (81/100), and 5 (88/100) were higher compared to the benchmark (75/100). In conclusion, it became clear that many viral and bacterial (co)infections occurred on so-called `healthy' farms, which mostly do not result in disease outbreaks. The clinical signs that were sometimes observed could be related to some (co)infections, environmental deviations, and biosecurity problems. Therefore, more e orts should be made on `healthy' farms to reduce the number of infections and to improve environmental conditions and biosecurity. This work forms an ideal reference for future analysis on farms with clear respiratory and enteric problems.

Keywords:Pig farms, Biosecurity, High-tech approach

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Novel strategies to enhance vaccine immunity in neonatal livestock

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Abstract

The NEOVACC project addresses the signi cant challenge of e ective vaccination of neonatal animals in the presence of maternally derived antibodies (MDA). This is critically important since MDA inhibits immune responses of neonates to vaccination and as MDA wanes immunised animals remain vulnerable to pathogen challenge. The NEOVACC project is evaluating novel vaccine strategies designed to enhance immune responses in neonatal animals with MDA. We are focussed on bovine respiratory syncytial virus (BRSV) and porcine reproductive and respiratory syndrome virus (PRRSV), as prime examples of endemic livestock diseases that require next-generation vaccines to improve control. We are exploring a state-of-the-art structural vaccinology approach to design novel immunogens based on the BRSV pre-fusion (preF) protein for neonatal calves. We are determining whether discrete di erences exist between maternal and calf antibody binding to epitopes on BRSV preF. We will then design and select BRSV preF epitope sca olds which would not be recognised by MDA and evaluate their vaccine potential. The second approach aims to exploit DNA vaccination to overcome MDA interference. We are evaluating novel DNA-based vaccines encoding well-de ned PRRSV antigens fused to moieties that target antigen to professional antigen-presenting cells and assessing their ability to prime immune responses in piglets with MDA and augment a subsequent modi ed live vaccine (MLV) boost. We are conducting a parallel in vitro investigation to better understand how MDA interfere with PRRSV vaccination. Finally, we are evaluating the potential of peptide-based immune checkpoint inhibitors (ICIs) to adjuvant responses to PRRSV MLV. By transiently blocking negative immunoregulation, we hypothesise that PRRSV MLV genetically engineered to express ICIs would allow neonatal animals to mount more robust immune responses. In addition to developing novel BRSV and PRRSV vaccine candidates, the approaches employed to design vaccine antigens, to deliver DNA encoded antigens in a targeted manner to augment B cell responses, and to potentiate responses through immune checkpoint inhibition may be more broadly applied, including to human clinical settings.

Keywords: Vaccines, Neonatal immunisation, Maternal antibody, Bovine respiratory syncytial virus, Porcine reproductive and respiratory syndrome virus

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Understanding the dynamics and evolution of swine influenza viruses in Europe: relevance for improved intervention in enzootically infected pig herds

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Abstract

In recent years, swine in uenza A virus (swIAV) infections have changed from epizootic to enzootic patterns in many industrial European pig herds. These self-sustaining forms of swine in uenza adversely a ect animal welfare and production economics, implying increased public health risks. The conditions sustaining recurrent swIAV infections, by in uencing swIAV transmission and spread, may depend on factors such as production systems, biosecurity level, housing conditions, co-infections, vaccination protocols, vaccine strain composition, and pre-existing herd immunity, but many of them are poorly understood. Thus, an urgent need is to increase knowledge of within-herd virus dynamics and evolution to design intervention and prevention measures to limit swIAV persistence in intensive herds and counteract impaired animal welfare, production losses, and the emergence of new swIAVs. As part of the PIGIE project, the within-herd swIAV infection dynamics have been compared in six European countries. Herds with continuous swIAV circulations were identi ed in Denmark, France, Germany, Italy, Spain, and the United Kingdom. In each country, longitudinal studies were completed in two herds, including two cohorts of sows and piglets per herd, according to a standard sampling frame. In addition, data on herd management and biosecurity activities were collected. Circulating swIAV was detected in the 24 monitored batches. The incidence in each age group varied between herds, batches, and countries. Viral RNA was detected in nasal swabs for more than 50% of the piglets sampled on at least one of three occasions between 3 and 10 weeks of age in each country. Prolonged shedding or re-infection was detected in some animals. Several swIAV lineages were identi ed in each country. They included H1avN2 (HA-1C) in ve countries, H1avN1 (HA-1C) in three countries, H1N1pdm (HA-1A) in ve countries, and H1huN2 (HA-1B) in three countries. Successive or mixed infections with di erent lineages were observed in 7/12 herds in four countries. High proportions of sows (vaccinated or not) were swIAV seropositive one week after farrowing in all farms. At that time, more than 50% of piglets were also found seropositive in 75% of batches, having acquired maternally derived antibodies (MDA). However, subsequent infections of MDA-positive piglets led to di erent serological pro les within the study period. Thus, while the decay of MDAs was observed in most herds with a signi cant decrease after weaning, it was observed that herds experiencing swIAV infections late in the nursery maintained high antibody levels. This was the rst coordinated longitudinal study on swIAV dynamics performed in parallel in several European countries, facilitating comparisons. The results emphasized that swIAV is highly prevalent, and strain subtyping further underlined the complexity of the swIAV dynamics, pathogenesis, and immunological correlates of protection. All data have been compiled to identify potential farm-speci c intervention measures. When possible, they have been implemented, and their e ciency in controlling swIAV infection will be evaluated in a second round of longitudinal studies.

