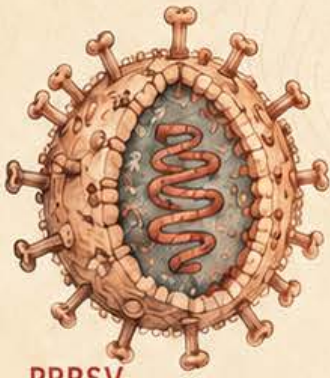




PRRSV

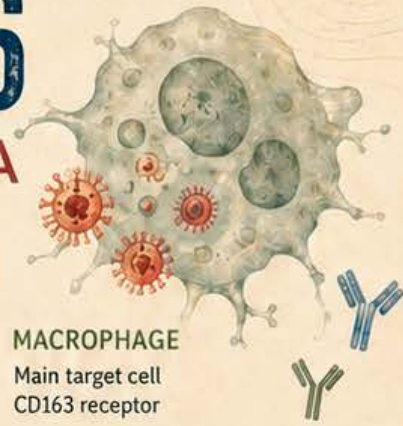
Porcine Reproductive
and Respiratory
Syndrome Virus



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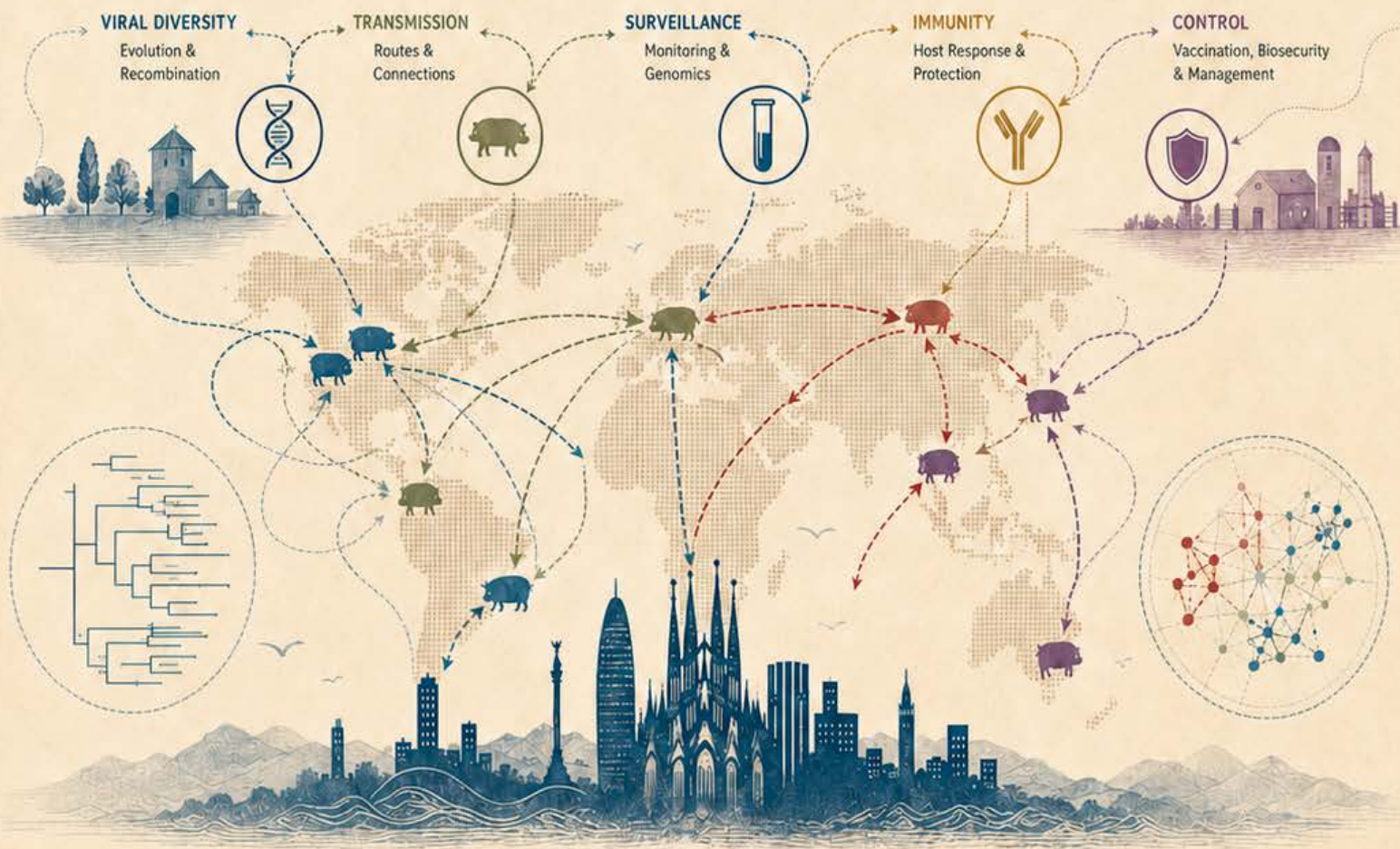
From genomes to global control
of viral diseases of swine



MACROPHAGE
Main target cell
CD163 receptor

IMMUNE RESPONSE
Neutralizing
antibodies

PRRSV GENOME (~15 kb)



ABSTRACT BOOK

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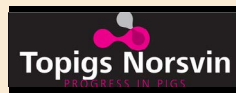
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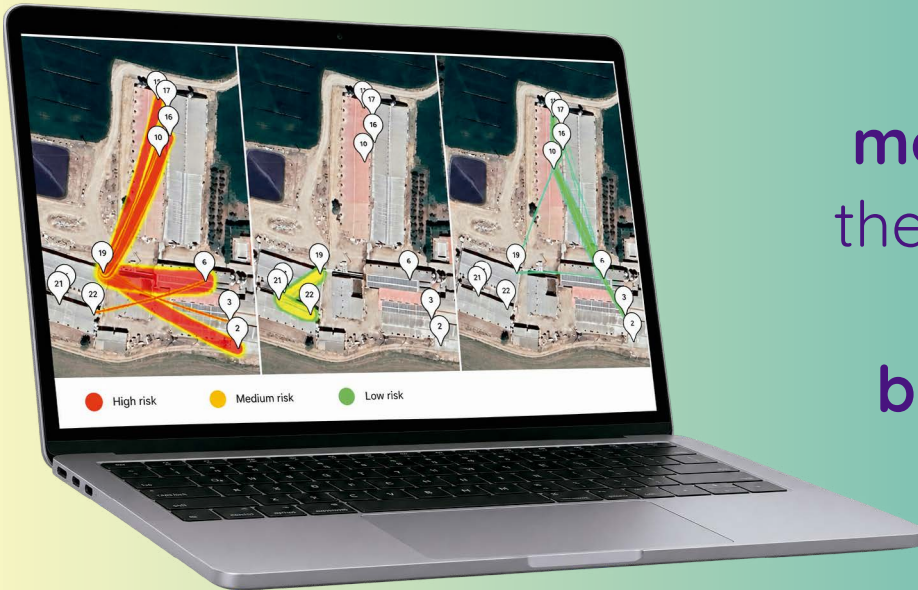


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FOREWORD

WELCOME TO IPRRS 2026

Benvinguts a l'IPRRS 2026 a Barcelona.

És un privilegi reunir una comunitat internacional compromesa amb la recerca, el maneig, la prevenció i el control del PRRS i d'altres malalties víriques del porc. Investigadors, veterinaris, professionals del sector i responsables sanitaris compartim el repte de transformar el coneixement científic en solucions que millorin la sanitat, el benestar i la sostenibilitat de la producció porcina.

Esperem que aquest simposi sigui un espai per compartir resultats i experiències, debatre noves idees, establir col·laboracions i impulsar avenços davant dels reptes presents i futurs de la virologia porcina.

Us desitgem un congrés estimulant i profitós, així com una estada memorable a Barcelona.

Bienvenidos al IPRRS 2026 en Barcelona.

Es un privilegio reunir a una comunidad internacional comprometida con la investigación, el manejo, la prevención y el control del PRRS y de otras enfermedades víricas del cerdo. Investigadores, veterinarios, profesionales del sector y responsables sanitarios compartimos el reto de transformar el conocimiento científico en soluciones que mejoren la sanidad, el bienestar y la sostenibilidad de la producción porcina.

Esperamos que este simposio sea un espacio para compartir resultados y experiencias, debatir nuevas ideas, establecer colaboraciones e impulsar avances frente a los retos presentes y futuros de la virología porcina.

Les deseamos un congreso estimulante y provechoso, así como una estancia inolvidable en Barcelona.

Welcome to IPRRS 2026 in Barcelona.

It is a privilege to bring together an international community committed to the research, management, prevention and control of PRRS and other viral diseases of swine. Researchers, veterinarians, industry professionals and animal health authorities share the challenge of translating scientific knowledge into solutions that improve the health, welfare and sustainability of pig production.

We hope this symposium will provide a forum for sharing results and experiences, discussing new ideas, building collaborations and driving progress in addressing the present and future challenges of swine virology.

We wish you a stimulating and rewarding symposium, as well as a memorable stay in Barcelona.

The fight against **PRRS** goes on

**AS DOES OUR
COMMITMENT**

More than 10 years
facing PRRS
together



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PROGRAM

TUESDAY 6 October

09:00 Swine Health Forum (in Spanish)

11:00 Registration

16:00 Welcome and opening remarks

KEYNOTE LECTURES

[View abstract](#) **16:15** The porcine reproductive and respiratory syndrome virus (PRRSV) saga: using macrophages as targets is ingenious but challenging for a virus with an evolutionary plan.
Prof. Hans Nauwynck · *Ghent University*

[View abstract](#) **17:00** Veterinary and public health challenges of swine influenza viruses.
Prof. Lars E. Larsen · *University of Copenhagen*

17:45 Welcome reception

WEDNESDAY 7 October · MORNING

INVITED LECTURES

[View abstract](#) **08:30** The evolutionary dynamics behind epidemic waves of PRRSV.
Prof. Kimberly VanderWaal · *University of Minnesota*

[View abstract](#) **09:00** PRRS epidemiology in practice.
Prof. Giovanni Trevisan · *Iowa State University*

ORAL COMMUNICATIONS

[View abstract](#) **09:30** Whole-genome classification system for porcine reproductive and respiratory syndrome virus type 2 (Betaarterivirus americanus) strains combining ORF5, nsp3-4-5 and indels.
Vicent Baby · *Université de Montréal*

[View abstract](#) **09:45** Known enemy or new threat? A tussle between introduction, persistence and recombination of PRRSV strains on pig farms.
Harsh Bardhan Gupta · *Utrecht University*

[View abstract](#) **10:00** Assessment of the mutation, selection, substitution and recombination patterns and dynamics of 'classical' versus 'Rosalía and Rosalía-like' Spanish PRRSV-1 strains.
Martí Cortey · *Universitat Autònoma de Barcelona*

[View abstract](#) **10:15** An artificial intelligence (AI) agent-based support system for porcine reproductive and respiratory syndrome (PRRS) health management in Colombia.
Francisco Gómez · *Universidad Nacional de Colombia*

10:30 COFFEE BREAK

INVITED LECTURES

[View abstract](#) **11:00** Molecular mechanisms of PRRSV persistence: challenges for elimination.
Prof. Ying Fang · *University of Illinois at Urbana-Champaign*

[View abstract](#) **11:30** Genome editing for disease resistance in pigs.
Dr. Christine Tait-Burkard · *The Roslin Institute, University of Edinburgh*

ORAL COMMUNICATIONS

[View abstract](#) **12:00** A new reverse-genetics system for PRRSV identifies a GP3 trafficking signal that promotes viral replication.
Dongwan Yoo · *University of Illinois Urbana-Champaign*



PROGRAM

- [View abstract](#)
- 12:15** Porcine reproductive and respiratory syndrome virus nsp1α degrades ribosomal components to inhibit cellular translation.
Qiongqiong Zhou · *China Agricultural University*
- [View abstract](#)
- 12:30** CD169 blockade as an *in vitro* model to discriminate PRRSV-1 isolates with different virulence profiles.
Francisco Javier Martínez-Lobo · *Universitat de Lleida*
- [View abstract](#)
- 12:45** Highly virulent PRRSV-1 Rosalía strain induces enhanced CTLA-4 expression and immune dysregulation in piglets during the early and late stages of infection.
Jaime Gómez-Laguna · *Universidad de Córdoba*

13:00 LUNCH

WEDNESDAY 7 October · AFTERNOON

INVITED LECTURES

- [View abstract](#)
- 14:00** Immunopathogenesis of the reproductive form of PRRS.
Prof. Andrea Ladinig · *University of Veterinary Medicine Vienna*
- [View abstract](#)
- 14:30** Emerging vaccine platforms for swine viral diseases.
Prof. Xiang-Jin Meng · *Virginia Tech*

ORAL COMMUNICATIONS

- [View abstract](#)
- 15:00** Towards an improved understanding of adaptive immune dysregulation during arterivirus infection.
Jane Edwards · *The Pirbright Institute*
- [View abstract](#)
- 15:15** Systems vaccinology reveals mechanistically distinct immune profiles of interferon-augmented PRRSV vaccines.
Laura Miller · *Kansas State University*
- [View abstract](#)
- 15:30** Generation of PRRSV epitope–MHC class I tetramers to investigate CD8 T cells at the maternal–foetal interface in infected sows.
Mahsa Rafieiyan · *The Pirbright Institute*
- [View abstract](#)
- 15:45** Mucosal induction of trained immunity reduces susceptibility to PRRSV infection in pigs.
Aida Tort Miró · *IRTA-CReSA*
- [View abstract](#)
- 16:00** PRRSV-anergic status in vaccinated sows: impact on vaccine efficacy and piglet challenge outcomes.
Marylène Tignon · *Sciensano*
- [View abstract](#)
- 16:15** Isolation and characterisation of naturally occurring PRRSV-neutralising monoclonal antibodies to support structural vaccinology.
Simon Graham · *The Pirbright Institute*

FLASH TALKS

- [View abstract](#)
- 16:30** Evaluating the economic impact of early interventions against PRRSV using multivariate syndromic surveillance in Spanish swine breeding herds.
Mafalda Pedro Mil-Homens · *Universitat Autònoma de Barcelona*
- [View abstract](#)
- 16:35** Estimation of PRRS hazard and vulnerability through a health risk index based on data analytics.
Fausto Moreno · *Universidad Nacional de Colombia*
- [View abstract](#)
- 16:40** Prevalence and epidemiological role of porcine respiratory coronavirus, porcine haemagglutinating encephalomyelitis virus and influenza D virus in Spanish herds.
Menjun Wang · *Universitat Autònoma de Barcelona*
- [View abstract](#)
- 16:45** Comparison of cell systems for isolation and replication assessment of porcine parainfluenza virus 1.
Ge Ye · *Universitat Autònoma de Barcelona*

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PROGRAM

- [View abstract](#)
- 16:50** **A novel swine trophoblast organoid system to investigate PRRSV–placenta interactions.**
Abigail Williams · *North Carolina State University*
- [View abstract](#)
- 16:55** **Impact of formalin fixation time on PRRSV-1 detection by RNAscope® in situ hybridisation combined with deep-learning-based digital pathology.**
Giuditta Capaldi · *Universidad de Córdoba*
- 17:00** **European PRRS Research Awards**
- 20:00** **GALA DINNER**

THURSDAY 8 October · MORNING

INVITED LECTURES

- [View abstract](#)
- 08:30** **Overview of viral swine diseases worldwide.**
Dr. Gregorio Torres · *Head of the Science Department, World Organisation for Animal Health*
- [View abstract](#)
- 09:00** **Investigations into influenza A virus reassortment in commercial pigs.**
Prof. Montserrat Torremorell · *University of Minnesota*

ORAL COMMUNICATIONS

- [View abstract](#)
- 09:30** **Validation of genomic prediction for disease resilience traits and their genetic relationship with selection-index traits.**
Claudia Sevillano · *Topigs Norsvin*
- [View abstract](#)
- 09:45** **Enhancing biosecurity in swine operations: investigating wildlife interactions at mortality-management structures on swine farms.**
Rafael Medeiros de Avila Melo · *University of Minnesota*
- [View abstract](#)
- 10:00** **Road-level transmission risk of PRRS in the United States swine network: a spatiotemporal Bayesian framework linking animal movement to farm infection.**
Nicolás Cárdenas · *North Carolina State University*

FLASH TALKS

- [View abstract](#)
- 10:15** **A structural bioinformatics framework for precision host-factor engineering towards influenza-resistant pigs.**
Joaquín Gadea · *Universidad de Murcia*
- [View abstract](#)
- 10:20** **Monitoring PRRSV dynamics in different farm environments: evaluating diagnostic samples in farrowing and nursery units.**
Laura Junquera · *Universidade de Santiago de Compostela*
- [View abstract](#)
- 10:25** **Interlaboratory comparison of PRRSV genome and antibody detection across diagnostic laboratories in Central Europe**
Kristin Heenemann · *Leipzig University*

10:30 COFFEE BREAK

ROUND TABLE · INVITED SPEAKERS

- 11:00** **ROUND TABLE. Experiences in the control and eradication of PRRS.**
Moderator: Dr. Tijs Tobias · *Royal GD, Deventer*
- [View abstract](#)
- Danish PRRS reduction strategy: background, progress and lessons learned.**
Nicolai Rosager Weber · *Danish Agriculture & Food Council*
- [View abstract](#)
- PRRS control and eradication experiences in the USA.**
César Corzo · *University of Minnesota*
- [View abstract](#)
- Diverse PRRSV and its control and elimination strategies in China.**
Prof. Hanchun Yang · *China Agricultural University*

13:00 LUNCH

[CONTINUE >](#)



PROGRAM

THURSDAY 8 October · AFTERNOON

INVITED LECTURES

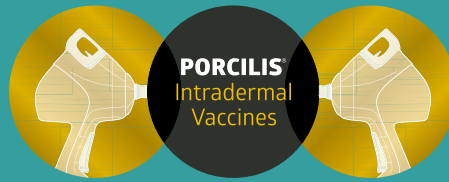
- [View abstract](#)
- 14:00** African swine fever and eradication in the European context.
Spanish Ministry of Agriculture, Fisheries and Food
- [View abstract](#)
- 14:30** Protective immunity associated with viral control in PRRS.
Prof. Jun Han · China Agricultural University
- [View abstract](#)
- 15:00** New PEDV epidemic patterns in China: virulent G2c variants and S-INDEL clade-2 recombinants and their implications for global PEDV evolution.
Prof. Yao-Wei Huang · South China Agricultural University

FLASH TALKS

- [View abstract](#)
- 15:30** A highly virulent porcine reproductive and respiratory syndrome virus strain exhibits macrophage and endothelial cell tropism in infected piglets.
Paula Curto · Universitat de Lleida
- [View abstract](#)
- 15:35** Comparison of infection by PAM- and MARC-145-passaged PRRSV isolates in PK15CD163+ and PK15Sialoadhesin+CD163+ cell lines.
Wenjing Su · Ghent University
- [View abstract](#)
- 15:40** Lipid nanoparticle encapsulation enhances DNA vaccine efficacy against highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b.
Hiep Vu · University of Nebraska–Lincoln
- [View abstract](#)
- 15:45** Local viral load shapes T-cell composition and differentiation at the maternal–foetal interface during PRRSV infection
Melissa Stas · University of Veterinary Medicine Vienna

ORAL COMMUNICATIONS

- [View abstract](#)
- 15:50** Impact of persistent infection with an attenuated African swine fever virus on viral population dynamics following subsequent inoculation with virulent strains.
Tomoya Kitamura · NIAH–NARO
- [View abstract](#)
- 16:05** Clinical, histomorphological and molecular profiling of swine enteric coronavirus infection: insights from microRNA and target-gene expression in piglets.
Carmen Álvarez Delgado · Universidad de Córdoba
- [View abstract](#)
- 16:20** Non-canonical TRS usage and functional analysis of the ORF6 region of a type I PRRSV strongly suggest translation of a novel essential protein, 6a.
Eszter Göttl · HUN-REN Veterinary Medical Research Institute
- [View abstract](#)
- 16:35** Evaluation of the dose-dependent virulence of PRRSV-1 ‘Rosalía’ in weaned piglets and the modulating role of maternal antibodies.
Carles Vilalta · IRTA-CReSA
- 16:50** IPRRS 2026 Awards
- 17:00** Farewell message



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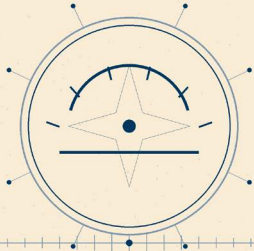
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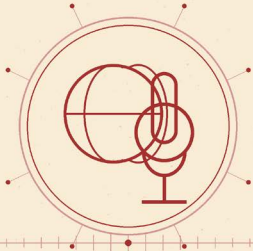
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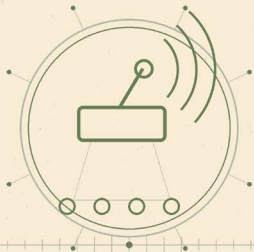
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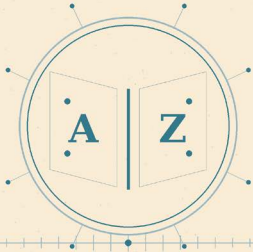
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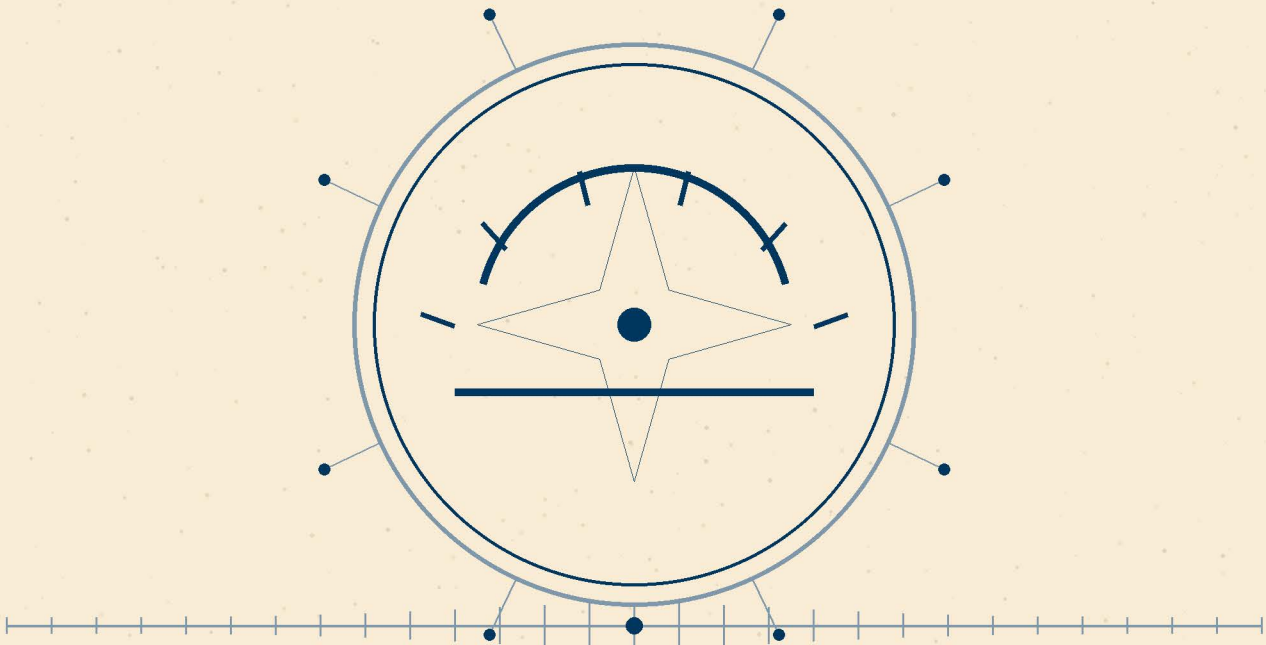
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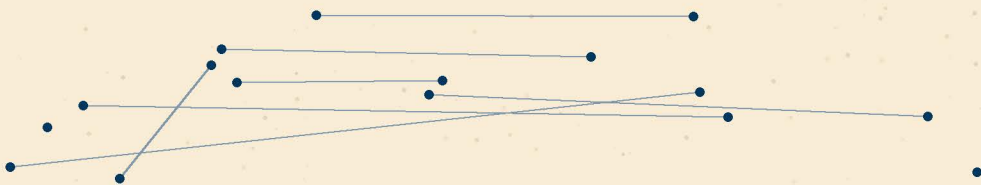
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OPENING LECTURES



Every piglet, a reason

Piglets made us
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KL.01

THE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) SAGA - USING MACROPHAGES AS TARGETS IS INGENIOUS BUT CHALLENGING FOR A VIRUS WITH AN EVOLUTIONARY PLAN

Hans Nauwynck

Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University

History

“Once upon a time, there was an arterivirus that was living happily together with wild pigs in pristine forests somewhere in Eurasia. It did not want to damage tissues during replication and make its host ill. Therefore, it replicated at low levels in a small population of macrophages with limited importance in its host. The infected cells died through apoptosis, inducing little inflammation and leaving all tissues intact. As this restricted replication was not ideal for transmission, the virus had chosen to persist by evading innate and adaptive immunity and to be transmitted in a very efficient way, via semen during intercourse (without destroying embryos) and via saliva during close contact. Everything went well until the virus decided to jump into the domestic pig. Then, the entire situation turned into an unintended biological disaster...”