Keywords: Dynamics, Evolution, Swine in uenza viruses, Europe, Pig herds

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Deciphering the role of Influenza D virus in bovine and human respiratory diseases in Europe

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Abstract

Recent studies in the USA, Asia and our preliminary work in Europe have identified a new genus of the *Orthomyxoviridae* family named Influenza D virus (IDV). This novel virus was shown to infect farm animals, including swine and cattle, and to replicate and transmit in ferrets efficiently, the animal model of choice for transmission of many zoonotic pathogens, including influenza A virus (IAV), to humans. Our objective is to develop an integrated approach to not only assess the emergence threat associated with influenza D viruses circulating in Europe but also the role played by the virus in cattle respiratory disease complex and the risk it may play for humans. By promoting the transfer and exchange of knowledge and expertise between the partners, we will pave the way towards scientific-based decision-making and developing effective strategies for cattle respiratory disease control and risk assessment for Influenza D virus infections in Humans. The first question relates to the role of IDV among respiratory pathogens of cattle and humans. A first work package will therefore be to survey IDV occurrence and prevalence in the two species in Europe and collect field data (samples for respiratory pathogens detection, bioaerosols, cloths from farm premises, but also questionnaires on biosecurity and mitigation measures) to understand IDV's place within its pathogens counterparts. In field samples collected at a given time, it will be impossible to understand the sequence of infection (which pathogen is more likely to infect first/second) nor whether the co-circulating pathogens act in synergy or antagonism in the host. Therefore, a second work package will enable answering more mechanistic questions using *in-vitro* and *ex-vivo* culture methods to understand the field situation better. All parts of the projects will support the models for risk assessment (third work package) with clear benefits for animals (cattle) and public health. To estimate the IDV human risk exposure through aerosols in cattle farms at risk (viral circulation), a quantitative risk assessment modeling will be performed and refined using field (WP1) and experimental (WP2) data. Based on prospective scenario analysis, the previous modeling will evaluate the effect of medical (vaccination) and sanitary (biosecurity) mitigation measures. This project addresses the need for capacity building at the EU level to improve the EU's scientific assessment capacity and international competitiveness. We will achieve this goal by promoting cross-disciplinary cooperation between the partner institutes representing four European countries and a non-EU member State. The project will allow the sharing of knowledge, skills, competencies, and expertise in the field, enabling capacity building within Europe. The project's output will enhance European cooperation and generate a sustainable network necessary for detecting, preventing, and responding to an emerging animal disease that could threaten not only animal health and welfare but also European food production and, directly or indirectly, human health.

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RodentGate: future rodent management for pig and poultry health

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Abstract

Apart from consuming and spoiling animal feed and damaging infrastructure in and around farm buildings, rodents are a considerable threat to animal health and One Health. They can cause direct stress to pigs and poultry but are mainly important as carriers of pathogens. These include economically signi cant diseases like Swine dysentery, Aujeszky's Disease, PCV2, and Encephalomyocarditis. They can act as a primary or secondary host for diseases such as in uenza A and avian in uenza or as a mechanical reservoir for diseases like African swine fever and play a role in the epidemiology of leptospirosis and salmonellosis and the spreading of antibiotic-resistant bacterial strains. They can pick up the infection from infected livestock, spread it within and between farms, and act as a bridge between wild fauna and livestock. Therefore, they can maintain an infection locally after a farm is emptied and decontaminated after a disease outbreak or livestock turnover. Thus, there are very good reasons for rodent management on pig and poultry farms. Current rodent control is mainly done by using rodenticides. However, concerns about the environmental safety of the most common rodenticides and resistance against them have led to changes in the European and national regulations aimed to restrict their use, which poses new challenges for e cient rodent management on farms. This project, RODENTGATE, will investigate the rodent-related risks for animal health in the pig and poultry industry and how this might change with altered rodent control. Ecologically based rodent management is a strategy that combines an Integrated Pest Management approach with a thorough knowledge of rodent ecology, enabling interventions to be precisely targeted in time and space while being ecologically and economically sustainable. This requires a thorough understanding of rodent demography, space use, dispersal capacities, and appropriate documentation of the rodent population's pathogen presence and transmission patterns. Understanding the transmission mechanisms is crucial since killing hosts may have unexpected e ects on spreading an infection. RodentGate's objectives are (1) to document changes in disease risk for pigs and poultry when classical rodent management is prevented, leading to changes in the rodent population, and 2) to propose appropriate economically sustainable, evidence-based strategies for the ecologically based management of rodents and rodent-borne infections around farms. To achieve this, we will (i) document the current status, prevalence, and diversity of rodent-borne pathogens in livestock and wild rodents in and around pig and poultry farms in di erent parts of the EU and determine the role of rodents in spreading pathogens between farms. (ii) Investigate how pig and poultry health is a ected by changes in population composition and abundance following a ban on rodenticides and how the e ciency of rodent management practices on farms can be maintained under restricted or no-rodenticide situations. These questions will be addressed by a multidisciplinary consortium of scientists from Belgium, the UK, Germany, The Netherlands, and Poland, using a combination of analysis of existing data, sampling rodents, environment, and livestock on farms, molecular diagnosis of pathogens, eld work on rodent population biology and movements, ecological modeling, control strategy development and communication with the pig and poultry industry and pest control industry.

Keywords: Rodent, Management, Swine health, Poultry health

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