This could be the beginning of one of the greatest fairy tales ever written in virology. Although uncertainties remain regarding geography and timing, this story may well reflect reality. Consequently, we refer to it as the PRRSV saga. One thing is certain: the virus has co-evolved with its host for a very long time. The story of the disease, porcine reproductive and respiratory syndrome (PRRS), began in the late eighties, beginning of the nineties. However, the virus was already present in pig populations before in northern America¹ and in Europe behind the former iron curtain². The virus and the clinical outcome of its infection only attracted attention once overt disease appeared, characterized by reproductive and respiratory problems. Because the viruses circulating on these two continents were genetically distinct (approximately 60% sequence homology), they came to be regarded as two separate viruses with their own evolutionary trajectories: PRRSV-1 (originating from Europe) and PRRSV-2 (originating from northern America). Their epidemiology differed markedly. From the outset, PRRSV-2 was a highly efficient spreader³. In contrast, PRRSV-1 spread much more slowly; it took an entire fattening period of approximately 3 months before all animals in a herd became infected (own observations). It therefore became clear that the two viruses possessed distinct biological characteristics.

Virus-macrophage interactions explain viral behavior

In the pig, PRRSV infects macrophages. Macrophages are highly diverse and consist of numerous subpopulations⁴. They originate from myeloid blast cells that are mainly located in the red bone marrow. Monocyte/macrophage homeostasis is finely regulated and involves exit from proliferation areas, entry in the blood circulation, and migration towards target tissues on call. These processes are coordinated by chemokines and adhesion molecules. The recruitment of new monocytes may occur under physiological or pathological conditions. Macrophages ultimately settle in specific tissues/organs and the local environment determines the direction of maturation. Like chameleons, macrophages adapt to their surroundings through interactions between cell surface molecules and tissue components. This gives rise to a highly plastic continuum of macrophage phenotypes, making it impossible to classify all macrophages into discrete categories. Many differentiation molecules are present on the macrophage surface and may participate in initiating the PRRSV replication cycle. Consequently, it is impossible to speak of a single PRRSV receptor (‘the’ receptor). Indeed, numerous molecules have already been shown to contribute to viral entry. When studying virus-macrophage interactions, it is important to use macrophage-grown virus. In general, PRRSV-1 does not grow in MARC cells⁵. Only after the selection of specific mutants, growth may become possible. Many PRRSV-1 isolates cannot be adapted, even after many attempts (e.g. Rosalia strain). For PRRSV-2, it is easier to grow PRRSV in MARC cells. However, half of the PRRSV-2 viruses also do not grow on MARC cells without adaptation/selection. Because PRRSV mutates easily during passages on MARC cells, results obtained using MARC-grown viruses should be interpreted with caution. Therefore, it is preferable to work with viruses originally propagated in macrophages. As with many viruses, the first interactions of PRRSV with host cells are mediated by sugars. The PRRSV M protein interacts with heparan sulfate moieties on the cell surface, whereas the sialic acids located at the termini of glycan chains on the GP5 ectodomain interact with sialic acid-binding proteins, such as Siglec-1 (=sialoadhesin), Siglec-10 and DC-SIGN^{6,7,8,9}. Considerable variation exists among PRRSV strains in their use of the different sialic-acid

[CONTINUE >](#)

binding proteins and it is highly likely that more sialic acid-binding proteins are involved but still need to be discovered. These binding receptors do not have internalization motifs in their cytoplasmic tail and therefore cannot internalize virus particles by themselves. Because PRRSV is intentionally inducing apoptosis at the end of the replication cycle, phosphatidylserine becomes incorporated into the viral envelope of newly formed particles which interacts with the TIM receptor that is present on the surface of most macrophages (and also on PK15 cells)¹⁰. There is still a lot of debate on the role of CD163 at the level of binding and internalization. CD163 alone is very inefficient in binding PRRSV compared to sialoadhesin¹¹. In recent experiments, we confirmed that PRRSV-1 is replicating 5 to 50 times better in PK15Sialoadhesin+CD163+ cells than in PK15CD163+ cells. Thus, CD163 by itself is very inefficient to bind and internalize the virus in the absence of sialic acid-binding molecules. More experiments are ongoing to see if the situation is the same for PRRSV-2 strains. Following entry, endosomal acidification and serine proteases are essential for coordinating the fusion process between GP2a/3/4 and CD163^{12,13}. What exactly occurs at this stage of the replication cycle remains unclear and warrants further investigation. Following release of the viral genome, viral protein expression begins approximately 6-9 hours post-inoculation¹⁴. During the early stages of infection (0-8 hpi), macrophages remain in an anti-apoptotic state; subsequently, the virus activates apoptosis. Viral egress begins between 9 and 12 hpi.

Survival of the fittest: Darwinian evolution driven by transmission efficiency

Following their emergence in densely populated pig-producing regions, PRRSV-1 and PRRSV-2 evolved extremely rapidly through mutation. This led to the appearance of fitter (more virulent) viruses that became increasingly pathogenic. For PRRSV-1, it was important to increase its array of macrophages that can be infected and to increase the virus yield. The original LV-like strains replicated poorly in nasal macrophages, explaining their slow spread between pigs. This rapidly changed resulting in improved transmission efficiency¹⁵. The use of nasal macrophages even increased progressively over time. In contrast, PRRSV-2 was already very fit in replicating in nasal macrophages in the early nineties, explaining some of the major differences between PRRSV-1 and PRRSV-2. However, PRRSV-2 replication in nasal macrophages also increased further over time.

The level of viremia reflects the level of PRRSV replication in tissues. The explanation is straightforward: as monocytes adhere to endothelial cells and initiate diapedesis, they scan the environment outside the blood vessel via a filopodium and rapidly differentiate, expressing different molecules (such as CD163 and Siglec-1) that may be used by PRRSV to infect the macrophage¹⁶. These differentiating macrophages become a target for the virus. Through evolutionary expansion of receptor usage, increasing numbers of intravascular endothelium-attached macrophages become infected in the blood circulation, resulting in higher viral titers in serum. Viruses with a restricted macrophage tropism (infects only a few macrophage subpopulations), replicate to a low level in the intravascular endothelium-attached macrophages, resulting in a low-level viremia. This was observed with the older PRRSV-1 LV-like strains (102-3 TCID₅₀/ml). Comparison of PRRSV-1 strains over time revealed progressively higher levels of viremia, most likely due to infection of an increasing number of macrophage subsets: 102-4 TCID₅₀/ml for 07V063 (Belgian isolate from 2007); 103-5 TCID₅₀/ml for Flanders 13 (Belgian isolate from 2013)¹⁷. The subtype 3 PRRSV-1 strain Lena is currently the most virulent PRRSV-1 strain that is described up till now. It replicates in a broad range of macrophage subsets (even macrophages in the nervous system), resulting in very high viremia levels (104-6 TCID₅₀/ml). This infection causes fever for 3-4 weeks, anorexia, respiratory, reproductive and neurological disorders. PRRSV-2 already replicated to higher levels in pigs than PRRSV-1 during the early years of the emergence of PRRS. High fever strains that appeared in 2006 in China are currently also present in the US. They are replicating to very high levels in different subsets of macrophages, resulting in a very high viremia. These infections give a severe disease, similar to that of Lena.

Immune evasion has been developed a long time before the first description of PRRS

Most immune evasion mechanisms were already present in the historical PRRSV strains LV (PRRSV-1) and VR2332 (PRRSV-2), reflecting extensive prior co-evolution with their host. In summary: (i) PRRSV poorly induces interferon¹⁸, (ii) PRRSV binding and entry by sialic acids on GP5 (interacting with sialic acid binding proteins) and phosphatidylserine (interacting with TIM receptors) cannot be blocked by antibodies, (iii) antibodies against the GP2a/3/4 fusion complex are neutralizing, but this complex readily escapes through random mutation¹⁹, (iv) PRRSV-infected cells escape from antibody-dependent complement- and cell-mediated cell lysis because infected macrophages do not express viral envelope protein complexes M/GP5 and GP2a/3/4 on their outer membrane owing to a retention mechanism²⁰, (v) PRRSV-infected macrophages are not lysed by conventional cytotoxic T-lymphocytes and NK cells²¹.



Conclusion

Clinical forms of PRRS were first recognized during the late eighties/early nineties. The PRRSV strains that were circulating at that time were already highly immune evasive as a consequence of prolonged co-evolution with their host. Subsequently, viral evolution focused increasingly on improving replication efficiency and accelerating transmission between pigs. Unfortunately, this evolutionary success came at a cost: the emergence of increasingly severe clinical disease. If we look into our crystal ball, we might expect the virus eventually to evolve towards a coexistence with its host while exerting a smaller impact on the physiology and well-being of the pig.

If this were a fairy tale, it would end with:

'PRRSV and pigs lived happily together ever after'.

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KL.02

SWINE INFLUENZA A IN THE CONTEXT OF THE H5N1 PANZOOTIC

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Influenza A viruses (IAVs) are characterized by extensive genetic diversity, broad host range, and a remarkable capacity for cross-species transmission. Based on the antigenic properties of their hemagglutinin (H) and neuraminidase (N) surface glycoproteins, influenza A viruses are classified into multiple subtypes that circulate in avian, mammalian, and human populations. Among domestic animals, swine occupies a unique ecological position, as they are susceptible to both avian- and mammalian-origin influenza viruses and may facilitate viral adaptation and reassortment. In the context of the ongoing H5N1 highly pathogenic avian influenza (HPAI) panzootic, renewed attention has been directed toward the role of pigs as potential intermediate hosts at the animal-human interface.

Swine influenza A virus (swIAV) remains endemic in pig populations worldwide, with epidemiology driven by continuous viral evolution, herd demographics, animal movement, and regional production practices. Multiple lineages and subtypes, including H1N1, H1N2, and H3N2, co-circulate in swine populations, resulting in substantial genetic and antigenic diversity. Frequent reassortment events contribute to the emergence of novel strains, complicating surveillance and control efforts. The introduction of human and avian-origin influenza viruses into swine populations, including incursions associated with the H5N1 panzootic, highlights the dynamic nature of influenza ecology and the importance of integrated monitoring systems.

From a veterinary perspective, swIAV infections are associated with acute respiratory disease, impaired productivity, and significant economic losses. Clinical severity is influenced by viral strain characteristics, host immunity, co-infections, and management factors. Effective diagnosis relies on a combination of clinical assessment, laboratory testing, and molecular surveillance to detect both endemic and emerging influenza strains. The increasing diversity of influenza viruses at the livestock-wildlife interface underscores the need for enhanced biosecurity and early detection strategies. Control of swine influenza requires a comprehensive approach integrating biosecurity, surveillance, herd management, and vaccination. Vaccination can reduce clinical disease, although the effectiveness on reduction of viral shedding may be limited due to antigenic drift, emergence of novel variants and the vaccine types used that induce ineffective mucosal responses.

Apart from being an important veterinary pathogen, swIAV constitute a significant zoonotic threat because pigs can serve as both reservoirs and mixing hosts for influenza viruses originating from humans, birds, and swine. Bidirectional transmission between humans and pigs is well documented, resulting in the establishment of human-derived lineages in swine populations and the occasional spillover of swine-adapted viruses into humans. Most human infections occur in individuals with direct exposure to pigs, including farmers, veterinarians, transport workers, and abattoir personnel; however, limited human-to-human transmission has been reported. The public health importance of swine influenza was highlighted by the emergence of the 2009 pandemic H1N1 virus, which contained gene segments derived from multiple swine influenza lineages and demonstrated the pandemic potential of reassortant viruses originating in pigs. In the context of the ongoing H5Nx highly pathogenic avian influenza panzootic, increasing concern has arisen regarding the introduction of avian-origin viruses into swine herds. Experimental studies indicate that pigs are susceptible, although variable, to certain H5N1 strains, creating opportunities for viral adaptation, reassortment with endemic swine influenza viruses, and the generation of novel genotypes with altered host range or transmissibility. While sustained circulation of HPAIVs in pigs has not been documented, the unprecedented geographic spread of H5N1 and its increasing incidence in numerous mammalian species have expanded opportunities for interspecies transmission at the livestock-wildlife-human interface. A One Health framework that connects animal, human, and environmental health sectors is essential to mitigate the risks posed by influenza A viruses and to strengthen preparedness for future zoonotic and pandemic threats. Consequently, systematic surveillance of swine populations, genomic characterization of emerging influenza viruses, and integration of veterinary and public health monitoring systems are critical components of pandemic preparedness within a One Health framework.



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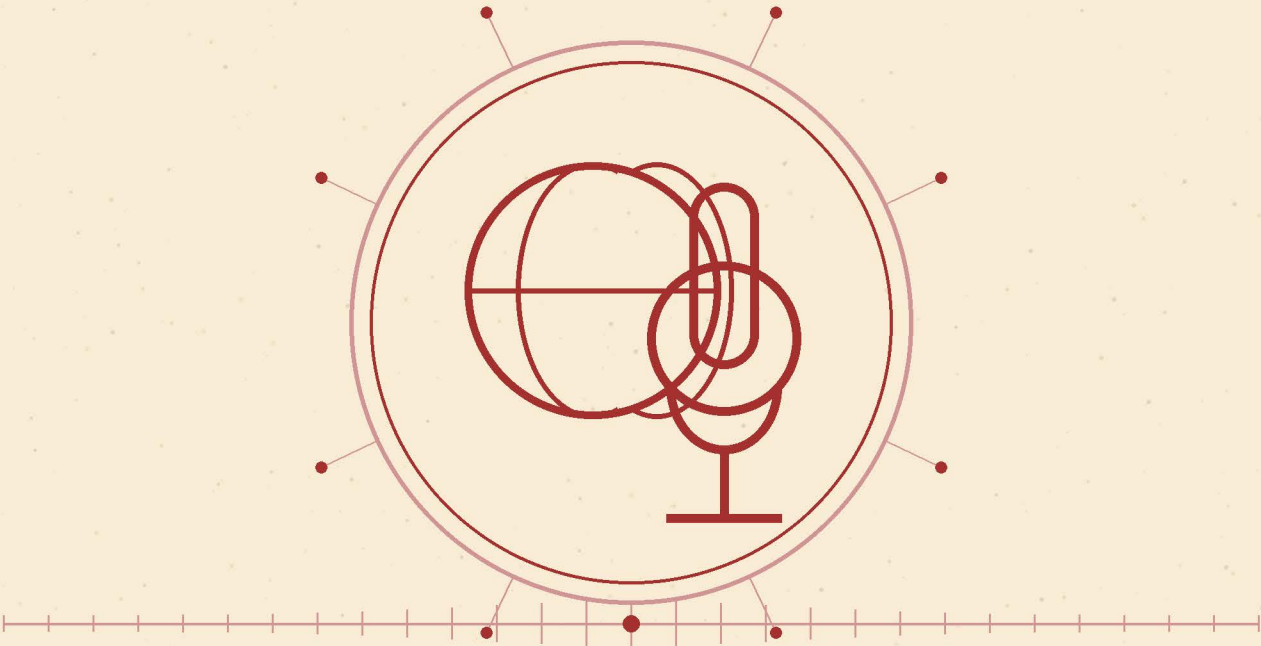
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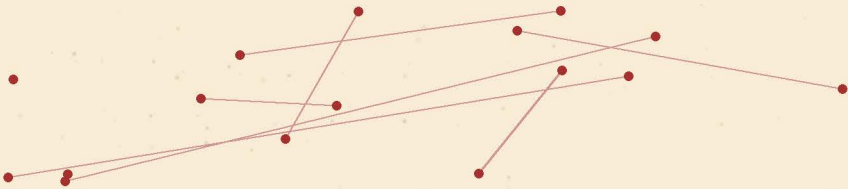
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IL.01

THE EVOLUTIONARY DYNAMICS BEHIND EPIDEMIC WAVES OF PRRSV-2

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Introduction

Over the past 25 years, we have seen the repeated emergence of novel genetic variants of PRRSV-2 in the U.S. and globally, including some that exhibit rapid epidemic-like spread that have been catastrophic for the industry. While such patterns are characteristic of rapidly evolving multi-strain RNA viruses such as PRRSV-2, the immense genetic diversity of the virus complicates control efforts. Here, we review the evolutionary dynamics of PRRSV-2 lineages and variants, focusing on immune-mediated selection pressures and whether PRRSV-2 evolutionary dynamics can be predicted.

Evolutionary dynamics of PRRSV-2 lineages and variants

PRRSV-2 genetic classification based on lineages (genetic distance <11%), sub-lineages (<8.5%), and variants (~2.5%) based on ORF5 phylogenetic relationships has been widely implemented in the U.S. and globally¹. Based on national-level sequence databases of >50,000 sequences, we can document that major emergence events of novel PRRSV-2 clades occur every few years., which are followed by a period of epidemic-like spread across the industry. The results in a new dominant sub-lineage or variant every three years extending back to the late 1990s, with turnover in the dominant clade likely related to immune-driven evolutionary dynamics².

Immune-mediated competition and selection

To better understand the antigenic significance of evolutionary change, we present new data on how genetic differences impacts cross-neutralization *in vitro*³ and clinical cross-protection in the field. While these studies concretely demonstrate the antigenic diversity of PRRSV-2, causal evidence of vaccine-induced evolutionary pressure remains limited. In a recent study⁴, our team tracked the evolution of PRRSV-2 from infection chains of sequentially infected pigs under different immune conditions. We showed that vaccinated groups exhibited over twice the genetic divergence from the original challenge virus (0.3%–0.4% mean nucleotide distance over 84 days) compared to the non-immunized group (0.15%), and deep sequencing revealed more rapid shifts of viral quasispecies composition in the vaccinated pigs. These findings demonstrate that MLV vaccination can exert substantial evolutionary pressure on PRRSV-2, driving genetic diversification and highlighting the need for continuous PRRS monitoring and adaptive control strategies.

Predicting PRRSV-2 evolution

PRRSV-2 continues to rapidly evolve, and new genetic variants of the virus are constantly emerging. Evolution creates winners and losers, and more proactive control requires the ability to anticipate which variants will have high fitness. Our team has developed and continues to update a machine learning model that predicts the future fitness of each circulating variant, aiming to provide early indicators of which variants are most likely to show accelerating incidence and expanding geographic range⁵. This creates the opportunity for targeted control measures of high-consequence genetic variants, and forms the basis for enhanced epidemiological reports produced by our team and distributed to the industry on a monthly basis. Altogether, this system allows for near real-time monitoring of PRRSV variant emergence and improved situational awareness, enhancing disease surveillance, preparedness, and communication.

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IL.02

PRRS EPIDEMIOLOGY IN PRACTICE

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the costliest disease for U.S. swine production, causing losses of over \$1 billion per year. Both PRRS virus (PRRSV) species, PRRSV-1 and PRRSV-2, circulate in the U.S., and a producer-lead initiative named “National Swine Health Strategy” is requesting work towards PRRSV elimination. The broad distribution of PRRSV across the country and the emergence of more aggressive strains have required further development and implementation of tools and programs to generate information ranging from macroepidemiological detection to outbreak-level assessments of the status quo of PRRSV and its associated PRRS disease implications. Such programs and tools generate information that can be used in the decision-making process to design and implement interventions to contain further spread of the virus or improve the animal health of the affected population. A brief description of epidemiological programs housed at Iowa State University is provided.

Macroepidemiological level of detections

At the national level, PRRS consistently ranks among the most frequently diagnosed diseases. The Swine Disease Reporting System (SDRS, <https://fieldepi.org/sdrs/>) brings together a national swine-centric laboratory network, from various U.S. institutions, for real-time monitoring of data on the detection and diagnosis of endemic diseases. SDRS has become a fundamental pillar for the rapid identification of disease activity using anonymized diagnostic data and for transferring information to decision-makers within production systems, enabling them to respond to emerging or re-emerging diseases. Anonymized data contain no information identifying the farm, producer, production system, or veterinarian. Once aggregated, the information is presented to the public via monthly PDF, audio, and video reports, and daily updated online dashboards. Diagnostic data, combined with expert insights from a group comprising veterinarians and producers, are used to compile monthly reports on endemic disease activity. The resulting information serves as a starting point for future investigations into factors associated with emerging or re-emerging diseases. Key PRRSV megatrend learnings from aggregated diagnostic data include:

- a) Cyclic pattern of detection with higher PRRSV occurring during colder months, with year-over-year increased detection since 2014;
- b) PRRSV detection in the wean-to-market age category precedes the increase in the detection of PRRSV in adult/sow farms during the second half of the year;
- b) Rise in the usage of population-based specimens, e.g., oral fluids, processing fluids, for PRRSV monitoring in the last 15 years;
- c) Increased PRRSV genotyping testing (ORF-5 Sanger sequencing), documenting the discomfort of producers and veterinarians with the pathogen’s activity in the field, use of modified-live virus vaccines, and emergence of new and more aggressive strains such as the 1C.5 and 1C.2 variants;
- d) Documentation through real-time spatial-temporal analysis of significant shifts over time (year after year) and space (defined as US states) of predominant PRRSV strains, e.g., different variants within each of the wild-type PRRSV genotypes.

Porcine Regional Information Management Ecosystem

Another highly valuable initiative is the Porcine Regional Information Management Ecosystem (PRIME, <https://fieldepi.org/pips/>). PRIME integrates data on facilities, diagnostics, and animal movements from participating swine production systems to monitor disease activity in real time at a site level. The generated information becomes available to participants, enabling them to make data-driven decisions regarding swine health management. Key contributions of regional information management include:

- a) Reduces the time required to identify emerging and re-emerging infectious diseases at a site level;
- b) Provides the ability to infer and predict, based on diagnostics and movement-derived site status, and animal health changes;
- c) Based on genomic surveillance, PRRSV ORF5 sequencing, and animal movements, identify communities and indirect connections between geographically separated production systems but connected through other channels, e.g., animal movements;
- d) Identify and quantify regional spots of viral circulation and spread.
- e) Benchmark interventions post-weaning against PRRSV between participants.

CONTINUE >

Farm-Level PRRS Outbreak Management

At the farm level, the PRRS Outbreak Management Program (POMP, <https://fieldepi.org/pomp/>) is an ongoing epidemiological platform that gathers data and provides comprehensive insights to assess PRRS outbreaks from the onset through response and recovery. Key contributions of regional information management include:

- The usage of live virus inoculum (LVI) for herd immunization is associated with higher total piglet losses per 1,000 sows but contributes to faster time to stability when compared with modified live vaccine usage (MLV);
- The sooner herds start herd closure after the outbreak, the faster they achieve stability;
- Herds that don't seek elimination had lower production losses at the time of outbreak, but more frequent rebreake, contributing to constant losses;
- PRRSV variants from 1C.5 and 1C.2 groups are the most aggressive and contribute to higher production losses.

Farm-Level Breeding Herd and Downstream Flow

The Predictors of Swine Performance (PROSPER, <https://fieldepi.org/prosper/>) aggregates various breeding-herd and downstream flow data streams, such as disease status, diagnostic results, productivity, infrastructure, and pig movement, into consolidated databases. Once the data is assembled, it enables users to explore whole-herd trends and performance predictors. Key contributions of regional information management include:

- Allows to predict expected downstream mortality based on the breeding herd characteristics;
- Creates a platform to develop and implement a piglet wean quality score, allowing to predict expected mortality and apply interventions to ameliorate the health of the animals or to extract the maximum potential of high-quality weaned piglets;
- Quantify the production impact and performance of groups of animals affected by different diseases.

Genomic surveillance

The PRRSV open reading frame 5 (ORF5) gene has been widely used for PRRSV surveillance. ORF5 accounts for only 4% of the PRRSV genome and cannot explain the remaining 96%. Increasing availability and reduced prices for next-generation sequencing (NGS) testing contributed to advances and a better understanding of the use of whole-PRRSV genome information in PRRSV epidemiological investigations. Key advancements include:

- Developing guidelines on how to use population samples, whole-PRRSV genomes, and assembled NGS genome fragments, i.e., contigs, for farm or production system-specific PRRSV epidemiological investigations;
- Understanding the genetic evolution of the virus and the implications of recombination events for the emergence of more aggressive strains, e.g., 1C.5 for PRRSV-2 and Rosalia for PRRSV-1, which originated from wild-type and wild-type recombination;
- Identify and measure the viral diversity present in a farm, or the viral and bacterial diversity present in live virus inoculum materials used for PRRSV exposure in herds that faced an outbreak.
- Quantify the diversity of the PRRSV genome regions. Based on currently U.S. circulating wild-type PRRSV-2 and representing 95% of wild-type diversity: a) across the non-structural portion of the genome, nsp8 is the most conserved region and nsp2 the most diverse; b) across the structural portion of the genome, ORF5a is the most conserved and ORF3 the most variable.

Conclusion

PRRSV remains the costliest disease in U.S. swine production, driving the need for better surveillance, management, and control strategies. The development of built-for-purpose tools has furthered the understanding of PRRSV epidemiology. A diverse set of tools and programs are available, each designed to answer a specific question. Key learnings include:

- Early detection and monitoring are critical for identifying PRRSV trends, emerging strains, and disease hotspots.
- Data integration (diagnostics, animal movements, production records, and facility information) improves the ability to predict and manage disease spread.
- Farm-level analytics help measure the impacts of outbreaks and identify factors affecting animal health and productivity.
- Genomic surveillance is enhancing understanding of viral evolution, diversity, and the emergence of more aggressive PRRSV variants.

Together, these tools support evidence-based decision-making and advance industry efforts toward PRRSV control and eventual elimination.

IL.03

MOLECULAR MECHANISMS OF PRRSV PERSISTENCE: CHALLENGES FOR ELIMINATION

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically important swine pathogens in the global swine industry. The extensive challenges faced in the development of effective control strategies reflect our inadequate knowledge of viral biology and pathogenesis. PRRSV infection can be divided into at least three stages: acute infection, persistence, and extinction. After initial acute infection, PRRSV can persist in infected pigs for several months and viral RNA can be detected in lymphoid tissues for more than 200 days^{1,2}. The ability to maintain a long-term nonclinical persistent infection is one of the hallmarks of PRRSV infection. Under certain conditions, PRRSV persistent pigs can transmit the virus to naïve animals^{3,4}. Current PRRSV diagnostic methods are unable to specifically identify persistently infected animals. Thus, PRRSV persistence is a significant problem that impedes disease control and prevention. Development of effective strategies requires an understanding of molecular mechanisms of viral persistence. In our study, we established an *in vitro* cell culture model, as well as *in vivo* pig model systems, to study the in-depth mechanisms of PRRSV persistence. The ultimate goal is to apply new fundamental knowledge to develop better strategies for PRRSV diagnostics, control, and prevention.

Viral signatures associated with PRRSV persistence

Initially, we established a cellular model of a persistently infected cell line. Using this model system, viral dsRNA was revealed to function as a mediator for PRRSV persistence⁵. The viral dsRNA was identified to be associated with an alternative pathway for intercellular spreading of PRRSV infection, in which viral infectious materials can be transmitted through intercellular nanotube connections^{6,7}. Utilizing this pathway to directly access the cytoplasm of a naïve cell presents an efficient spreading route for the virus to bypass many of the otherwise critical assembly, budding, and cell entry steps that allow the virus to escape the host immune responses, which could contribute to the pathogenesis of viral infection and persistence. Pig models have been used to confirm the findings in the cell culture system. PRRSV dsRNA was detected persisting in lymphoid tissues of infected pigs. Importantly, the germinal center of the lymphoid tissue was identified as a potential reservoir for dsRNA persistence, in which dsRNA had limited ability to stimulate host antiviral responses, suggesting that viral dsRNA persistence in the germinal center allows the virus to escape antiviral immune responses. Specific mutations in the viral genome were further identified from the lymphoid tissues of persistently infection pigs, which were associated with the inactivation of viral replication complex⁸.

Host cell responses to PRRSV persistence in infected pigs

Using our established pig model system and cutting-edge technologies, the specific type of host cells and cellular markers correlated to PRRSV persistence were identified⁹. We used single cell RNA sequencing to characterize blood cells collected at 14- and 84-days post-infection (DPI) from pigs infected with PRRSV. Infected pigs were grouped as persistently infected (PI) or virus extinction (VE) phenotypes based on viral RNA detection in blood and tissue samples at 84 DPI, along with mock infected pigs. At 14 DPI, gene expressions in monocytes in VE pigs displayed activation of antiviral and interferon responses, reflecting a controlled, effective immune response promoting viral clearance, while monocytes from PI pigs were characterized by metabolic dysregulation. By 84 DPI, VE pigs showed a transcriptional gene profile towards immune homeostasis, whereas PI pigs had gene profile consistent with chronic inflammation, suggesting that major differences between PI and VE arise from immune resolution processes that are uniquely activated in VE pigs. To explore unique early and late PRRSV immune responses, we performed comparative enrichment and pathway analyses on monocyte differentially expressed gene (DEG) profiles from PI and VI pigs. At 14DPI, 592 DEGs were found to be activated only in VE pigs, which involve in tuning immune signals to resolve inflammation. These results demonstrate that PRRSV infection induces early cell-type-specific DEGs that facilitate PRRSV clearance in infected pigs. Taken together, this study provides a framework for mining blood cell transcriptomes to identify predictive markers of infection outcomes, which paves the road for integrating cell-type-resolved immune signatures into strategies for PRRSV persistence control and prevention.

CONTINUE >

Conclusion

The ability of PRRSV to invade the host immune system and establish persistent infection significantly impedes our efforts to eliminate PRRS. Novel mechanisms of PRRSV persistence revealed in our studies provide fundamental knowledge and new directions for developing novel diagnostic assays, therapeutics, and other control and prevention strategies.

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IL.04

FROM LAB TO FARMYARD: GENE EDITING FOR DISEASE RESISTANCE IN PIGS

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Infectious disease remains one of the major constraints on pig health, welfare and sustainable production. Genome editing provides an opportunity to complement conventional disease-control measures by making precise changes to host genes required for infection, potentially creating resistance that is inherited from one generation to the next. Our work at the Roslin Institute has focused on translating this concept from identifying virus–host interactions through to disease-resistant animals and, ultimately, its implementation in commercial pig breeding.

Porcine reproductive and respiratory syndrome virus (PRRSV) provides a compelling example. PRRSV requires the macrophage receptor CD163 for infection, with scavenger receptor cysteine-rich domain 5 (SRCR5) playing a critical role in the viral life cycle. Rather than removing CD163 completely, we used genome editing to delete exon 7, encoding SRCR5, while retaining expression and biological function of the remainder of the protein. Macrophages from these pigs were resistant to PRRSV-1 and PRRSV-2 isolates¹. Subsequent *in vivo* challenge demonstrated complete resistance to a highly virulent PRRSV-1 strain in pigs carrying the Δ SRCR5 modification, with no viraemia, antibody response, clinical disease or virus-associated pathology². This work established that targeted alteration of a discrete functional domain of a host protein can prevent viral infection without requiring complete loss of an otherwise biologically important protein.

Subsequent work by Genus plc and its pig-breeding business PIC demonstrated that this resistance extends to PRRSV-2 *in vivo* and can be incorporated into commercial breeding populations. A scaled genome-editing programme introduced the CD163 exon 7 deletion into four genetically diverse elite pig lines, with multigenerational studies demonstrating stable inheritance, PRRSV resistance and no detected detrimental effects on important production traits³. Importantly, CD163 Δ E7 pigs were resistant to a broad panel of PRRSV-1 and PRRSV-2 isolates, including contemporary and emerging lineages representing the great majority of circulating viral diversity in the US⁴. The notable exception was PRRSV-2 isolate KS06-72109, a lineage 6 virus against which Δ E7 did not provide complete protection, although this lineage was not detected at appreciable levels in the US commercial population during the period evaluated⁵. Together, these findings support the potential durability and commercial application of the Δ E7 strategy, while highlighting the need for continued viral surveillance. They also illustrate the importance of collaboration between academic researchers, breeding companies and industry partners in translating disease resistance from experimental animals into genetically diverse, high-performing breeding populations.

We are now applying the same principle to other major viral diseases of pigs. Recent collaborative work between the Roslin Institute, the Animal and Plant Health Agency, the University of Lübeck and Genus plc identified the host protein DNAJC14 as a target for resistance to classical pestiviruses. Genome-edited pigs carrying altered DNAJC14 produced primary cells that did not support replication of either classical swine fever virus (CSFV) or bovine viral diarrhoea virus, while *in vivo* challenge demonstrated complete resistance to CSFV infection⁶. This provides a second example of rationally engineering the host rather than targeting the virus and raises the possibility of extending this approach to other pestiviruses and livestock species.

These developments exemplify the vision of “Livestock 2.0”: moving genome editing from a tool for functional genomics towards a component of modern animal breeding⁷. Genome-edited disease resistance should not replace established disease-control measures, but provide an additional tool within an integrated approach to animal health. Combining genetic resistance with vaccination, biosecurity, pathogen surveillance, good husbandry and appropriate veterinary interventions could provide multiple layers of protection while reducing reliance on any single control measure.

The challenge is therefore not simply whether we can produce disease-resistant animals, but how resistance traits can be responsibly integrated into diverse breeding populations and wider disease-management strategies. PRRS-resistant pigs provide a tangible example of this journey from lab to farmyard: from understanding a virus–

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host interaction and designing a resistance allele, through validation against diverse viral strains, to incorporation into elite commercial breeding populations. Together with emerging examples such as CSFV resistance, this demonstrates how genome editing can complement existing disease-control approaches and contribute to healthier, more resilient and sustainable livestock production.

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IL.05

IMMUNOPATHOGENESIS OF THE REPRODUCTIVE FORM OF PRRSAndrea Buzanich-Ladinig¹; Melissa Stas¹; Wilhelm Gerner²

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) remains a substantial economic and animal welfare burden for global pig production. Research on PRRS pathogenesis, immunity and vaccine development has mainly focused on the respiratory form of the disease, while mechanisms of PRRS virus (PRRSV) induced reproductive failure remain incompletely understood. Although the majority of economic losses occur in the nursery/grow/finishing stage (Osemeke *et al.*, 2025), the reproductive form also accounts for substantial economic losses, and preventing vertical transmission is of utmost importance to control PRRS within a herd.

Reproductive PRRS

The outcome of PRRSV infection of pregnant gilts and sows varies greatly throughout gestation. The mechanisms underlying the differences in transplacental transmission throughout gestation are not entirely understood, but permissiveness of local macrophage populations in the uterus has been proposed as an explanation (Karniychuk and Nauwynck 2013).

Even in late gestation, when PRRSV has the greatest impact, fetal outcome within a litter is highly variable. While some fetuses die, others are infected without changes in preservation, and some escape infection even though virus might be present in the endometrium (Pasternak *et al.* 2020b; Van Goor *et al.* 2020). Viral load which varies amongst fetuses within a litter from uninfected to high, seems to be a key factor influencing fetal outcome (Ladinig *et al.* 2014; Malgarin *et al.* 2019).

Therefore, local events occurring in the maternal-fetal interface (MFI) are crucial for the outcome of reproductive PRRS.

Immune responses in the maternal-fetal interface

Local immune cells at the maternal-fetal interface must be capable of combatting infections while tolerating the semi-allogenic fetus. Although the roles of uterine natural killer (uNK) and T cells have been studied intensely in humans and mouse models (Schumacher *et al.* 2018), little is known about the phenotype and function of local immune cells of pregnant swine. As pigs have an epitheliochorial placenta rather than the hemochorial placenta found in humans, direct comparisons between the two species are limited. In the pig, research addressing reproductive immunology at the maternal-fetal interface is sparse and has predominantly focused on the earlier stages of gestation, while the knowledge on phenotypic and functional characterization of local leukocytes during late gestation is limited.

We have developed methods tailored to the porcine epitheliochorial placenta, which allowed us to isolate mononuclear cells separately from the maternal endometrium (ME) and fetal placenta (FP) of late-term pregnant sows to phenotypically characterize immune cells by flow cytometry (Stas *et al.* 2020). We have shown that T cells with a highly differentiated phenotype (including CD27–perforin+ CD8 T cells and T-bet+CD8α+CD27–effector memory CD4 T cells) prevail in the ME, while T cell populations with a naive phenotype (including CD27+perforin– CD8 T cells and T-bet–CD8α–CD27+ CD4 T cells) dominate in the FP. More recently, we applied these methods to investigate immune cell phenotypes at the MFI 21 days after PRRSV infection in late-term pregnant gilts, either vaccinated or non-vaccinated with a commercial modified live virus (MLV) vaccine (Stas *et al.* 2022). We used a highly virulent and a low virulent strain of PRRSV to infect gilts in the last trimester of gestation. We found a vast increase in CD8 T cells with CD8α+CD27dim early effector phenotype in the ME for fetuses from the non-vaccinated infected gilts, compared to non-treated and vaccinated-only controls. Fetuses from gilts infected with the highly virulent strain also showed significant increases of lymphocytes with effector phenotypes in the FP, including CD8α+CD27+ CD8 T cells and CD8α+CD27+/dim CD4 T cells. In vaccinated animals, this activation of effector phenotypes was more confined. Impaired fetal preservation and fetal infection were found in non-vaccinated gilts infected with the highly virulent strain, but not in other groups of gilts.

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In our latest experiment, we could show an association between viral load and T cell responses within the MFI with CD8β+ T cells showing the greatest response to PRRSV infection (manuscript in preparation). Median CD8β+ proportions increased from 17.9% in non-infected to 36.5–39.9% in infected ME, and from 7.3% in non-infected to 25.0% in high viral load FP. Phenotypic analysis demonstrated a marked expansion of proliferating CD8β+ T cells with an early effector perforin+CD27+ phenotype, particularly in high viral load tissues. The proportion of total CD4+ and total γδ+ T cells was lower in infected groups in both ME and FP. No differences were found with regards to Ki-67 expression in CD4+ and γδ+ T cells. Following restimulation with PRRSV AUT15-33 peptide pools, PRRSV-specific CD4+ and CD8β+ T cells were identified by staining for IFN-γ and TNF in the ME and the FP.

In the same experiment, we investigated functional properties of uNK cells isolated from ME and FP of PRRSV infected and non-infected gilts (Sawyer *et al.* 2025). Flow cytometry analyses showed that PRRSV infection did not significantly alter the total NK cell frequencies but affected NK cell proliferation. uNK cells with a high expression of the activating receptor NKp46 showed increased Ki-67 and perforin expression in samples from PRRSV infected gilts indicating potential immune responsiveness to PRRSV. CD107a degranulation capacity was lower in FP uNK cells, possibly due to the fetal immune environment’s immaturity. Despite PRRSV exposure, CD107a expression did not significantly change in uNK cells.

Conclusion

Our results suggest that the local activation and strong effector response of local immune cells within the MFI, particularly CD8 T cells, might have detrimental effects on fetal survival after PRRSV infection. Ongoing research will further investigate whether immune responses in the maternal endometrium and fetal placenta contribute to protective immunity or immunopathology and determine whether local adaptive immunity in the fetal placenta originates from the fetal or maternal immune system.

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IL.06

EMERGING VACCINE PLATFORMS FOR SWINE VIRAL DISEASES

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Introduction

Conventional vaccines such as modified live vaccine (MLV) and whole inactivated vaccines (WIV) have been invaluable for modern swine herd health against many important swine viruses, including porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), swine influenza virus (SIV) and porcine epidemic diarrhea virus (PEDV). These vaccines have some inherited challenges such as limited cross-protection against variant strains, reversion to virulence for MLV, weak cell-mediated immune response (CMI) for WIV, etc. Numerous emerging vaccine platforms, such as mRNA-based, nanoparticle protein-based, lipid nanoparticle DNA-based, multi-epitope-based, consensus antigen-based, synthetic attenuated virus engineering via codon-pair deoptimization, and novel vector-based vaccine platforms, offer alternative approaches for developing effective vaccines against swine viruses. Here in this talk, the focus will be on recent development in nanovaccine, broadly-protective and vectored vaccine platforms.

Nanovaccine platforms

Nanovaccine platform improves the safety and efficacy of conventional vaccines by incorporating nanotechnology for rationale antigen choice, design, display, adjuvant integration and targeted mucosal and immune cells deliveries. It takes advantage of many advanced delivery systems, including self-assembling protein scaffolds, protein nanocages, virus-like particles, lipid and polymeric nanoparticles, to enhance both CMI and humoral immune responses. The nanovaccine platform has been used to develop novel candidate vaccines for a number of economically-important swine viruses such as PRRSV^{1,2}, SIV³⁻⁵, African Swine Fever Virus (ASFV)^{6,7}, and PEDV⁸⁻¹⁰. Nanovaccines can encapsulate different forms of vaccine antigen including mRNA-based, DNA-based, protein or conserved epitopes-based to improve humoral immune response, CMI, mucosal immunity with chitosan-based system and targeted delivery to antigen-presenting cells.

Novel viral and bacterial vector platforms

Non-pathogenic viral or bacterial vector expressing viral antigen induces robust immune responses to augment vaccine coverage against variant strains of virus, improve vaccine safety and reduce vaccine production cost. Adenovirus-vectored vaccines, commonly used for respiratory and enteric viruses, have the added advantage of mucosal-based vaccine delivery and induction of local immune response against a number of important swine viruses such as ASFV^{11,12}, PRRSV^{13,14}, PEDV^{15,16}, and SIV^{17,18}. Hepatitis B virus core antigen have also been used as a vector to express viral antigens as PRRSV¹⁹ and PEDV²⁰ candidate vaccines. A recombinant tri-segmented viral vector based on non-pathogenic Pichinde virus was used to develop safer and more effective vaccines against for SIV^{21,22}. Novel bacterial vectors are also used to express viral antigens as vaccines. For examples, a killed genome-reduced bacterial vector-based vaccine platform utilizes an expression vector with an inducible gram-negative autotransporter to express viral antigen on the bacterial surface against PEDV²³. Lactobacillus species-based bacterial vector systems such as *L. reuteri*, *L. plantae*, *L. casei* and *L. johnsonii* have been used to develop vaccines against numerous major swine viral pathogens including PEDV^{24,25}, PRRSV²⁶, PCV2²⁷, SIV^{28,29}, and ASFV^{30,31}.

Universal or broadly-protective vaccines

Many swine viruses continue to evolve leading to emergence of variant strains that can evade vaccine-induced immunity. Novel strategies have recently been used to develop universal or broadly-protective vaccines by targeting highly conserved antigenic regions, utilizing mosaic antigens, employing consensus sequence or gene-shuffled antigens. For examples, epigraph-based vaccine design utilizes computer algorithm to identify most frequent epitopes and create a cocktail vaccine for broader vaccine coverage. Epigraph vaccines, which are similar to mosaic vaccines, have been used for developing broadly-protective vaccines against SIV^{18,32-34}. Molecular breeding via gene shuffling can increase vaccine coverage for improved heterologous protection against divergent PRRSV strains³⁵⁻³⁷ and PCV2 strains³⁸. Consensus antigen strategy has also been used to overcome the challenge of extensive genetic diversity of many RNA viruses for developing broadly protective vaccines against SIV^{39,40}, PCV2⁴¹, and PRRSV⁴².

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IL.07

BEYOND DISEASE CONTROL: THE ROLE OF THE WORLD ORGANISATION FOR ANIMAL HEALTH IN ADVANCING GLOBAL SWINE HEALTH

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The global swine sector is a cornerstone of food production, food security, trade and rural livelihoods. Pork remains one of the most widely consumed sources of animal protein worldwide, and pig production provides income, employment and nutrition to millions of people. In many regions, swine production is not only an economic activity but also a critical contributor to livelihoods, community resilience and food security.

As production systems intensify and trade continues to expand, maintaining healthy swine populations has become both an economic necessity and a global responsibility. At the same time, the sector faces growing pressures associated with climate change, environmental sustainability, evolving production systems and changing disease risks. These factors are reshaping the epidemiology of infectious diseases and reinforcing the need for resilient animal health systems capable of anticipating and responding to future challenges.

Swine diseases continue to be an important burden for the production systems worldwide, resulting in production losses, trade disruptions, significant economic consequences and environmental impact. Recent decades have demonstrated the devastating impact that transboundary animal diseases can have on the swine industry, national economies and food systems. Outbreaks of African swine fever (ASF), classical swine fever (CSF), foot and mouth disease (FMD), swine influenza, PPRS and other major viral diseases have highlighted the vulnerability of increasingly interconnected production systems and remain major constraints to productivity, animal welfare and sustainability in many parts of the world.

While these diseases differ in their epidemiology and control options, they share common challenges. Effective prevention and control require robust surveillance systems, reliable diagnostics, strong biosecurity, transparent reporting and sustained engagement from both the public and private sectors. In many cases, success also depends on access to effective vaccines and well-resourced Veterinary Services capable of translating scientific knowledge into practical action.

Experience from around the World demonstrates that successful disease control and eradication programmes are rarely the result of technical solutions alone. Disease control should be viewed not only as a scientific challenge, but also as a complex technical, social and adaptive challenge. It requires collaboration across disciplines and sectors, bringing together animal health professionals, researchers, social scientists, producers, industry and governments. Success ultimately depends on governance, trust, coordination and long-term commitment.

This lecture will provide an overview of the current global situation of the major viral diseases affecting swine populations, with particular emphasis on diseases included in the WOAH list of notifiable diseases. It will review recent epidemiological developments, highlight lessons learned from national, regional and global control programmes and discuss the challenges that continue to hinder disease prevention, control and eradication efforts.

The broader context in which swine disease control takes place will also be explored. Globalisation has reinforced the reality that transboundary animal diseases cannot be addressed in isolation. Animal movements, trade flows, wildlife interfaces and increasingly complex value chains create pathways through which disease risks can spread across borders. As repeatedly demonstrated by major animal health crises, weaknesses in one part of the system can rapidly become challenges for many others.

Science, transparency and multilateral collaboration and cooperation remain fundamental pillars of effective disease prevention and control. Science-based international standards, timely disease reporting, scientific collaboration and regional coordination mechanisms provide the foundation for informed decision-making and mutual trust. These elements are particularly important when disease control measures have implications not only for animal health, but also for food security, livelihoods and international trade.

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As the global authority on animal health and welfare, WOAHA contributes to the improvement of animal health globally through the development of international standards adopted by its Members, the collection and dissemination of official disease information through WAHIS, and the provision of scientific and technical support to strengthen national animal health systems. Through its scientific networks, including more than 300 Reference Centres worldwide, WOAHA helps facilitate access to expertise, innovation and scientific evidence, enabling Members to make informed decisions and implement effective disease prevention and control strategies.

Combating swine diseases cannot be the responsibility of a single actor. Strong partnerships between governments, academia, industry and producers are essential. Initiatives such as the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs) and the STAR-IDAZ International Research Consortium illustrate the importance of coordination in accelerating the development and adoption of solutions to shared animal health challenges.

Looking ahead, significant opportunities exist to transform the prevention and control of swine diseases. Advances in genomics, molecular epidemiology, next-generation diagnostics, novel vaccines, artificial intelligence and data-driven disease intelligence are creating new possibilities for earlier detection, improved preparedness and more effective response. However, technological innovation alone will not be sufficient. Continued investment in research, Veterinary Services, laboratory capacity and workforce development will be critical to ensure that innovation translates into sustainable impact.

Improving swine health is not solely an animal health and welfare objective. It is an investment in food security, livelihoods and the sustainability of livestock systems. By strengthening the foundations of disease prevention, preparedness and control, we are not only protecting pig populations today but also investing in a healthier, more resilient and more sustainable future for all.

IL.08

INVESTIGATIONS INTO INFLUENZA A VIRUS REASSORTMENT IN COMMERCIAL PIGS

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Introduction

Influenza is an important disease in pigs and people. The increased genetic diversity of influenza A virus (IAV) in pigs threatens producers' profitability, food security and pig and human health because new strains continue to emerge and spread making control of influenza more difficult. Because reassortment is central to the emergence of novel influenza virus in pigs, we investigated the occurrence of distinct influenza genotypes at the population and single pig level across different stages of production to further elucidate pig and population factors critical to the emergence of reassortant viruses. Ultimately, the goal is to control influenza infections more effectively and limit virus diversity and the emergence of novel viral reassortants.

Methods

In order to gain insight into the level of genetic diversity and reassortment occurring in commercial pigs, we sampled pigs throughout their production cycle from commercial farms known to be infected with multiple subtypes of IAV. Multiple influenza virions were isolated from single pigs by plaque purification and their whole genome sequenced using Illumina NextSeq platform, and analyzed to identify whether pigs harbored one or more distinct types of influenza viruses (i.e genotypes) including reassortant viruses. Phylogenetic analysis were conducted to identify gene segment lineages, and Phylopart was used to identify within sub-clustering of the lineages (sub-lineage) of closely related sequences within each gene segment based on pairwise phylogenetic distances¹. We considered a genotype as a unique combination of segment-specific lineages or sub-lineages across all eight segments. Network analysis were used to evaluate the relationships among observed IAV genotypes in which nodes represented unique genotypes and edges connected genotypes differing in one or two sub-lineage segment classifications. This information was used for population-level reconstruction of newly detected genotype constellations and determine most likely parental genotypes.

Information on pigs' age, production stage and cohort was also obtained to further assess population and management level factors that may contribute to influenza infections and reassortment. In total we investigated three cohorts of pigs, from weaning to near market, that were mixed at weaning and originated from three breeding herds. We sampled 720 pigs from which 62 influenza infected pigs yielded 428 viral plaques which after further characterization yielded 173 complete genomes and 237 incomplete genomes. We also conducted serological analysis to evaluate levels of protective immunity against the distinct influenza viruses known to be present in the herds using the hemagglutination inhibition test. Blood samples from the 720 pigs were tested against four distinct types of influenza viruses including representatives of the H1 and H3 subtypes.

Results

We identified extensive genetic diversity and reassortment of IAV at both, the population and single pig level. There were three subtypes (H1N1, H1N2 and H3N2) with multiple distinct gene segment constellations that resulted in 20 distinct genotypes. The genotypes included a mixture of swine-adapted H1 and H3 hemagglutinin lineages (H1.1A.1.1.3, H1.1A.3.3.3, H3.1990.4, H3.2010.1) and neuraminidase N1 and N2 lineages (N1.C.3.2, N2.1998B, N2.2002B) with internal genes of TRIG (triple reassortant) and pandemic origin. Viral diversity was highest in younger pigs with the number of genotypes decreasing as pigs became older. Viral diversity from the breeding herds was unequally represented in pigs at weaning with one breeding herd contributing nearly 90% of the genotypes, while another contributing only one genotype and another zero genotypes. Reconstruction of multiple influenza genomes at the single pig level revealed substantial viral diversity taking place in some animals with some genotypes being transient while others contributed generously to the pool of gene segments capable to transmit on-ward. Single pigs frequently harbored a rich pool of viral populations with the genetic composition capable to result in reassortment. Network analysis revealed massive exchange of gene segments among the viral isolates and identified that only a limited subset of genotypes were parental contributors of reassortment-compatible patterns. Loss of diversity and emergence of a new genotype could be observed simultaneously as the only genotype identified in the oldest pigs was new and had not been detected as a complete genotype

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in prior production stages. However, the new genotype consisted of gene segments present among viruses detected in earlier stages. In contrast, there were two genotypes where the majority of the gene segments were genotype specific and not identified among previously detected genotypes in the population. Furthermore, the landscape of protective immunity was variable among viral strains, cohorts, subpopulations and production stages. In general, humoral immunity against all hemagglutinins from IAV detected in the population under study was associated with less influenza infections. However, HA lineage-specific immunity was associated with a moderate decrease in detection of homologous HA-lineages but this association could only be observed for two of the lineages.

Conclusion

Our study demonstrates that IAV infected pigs harbor viral populations composed of complex genotype constellations that provide continuous opportunities for reassortment. This study helps identify target pig populations, and management and vaccination practices for interventions, emphasizes the importance of surveillance across production stages, helps with interpretation of diagnostic and sequence data in particular when new reassortant viruses are suspected, and provides a new way to think about flu control by targeting those viruses that are more likely to result in novel virus reassortants. Overall, this study contributes to knowledge necessary to decrease influenza infections, influenza genetic diversity and emergence of novel influenza reassortants in pigs.

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IL.09

DANISH PRRS REDUCTION STRATEGY - BACKGROUND, PROGRESS AND LESSONS LEARNED

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Objectives

The Danish national PRRS reduction programme, initiated in 2022, aims to reduce the prevalence of Porcine Reproductive and Respiratory Syndrome (PRRS) in a high-density pig production system. The primary objective is to increase the proportion of herds with a negative PRRS health status through coordinated, area-based eradication, thereby reducing infection pressure and improving animal health, productivity, and antimicrobial stewardship.

Methods

The Danish PRRS reduction programme is implemented as a nationwide, multi-level intervention combining regulatory measures, industry coordination, and data-driven surveillance. The strategy was developed through a formal collaboration between key stakeholders in the Danish pig sector, including the Danish Agriculture & Food Council, the Danish Veterinary Association, the Danish Veterinary and Food Administration, and the Association of Danish Swine Slaughterhouses.

The programme relies on coordinated regional eradication strategies, where the effectiveness of herd-level interventions is dependent on synchronised action among neighbouring herds to minimise the risk of re-infection. The national territory has been subdivided into 21 regional eradication areas designed to reflect pig density and geographical proximity. Each region is coordinated by designated administrators responsible for aligning herd-level interventions, including stabilisation, partial or total eradication strategies, and implementation of enhanced biosecurity measures.

The programme is based on mandatory PRRS health status declaration for all pig herds above a defined size threshold, ensuring near-complete population coverage. By January 2026, a total of 4,348 herds were included and assigned to geographically defined eradication regions. The surveillance framework integrates serological, clinical, and progressively virological monitoring. Herd-level PRRS status is primarily determined through antibody testing, supported by regular clinical assessments conducted by herd veterinarians and targeted PCR testing in connection with suspicion or eradication procedures.

Data for the present analysis were obtained from national SPF and CHR databases via the Danish PRRS monitoring system, with extraction performed in January 2026. These data enable comprehensive evaluation of herd-level PRRS status, temporal trends in prevalence, infection dynamics, and geographical variation across regions.

Results

The implementation of the programme has resulted in a consistent and measurable reduction in PRRS prevalence. By January 2026, 73.7% of herds had achieved a negative PRRS health status, whereas 20.8% remained positive, 3.3% were undergoing eradication, and 2.2% had an unknown status. This represents a continued increase from 68% PRRS-negative herds in 2025, indicating sustained programme effectiveness over time.

Substantial geographical variation persists, reflecting differences in regional infection pressure and progress of eradication efforts. Nevertheless, successful area-based elimination has been demonstrated, and the island of Bornholm became the first PRRS-free region in 2025.

The programme was associated with a marked reduction in infection incidence. In 2025, 23 sow herds became newly infected, corresponding to an annual infection risk of 2.4%, compared with pre-programme levels of 6–7%. Most infections in 2025 were caused by PRRS virus type 2.

Conclusions

The Danish PRRS reduction programme demonstrates that a coordinated, nationwide approach combining mandatory surveillance with regionalised eradication strategies can lead to significant and sustained reductions in disease prevalence.

The observed increase in PRRS-negative herds and the concurrent reduction in infection pressure indicate that large-scale control of PRRS is feasible in a complex and highly interconnected production system. These results underline the importance of coordinated regional action, as the success of herd-level eradication is strongly dependent on the infection status of neighbouring herds.

Implications

Despite substantial progress, complete elimination of PRRS has not yet been achieved. Structural dependencies between herds, heterogeneous regional progress, and the presence of herds not yet engaged in eradication efforts continue to represent constraints for further reduction.

Based on current projections, approximately 150 sow herds are expected to remain PRRS-positive by the end of 2026, indicating that continued progress will require sustained coordination and participation across regions.

Future Perspectives

Future implementation will focus on preventing re-introduction into PRRS-free regions through strengthened biosecurity measures and stricter regulation of live animal movements, including enhanced requirements related to gilt introduction, quarantine procedures, and documentation of herd health status prior to trade.

Regulatory developments are expected to include official declaration of PRRS status at regional level, restrictions on the movement of PRRS-positive pigs, and expansion of virological surveillance through systematic PCR-based monitoring. These measures will be integrated into a differentiated monitoring framework adapted to regional PRRS status and will further strengthen incentives for eradication.

Continued support for regional eradication programmes, together with the development of targeted contingency plans for infection events in PRRS-free areas, will be essential for consolidating current progress. Collectively, these efforts aim to facilitate a transition from reduction towards the ultimate goal of national elimination of PRRS in Denmark.

IL.10

PRRS CONTROL AND ERADICATION IN THE U.S.

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Introduction

The U.S. continues to be challenged by the Porcine Reproductive and Respiratory Syndrome virus (PRRSv) since its first detection in the mid 1980's. The virus has proved to be transmissible under different conditions, persist in pig populations and evolve through mutation and recombination. Due to these viral characteristics, the virus has become the most important disease in the industry as it has become the most economically important swine disease. Given the important losses this virus generates, veterinarians and producers learned promptly that this virus could not be fully controlled and generated persistent losses and thus, it needed to be eliminated from the herd. However, as the virus was eliminated from the sow herds, multiple re-infections occurred because of pig regional density which led to "area spread", suggesting that the virus moves from farm to farm.

As frustration built, veterinarians and producers began wondering whether a regional approach among neighboring farms could reduce viral circulation and thus reduce prevalence and incidence. Therefore, a small group of veterinarians, producers and researchers embarked into a proof-of-concept field experiment with the goal of controlling and eliminating the PRRSv at the regional level. The initiative was producer and veterinarian led and supported by veterinary epidemiologists such as Dr. Bob Morrison. While there were no formal regulatory framework, producers and veterinarians were expected to share data with goal of advancing regional swine health. These efforts proved to be worthwhile and today they continue to be relevant given that they demonstrated that regional elimination of PRRSv was possible.

The early stages of regional elimination of PRRSv

Regional elimination of PRRS can be traced back to the early 2000's when Dr. Morrison and others selected a region in the state of Minnesota known as Rice County in which producers and veterinarians were interested in testing the concept. While there was some progress in Rice County, the regional project that generated the most data and became famous for its success was the one conducted in Stevens County (Minnesota). The Stevens County project successfully confirmed that decreasing both the prevalence and incidence of the virus was possible and thus, the project was expanded to its surrounding six counties that thanks to its success, producers and veterinarians began discussing whether state-wide efforts could be entertained. A decision to expand the project was made but focused on the area north of highway 212 which is considered as the one with the lower pig density when compared to the area south of the same highway. Given this experience, other regional projects in the U.S. began and during the early 2010's, approximately 20 independent projects had been reported.

Regional project participation

As producers saw regional elimination projects as one more tool for disease prevention and as a complementary action to their biosecurity efforts, project buy in was in some cases quite straightforward but in some others, producers were reluctant to participate. Veterinarians play a key role as they provided producers with necessary information to make the decision of joining or not but also, they became the trusted intermediaries and coordinators. In addition, veterinarians helped the producer understand diagnostic testing results at the regional level and their implications which ultimately helped in the development of preventive or intervention plans with the overarching goal of contributing to maintaining the region free of PRRSv.

While regional participation was somewhat uniform among interested parties, reaching 100% participation was not achieved. The central social challenge was not simply persuading producers to join a program, it was actually maintaining meaningful participation while protecting confidentiality and ensuring that participants perceived a direct benefit from sharing information. Therefore, the level of participation in these kinds of programs proved to be both its greatest strength and vulnerability as without participation progress will not occur. An important ingredient for high participation and engagement was that the project coordinating body had to be neutral and trusted as a large amount of confidential information was managed. These regional experiences showed why a neutral project coordinator is important for the sustainability of the initiative. All these efforts became pivotal for the establishment of the Morrison Swine Health Monitoring Project (MSHMP) which began in 2011 as producers

and veterinarians began sharing PRRSv breeding herd status with the goal of understanding the epidemiology of PRRSv outbreaks. The MSHMP, led by the University of Minnesota (a third and neutral party) began providing epidemiological analysis, analysis, mapping and disease metrics for the industry to measure progress. While MSHMP was not a regional project per se, it demonstrated that voluntary participation could be sustained at a larger scale with the goal of learning and not blaming.

Success and lessons learned

Regional projects for PRRS elimination were in general successful. Success in this case was achieved at different levels such as reaching voluntary participation, reduction of PRRSv prevalence/incidence in some projects and producer/veterinarian collaborative efforts. Perhaps the largest success was the fact that what started as a proof-of-concept became a reality and that led to others to replicate such projects.

Through these projects, we learned that producer and veterinarian participation must be active and constant. While some producers/veterinarians participated, some did not contribute to discussions or provided diagnostic data. During the North 212 project, a large number of farms had been enrolled but a proportion of them remained with an unknown PRRSv status. In addition, regional projects will benefit from 100% participation, but the reality is that the likelihood of having all producers onboard is not necessary.

Another lesson is that while producers and veterinarians are willing to participate and share some data, confidentiality and trust are two necessary ingredients. If producers and veterinarians believe their data can be used against them by the group, the chances that they will opt out will increase. Therefore, managing data at the aggregate level will always contribute to maintaining a friendly environment while participants learn together from their shared data.

A constant influx of diagnostic data is important for the project as this will contribute to measure success and keep the outcome relevant. Diagnostic data contributed to maintaining transparency; however, diagnostic testing costs became a limiting factor in some cases as not all producers were willing to enroll in routine testing. However, later in the process we learned that diagnostic testing data was not as important as it was the commitment by the producer who controlled pig movement.

While there were multiple PRRSv regional elimination projects ongoing simultaneously at one time point, the number of active projects began declining. There are several reasons why this phenomenon occurred, the main one being an asymmetry between effort and immediate reward. As producers invested resources (e.g., time and money), the expectation of clearing the virus from the region was not being met at a reasonable pace and slowly producers began to retract themselves from this initiative.

A second factor that may have played against these projects is participation heterogeneity as some local producers would champion the initiative, but others played a back seat role. Eventually, champions lost momentum due to passive behavior and lack of engagement and also lack of funding, changes in ownership and historical communication challenges led to make these project vulnerable.

Current situation in the U.S.

The U.S. swine industry continues to fight the virus through the combination of different tools such as biosecurity (e.g., transport sanitation, D&D room, shower-in/shower-out, air filtration, feed mitigants, biosecure mortality removal, employee training), changes in pig flow (e.g., herd closure, depop/repop, batch farrowing, gilt introduction), vaccines (e.g., modified-live and killed) and the use of strategic live-virus inoculation during breeding herd elimination programs. There have been some attempts to assemble regional projects but at a much smaller scale with the goal of maintaining communication and monitoring rather than focusing on elimination only.

The industry faces a yearly epidemic that begins in October-November of each year and ends in April-May of the following year. Usually, late spring and summer seems to be the window of time when producers and veterinarians have a break when it comes to outbreaks; however, recently, new viruses have emerged and have shown their capability of transmitting during the summer.

Historically, the U.S. breeding herd PRRSv cumulative incidence ranges between 20-25% which indicates that every year 20-25% of the breeding herds in the MSHMP report a break. In addition, the prevalence of unstable herds has reached a “plateau” and it usually ranges between 25-35% which a cyclical fluctuation. The factors that trigger the onset of the epidemic during the fall season are still not well understood. There are several hypothesis including environmental temperatures leading to better viral viability, manure pumping and lack of

biosecurity. While it is difficult to attribute to a single factor, producers and veterinarians continue to invest in biosecurity. On the other hand, the emergence of highly virulent variants such as the lineage 1A, 1C.5, 1C.5.32 among others has got the industry wondering why and how these new variants emerging. In addition, these new variants are challenging the breeding herd elimination protocols as the time to eliminate the virus is apparently longer and, in some cases, herds that are undergoing elimination can go into a secondary break (also known as re-break) as the originally introduced virus changes and evades the already established herd immunity. Another phenomenon is that our current monitoring approaches through processing fluids, tongue-tip-fluids, due-to-wean piglet serum do not seem to be sensitive enough as in the later stages of monitoring within the elimination program, the herd yields multiple negative RT-PCR test results followed by a singleton positive test result.

Future perspective

The industry will continue to live with this virus until major changes are made to reduce the number of new infections but also keep breeding herds from becoming endemically infected with more than one variant. Swine dense regions will continue to exist but opportunities regarding the re-location of swine herds in these high dense regions need to be assessed in the long run. Currently, the National Pork Board through its National Swine Health Strategy has recently begun an industry-wide effort to improve swine health in which one of the goals and priorities is to reduce the domestic disease impact of PRRSV by advancing an elimination framework, research and also investing resources to understand gaps and feasibility of national elimination.

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IL.11

DIVERSE PRRSV AND ITS CONTROL & ELIMINATION STRATEGY IN CHINA

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) has experienced a long history of genetic evolution since it was recognized in China in early 1990s. Currently, with diversity, this virus remains a prominent threat to the Chinese swine industry, leading to usual economic losses. Different strategies including prevention, control and elimination have been practiced in order to reduce economic impact caused by PRRSV at the farm level.

Diversity of PRRSV

PRRSV-2 is the dominant species prevailing in China. Based on ORF5 gene, at least four distinct lineages (L8, L1 and L3) are present, together with the recombinant viruses among these lineages (Zhou *et al.*, 2024). Meanwhile, novel strains and revertant viruses from modified live vaccines (MLVs) of highly pathogenic PRRSV are usually emerged in the field, leading to clinical outbreaks (Wu *et al.*, 2025). PRRSV-1 infection and relevant clinical condition is also an issue in some areas.

Prevention and control

For PRRSV-free pig farm, negative status is sustained to prevent the introduction of PRRSV by external biosecurity system. In PRRSV-positive pig farm, a stable status is established through internal biosecurity measures, use of safe MLVs, acclimation of naïve gilt replacements, and control of secondary bacterial infections.

Elimination

Elimination is implemented in breeding pig farm with PRRSV-positive in China (Hu *et al.*, 2023). Main strategies include total depopulation/repopulation, partial depopulation and combination of herd closure and test & removal. Basic conditions and measures in relation to PRRSV elimination are involved in location and biosecurity of pig farm, air-filter system of pig house, gilt development unit (GDU), naïve breeding-gilt replacements, semen control, antibody and viral nucleic acid detection, and removal of antibody/virus- positive sows.

Conclusion

In China, PRRSV continues to bring economic burden for the pig production. Controlling its impact is a task for pig producers, PRRSV-elimination is an inevitable tendency for breeding pig farm.

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IL.12

AFRICAN SWINE FEVER AND ERADICATION IN THE EUROPEAN CONTEXT

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Spain had free status of African Swine Fever since 1995, when the historic outbreak that had affected the Iberian Peninsula since the 1950s was eradicated. Since then, strict preventive measures against the disease had been implemented, particularly following the entry of genotype II into the European Union in 2014 and its progressive spread across several European countries, primarily among wild boar populations. These measures included the progressive enhancement of epidemiological surveillance, biosecurity protocols on pig farms and during hunting activities, and extensive awareness campaigns, among many others. However, in November 2025, it was confirmed the detection of the virus into the province of Barcelona, Catalonia, following the detection of cases in wild boar within a moderately forest area surrounded by numerous residential developments and characterized by an initial average wild boar density. Epidemiological investigation was inconclusive regarding the source of introduction in the country. Following this detection, control measures included in EU regulations were implemented. These included the establishment of an Infected Zone (later regionalized into Zone I and Zone II in accordance with Implementing Regulation (EU) 2023/594), the suspension of non-essential activities in the area, the reinforcement of fencing to limit wild boar movements, and intensified searches for wild boar carcasses (with searches conducted by specialized dog teams proving particularly important). Additionally, traditional hunting was prohibited, and a white zone was subsequently established where population control measures—such as trapping and controlled drives—were carried out by personnel authorized by the Generalitat de Catalunya to minimize population levels. All wild boar cases were detected within the designated Zone II; no cases have been detected outside this area in the disease-free zone. Strict biosecurity and surveillance measures remain in place for active pig farms within the restricted zone, and to date, no cases have been detected in domestic pigs.

IL.13

PROTECTIVE IMMUNITY ASSOCIATED WITH VIRAL CONTROL IN PRRSV

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) remains a major threat to the global swine industry. Vaccination has remained a critical measure for PRRS control in the past three decades, and modified live vaccines (MLV), in particular, are widely used and can reduce viral tissue load and mitigate clinical symptoms. Unfortunately, PRRSV possesses an extraordinary genetic plasticity in the field, driving continuous emergence of variants, such as the Chinese highly pathogenic PRRSV (HP-PRRSV) and increasingly prevalent NADC30/34 strains. Consequently, cross-protection conferred by existing MLV against heterologous strains is often incomplete and unpredictable, striking an urgent need to define the immune determinants of vaccine-mediated protection and to understand how viral variations impact these responses.

Results

We applied integrated single-cell RNA sequencing and T cell receptor (TCR) profiling to characterize immune responses in a PRRSV vaccination–challenge model spanning complete, partial, and non-protection outcomes. We identified distinct CD8⁺ T cell subsets that were selectively enriched in protected animals vaccinated with modified live vaccine (MLV) and marked by clonal expansion, strong cytotoxic transcriptional programs, and enhanced functional activity, which correlated with rapid control of viremia after challenge. In contrast, non-protected animals accumulated dysfunctional CD8⁺ T cells expressing exhaustion-associated markers such as CTLA4 despite partial cytotoxic signatures. Mechanistically, the protection-associated responses were primarily driven by viral structural proteins (SP). Replacing the SP-coding region of a heterologous strain reshaped the CD8⁺ T cell landscape from a mixed cytotoxic/exhausted profile toward a protective program, accompanied by improved clinical outcomes. Further, optimal CD8⁺ T cell activation required macrophages/monocytes-derived innate signaling, including TLR4 and TLR8 pathways, and was enhanced by CD4⁺ T cell help.

Conclusion

Our work provides *in vivo*, single-cell-resolved insights into the immune responses associated with PRRSV vaccine-induced protection. Our findings define protection-associated CD8⁺ T cell states linked to viral control and support a model in which effective immunity involves coordinated interactions between viral antigen composition, innate immune activation, and CD8⁺ T cell differentiation. These results have implications for vaccine design, including optimization of antigen selection and the potential use of adjuvants that enhance beneficial innate signaling while limiting inhibitory pathways.

IL.14

NEW PEDV EPIDEMIC PATTERNS IN CHINA: VIRULENT G2C VARIANTS AND S-INDEL CLADE-2 RECOMBINANTS AND THEIR IMPLICATIONS FOR GLOBAL PEDV EVOLUTION

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Introduction

Porcine epidemic diarrhea virus (PEDV) continues to cause devastating economic losses to the global swine industry. Despite widespread vaccination, recurrent outbreaks with high neonatal mortality persist in China. The emergence of novel variants, particularly the highly virulent G2c subtype and newly dominant S-INDEL recombinant lineages, has reshaped the epidemiological landscape and raised critical concerns regarding vaccine efficacy and disease control strategies.

Epidemiological Shift

Surveillance of 69 PEDV S gene sequences collected across nine Chinese provinces during 2021–2024 revealed that G2c variants have become the predominant circulating strains, accounting for 69.57% (48/69) of isolates. Concurrently, phylogenetic analysis of 415 S-INDEL strains identified two distinct clades: clade 1, comprising early endemic strains circulating before 2019, and clade 2, a newly dominant recombinant lineage restricted to China since 2019. This dual shift — G2c dominance and S-INDEL clade 2 emergence — defines the current PEDV epidemic patterns in China.

Molecular Signatures and Recombination-Driven Evolution

Comparative amino acid analysis of the G2c spike (S) protein identified five signature substitutions (N139D, Y182H, I287M, F345L, L998M) distinguishing it from G2a/G2b subtypes. Three-dimensional structural modeling revealed that four of these mutations (N139D, I287M, F345L, L998M) induce conformational changes in critical antigenic domains, including the S10 neutralizing epitope region, the N-terminal domain, and the S2 subunit, potentially facilitating immune evasion. For S-INDEL clade 2 strains, 22 clade-specific amino acid changes were identified, with 11 located in the D0 domain. Notably, mutations at positively selected sites 113 and 114 (HN↗IG) within the D0 domain — a region associated with virulence — induced helix formation and may alter pathogenic potential. Recombination analysis revealed distinct evolutionary mechanisms driving S-INDEL diversity. Clade 2 strains emerged through both inter-genotypic recombination (involving G1b, G2, and S-INDEL parents) and unprecedented intra-genotypic/intra-clade recombination events, distinguishing their evolutionary trajectory from clade 1 strains. These findings indicate that recombinant clade 2 is forming localized transmission networks within China, warranting urgent investigation of its pathogenic potential.

Virulence and Pathogenicity

Pathogenicity assessment of a representative G2c strain (AHCZ02) in 3-day-old neonatal piglets demonstrated 100% mortality within 66 hours post-inoculation, accompanied by severe watery diarrhea, rapid weight loss, and marked jejunal villus atrophy. This hypervirulence profile underscores the clinical threat posed by contemporary G2c variants.

Currently-used Vaccine Efficacy and Immune Response

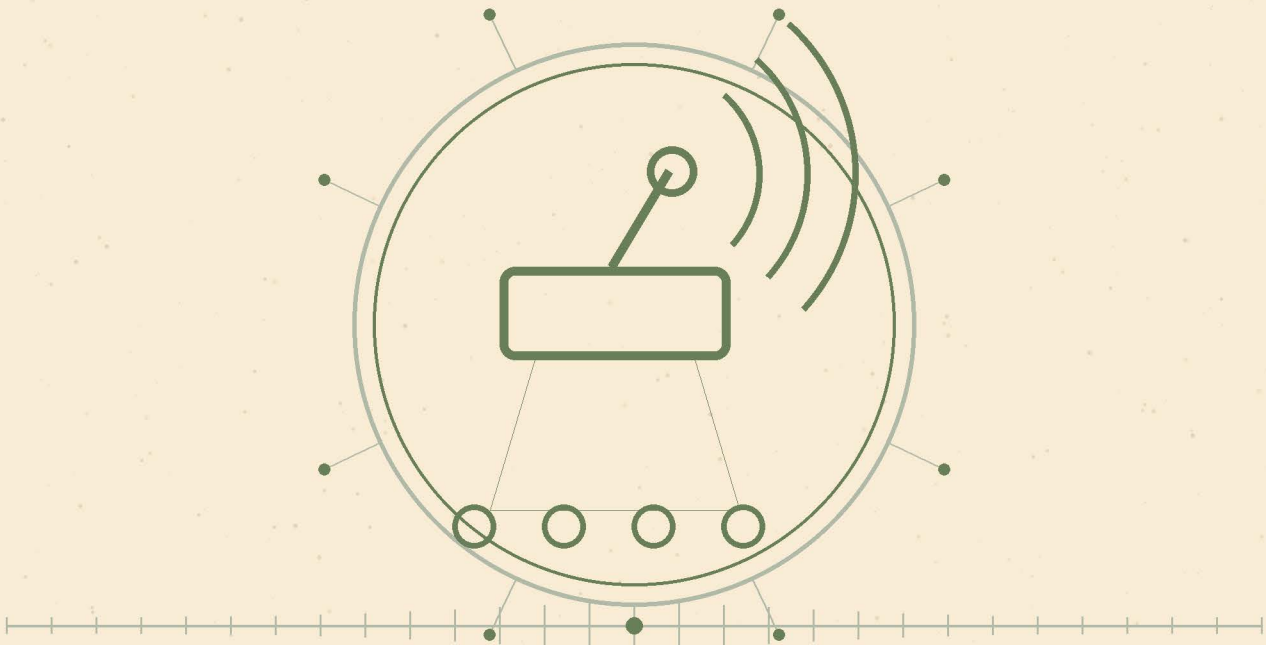
Cross-neutralization assays using the AHCZ02 strain revealed striking disparities in neutralizing antibody responses among different immunization strategies. G2c-based feedback exposure elicited geometric mean titers (GMT) of 1,893 — 3.9-fold higher than S-INDEL-based feedback (GMT = 480) and 12- to 189.3-fold higher than conventional vaccines (GMT = 10–40). These results highlight the profound antigenic divergence between circulating G2c variants and currently used vaccine strains (G1 strain CV777 or G2b strain AJ1102), and explain the limited cross-protection observed in vaccinated herds.

Conclusions

The PEDV epidemiological landscape in China is characterized by the dual emergence of hypervirulent G2c variants and recombinant S-INDEL clade-2 strains, driven by sustained mutations and recombination. Whether the identified mutations are indeed responsible for G2c virulence or immune escape is currently under investigation using PEDV reverse genetics. The diminished neutralizing capacity of conventional vaccines against these emerging variants underscores the urgent need for next-generation vaccines incorporating G2c-specific or clade 2-specific antigenic determinants, alongside strengthened molecular surveillance to monitor ongoing viral evolution at the global level.

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WHOLE GENOME CLASSIFICATION SYSTEM FOR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS TYPE 2 (BETAARTERIVIRUS AMERICENSE) STRAINS COMBINING ORF5, NSP3-4-5 AND INDELS

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Introduction and objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) disease was first observed in 1987 in the United States of America. This disease is responsible for the loss of billions of dollars worldwide in the pig industry. Since the discovery of its etiological agent, the virus denomination and classification method has often changed. The current PRRS virus classification system is based on the ORF5 viral gene phylogeny which represents only 4% of the viral genome. However, veterinarian practitioners are confronted with cases of unexpected severity while viral isolates are classified as vaccinal strains. As this virus evolves at high speed by combining an error prone RNA polymerase and a high recombination rate, the ONI classification system (ONI standing for ORF5, nsp3-4-5 and indel) is hereby proposed to better discriminate vaccinal strains from viral recombinants while remaining simple to use.

Methods

The ONI classification system was designed using a dataset of 1809 publicly available complete genome sequences from 14 countries including 130 newly deposited Canadian sequences. This system combines the current ORF5 classification with a phylogeny of the nsp3, nsp4 and nsp5 segments of ORF1a viral gene as well as groups based on insertions and deletions (indels) at the whole genome scale. Clusters of nsp3-4-5 sequences were determined using sequence identities and phylogeny. Indel groups were determined using hierarchical clustering inferred from a presence/absence matrix of each indel event observed in the dataset.

Results

The nsp3-4-5 clusters revealed 77 clusters distributed around the world. Most clusters were shown to be specific to a single country while 2 clusters associated with vaccinal, and highly pathogenic strains were shown to be widely distributed. Each sequence had an average of 3.68 indel events with most events observed in the nsp2 viral gene. However, some countries shown more an enrichment in indel events in other genes. For example, Canadian strains had a greater number of indel events in the nsp7 gene than any other country. The ONI system was also used to reclassify seemingly vaccinal strains based on their ORF5 lineage alone. For example, of 50 Canadian PRRS virus strains sharing the same ORF5 lineage as the Ingelvac MLV PRRS vaccinal strain, 22 (44%) were identified as different using the ONI classification, with 4 (8%) strains being viral genome recombinants. Finally, combining all components of the ONI classification, the reference strain ATCC VR-2332 would be classified as the 5A-n67-IDVR-2332 ONItype.

Discussion and conclusions

The ONI classification system offers a broader view of the PRRSV genome while retaining the information from the current ORF5 classification system. As more complete genome sequences or PRRSV will be made available, the ONI classification will be able to evolve and be refined. Bioinformatic tools were made available for anyone who would like to analyze their own sequences in the form of a Nextstrain community dataset and the IDindel suite of python scripts through Github.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

The authors declare that no ethical concerns were identified.

O.02 · ID93

KNOWN ENEMY OR NEW THREAT? A TUSSLE BETWEEN INTRODUCTION, PERSISTENCE, AND RECOMBINATION OF PRRSV STRAINS ON PIG FARMS

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) remains challenging to control due to its high mutation rate and extensive recombination potential. The viral dynamics on farms involve persisting resident strains, newly introduced strains, modified live vaccine (MLV) strains, and emerging recombinant strains. However, little is known about how frequently new strains are introduced, which factors determine whether they persist and become dominant or disappear, and how vaccine-induced immunity and cross-protection between genetically distinct PRRSV strains influence these processes. Additionally, it remains unclear how often successful recombinants emerge, become established, and eventually replace resident strains. As more European nations aim to eradicate PRRSV, a better understanding of these evolutionary and epidemiological dynamics is essential.

Methods

To quantify strain introductions, persistence, and their predictors, Bayesian discrete trait phylodynamic methods were applied to 597 ORF5 sequences collected through regular monitoring from 83 Dutch pig farms between 2018 and 2025. Of the 83 farms, thirty contributed ≥3 field sequences and were included in the farm-level analyses; sequences from the remaining farms served as background data. From the resulting highest-independent-posterior-subtree (HIPSTR), the time of the most common ancestor of a clade per farm was interpreted as the time of introduction and used to calculate strain persistence. Additionally, to study the emergence and persistence of recombinants, 241 ORF2-7 sequences collected between 2023 and 2025 from the same farms were analysed with the RDP5 recombination detection method to detect recombinants, breakpoints, parents, and descendants. Finally, the corresponding ORF5 sections of these ORF2-7 sequences were extracted for comparison of recombinant detections.

Results

Twenty of Thirty farms experienced at least one introduction, and 12 of those experienced multiple introductions. Overall, strains were introduced at a median rate of 0.49 (IQR=0.801) per farm per year. However, not all introduced strains persisted and displaced resident strains (no-takeover). In total, fifteen introduced strains successfully took-over and persisted for a median of 1.30 years. Six farms had a single strain that persisted for ≥4 years. Among all inferred farms, detected strains with ≥3 sampled sequences persisted for a median of 2.99 years (IQR=2.49). Recombination analysis showed that OF2-7 sequences detected 5.5 times more recombinants than the corresponding ORF5 sequences. Around 22-25% of field sequences were identified as recombinants, 68% of which were field-x-vaccine recombinants. Another 20-25% represented descendants of these recombinant strains, which persisted for a similar duration as other field strains (0.47-1.82 years) within farms, indicating sustained transmission and spread of field-x-vaccine recombinant strains. Ongoing analyses aim to identify predictors for strain takeover, including antigenic distance between resident, introduced, and vaccine strains.

Discussion and conclusions

Our results demonstrate that routine PRRSV sequencing can distinguish long-term persistence from new farm introductions, providing valuable information for targeted biosecurity and control. Our analysis reveals that relying on ORF5 sequencing alone substantially underestimates the recombination rate. Also, investigating the epidemiological conditions that enable field-x-vaccine recombinants is necessary due to their high frequency and persistence. Overall, our findings highlight the importance of integrating phylodynamic and recombinant analyses to understand PRRSV dynamics and to support effective eradication programmes.



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Conflicts of interest

None

Ethics statements

existing anonymized-data was reused

Financial support and Acknowledgements

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Keywords

Phylogenetics • Recombination • Introduction • Persistence

O.03 • ID52

ASSESSMENT OF THE MUTATION, SELECTION, SUBSTITUTION AND RECOMBINATION EVOLUTIONARY PATTERNS AND DYNAMICS OF “CLASSICAL” VERSUS “ROSALIA AND ROSALIA-LIKE” SPANISH PRRSV-1 STRAINS

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Introduction and objectives

In Spain, the epidemiological landscape of PRRSV-1 has been profoundly altered over the last six years by the emergence and spread of a highly virulent strain, so-called “Rosalia”. Since its appearance, this strain is estimated to have infected tens of millions of pigs. The objective of this study was to compare the evolutionary patterns and dynamics of “classical” PRRSV-1 strains and “Rosalia” strains, including mutation, selection, substitution and recombination analyses, and to assess their implications for classification, diagnosis and understanding of PRRSV-1 evolution.

Methods

We assembled a dataset of 235 PRRSV-1 genomes, including 111 “classical” PRRSV-1.1 sequences, 107 “Rosalia” isolates, 13 PR40-clade isolates and four 1.2 and 1.3 sequences. Ninety-six genomes were retrieved from GenBank, whereas 139 come from our laboratory. A Neighbor-Joining phylogenetic tree was initially generated and used to prune redundant sequences. Also, ORF5 sequences were classified using Nextstrain. Recombinant sequences were identified using RDP 5.0, and potential breakpoints were individually inspected. The dataset was then split into recombinant and non-recombinant “classical” and “Rosalia-like” subsets. Mutation, selection and evolutionary analyses were performed using MEGA12 and BEAST. In addition, RNA secondary structure was examined using complementary approaches, including LinearAlifold, RNAalifold and RNAplfold.

Results

A considerable proportion of genomes showed evidence of recombination, with 20.4% of sequences affected overall (48/235). Recombination was especially frequent among “Rosalia”-like strains, where 37.8% of genomes were classified as recombinant (40/107). Breakpoints were unevenly distributed across the genome, with potential recombination hotspots in nsp1, nsp2, nsp9, ORF3 and ORF5. However, recombination breakpoints were not clearly associated with differential RNA secondary-structure stability. Clear differences were also observed in selection patterns. “Classical” strains showed a higher proportion of sites under purifying selection and lower overall dN/dS values than “Rosalia” strains, indicating stronger evolutionary constraint. In agreement with this, substitution rates were higher in the “Rosalia” dataset. When examined by protein-coding region, substitution rates in “Rosalia” isolates were approximately three- to eight-fold higher than those estimated for classical isolates. Although the total number of variable positions was higher among “classical” strains, this likely reflects their much longer sampling period, spanning approximately 35 years compared with only five years for “Rosalia” isolates. Therefore, mutations appear to be generated and accumulated more rapidly in “Rosalia” strains. Finally, 82% of isolates classified as “Rosalia-like” by whole-genome phylogeny were assigned to clade L1.17 by Nextstrain.

Discussion and conclusions

Overall, these results indicate that “Rosalia” strains represent a rapidly evolving PRRSV-1 lineage characterized by increased recombination frequency, relaxed purifying selection and accelerated substitution rates. The high replicative capacity of these high-virulence strains, together with their extensive and rapid spread in the pig population, has probably contributed to this accelerated evolution. Understanding the selective pressures acting on these viruses and the biological relevance of emerging variants is therefore essential. In the current epidemiological context, recombination appears to be a major evolutionary force. Furthermore, the discrepancy between whole-genome and ORF5-based classification highlights that, under certain circumstances, ORF5 alone may provide a misleading picture of PRRSV-1 diversity and evolution.

Conflicts of interest

N/A

Ethics statements

Samples were submitted for diagnostic purposes not requiring ethics committee approval

Financial support and Acknowledgements

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O.04 • ID106

SUPPORT SYSTEM OF AN ARTIFICIAL INTELLIGENCE (AI) AGENT FOR THE HEALTH MANAGEMENT OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) IN COLOMBIA

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Introduction and objectives

PRRS management is complicated by territorial heterogeneity, animal mobilization, and the coexistence of production systems with varying biosecurity levels, necessitating the use of tools that enable effective health management. This study aimed to develop an AI agent-based decision-support system that integrates quantitative risk modeling and knowledge-assisted reasoning to strengthen PRRS health management at the municipal level.

Methods

The system integrated a hierarchical Health Risk Model that combines Hazard and Vulnerability components to generate a Sanitary Risk Index (SRI). It also incorporated fifteen Mitigation Capacities recognized as relevant by Expert Criteria, identified from six repositories with 210 files that included research articles, review articles, interviews, and chapter podcasts. The AI architecture comprised two agents with independent Retrieval-Augmented Generation (RAG) pipelines to improve the accuracy of large language models and guarantee responses based on retrieved evidence, reducing hallucinations. The first agent explains municipal PRRS risk by interpreting the quantitative model outputs and retrieving supporting evidence on the highest contributing factors from a curated, risk-oriented knowledge base built upon 38 scientific references. The second agent generates actionable mitigation recommendations by retrieving prevention protocols and biosecurity guidelines from a separate knowledge base supported by 21 mitigation-focused records. Both agents were deployed on Amazon Bedrock using Claude 3.5 Sonnet with Titan Embeddings V2 and OpenSearch Serverless, ensuring evidence-grounded responses with source attribution and expert validation. For prediction, thematic evaluation was conducted by the Colombian swine industry, determining a certainty of 95.20% regarding the non-occurrence.

Results

The Risk Explanation Agent can generate up to 824 individualized explanations, one for each municipality with commercial swine production, ensuring full territorial interpretability of the SRI (53.07%). The Mitigation Agent produces at least five context-specific recommendations per user query within seven seconds.

Discussion and conclusions

The tool transforms numerical risk metrics from the Health Risk Model into actionable decision-support components, in a timeframe that strengthens its responsiveness. Those recommendations, based on technical literature and expert knowledge, do not have a uniform effect, as the impact depends on the type of risk being targeted for reduction. Integrating Health Risk Models with knowledge-based A.I. tools reduces dependence on manual analysis, supports decision-making and contributes to more efficient, targeted, and adaptive epidemiological surveillance for Colombian swine production.

Conflicts of interest

The authors state there are no conflicts of interest.

Ethics statements

The authors declare that the work presented here was conducted with full transparency and integrity, in accordance with Colombian regulations. All individuals involved disclosed any conflicts of interest and safeguarded the confidentiality of private or sensitive data. Accordingly, the academic methodological approach adopted is grounded in honesty, accountability and respect. This yields a high-quality solution, acknowledges collaborators’ work and addresses challenges objectively and fairly.

Financial support and Acknowledgements

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Keywords

Artificial Intelligence Agent • PRRS Health Management

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O.05 • ID37

NEW REVERSE GENETICS SYSTEM FOR PRRSV IDENTIFIES A GP3 TRAFFICKING SIGNAL THAT PROMOTES VIRAL REPLICATION

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) glycoprotein 3 (GP3) associates with GP2 and GP4 to form an envelope glycoprotein complex required for virion assembly. However, the intracellular transport of GP3 and its contribution to productive viral replication remain incompletely understood. GP3 contains two conserved tyrosine-based motifs (YxxΦ) that may recruit intracellular sorting machinery. Conventional analysis of these motifs in the viral genome is limited by the laborious manipulation of large infectious-clone plasmids. This study aimed to establish a bacteria-free reverse-genetics platform for PRRSV and use it to determine how the two GP3 sorting motifs affect intracellular trafficking and viral replication.

Methods

A Linear Overlapping Infectious Polymerase Amplicon (LOIPA) system was developed by amplifying the PRRSV genome as overlapping PCR fragments. A synthetic linker carrying an upstream promoter and a downstream polyadenylation signal was included to drive full-length viral RNA transcription in transfected cells. Targeted mutations were introduced into the two GP3 YxxΦ motifs during PCR amplification. The overlapping PCR fragments were then cotransfected into BHK-21 cells to reconstitute full-length viral genomes, and the recombinant viruses were subsequently rescued and amplified in porcine macrophage cells. Viral infectivity and maintenance of the engineered substitutions were evaluated by RT-PCR, immunofluorescence, and sequencing. GP3 localization was examined using intracellular compartment-specific markers, and replication efficacy was assessed by RT-qPCR and TCID50.

Results

The LOIPA system enabled the recovery of recombinant PRRSV carrying targeted substitutions within either GP3 YxxΦ motif. Substitution of Y108 within the 108-YAWL-111 motif produced the most pronounced phenotype. Y108A abolished GP3 localization in the ER–Golgi intermediate compartment (ERGIC) and Golgi apparatus, indicating impaired transport through the early secretory pathway. In comparison, substitutions within the 136-YVDI-139 motif did not substantially alter GP3 compartmental localization. Despite the trafficking defect, the Y108A virus remained fully infectious with fewer copies of viral genome and lower infectious titers than those of wild type virus. Our results demonstrate that Y108 is nonessential for viral infectivity but required for efficient replication and infectious virion production.

Discussion and conclusions

These findings identify YAWL as the functional tyrosine-based sorting motif in PRRSV GP3 glycoprotein, whereas YVDI appears to have a limited role in GP3 compartmental targeting under the conditions examined. The loss of ERGIC and Golgi localization following Y108 substitution suggests that this residue facilitates GP3 transport through the secretory pathway and its delivery to intracellular sites involved in virion formation. The accompanying reduction in viral RNA and infectivity further indicates that altered GP3 trafficking compromises productive replication, potentially by affecting glycoprotein maturation, assembly, or incorporation into virions. Additionally, the LOIPA system provides a rapid, bacteria-free platform for recombinant virus construction, targeted genome manipulation, and functional analysis of viral genetic elements.

Conflicts of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statements

Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.



Financial support and Acknowledgements

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Keywords

Reverse genetics • GP3 • Intracellular trafficking • Tyrosine sorting signal • PRRSV-2

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O.06 • ID50

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS NSP1α DEGRADES RIBOSOMAL COMPONENTS TO INHIBIT CELLULAR TRANSLATION

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Introduction and objectives

Efficient utilization of the intracellular protein translation machinery to synthesize viral proteins represents a rate-limiting step for viruses establishing infection within host cells. It is particularly critical for RNA viruses with relatively small genomic sequences, such as Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), a member of the Nidovirales, which pose a devastating threat to the global swine industry. The mechanisms by which PRRSV infection modulates the limited protein synthesis resources—such as ribosomal components—and thereby ensure efficient viral replication remain largely unknown. The objective of this article is to investigate the mechanisms by which PRRSV suppresses host cell translation and selectively utilizes translation initiation factors and ribosomal components to initiate its own efficient translation. This study aims to elucidate a unique immune evasion strategy employed by the PRRSV, thereby providing promising targets for antiviral drug development and vaccine design.

Methods

The PRRSV RNA translation manner within cells was investigated using indirect immunofluorescence (IFA), small molecule inhibitors, RNA interference (siRNA), and the construction of monocistronic and bicistronic luciferase reporter gene systems. The cap-binding activity of nsp1α was examined through sucrose density gradient centrifugation, m7G pulldown, GST pulldown, RNA pulldown, and RNA immunoprecipitation (RIP) assays. The proviral functions of RPS25 were characterized employing immunoprecipitation-mass spectrometry (IP-MS), co-IP, siRNA, and CRISPR/Cas9 knockout, with viral kinetics assessed via Western blotting, TCID50, and IFA. The viral RNA translation mechanism was validated using RIP, RIP-sequencing, *in vitro* transcription, siRNA, and dual-luciferase reporter gene systems. Nsp1α-mediated degradation pathways of RPS27 were analyzed through co-IP, construction of truncation mutants, ubiquitination assays, and Western blotting. Finally, point mutant viruses were generated using a reverse genetics system, and IFA and RT-qPCR were employed to evaluate the replication levels of both wild-type and point mutant PRRSV strains.

Results

This study reveals that PRRSV facilitates efficient translation of viral RNA through a cap-dependent mechanism that operates independently of eIF4E and eIF2α, even under conditions of pronounced suppression of host cell translation. Furthermore, we identified that under stringent inhibition of the host translation level, the PRRSV nsp1α functions as a cap-binding protein. It promotes viral RNA translation and replication by recruiting the host non-essential ribosomal protein RPS25. Additionally, PRRSV nsp1α suppresses host cell translation by targeting the host essential ribosomal protein RPS27 for degradation via the ubiquitin-proteasome pathway. Notably, the critical amino acid residues responsible for nsp1α’s cap-binding activity closely coincide with those mediating RPS27 degradation; these residues spatially form a concave pocket that constitutes the functional core of nsp1α.

Discussion and conclusions

This study innovatively elucidates the dual function of the PRRSV replicase nsp1α, which inhibits host cell translation and recruits specific ribosomal components to facilitate viral RNA translation. These findings uncover a unique viral immune evasion strategy and provide promising targets for antiviral therapeutics against PRRSV.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethics statements

The protocol for primary PAMs preparation was approved by the Laboratory Animal Ethical Committee of China Agriculatural University, with the approval No. AW30115202-2-01.

O.07 • ID131

CD169 BLOCKADE AS AN *IN VITRO* MODEL TO DISCRIMINATE PRRSV-1 ISOLATES WITH DIFFERENT VIRULENCE PROFILES

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Introduction and objectives

PRRSV-1 strains differ markedly in virulence, although the mechanisms underlying these differences have not been completely elucidated. Previous studies have showed that highly virulent PRRSV-1 strains (HV-PRRSV-1) can replicate in some macrophage populations with low or absent CD169 expression, suggesting that the presence of CD163 alone may be sufficient to confer permissibility to infection by those HV-PRRSV-1. Preliminary experiments by our group have showed that HV-PRRSV-1 isolates replicate to higher titers and reach the maximum titer earlier post-infection than low-virulence isolates in splenic CD163+ macrophages (sCD163+MOs), which express very low CD169 levels. These observations suggest a reduced dependence of these HV-PRRSV-1 of CD169 as a coreceptor. This study expands previous experiments and evaluates whether CD169-blocking assays can be used as an *in vitro* model to discriminate PRRSV-1 isolates with different virulence profiles, based on the hypothesis that low-virulence PRRSV-1 isolates (LV-PRRSV-1) are more CD169-dependent than highly virulent PRRSV-1 isolates (HV-PRRSV-1).

Methods

Nine European PRRSV-1 isolates characterized *in vivo* were used in this study: four LV-PRRSV-1 and five HV-PRRSV-1. Lena was included as a HV-PRRSV-1 control. Porcine alveolar macrophages (PAMs), undifferentiated bone marrow-derived cells (BM-DC) and sCD163+MOs from PRRSV-negative piglets were inoculated with the PRRSV isolates used in the study at a multiplicity of infection of 0.01. Viral replication was evaluated by titration and flow cytometry through intracellular PRRSV nucleoprotein detection. CD163 and CD169 expression was characterized in PAMs and sCD163+MOs, and Siglec-10 expression in sCD163+MOs. To assess PRRSV-1 CD169 dependence, PAMs were incubated with an anti-CD169 monoclonal antibody before and during infection and infection was evaluated at 48 and 72 h post-infection. Comparisons were performed using one-way ANOVA with Bonferroni correction.

Results

HV-PRRSV-1 reached higher viral titres earlier post-infection than LV-PRRSV-1, with the clearest differences observed in sCD163+MOs and undifferentiated BM-DC. sCD163+MOs expressed CD163 but showed very low or undetectable CD169 expression, while Siglec-10 was detected only in a very low proportion of cells. In PAMs, CD169 blockade reduced significantly the infection by most of the LV-PRRSV-1 tested. In contrast, most of the HV-PRRSV-1 still replicated after CD169 blockade, particularly at 48 and 72 h post-infection, suggesting greater ability to overcome inhibition of the CD169-dependent pathway. However, not all isolates followed this pattern. LV-PRRSV-1 Sp-22 showed a faster profile than other LV-PRRSV-1, whereas HV-PRRSV-1 EU-10 displayed a profile slower than most HV-PRRSV-1.

Discussion and conclusions

These results support that PRRSV-1 isolates with different virulence profiles differ not only in replication capacity, but also in their dependence on the use of CD169 as a coreceptor and, consequently, in their target cell range. LV-PRRSV-1 appeared to rely more strongly on CD169, whereas HV-PRRSV-1 can replicate in PAM after CD169 blockade. Lena behaved consistently with its highly virulent phenotype and also replicated without functional CD169, in agreement with previous findings in explant models¹. CD169-blocking assays, combined with restrictive macrophage models, may help discriminate *in vitro* PRRSV-1 isolates with different pathogenic potential.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

Animals were used only as tissue donors. Therefore, no specific animal experimentation approval was required under directive 2010/63/EU.

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O.08 • ID130

HIGHLY VIRULENT PRRSV-1 ROSALÍA STRAIN INDUCES ENHANCED CTLA-4 EXPRESSION AND ENHANCED IMMUNE DYSREGULATION IN PIGLETS ALONG EARLY AND LATE STAGES OF INFECTION

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Introduction and objectives

The pathogenesis of the immune dysregulation in porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) infection is not fully understood yet. In this context, immune checkpoints have been identified as pivotal regulators of inflammatory and immune responses. This study evaluated the expression of CTLA-4 at early and late stages of PRRSV-1 infection in piglets with two strains of different virulence (3249 and Rosalía).

Methods

Fifty four piglets were allocated in three experimental groups: control (n=18), 3249 strain (n=18), and Rosalía (n=18). Animals were monitored for clinical signs and rectal temperature along the study. Pigs were euthanised at 7, 14 and 42 dpi to carry out postmortem examination and serial tissue sample collection for histopathological, immunological, and molecular biology studies. At the same time points, blood samples were collected to determine the viraemia and seroconversion and to isolate PBMCs and analyse the phenotype of circulating T cells as well as the expression of the immune checkpoint CTLA-4 by flow cytometry.

Results

Animals infected with the highly virulent Rosalía strain showed more severe clinical signs and a significantly reduced average daily gain. In addition, they presented more severe lung lesions in the early stages of the infection, characterised by interstitial pneumonia, with marked thymus atrophy and enlargement of the tracheobronchial lymph nodes. Although seroconversion was similar in both groups, Rosalía induced earlier and higher viraemia, as well as higher viral loads in the thymus, where the infection persisted for up to 42 days. Gene expression analysis revealed up-regulation of CTLA4 and TIM3 in the lung, tracheobronchial lymph node, and thymus during the early stages of infection, particularly in Rosalía-infected animals, decreasing until the advanced stage in the lung. This was associated with a higher frequency of CD4⁺CTLA-4⁺ and CD8 β ⁺CTLA-4⁺ T cells in peripheral blood and lung, which persisted, although to a lesser extent, until 42 dpi in animals infected with Rosalía strain.

Discussion and conclusions

Our findings support the high virulence of Rosalía strain and point out to a role of CTLA-4, as well as other immune checkpoints, such as TIM-3, in modulating the host's immune response.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. PRRS; Rosalía; Immunopathogenesis; Immune checkpoints; CTLA-4; T cells.

Ethics statements

This experiment was performed according to the guidelines of the European Union (Directive 2010/63/EU) and approved by the IRTA Ethics Committee and by the Catalan Autonomous Government (Project 12055; FUE-2023-03053650).

Financial support and Acknowledgements

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Keywords

PRRS • Rosalía • Immune checkpoints • CTLA-4 • T cells • Immunopathogenesis

O.09 • ID111

TOWARDS AN IMPROVED UNDERSTANDING OF ADAPTIVE IMMUNE DYSREGULATION DURING ARTERIVIRUS INFECTION

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Introduction and objectives

Porcine reproductive and respiratory syndrome viruses (PRRSV) are poorly controlled by the porcine immune system in part due to a delay in neutralising antibody responses, which are also restricted in both titre and breadth. High affinity, class switched B cell responses are shaped in germinal centres (GC), driven by T follicular helper (Tfh) cells and regulated by T follicular regulatory (Tfr) cells. Understanding T and B cell dynamics in secondary lymphoid tissues of infected animals could hold the key to understanding PRRSV-related adaptive immune dysregulation. In this study, we developed tools to better understand GC responses in PRRSV-infected pigs and compared these to mice infected with the closely related lactate dehydrogenase elevating virus (LDV), as a more tractable model.

Methods

Pigs were experimentally infected with PRRSV-1 and -2 and mice experimentally infected with LDV. Blood (both species), spleen (mouse) and lymph node and tonsil samples (pig) were collected longitudinally. High parameter flow cytometry was used to assess the phenotype, frequency and responses of different T and B cell populations. Viraemia and tissue loads were inferred using RT-qPCR; and serum antibody responses measured by GP3 and N protein ELISAs, and virus neutralisation tests.

Results

In PRRSV-1 infected pigs, viremia was observed as early as 3 days post infection (dpi) but was cleared by 28 dpi in most animals, while virus was still detected in the lung. PRRSV N protein specific antibody responses were detected from 7 dpi and were maintained to the end of the study. Flow cytometric analysis of lymph node cells revealed a rapid increase in the total frequency of type 1 Tfh cells with little change in the frequency of Tfr cells. GC B cells did not increase until 21 dpi in both PRRSV-1 and -2 infections, while non-GC atypical Tbet+ B cells emerged from 10 dpi and remained high throughout the study. Clear parallels were observed in LDV infected mice, which also demonstrated a high frequency of Tfh-1 cells and a comparable emergence of atypical Tbet+ B cells. Additionally, in both LDV and PRRSV-2 infections, there was a delay in the expression of activation induced markers associated with Tfh cells.

Discussion and conclusions

The transcription factor T-bet is the master regulator of Th1 immunity and our data suggests arterivirus infection induces a strong Th1 response. Tbet+ B cells expanded in both LDV and PRRSV infections. Often believed to represent a first line of defence, the consequence of the continued prevalence of Tbet+ B cells during arterivirus infection is currently unknown, although their low levels of somatic hypermutation and prevalence outside of the GC may, in part, explain the delayed/absence of high affinity neutralising antibody responses. Collectively, these data suggest similarities in the murine and porcine immune responses to LDV and PRRSV, respectively, and support the further application of the LDV mouse model to interrogate the mechanisms that lead to ineffective immunological control of arterivirus infection.

Conflicts of interest

No conflicts of interest

Ethics statements

Animal studies were performed in accordance with the Animals (Scientific Procedures) Act 1986, UK.

O.10 • ID137

SYSTEMS VACCINOLOGY REVEALS MECHANISTICALLY DISTINCT IMMUNE PROFILES OF INTERFERON-AUGMENTED PRRSV VACCINES

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Introduction and objectives

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes major economic losses, yet vaccine development remains constrained by incomplete understanding of protective immune mechanisms against heterologous strains. Our previous study demonstrated that IFNmixMLV, a multi-interferon-expressing PRRSV vaccine candidate, confers protection against heterologous PRRSV challenge equivalent to that of commercial modified live virus vaccines (ComMLV2). However, the mechanistic basis for this functional equivalence remained unresolved: specifically, whether IFNmixMLV and ComMLV2 engage fundamentally distinct immune architectures, and whether the adaptive immune programming induced by IFNmixMLV is sufficient to support durable protection.

Methods

We conducted a randomized trial using 49 pigs across five groups: sham control, challenge-only (heterologous PRRSV strain NADC34), IFN- ω 5-expressing MLV (IFN ω 5MLV), multi-interferon-expressing MLV (IFNmixMLV), and commercial vaccine (ComMLV2). Longitudinal whole-blood RNA sequencing at days 2, 7, and 14 captured early innate activation, adaptive transition, and protective consolidation phases, generating 137 quality-controlled libraries. Differential expression analysis was integrated with blood transcription module enrichment, rescue profiling, co-expression network modelling along with a pre-specified six-domain Integrated Vaccine Performance Index encompassing Clinical Efficacy (CES), Cytokine Coordination (CCS), Transcriptomic Programming (TPS), Gene Network Rewiring (GNRS), Temporal Transcriptional Entropy (TTE), and Temporal Effector Advantage (TEA) to determine whether the vaccine platforms achieve protection through shared or divergent biological programs.

Results

IFNmixMLV achieved the highest early clinical protection score and superior viral control trajectory, while ComMLV2 achieved stronger cytokine coordination, B-cell memory precursor formation, and adaptive network synchrony. Despite equivalent immunogenicity magnitude, five independent transcriptomic analytical layers established that the two platforms pursue mechanistically opposed immune strategies. IFNmix deployed a sustained cellular effector program characterized by early CD4⁺ T helper enrichment, coordinated myeloid-T cell crosstalk, and rapid transcriptional resolution. ComMLV2 drove IL-12-mediated humoral mobilization with stronger adaptive consolidation but inferior acute viral control. Multi-domain integration revealed that acute protection and adaptive network consolidation are dissociable biological properties that current platforms achieve at each other's expense. The molecular axis separating B-cell recovery velocity during the day 2 to day 14 consolidation window was identified as the precise optimization target for next-generation IFNmixMLV formulation development.

Discussion and conclusions

The first multi-domain mechanistic characterization of PRRSV vaccine response demonstrates that IFNmixMLV and ComMLV2 represent complementary biological blueprints whose failure modes together define the full specification of durable heterologous protection. This mechanistic precision reduces the optimization requirement from broad platform redesign to a single tractable intervention (accelerating B-cell compartment engagement during the consolidation window without sacrificing IFNmixMLV's acute effector advantage) and establishes a replicable framework for rational immune architecture design applicable across veterinary vaccine development.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported.

Ethics statements

All animal work was performed under the approval of the USDA-ARS National Animal Disease Center Institutional Animal Care and Use Committee (ACUP#ARS-2015-2809 and ACUP#ARS-2019-818) and laboratory analysis under the approval of Kansas State University Institutional Review Board (IBC 1732).

Financial support and Acknowledgements

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Keywords

Systems Vaccinology • PRRSV • Interferon • Innate immunity • Adaptive Immune Signatures • Host-Pathogen interaction

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O.11 • ID78

GENERATION OF PRRSV-EPITOPE MHC CLASS I TETRAMERS TO INVESTIGATE CD8 T CELLS AT THE MATERNAL-FOETAL INTERFACE IN INFECTED SOWS

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Introduction and objectives

A substantial part of porcine reproductive and respiratory syndrome (PRRS) research has focused on the respiratory form of the disease. However, the reproductive form accounts for considerable economic losses in the swine industry. Effector CD8 T cells have been reported to be significantly enriched in the foetal placenta following PRRSV-1 AUT15-33 infection in late gestation, raising the question of the origin of these cells and their role in protection versus immunopathology. This project aims to understand the PRRSV-induced reproductive failure by characterising the virus-specific CD8 T-cell response at the maternal-foetal interface. For this, we are developing major histocompatibility complex class I (MHC-I) tetramers to identify CD8 T cells targeting PRRSV-1 AUT15-33 (GenBank MT000052) in inbred Babraham pigs, which are homozygous for their MHC.

Methods

Pregnant Babraham sows were infected intranasally with PRRSV-1 AUT15-33 at day 89 of gestation and euthanised 20 days later. Peripheral blood mononuclear cells were isolated and cryopreserved. In a broad, unbiased screening approach, pools of 15-mer peptides overlapping by 10 amino acid residues derived from PRRSV-1 AUT15-33 non-structural proteins (NSP) 1-12 and the structural M protein were assessed for T-cell reactivity.

Results

Initial IFN- γ ELISpot assay screening identified four peptide pools (1, 3, 4, and 11) that induced a strong response. These pools were further analysed by intracellular cytokine staining (ICS) to characterise CD8 T cell responses. Most peptide pools were efficiently recognised by CD4 and CD8 T cells, resulting in the production of IFN- γ and TNF. This motivated us to screen a set of overlapping 15-mer peptides derived from the M protein by ELISpot which led to the identification of three putative epitopes that are currently being tested for CD4 and CD8 T-cell reactivity. In parallel, we used the NetMHCpan-4.2 and IEDB prediction tools to identify NSP and M protein derived peptides with the capacity to bind to the Babraham class-I SLA-1*14:02 and SLA-2*11:04 alleles. Given the superior T-cell reactivity to peptide pool 1 (representing NSP1 and 2) and previous identification of promiscuous T cell epitopes on the M protein, we focused on those regions and identified in silico 26 9-mer peptides which are currently in synthesis and will be tested by IFN- γ ELISpot and ICS assays.

Discussion and conclusions

Based on these results, we will generate SLA-1*14:02- and SLA-2*11:04-based tetramer reagents to track CD8 T cells in the maternal endometrium and foetal placenta of AUT15-33-infected sows, with the aim of identifying the origin of PRRSV-reactive CD8 T cells isolated from the foetal compartment. These experiments will be complemented by high-dimensional flow cytometry, single-cell RNA-seq and spatial transcriptomics analyses of the maternal-foetal interface in PRRSV-infected Babraham sows to generate a comprehensive, spatially resolved cell atlas and provide a detailed picture of the PRRSV-driven immune response at this critical interface.

Conflicts of interest

The authors declare no competing interests.

Ethics statements

Animal experiments were approved by the ethical review processes of The Pirbright Institute and were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

O.12 • ID80

MUCOSAL INDUCTION OF TRAINED IMMUNITY REDUCES SUSCEPTIBILITY TO PRRSV INFECTION IN PIGS

Aida Tort Miro

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Introduction and objectives

The swine production industry is constantly challenged by multiple infectious diseases. To minimize their economic impact, immunostimulants have emerged as a promising strategy to enhance animal disease resilience. In particular, immunostimulants that induce innate immune memory (trained immunity) can enhance non-specific responses against secondary infections. In this line, bacterial-based immunostimulants have proven to be cost-effective inducers of trained immunity. We have previously demonstrated *in vitro* that the heat-inactivated bacterium *Rothia nasimurium* (HI-Ro) confers a long-term antiviral state in porcine alveolar macrophages (PAMs). However, the underlying mechanisms of this response were not investigated.

Methods

Here, we aimed to confirm that *in vitro*-treated cells acquire a trained immunity phenotype and to evaluate whether intranasal administration of HI-Ro reduces susceptibility to porcine reproductive and respiratory syndrome virus (PRRSV), one of the most impactful diseases in swine worldwide.

Results

We demonstrate that priming of porcine macrophages with HI-Ro results in an enhanced cytokine response upon LPS restimulation, which is abrogated by inhibition of metabolic and epigenetic pathways associated with trained immunity. Importantly, these cells also showed an increased cytokine response upon PRRSV infection *in vitro*. We then performed a dose-response study *in vivo* and identified an effective intranasal dose that induces a trained immunity phenotype in treated pigs, as evidenced by ex vivo stimulation and infection of PAMs. Finally, a proof of concept study using the selected HI-Ro dosing regimen showed that treated pigs are less susceptible to challenge with the virulent PRRSV strain Rosalia. Specifically, HI-Ro-treated animals showed reduced mortality, lower viral loads, diminished pulmonary damage, and improved average daily gain compared with untreated controls. Importantly, HI-Ro treatment also enhanced PRRSV-specific antibody and cellular immune responses following challenge.

Discussion and conclusions

Altogether, these results demonstrate that HI-Ro induces trained immunity in porcine macrophages, leading to a long-lasting protective effect against PRRSV infection. This study highlights the potential of trained immunity-based treatments as a complementary strategy to control PRRSV and improve swine health.

Conflicts of interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics statements

All animal experiments were conducted in accordance with the European Directive 2010/63/EU for animals experiments and the Spanish legislation (RD 53/2013) on the protection of animals used for scientific purposes.

Keywords

trained immunity • porcine alveolar macrophages

O.13 • ID60

PRRSV-ANERGIC STATUS IN VACCINATED SOWS: IMPACT ON VACCINE EFFICACY AND PIGLET CHALLENGE OUTCOMES

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1. Sciensano; 2. Ughent

Introduction and objectives

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) remains a significant threat to swine production globally. While vaccination is widely implemented, its effectiveness can be compromised by maternally-derived antibodies (MDAs). Field observations revealed the presence of repeatedly vaccinated sows that remain PRRSV-seronegative despite multiple vaccination attempts. This study aimed to evaluate how the immunological non-responsiveness of these sows influences MDA levels and subsequent PRRSV vaccination and infection outcomes in their offspring.

Methods

Two experimental studies were conducted. In Study 1, piglets from vaccinated PRRSV-seropositive sows (E+; with MDAs) and PRRSV-seronegative or weakly seropositive sows (E-; without MDAs) were challenged with PRRSV-1. Viral replication, shedding, antibody responses, and interferon-gamma production were monitored. In Study 2, piglets from both groups were vaccinated against PRRSV prior to viral challenge. Post-vaccination antibody production and post-challenge viremia and nasal shedding were assessed.

Results

In Study 1, E- piglets displayed significantly higher viral replication and shedding early post-infection compared to E+ piglets, indicating reduced initial protection. However, E- piglets demonstrated stronger immune responses, including earlier seroconversion, higher antibody titers, and greater IFN- γ production. In Study 2, vaccination efficacy differed markedly between groups. Among vaccinated E+ piglets, 7 of 15 failed to produce antibodies post-vaccination, whereas nearly all E- piglets responded robustly. Post-challenge, vaccinated E- piglets and E+ piglets with observed antibody responses exhibited lower viremia and nasal shedding, demonstrating enhanced protection. Conversely, E+ piglets that failed to respond serologically showed reduced protection against challenge infection.

Discussion and conclusions

These findings underscore the challenges inherent in managing PRRSV vaccination in herds with heterogeneous MDA populations. While maternal antibodies provide short-term passive protection, they simultaneously interfere with active vaccine-induced immunity in some piglets. The immunological non-responsiveness of certain sows creates populations of piglets with varying MDA levels, resulting in differential susceptibility to infection and variable vaccine responses. Piglets lacking MDAs face increased viral exposure early in life, potentially amplifying herd transmission rates. Conversely, high MDA levels can suppress vaccine efficacy through antibody-mediated interference. These findings highlight the urgent need for refined vaccination strategies that account for MDA heterogeneity, such as modified vaccination timing or alternative vaccine formulations, to ensure robust immunity across all piglets and improve overall herd health management and PRRSV control.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

All animal procedures were conducted in accordance with institutional animal care and use guidelines and approved by the relevant ethical review board.

Financial support and Acknowledgements

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Keywords

Immunology • PRRSV • Maternal immunity

O.14 • ID109**ISOLATION AND CHARACTERIZATION OF NATURALLY OCCURRING PRRSV NEUTRALIZING MONOCLONAL ANTIBODIES TO SUPPORT STRUCTURAL VACCINOLOGY**

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Introduction and objectives

Current vaccines provide variable cross-protection against the extensive genetic diversity of circulating porcine reproductive and respiratory syndrome virus (PRRSV) strains. Structural vaccinology offers a promising strategy for the rational design of broadly protective vaccines through the identification of conserved neutralising epitopes. To support this approach, we aimed to isolate monoclonal antibodies (mAbs) from hyperimmunised pigs whose sera were capable of broad neutralisation.

Methods

Three sows with broadly neutralising serum activity against both PRRSV-1 and PRRSV-2 were boosted with a PRRSV-1 modified live virus vaccine. Serum reactivity against recombinant PRRSV-1 soluble (s)GP2, sGP3 and sGP4, as well as cross-reactivity against the corresponding PRRSV-2 glycoproteins, was confirmed by ELISA. Recombinant GPs were assembled into fluorescent tetramers and used as bait to isolate antigen-specific single B cells by flow cytometric sorting. Immunoglobulin heavy and light chain variable regions were amplified by PCR, cloned into expression vectors containing porcine antibody constant regions, and expressed as recombinant mAbs in CHO cells. Antibody specificity was assessed by ELISA using recombinant glycoproteins and purified PRRSV-1 virions, followed by virus neutralisation assays in MARC-145 cells.

Results

A total of 54 recombinant mAbs were successfully expressed, of which six specifically recognised recombinant PRRSV-1 sGP3 and bound purified PRRSV-1 virions, demonstrating recognition of the native viral antigen. Importantly, three of these sGP3-specific mAbs exhibited neutralising activity against PRRSV-1 *in vitro*.

Discussion and conclusions

These findings validate this strategy for the isolation of PRRSV-specific mAbs from pigs and identify the PRRSV GP3 ectodomain as a target of naturally occurring neutralising antibodies. These mAbs can now serve as valuable tools to elucidate epitopes and support structure-guided vaccine design.

Conflicts of interest

None.

Ethics statements

Animal work was approved by the Ethical Committee of the University of Lleida and the Government of Catalunya (Departament d'Acció Climàtica, Alimentació i Agenda Rural) under Project Licence 12028 and conducted in accordance with Directive 2010/63/EU.

Keywords

PRRSV • Neutralisation • Monoclonal antibody • glycoprotein

O.15 • ID36

VALIDATION OF GENOMIC PREDICTION FOR DISEASE RESILIENCE TRAITS AND THEIR GENETIC RELATIONSHIP WITH SELECTION INDEX TRAITS

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Introduction and objectives

Breeding pigs for enhanced, general resilience to disease is an attractive solution for disease control. Since 2018, Topigs Norsvin collects data from crossbred animals of various genetic lines under disease-challenged conditions, including exposure to PRRSV and other pathogens. These data show a heritable host response to PRRS challenge, supporting the use of genomic selection to breed for enhanced, natural disease resilience. However, facilitating genomic selection for enhanced disease resilience requires on-going data collection under disease-challenged conditions. To assess the value of the current method of data collection, the objectives of this study were to 1) evaluate the accuracy of genomic prediction for disease resilience traits using data collected across genetic lines, and under various challenged conditions, and to 2) understand the potential impact of selection for improved disease resilience on performance under non-challenging conditions, by estimating correlations between disease resilience traits and production traits.

Methods

Phenotypic data were available for six disease challenge trials and used to estimate two disease resilience traits: average daily gain (ADG) and mortality (MOR) post-challenge, both estimated using data collected within the first 28 days post-infection. Each pig was genotyped using a custom 25K single nucleotide polymorphism chip. For the first objective, accuracy of breeding value estimation was assessed for ADG and MOR using the linear regression (LR) method, where the most recent challenge trial was used as validation dataset, and prior challenge trials were used as training dataset. For the second objective, the genomic relationship matrix was enlarged to include purebred ancestors. Breeding values for ADG and MOR were then correlated to breeding values for 106 other traits, available from routine genomic evaluation. The assessment of the correlations included 14,906 relatives covering three generations.

Results

Results of the LR validation showed a prediction accuracy of 0.49 for ADG, with a small upward bias (2.30 grams) but no evidence of dispersion (0.92). For MOR, prediction accuracy was 0.64, with no evidence of bias (−0.06) or dispersion (0.97). Correlations between ADG and MOR and 106 other traits from routine evaluations were small. The two disease resilience traits were favorably correlated with each other (−0.30), where greater ADG was associated with lower MOR. Slightly favorable correlations were found between breeding values for MOR with piglet vitality (−0.10), and for ADG with sow longevity (0.12). A slightly unfavorable correlation was found between MOR and total feed conversion (−0.12) and between ADG and gestation length (0.12).

Discussion and conclusions

Results from this study demonstrate that data collected across diverse genetic lines and challenge conditions can be used as a reference population to facilitate genomic selection for enhanced resilience to PRRS and other diseases. Correlations between disease resilience traits with other traits in the breeding program were low. As such, selection based on ADG and MOR presents a promising opportunity to select pigs for enhanced resilience to disease without compromising performance under non-challenged conditions.

Conflicts of interest

CAS, SW, JD, LV, and EG were employed by the Topigs Norsvin Research Center (‘s-Hertogenbosch, Netherlands).

Ethics statements

All data was collected according to Pork Quality Assurance (PQA) guidelines, and with veterinary oversight.

Keywords

PRRSV • breeding • growth • mortality • resilience

O.16 • ID134

ENHANCING BIOSECURITY IN SWINE OPERATIONS: INVESTIGATING WILDLIFE INTERACTIONS ON MORTALITY MANAGEMENT STRUCTURES OF SWINE FARMS

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Introduction and objectives

Wildlife activity around swine mortality management structures is often reported; but little is known about species, frequency, and behaviors exhibited around these sites, limiting our understanding of their role in farm biosecurity. Wildlife potentially interacting with carcasses has implications for endemic diseases and emerging diseases such as African swine fever (ASF), which continues to occur in Europe. Beyond this, wildlife may serve as a source for pathogen spillover, including avian influenza (H5N1) and other pathogens. Documenting and understanding wildlife interactions with mortality management structures may help identify preventive measures industry stakeholders can take to mitigate these risks. The objective of this study was to monitor wildlife visiting swine mortality management structures and characterize the species, frequency, and behaviors observed around these sites.

Methods

Solar-powered, motion-activated, cellular network-connected cameras were installed facing mortality management structures (mostly composting bins and dead-boxes used for rendering) on 30 commercial US swine farms in four different states. Cameras were installed from Nov 2025 to Jan 2026 and will remain capturing data up to Dec 2026. Here, we present results up to Feb 13th, 2026. The number of videos recorded per camera per day was used to define days as atypical (those exceeding the $\bar{x} + 2$ SD) or typical. To identify wildlife species and characterize observed behaviors, we watched all recordings from typical days in search for wildlife recordings, while 50 random recordings from atypical days were watched. Wildlife was classified broadly according to species and we here report the frequency in which recordings from animals of different species were made, as well as the behavior they displayed while interacting with the mortality handling structures.

Results

As of February 13th, 2026, a total of 100,043 videos (roughly 200 hours of footage) captured from 1,576 camera-days were recorded. Wildlife activity was detected in 13.3% of recordings. The most frequently observed species were black and turkey vultures (*Coragyps atratus* and *Cathartes aura*), coyotes (*Canis latrans*), rodents (likely *Rattus norvegicus*), crows (*Corvus* spp.), opossums (*Didelphis* spp.), skunks (*Mephitidae* spp.), bald eagles (*Haliaeetus leucocephalus*), cattle (*Bos taurus*), domestic animals (cats and dogs), owls (*Strigiformes* spp.), followed by other less common species. Wildlife activity occurred predominantly during the night. Direct interaction with carcasses, such as feeding and dragging were documented. Animals were also recorded climbing and digging on compost piles. Birds were recorded both consuming carcasses, as well as flying from the mortality management structures towards nearby barn ventilation systems.

Discussion and conclusions

A diverse set of wildlife species was observed accessing swine mortality management structures and engaging in behaviors that could mechanically transport contaminated material within and potentially between farms. Understanding the frequency, timing, and nature of these interactions is essential for evaluating how wildlife may contribute for pathogen spread risks and informing future risk assessments for endemic and emerging swine diseases, including African swine fever. These findings highlight the need for enhanced wildlife prevention around mortality management systems, potentially reducing opportunities for pathogen dissemination.

Conflicts of interest

The authors did not have any conflict of interest.

Ethics statements

N/A

Financial support and Acknowledgements

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Keywords

Wildlife • Biosecurity • Pathogen transmission • Swine mortality management • African Swine Fever

O.17 • ID76

ROAD-LEVEL TRANSMISSION RISK OF PRRS IN THE UNITED STATES SWINE NETWORK: A SPATIOTEMPORAL BAYESIAN FRAMEWORK LINKING ANIMAL MOVEMENT TO FARM INFECTION

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Introduction and objectives

Porcine reproductive and respiratory syndrome (PRRS) represents the most economically significant endemic disease affecting U.S. swine. Although contaminated vehicle road pathways are a recognized route for between-farm transmission, their contribution to disease occurrence is rarely quantified. Conventional surveillance approaches treat the farm as the primary unit of risk, leaving the role of the contaminated road network and infected vehicle movement largely unmeasured. The extent to which routable animal-movement traffic near a premises predicts PRRS risk, and the identification of corridors that concentrate this risk over time, remain poorly characterized. Thus, this work is aimed to elucidate the relation between transportation roads and PRRS by quantifying the association between road-traffic exposure and farm-level PRRS occurrence in North Carolina, United States, and translated the fitted farm risk into an operational road-segment-level transmission-risk surface.

Methods

Our data comes from the Rapid Access Biosecurity application (RABapp™), which involves academic researchers, livestock producers, state animal health officials, and industry veterinarians. The dataset included 1,868 swine premises and 74,907 pig movements recorded from January 2024 to June 2026. Outbreak data corresponded to a monthly binary outcome, defined as at least one reported PRRS event (412 events and 299 affected farms). Pig shipments were routed across 8,021 road segments retrieved from OpenStreetMap, and shortest-path algorithms were used to derive per-segment monthly pig flow. For each farm, a 10 km buffer was calculated to capture covariates: the maximum number of pig heads transited per month on each road segment (distance-decayed buffer road pressure), distance to the nearest road, and herd capacity. A Bayesian hierarchical spatial model was fitted using the Integrated Nested Laplace Approximation (INLA), modeling PRRS-positive status as a Bernoulli response with a logit link function, with covariates computed from our data and a spatial random effect specified via a stochastic partial differential equation. We used the fitted model to estimate farm-level positivity probabilities and then assessed proximity to road segments to identify risky road segments for PRRS transmission.

Results

The final model identified distance-decayed buffer road pressure as significant with an odds ratio of 1.30 (95% CI: 1.02–1.67). In contrast, maximum-segment pressure, road distance, and herd capacity were not statistically significant. Projections indicated that PRRS risk peaks in February and reaches its minimum in September. Furthermore, 5.27% of all road segments were classified into the highest-risk category, with an average nearby-farm PRRS prevalence of 0.80%. The analysis also revealed pronounced spatial clustering of risk in the southeastern area of North Carolina.

Discussion and conclusions

We demonstrated that distance-weighted animal-movement traffic near a farm was independently associated with higher odds of PRRS in North Carolina. Cold months increase the risk of roads being risky for PRRS. These findings present an opportunity to implement practical means to time and target biosecurity (truck sanitation, route planning, surveillance).

Conflicts of interest

Authors confirm that there are no conflicts of interest to declare.

Ethics statements

Since this work did not involve animal sampling or questionnaire data collection by the researchers, there was no need for ethics permits.

Financial support and Acknowledgements

SWINE HEALTH INFO CENTER 24-096 SHIC

Keywords

PRRS • Integrated Nested Laplace Approximation (INLA) • Animal-movement traffic • Risk analysis • Geoprocessing • Spatial epidemiology

O.18 • ID18

IMPACT OF PERSISTENT INFECTION WITH AN ATTENUATED AFRICAN SWINE FEVER VIRUS ON VIRAL POPULATION DYNAMICS FOLLOWING SUBSEQUENT INOCULATION WITH VIRULENT STRAINS

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Introduction and objectives

African swine fever (ASF) is a hemorrhagic infectious disease affecting domestic pigs and wild boars, caused by the African swine fever virus (ASFV). Public attention has largely focused on highly virulent strains due to their high mortality rates; however, chronic and asymptomatic forms of ASF associated with attenuated virus infections also occur in endemic regions. Moreover, prolonged viral shedding accompanied by chronic clinical manifestations, such as dermatitis, arthritis, abortion, and stillbirth, is frequently reported in pigs inoculated with licensed live-attenuated ASF vaccines. In this study, we established a persistent infection model using an attenuated ASFV and investigated viral population dynamics following subsequent inoculation with virulent ASFV strains.

Methods

We generated an attenuated ASFV by deleting the D250R gene from a virulent genotype II strain, AQS-C-1-22, and evaluated its pathogenicity in pigs. Based on its attenuated phenotype and ability to induce prolonged viremia, the D250R gene-deleted ASFV (AQSΔD250R) was used to establish a persistent infection model. 28 days after inoculation with this virus, the animals were subsequently inoculated with either a genotype-matched virulent strain, AQS-C-1-22, or a genetically distinct virulent genotype I strain, Esp75. Viral population profiles in the blood of inoculated pigs were monitored, and the biological characteristics of isolated viruses were analyzed.

Results

Pigs inoculated with the D250R gene-deleted ASFV exhibited no apparent clinical signs but developed prolonged viremia. After inoculation with the homologous genotype II virulent ASFV, the virulent virus was maintained at a low level in the pig body until the end of the experiment. In contrast, inoculation with the heterologous genotype I virulent ASFV resulted in marked alteration in virus population in the inoculated pigs. Furthermore, in these pigs, novel recombinant viruses emerged, exhibiting mosaic genomes composed of genetic material from both the persistently infecting attenuated virus and the co-inoculated virulent strain.

Discussion and conclusions

These findings indicate that persistent infection with attenuated ASFV enables prolonged viral replication in pigs, and that subsequent inoculation with virulent strains may facilitate viral persistence and genome exchange, thereby increasing viral diversity. Such processes may have important implications for ASFV population dynamics during outbreaks, particularly in regions where attenuated ASFV strains are prevalent, including those derived from live-attenuated vaccines.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

All animal and recombinant DNA experiments were approved by the relevant institutional committees of the National Institute of Animal Health, NARO, and were conducted in accordance with institutional guidelines. Approval numbers: R5-I047-NIAH and 5文科振第1079号

Financial support and Acknowledgements

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Keywords

African swine fever virus (ASFV) • persistent infection • superinfection • chimeric (recombinant) ASFV

O.19 • ID123

CLINICAL, HISTOMORPHOLOGICAL AND MOLECULAR PROFILING OF SWINE ENTERIC CORONAVIRUS INFECTION: INSIGHTS FROM MICRORNA AND TARGET GENE EXPRESSION IN PIGLETS

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Introduction and objectives

Swine enteric coronaviruses are responsible for enteric disease in piglets and continue to represent a major challenge for the swine industry. While their clinical and pathological consequences have been extensively investigated, less is known about the coordinated molecular mechanisms underlying intestinal immune responses, particularly the contribution of microRNAs to the regulation of inflammation and mucosal homeostasis. Therefore, this study aimed to characterize the response to PEDV, SeCoV, and rPEDV–SeCoV infection by integrating clinical data, growth, histomorphometry, viral load, and immune-related gene and microRNA expression.

Methods

Forty-eight three-week-old piglets were randomly allocated into four experimental groups: control (A), PEDV (B), SeCoV (C), and rPEDV–SeCoV (D). Animals were orally inoculated and subsequently subjected to homologous (D) or heterologous (B, C) re-challenge 20 days after the initial infection. Clinical signs, growth performance (ADG), histomorphometric parameters, and viral load in duodenum and jejunum were assessed at 3 and 6 dpi (primoinfection), and at 23 and 26 dpi (after re-challenge). Relative expression of selected target genes (TJP1, IL8, IL10, TNF, TGFB1, FOXP3, IFNG), together with ten miRNAs (miR-21, miR-124, miR-15b, miR-19a, miR-17, miR-146a, miR-155, miR-223, miR-375 and miR-27b) were analyzed by RT-qPCR and statistically processed.

Results

Challenged groups exhibited transient diarrhea, reduced ADG, and lower villus and mucosal height, partially recovering by 26 dpi. Viral RNA peaked at 3 dpi and persisted longer in heterologous re-challenge groups. Anti-inflammatory miRNAs were upregulated after re-challenge, showing moderate positive correlations with cytokines: miR-146 and miR-223 with IL8 in the duodenum ($r=0.5$; $p=0.0003$), and miR-27b with IFNG in the jejunum ($r=0.5$; $p=0.0002$). Pro-inflammatory miRNAs also increased, with miR-15b correlating with IFNG (duodenum: $r=0.53$, $p=0.0001$; jejunum: $r=0.53$, $p<0.0001$), and miR-155 correlating with IFNG in jejunum ($r=0.53$; $p=0.0001$).

Discussion and conclusions

Swine enteric coronavirus infection induced clinical disease, impaired growth, and intestinal damage with distinct viral kinetics between homologous and heterologous re-challenge. Specific miRNAs (miR-146, miR-223, miR-27b, miR-15b, and miR-155) correlated with cytokines, suggesting roles in inflammatory regulation. These findings highlight the need for mechanistic studies on miRNA-mediated mucosal immunity to clarify their potential as biomarkers and therapeutic targets.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethics statements

All experimental procedures involving animals were approved by the Institutional Animal Ethics Committee of the University of León (Reference Numbers OEBA-ULE-006-2019 and OEBA-ULE-013-2020). Animal handling and experimental protocols were conducted in accordance with current European legislation on animal welfare and the protection of animals used for scientific purposes.

Keywords

Swine enteric coronavirus • Piglet • Intestine • microRNA • Immunity

O.20 • ID70

NON-CANONICAL TRS USAGE AND FUNCTIONAL ANALYSIS OF THE ORF6 REGION OF A TYPE I PRRSV STRONGLY SUGGESTS THE TRANSLATION OF A NOVEL ESSENTIAL PROTEIN 6A

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Introduction and objectives

Literature data indicate that a subgenomic RNA (sgRNA) is transcribed from the open reading frame 6 (ORF6) region of type II PRRSV, potentially enabling the translation of ORF6a in an alternative frame. We hypothesized that ORF6a is also present in type I PRRSV and is preceded by a functional transcription regulatory sequence (TRS). **Objective:** Identification of non-canonical TRS sites in the ORF6 region of a type I PRRSV genome, as well as studying the functionality of the potential 6a ORF through mutation analysis on a type I PRRSV infectious clone.

Methods

The presence of TRS sites associated with ORF6 and 6a was determined by using RT-PCR, sequencing and sequence analysis. To map the 5' end of the ORF6 region, nested RT-PCR was performed using primers specific to the 5' UTR and ORF6. The resulting amplicons were sequenced on an Illumina platform to identify and quantify sgRNAs capable of initiating translation within the 6a region. In parallel, a type I PRRSV infectious clone was used to evaluate whether the integrity of ORF6a is essential for virus replication.

Results

As expected, a high volume of reads (216,245) mapped to the canonical ORF6 TRS. Twelve novel TRS sites (TRS-H1-12) were identified, ten of which were at low abundance and two were at moderate abundance. The substantial read counts for TRS-H2 and TRS-H9 (>11,000) strongly suggest the translation of both a 30-amino-acid ORF6a protein and a truncated version of the M protein, shortened by 29 amino acids. To assess whether modifications to the ORF6a sequence or its encoded protein are lethal to the virus, we generated an ORF6a knockout mutant and mutants with modified ORF6a amino acid sequences ensuring that the nucleotide alterations maintained the integrity of the overlapping M protein sequence. These mutants were not viable.

Discussion and conclusions

Our results indicate that type I PRRSV expresses an ORF6a protein from an additional sgRNA. Mutation analysis of the ORF6a strongly suggests that protein 6a is essential for type I PRRSV replication.

Conflicts of interest

Zoltán Zádori is the owner of Avirtransvac Ltd., which is a collaborator and a contractual partner of MSD Animal Health.

Ethics statements

Animal housing complied with European Directive 2010-63 on the protection of animals used for scientific purposes (<https://eur-lex.europa.eu/eli/dir/2010/63/oj>). Each animal trial was performed in compliance with the Dutch law on Animal Experiments. In addition, the protocol of each animal trial was checked against the license and animal welfare criteria by the Animal Welfare Body of MSD Animal Health.

Keywords

PRRSV • ORF6 • ORF6a • essential protein

O.21 • ID16

EVALUATION OF DOSE-DEPENDENT VIRULENCE OF PRRSV-1 “ROSALÍA” IN WEANED PIGLETS AND THE MODULATING ROLE OF MATERNAL ANTIBODIES

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Introduction and objectives

The highly virulent PRRSV-1 Rosalía strain has caused unprecedented clinical impact in Spain since 2020, highlighting the need for robust challenge models to assess vaccine efficacy. Determining how different challenge doses interact with pre-existing maternal antibodies is particularly relevant in weaned piglets, as maternally derived immunity can substantially shape early infection dynamics. This study evaluated three intranasal challenge doses of PRRSV-1 Rosalía and examined the extent to which maternal antibodies modulate viral replication, clinical expression, and pathological outcomes.

Methods

Twenty-seven piglets were assigned to three infected groups (10⁴, 10⁵, or 10⁶ TCID₅₀) and one control group. Pre-challenge ELISA status (maternal antibody–positive vs. negative) was used as a stratifying variable. Animals were monitored for 14 days for clinical signs, temperature, growth, viremia, nasal shedding, seroconversion, and lung pathology. Viral RNA was quantified via qPCR; antibodies were measured using IDEXX PRRS X3 ELISA.

Results

All challenge doses established infection, with serum viral loads peaking at day 7. Differences between challenge doses were modest; however, maternal antibody status produced consistent and biologically meaningful contrasts. Antibody-negative piglets showed lower Ct values (higher viral load) at peak viremia—particularly in the 10⁶ TCID₅₀ group—and exhibited accelerated seroconversion kinetics. Clinical expression was also modulated by maternal immunity: although fever patterns were similar, growth suppression was markedly greater in antibody-negative animals, again most evident under high-dose challenge. Lung-to-body weight ratios and histopathology confirmed a dose-dependent inflammatory response, but maternal antibody–positive pigs consistently showed milder lesions.

Discussion and conclusions

These findings suggest that maternal antibodies blunt early viral replication and reduce downstream inflammation, even when facing a highly virulent PRRSV-1 strain. Importantly, high-dose challenge amplified these contrasts, increasing the sensitivity of detecting immunity-associated differences. Maternal antibodies significantly modulate infection outcomes under PRRSV-1 Rosalía challenge, reducing viral load, lung inflammation, and growth loss. High-dose challenge (10⁶ TCID₅₀) most reliably differentiates immune-status groups and is recommended for PRRSV vaccine efficacy studies in weaned piglets.

Conflicts of interest

This study was funded by Boehringer Ingelheim.

Ethics statements

The experimental protocol was approved by the Ethics Committee for Animal Experimentation of IRTA-CReSA and authorized by the competent authority of the Government of Catalonia (protocol no. XXX). All procedures complied with Directive 2010/63/EU. Experimental challenges were conducted in BSL-2 facilities under strict biosafety and animal welfare conditions.

Financial support and Acknowledgements

This study was funded by Boehringer Ingelheim.

Keywords

PRRS • Rosalia • Infection model • Maternal antibodies



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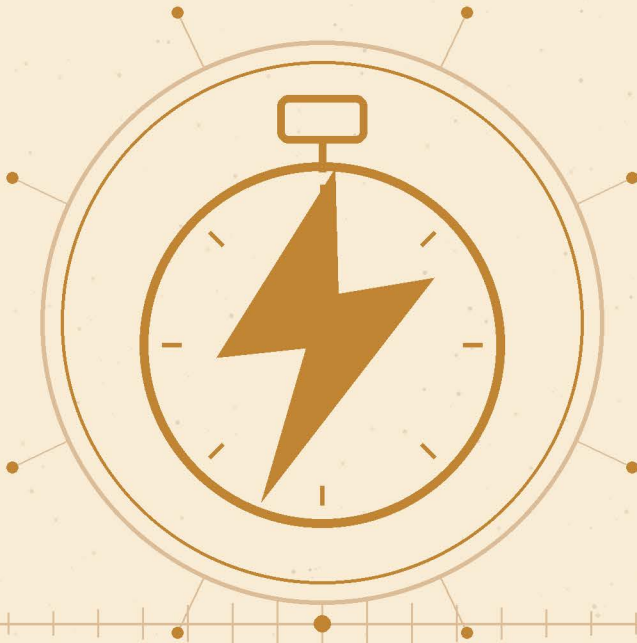
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F.01 • ID46

EVALUATING THE ECONOMIC IMPACT OF EARLY INTERVENTIONS AGAINST PRRSV USING MULTIVARIATE SYNDROMIC SURVEILLANCE IN SPANISH SWINE BREEDING HERDS

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus causes major economic impacts, estimated at 380.82 million USD annually in the breeding phase alone. Early detection through syndromic surveillance can provide an actionable intervention window to control outbreaks and limit losses at the source farm and in downstream wean-to-finish operations. This study aimed to apply multivariate time series analysis with change-point detection to identify early signs of outbreaks in swine breeding herds and quantify the economic impact of early intervention.

Methods

Weekly production data and diagnostic results from two Spanish swine breeding herds (farm A: average 3517 sows, and farm B: average 3248 sows) experiencing PRRSV outbreaks were analysed from week 1 of 2023 to week 17 of 2026. Fifteen key performance indicators were monitored, including dead sows and abortions. PRRSV status was checked weekly by PCR (farm A: 74 positive, 86 negative weeks; farm B: 53 positive, 70 negative weeks). An outbreak was considered detected if at least one alarm occurred within the 8 weeks before outbreak confirmation, following approaches used in previous studies. A per-farm baseline model (Principal Component Analysis (PCA)) was fitted on a training subset of PRRSV-free weeks. Change-point detection methods were performed using parametric (i.e., Pruned Exact Linear Time (PELT) algorithm) and non-parametric tests (i.e., E-Divisive method). In addition, an economic analysis was performed using farm-derived outbreak losses, intervention costs, and disease-spread costs on downstream farms. Net benefit and return on investment (ROI) for a scenario in which redirecting animal flow was estimated.

Results

The results showed that the model with the best performance was PELT with a sensitivity of 0.90, a specificity of 0.71, and an average early detection of 5.6 weeks. The E-Divisive was the second best with a sensitivity of 0.75, a specificity of 0.82, and an average early detection of 5.8 weeks. The average lead time observed across the farms evaluated was 6 weeks, at which point the average net benefit reached 638,010 euros and ROI reached 15,918%. The economic analysis also showed that increasing the lead time for detection from 0 to 8 weeks increased the average net benefit from a loss of 1,150 euros to a gain of 851,064 euros, while ROI increased from -100% to 17,155%.

Discussion and conclusions

Multivariate syndromic surveillance can aid in detecting PRRSV outbreaks early, and the average 6-week alarm anticipation can provide a clinically and economically meaningful intervention window. The net benefit reflected the downstream cost avoided by halting positive pig shipments to nurseries or fattening units early, even when source farm losses continue. The findings support the integration of automated production surveillance into PRRSV management protocols in commercial swine breeding herds.

Conflicts of interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this abstract.

Ethics statements

At this stage, the research is entirely methodological and does not involve direct animal experimentation, farm visits, or surveys with human participants. The production system has agreed to provide retrospective production and diagnostic data, provided that the farms remain fully anonymised.

Financial support and Acknowledgements

The study was funded by the Boehringer Ingelheim PRRSV Research Award. The authors would like to thank CEFUSA and the Department of Physiology of the University of Murcia, Spain.

Keywords

Anomaly detection • Early detection • PRRSV • Economic impact

F.02 • ID104

ESTIMATION OF THE HAZARD AND VULNERABILITY DUE TO PRRS THROUGH A HEALTH RISK INDEX BASED ON DATA ANALYTICS

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Introduction and objectives

Health Risk analysis is an process designed to estimate the probability of an unwanted biological event. It uses a methodology to transform uncertainty into measurable metrics. This study aims to quantify the Hazard and Vulnerability for the Health Risk of PRRS in municipalities with registered pig production in the Commercial Stratum in Colombia.

Methods

A multivariate model integrating the Hazard and Vulnerability Components was developed, called the Sanitary Risk Index (SRI). Following a Systematic Information Review, Risk Categories and Factors were defined, weighted, and prioritized using Expert Criteria. This process integrated 68 datasets with 433.988.574 data points associated with 5.181 commercial farms which had 9.428.558 pigs (85% of the total population) by the year 2.024. The resulting values for Factors, Categories and Components were normalized to a probability index from 0.00 to 1.00, then spatialized at the municipal level.

Results

Six Categories comprising 28 Risk Factors were defined and spatialized: two Categories for the Hazard Component and four Categories for the Vulnerability Component. SRI ranges from 0.30 to 0.84, with an average of 0.52 for 5.181 farms included in the study. Of these, 0.00% face 0.00-0.40 and 0.80-1.00 risk levels; 3.70% face 0.60-0.80 risk levels in 1 department (1 municipality) with 1.408 animals of the 9.428.558 pigs registered; 96.30% face 0.40-0.60 risk levels in 26 departments (806 municipalities) with 9.427.150 pigs. The Hazard Component ranges from 0.03 to 1.00, with an average of 0.45. The Vulnerability Component ranges from 0.42 to 0.85, with an average of 0.57.

Discussion and conclusions

Regions with SRI between 0.40 and 0.60 amplify virus circulation during an outbreak and facilitate its territorial expansion by acting as epidemiological transition zones. Although they do not reach the critical levels of the main nodes, they have sufficient swine density and connectivity to serve as transmission corridors to other areas. There are no areas with low Risk levels, suggesting that implementing adaptive capabilities and effective knowledge management moves producers toward higher levels of health security, even in challenging environments. The values of the Hazard and Vulnerability Components are close, but Vulnerability is higher due to informal production and biosecurity deficiencies. The Hazard Component is driven by viral circulation and the intense movement of animals. These results highlight opportunities for improvement in biosecurity and management in regions with a high concentration of animals under medium-risk conditions. Health Risk Quantification is a strategic tool that enables the identification of farms, municipalities and departments at different PRRS risk levels. It is an instrument that strengthens and supports strategic decision-making to improve biosecurity measures and epidemiological surveillance and to optimize human and economic resources.

Conflicts of interest

The authors state there are no conflicts of interest.

Ethics statements

The authors declare that the work presented here was conducted with full transparency and integrity, in accordance with Colombian regulations. All individuals involved disclosed any conflicts of interest and safeguarded the confidentiality of private or sensitive data. Accordingly, the academic methodological approach adopted is grounded in honesty, accountability and respect. This yields a high-quality solution, acknowledges collaborators' work and addresses challenges objectively and fairly.

CONTINUE >

Financial support and Acknowledgements

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Keywords

Hazard • Vulnerability • Health Risk • PRRS

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F.03 • ID64

PREVALENCE AND EPIDEMIOLOGICAL ROLE OF PORCINE RESPIRATORY CORONAVIRUS, PORCINE HEMAGGLUTININATING ENCEPHALOMYELITIS VIRUS AND INFLUENZA D VIRUS IN SPANISH HERDS

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Introduction and objectives

Porcine respiratory disease complex (PRDC) is one of the costliest health problems for commercial pig farms. In this study, we aimed to investigate the prevalence of three neglected swine respiratory viruses—porcine hemagglutinating encephalomyelitis virus (PHEV), porcine respiratory coronavirus (PRCV) and influenza D virus (IDV)— in samples submitted for diagnosis or monitoring of porcine reproductive and respiratory syndrome virus (PRRSV) or swine influenza A virus (IAV). The relationship of these viruses with the development of respiratory disease and the interactions with other agents were examined.

Methods

Nasal swabs were collected from farrowing units and/or nurseries on farms (n=82) either experiencing respiratory disease (n=38) or undergoing routine respiratory pathogen monitoring (n=44). Overall, the dataset included 39 farrowing units and 50 nurseries. Diagnostic sampling consisted of at least 20 nasal swabs from sick animals and 20 from healthy counterparts, while monitoring sampling consisted of 20 randomly collected samples from farrowing units and/or nurseries. Samples were analyzed by RT-qPCR for the presence of PRRSV, IAV, PHEV, PRCV, and IDV. RT-qPCR results and farm-level data, including respiratory disease status and age category, were used for statistical analyses to identify factors associated with respiratory disease and potential pathogen interactions.

Results

At the farm level, PHEV showed the highest prevalence (79.3%), followed by IAV (72.0%), PRCV (67.1%), and PRRSV (61.0%). All samples were negative for IDV. For all pathogens except PRRSV, the proportion of positive farrowing units and nurseries was similar. In the case of PRRSV, the probability of nurseries being positive was 1.8 times higher than that of farrowing units. Farms experiencing respiratory disease outbreaks were more likely to be positive for IAV (1.55-fold) and PRCV (1.35-fold) (p<0.05). In univariate analysis, IAV showed the strongest association with disease (OR=6.5; 95% CI: 1.9–18.9). Analysis of pathogen combinations showed an additive effect of pathogen number, with each additional detected pathogen increasing the odds of a respiratory outbreak by 2.47-fold. Co-detection of IAV+PRRSV+PHEV was enriched in farms with respiratory disease outbreaks. In these farms, a generalized linear mixed model showed that PHEV was significantly associated with sick status (OR=2.2; 95% CI: 1.2–4.0; p<0.05) at the individual level. In contrast, PRCV was frequently detected but was not significantly associated with sick status at the individual level.

Discussion and conclusions

This study shows that PHEV and PRCV are widespread in Spanish farms. Despite their high detection rates data are not supporting a primary role but rather an additive role with other agents, particularly IAV or acting as agent causing only subclinical infections. Overall, the role of PHEV and PRCV in PRDC of the examined Spanish farms seems secondary but further case investigation is required.

Conflicts of interest

None to be declared.

Ethics statements

Samples were sent for diagnostic or monitoring purposes. Project 5643 (approved by the Ethics Committee of UAB) also covered sampling schemes for the epidemiological study of respiratory disease in farrowing units and nurseries.

Financial support and Acknowledgements

We gratefully acknowledge the pig-producing companies that submitted the diagnostic or monitoring samples used in the present study.

Keywords

Porcine respiratory disease complex • influenza A • PRRSV • porcine hemagglutinating encephalomyelitis virus • porcine respiratory coronavirus

F.04 • ID94

COMPARISON OF CELL SYSTEMS FOR ISOLATION AND REPLICATION ASSESSMENT OF PORCINE PARAINFLUENZA VIRUS 1

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Introduction and objectives

Porcine parainfluenza virus 1 (PPIV1) has been increasingly detected in pigs with respiratory disease; however, its precise role as a respiratory pathogen remains poorly understood. One limitation for establishing experimental PPIV1 infection models is the lack of standardized viral isolation protocols and systematic evaluation of viral replication in different cell systems. This study aimed to compare the efficiency of PPIV1 isolation in porcine cell lines and primary cells and to evaluate viral replication kinetics in these platforms.

Methods

Fifty-three low Ct (<32) PPIV1-positive field samples were inoculated onto ST, LLC-MK2, primary porcine nasal epithelial cells (PNE), and primary porcine tracheal epithelial cells (PTE) to assess the efficiency of these different platforms for supporting viral isolation. RT-qPCR was used to determine viral replication and, in parallel, development of cytopathic effect was recorded. Cultures were blindly passed up to three times before reporting a negative result. Replication kinetics in the different cell platforms were further evaluated and monitoring Ct values over a 7-day incubation period.

Results

Among the four cell systems, ST cells showed the highest PPIV1 isolation efficiency, with 27 isolates out of 53 samples (50.9%), followed by LLC-MK2 cells with 20/53 (37.7%), PNE cells with 9/53 (17.0%), and PTE cells with 5/53 (9.4%). Interestingly, for the continuous cell lines, more than half of the isolates were obtained in the second passage. Thus, for ST cells 14/27 (51.9%) isolates were obtained in the second passage, and 11/20 (55.0%) for LLC-MK2 cells. In contrast, for primary cells, most isolations were obtained in the third passages (7/9 for PNE cells and 5/5 in PTE cells). Visible CPE development varies considerably between cell types. ST and PNE cells showed the highest overall CPE development with visible CPE observed in 22/27 (81.5%) and 8/9 (88.9%) isolates, respectively. In contrast, CPE was rarely observed in LLC-MK2 cells, with only 1/20 (5.0%) isolates showing visible CPE, while no CPE was observed in PTE cells. Growth curve analysis based on Ct-value changes showed the highest PPIV1 yields were produced by ST and LLC-MK2 cells followed by PTE cells.

Discussion and conclusions

The results of this study show that PPIV1 isolation efficiency varies markedly depending on the cell system used. Among the platforms evaluated, ST cells provided the highest isolation rate, followed by LLC-MK2 cells, whereas primary nasal and tracheal epithelial cells showed lower isolation efficiency. The need for two- or three-blind passages highlights the importance of extended culture protocols before declaring samples negative. Somewhat surprisingly, primary cells were less efficient for viral isolation. Further studies are needed to determine whether these differences are related to isolate-specific receptor usage or adaptation to different cell systems. CPE development was also highly cell-type dependent, indicating that CPE alone is not a reliable indicator of successful isolation. Overall, ST cells appear to be the most practical platform for PPIV1 isolation, while primary epithelial cells may provide complementary models for studying host-cell tropism and replication kinetics.

Conflicts of interest

No competing interests

Ethics statements

Cells were obtained from animals used in other studies (approval 6171 of the Ethics Committee of UAB).

Keywords

Porcine parainfluenza virus 1 • virus isolation • cell culture • cytopathic effect • RT-qPCR

F.05 • ID75

NOVEL SWINE TROPHOBLAST ORGANOID SYSTEM TO INVESTIGATE PRRSV-PLACENTA INTERACTIONS

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Introduction and objectives

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is one of the most economically devastating diseases affecting the global swine industry, with annual losses up to \$1.2 billion in the United States. PRRSV infection causes severe reproductive failure characterized by early farrowing, late-term abortions, mummified fetuses, weak-born piglets; as well as respiratory disease in weaned and growing pigs. Reproductive failure is restricted from mid-to-late gestation, yet the mechanism by which PRRSV crosses the placenta barrier is not well understood. Swine trophoblast organoids (sTOs) are 3D models that recapitulate placental architecture and provide a relevant platform to study vertical transmission across the maternal-fetal interface. These organoids express high levels of several canonical trophoblast markers (GATA3, KRT18), with sc-RNAseq confirming sTOs differentiate into diverse cell populations native to the porcine placenta. Although sTOs express putative PRRSV markers, differences in receptor expression were observed amongst cell populations, suggesting that susceptibility may vary across trophoblast populations. Building on these findings, the objective of this study was to validate the sTO approach to investigate PRRSV-sTO interactions, and further characterize infection dynamics across sTO lines using multiple PRRSV-2 strains.

Methods

Six swine trophoblast organoid lines were derived from full-term placentas from gilts and sows of different parity. Cells were cultured in Matrigel domes and passaged every 5-7 days. For a 2D approach, sTOs were isolated from Matrigel, transferred to Ultra-low attachment plates for 48 hrs to induce polarity inversion, washed, and seeded to form 2D monolayers. Monolayers were incubated with PRRSV-2 inoculum for 2 hrs, washed, and incubated for 48 hrs. Supernatant was collected for TCID50 assay, and cell monolayer harvested for Bulk-RNAseq, RT-qPCR, or IPMA staining. For the 3D approach, cells were incubated with PRRSV-2 inoculum for 2 hrs, re-embedded into Matrigel domes, and incubated for 48 hrs. 3D cells were collected for Bulk-RNAseq or stained for porcine placental and PRRSV markers and analyzed via immunofluorescence.

Results

In 3D cultures, PRRSV viral load remained stable between 24 and 72 hrs post-infection (hpi), with altered sTO structure and visible CPE at 72 hpi. Specific PRRSV+ localized foci were identified via immunofluorescence within the 3D structure. Infection on 2D sTOs yielded stable viral load between 24 and 72 hpi. IPMA staining confirmed that epithelial cell line-adapted PRRSV-2 strains exhibited greater tropism for 2D sTOs when compared to recent field isolates grown on primary macrophages.

Discussion and conclusions

We have validated 2D and 3D sTO systems to investigate PRRSV-placenta interactions. Future experiments will assess PRRSV susceptibility across distinct sTO lines, measuring viral replication kinetics and productive infection via RT-qPCR and TCID50 assay. Bulk-RNAseq of uninfected 2D vs 3D sTOs, alongside infected vs uninfected organoids will compare the structural-driven and infection-induced genetic signatures. Immunofluorescence staining of porcine placental and PRRSV markers will detect alterations in structure and identify trophoblast subpopulations that serve as the main target for PRRSV entry. This study has established sTOs as an *in vitro* model for PRRSV infection, and will help dissect the mechanism responsible for vertical transmission at the swine maternal-fetal interface.

Conflicts of interest

The authors declare no conflicts of interest.

CONTINUE >

Ethics statements

N/A.

Financial support and Acknowledgements

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Keywords

PRRSV • Maternal-fetal interface • Placenta • Swine trophoblast organoids

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F.06 • ID128

IMPACT OF FORMALIN FIXATION TIME ON PRRSV-1 DETECTION BY RNASCOPE® IN SITU HYBRIDIZATION COMBINED WITH DEEP LEARNING-BASED DIGITAL PATHOLOGY

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Introduction and objectives

Prolonged formalin fixation can reduce nucleic acid preservation in formalin-fixed paraffin-embedded (FFPE) tissues, potentially compromising molecular detection of porcine reproductive and respiratory syndrome virus (PRRSV). RNAscope® in situ hybridization (ISH) provides highly sensitive and specific detection of viral RNA while preserving tissue architecture. This study evaluated the impact of different formalin fixation times on PRRSV-1 detection in FFPE tissues using RNAscope® ISH combined with deep learning based digital histopathology to quantify viral RNA preservation and spatial distribution.

Methods

Lung, tonsil, and tracheobronchial lymph node samples were collected from five four-week-old piglets experimentally infected with PRRSV-1 strain 3249 and euthanized at 14 days post-infection. Serial tissue samples were fixed in neutral-buffered formalin for 1, 2, 3, 7, 15, 30, or 60 days before paraffin embedding. RNAscope® ISH targeting the ORF7 region of the PRRSV-1 genome was performed. Whole-slide images were analyzed using the AI-powered digital pathology platform Visiopharm® to quantify positive area, signal density, and tissue distribution of viral RNA. Statistical analyses were performed using GraphPad Prism®.

Results

PRRSV-1 RNA was detected in all three tissues at every fixation interval. Hybridization signals appeared as multifocal dots and clusters mainly associated with macrophage-like and dendritic-like cells. In the lung, viral RNA was found mainly within areas of interstitial pneumonia and bronchopneumonia, whereas in the tonsil and tracheobronchial lymph node the highest signal density was observed in germinal centers. Quantitative analysis demonstrated a progressive decline in both RNA-positive area and signal density with increasing fixation time, with the highest reduction occurring during the first week of fixation. Despite this decrease, PRRSV-1 RNA remained consistently detectable after 60 days of formalin fixation.

Discussion and conclusions

These findings demonstrate that RNAscope® ISH, combined with deep learning based digital pathology, provides robust detection and quantitative spatial analysis of PRRSV-1 RNA in FFPE tissues, even after prolonged fixation. Although extended fixation reduces signal abundance, reliable viral detection is maintained for up to 60 days, supporting the application of this approach in routine diagnostics, retrospective studies, and veterinary pathology research.

Conflicts of interest

Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics statements

The present project was approved by the Commission on Ethics in Animal and Human Experimentation of the University of Córdoba.

Keywords

Digital Pathology • Formalin Fixation • RNAscope® In Situ Hybridization

F.07 • ID96

A STRUCTURAL-BIOINFORMATICS FRAMEWORK FOR PRECISION HOST-FACTOR ENGINEERING TOWARDS INFLUENZA-RESISTANT PIGS

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Introduction and objectives

Swine influenza remains a major challenge for animal production and global health, with economic impact and zoonotic potential. Current prevention relies mainly on vaccination and biosecurity, whereas genetic resistance remains underexplored. Most genome-editing approaches use complete knockout of individual host factors, which may compromise physiology when broadly expressed or essential genes are targeted. This study aimed to develop a structural-bioinformatics framework to guide precision engineering of porcine host factors involved in influenza infection, balancing antiviral efficacy with animal viability, postnatal development and reproductive safety.

Methods

Representative H1N1 and swine H3N2 hemagglutinin (HA) sequences were compared. Conserved receptor-binding regions were identified by multiple sequence alignment using M-Coffee. Structural analyses were conducted on porcine host factors involved in viral attachment and activation, including TMPRSS2 and respiratory membrane glycoprotein/receptor carriers predicted to display IAV-relevant sialylated glycans. ST6GAL1 and ST3GAL4 were considered pathway-defining enzymes for SAα2,6-Gal and SAα2,3-Gal receptor biosynthesis, but not direct editing targets. A glycan-aware pre-docking tissue-risk screening prioritized candidates according to membrane/extracellular localization, respiratory enrichment, predicted glycoprotein status, absence of strong reproductive/gametic enrichment and limited immune-essential function. The workflow also explored editable genomic regions predicted to disrupt virus-host interactions while preserving protein function, supporting selection of CRISPR/Cas9 knockout for non-essential genes, base editing for residue-specific substitutions, or HDR-based knock-in when precise sequence replacement is required.

Results

Comparative HA analyses identified conserved receptor-binding site residues and surrounding regions. In the H1N1 reference framework, Y95, W154, H184 and D191 were conserved across analyzed strains, whereas Q227 showed variable conservation. The region corresponding to G229 in H1N1 was detected in both H3N2 strains at shifted alignment positions, supporting conservation of functionally related receptor-binding architecture despite subtype-specific variation. Porcine TMPRSS2 modeling and comparison with human TMPRSS2 supported conservation of catalytic residues associated with HA activation. Docking analysis suggested that K344 of porcine TMPRSS2 may contact the H1N1 HA interaction region, highlighting a candidate residue-level interface for precision-editing assessment. Glycan-aware pre-docking screening prioritized CLDN18 and GPRC5A as respiratory-enriched membrane candidates, while AGER/RAGE and PDPN were retained as conditional candidates because of inflammatory-related risks. The framework separates an attachment layer, focused on candidate carriers of HA-recognized sialylated glycans, from an activation layer centered on TMPRSS2 and related host factors.

Discussion and conclusions

This study presents a bioinformatics-guided framework for precision engineering of influenza-resistant pigs. Rather than applying uniform knockout strategies, it matches each host factor with the least disruptive editing strategy. The framework aims to reduce IAV susceptibility while minimizing developmental and reproductive risks. Furthermore, the prioritized targets can be rapidly evaluated in genome-edited porcine cell models to investigate virus–host interactions *in vitro* before generating genetically modified animals, substantially reducing experimental time, costs and the number of animals required for subsequent validation studies.

Conflicts of interest

The authors declare no conflicts of interest.

CONTINUE >

Ethics statements

This work was based on in silico analyses. No animals were used in this stage of the study.

Financial support and Acknowledgements

This work received no specific financial support.

Keywords

Bioinformatics; guide precision genome editing

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F.08 • ID90

MONITORING PRRSV DYNAMICS IN DIFFERENT FARM ENVIRONMENTS: EVALUATING DIAGNOSTIC SAMPLES IN FARROWING AND NURSERY UNITS

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Introduction and objectives

Monitoring PRRS virus (PRRSV) infection is essential for understanding transmission dynamics. While serum sampling is the benchmark, alternative matrices such as oral fluids and air samples may provide valuable information on viral circulation at the population level. The objective was to monitor PRRSV infection using different samples (serum, oral fluids, stillborn tongues, air) and evaluate their temporal diagnostic patterns.

Methods

Two farms (A and B) comprising farrowing and nursery units were sampled every three weeks over a four-month period (5 samplings). The following samples were collected: Farrowing rooms: Serum from 30 suckling piglets (10–15 days old); tongues from stillborn piglets; and one air sample. Nursery rooms: Serum from 30 piglets (35–45 days old); one oral fluid sample; and two air samples. Air samples (6 m3 of air) were collected with an MD8 Airport using 50 L/min. At the laboratory, serum samples were pooled (5/pool) and gelatin filters dissolved in 5 mL of PBS. RNA extraction was performed from pools or individual samples. All extracted RNA samples were analyzed for PRRS virus by qPCR.

Results

Farm A (approaching stability in farrowing; circulation in nursery). The farrowing unit showed a trend toward stability, with only 5 positive serum pools (samplings 1-2, Cq 21.96–39.67). All air samples were consistently negative. In the nursery unit, all air and oral fluid samples tested positive (Cq 33.10–36.98 and 29.30–32.50, respectively). Viremia varied over time from absence at sampling 3 to 100% positivity at samplings 2 and 4. All sample types were negative at sampling 3. Farm B (instability in farrowing and widespread circulation in nursery). The farrowing unit remained unstable throughout the study, with all stillborn tongue samples testing positive (Cq 19.12–33.03). Most serum pools were also positive, except at sampling 2 (50 % negative). Similarly, airborne detection was successful (Cq 34.76–39.80), except at sampling 2. In the nursery unit, all serum, air and oral fluids tested positive at every sampling (Cq: 20.73-33.10; 34.21-38.40; and 29.13-31.52, respectively).

Discussion and conclusions

The results show mostly temporal agreement among sample types in the nursery unit. On Farm A, clearance of viremia at sampling 3 coincided with cessation of PRRSV detection in oral fluids and air. Conversely, airborne detection appeared less sensitive in farrowing units. Air samples remained negative on Farm A despite occasional viremic piglets, and failed to detect PRRSV on Farm B farrowing units when serum prevalence declined at sampling 2. This lower efficiency could reflect a dilution effect: the low biomass of suckling generates lower concentrations of airborne viral particles, reducing the probability of detection in the larger air volume of farrowing rooms. Under the limitations of this pilot study, our findings suggest a consistent temporal correlation between viremia and the presence of PRRSV RNA in population-based matrices. That suggests that air surveillance might be of interest in nursery. Airborne monitoring seems to exhibit diagnostic limitations in farrowing units.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

The protocols employed were approved by the Bioethics Committee of the University of Santiago de Compostela.

Financial support and Acknowledgements

This project was funded by the Spanish government (Ministerio de Ciencia e Innovación: “Proyectos de Generación de Conocimiento”. Ref. PID2022-142212OB-I00)

Keywords

Air sampling • Surveillance • Farrowing and nursery unit • Epidemiolocal monitoring

F.09 • ID89

INTERLABORATORY COMPARISON OF PRRSV GENOME AND ANTIBODY DETECTION ACROSS DIAGNOSTIC LABORATORIES IN CENTRAL EUROPE

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Introduction and objectives

Reliable laboratory diagnostics are essential for surveillance, control and eradication programmes targeting Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). Interlaboratory comparisons provide an opportunity to evaluate diagnostic performance and identify potential sources of variation between laboratories and test systems. The objective of this study was to assess the comparability and accuracy of PRRSV genome and antibody detection among participating veterinary diagnostic laboratories.

Methods

An interlaboratory proficiency test was conducted during winter 2025/2026 including 31 veterinary diagnostic laboratories from Germany, Austria, Switzerland and France. Thirty laboratories participated in PRRSV genome detection and 26 laboratories performed antibody testing. For genome detection, vaccine strains representing PRRSV type 1 and type 2 were propagated, diluted in viral transport medium and aliquoted into six blinded samples containing defined combinations of PRRSV-1 and PRRSV-2 positive and negative material. Samples were supplemented with phosphate-buffered saline and porcine lung or lymph node cells and shipped without cooling. Participating laboratories analysed the samples using their routine conventional reverse transcription polymerase chain reaction (RT-PCR) and/or real-time RT-PCR protocols (qRT-PCR). For serology, pooled PRRSV antibody-positive and antibody-negative porcine sera were prepared and distributed as six blinded enzyme-linked immunosorbent assay (ELISA) samples.

Results

Conventional RT-PCR was performed by six laboratories. Four laboratories generated results fully consistent with the reference panel, while one laboratory reported an equivocal result and one approach did not differentiate between PRRSV types. Two laboratories omitted testing of selected negative samples after prior qRT-PCR screening. qRT-PCR was performed by 30 laboratories, of which 24 achieved fully correct results across all samples. Individual laboratories reported non-evaluable or equivocal results, while false-positive and false-negative findings occurred sporadically. One assay did not allow differentiation between PRRSV-1 and PRRSV-2. The most frequently used commercial qRT-PCR systems showed overall robust performance, with isolated laboratory-specific deviations requiring local verification. Antibody detection by ELISA was performed by 26 laboratories, and all participating laboratories correctly classified all serum samples independent of the test system used.

Discussion and conclusions

This interlaboratory comparison demonstrated overall high diagnostic agreement for PRRSV detection across participating laboratories, particularly for serological testing. Genome detection by qRT-PCR showed good robustness but also revealed isolated discrepancies and differences in assay capabilities, especially regarding type differentiation and occasional false classifications. Regular proficiency testing remains an important tool for external quality assurance and contributes to maintaining reliable PRRSV diagnostics to support surveillance and control programmes.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

This study was based on an interlaboratory proficiency testing scheme and did not involve animal experimentation. Samples used for preparation of the proficiency panels originated from existing biological material and were handled in accordance with applicable institutional and national regulations.

Keywords

PRRSV • Interlaboratory comparison

F.10 • ID120

A HIGHLY VIRULENT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS STRAIN EXHIBITS MACROPHAGE AND ENDOTHELIAL CELL TROPISM IN INFECTED PIGLETS

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) remains one of the most economically important pathogens affecting swine production worldwide. The emergence of highly virulent PRRSV-1 strains, such as the Rosalia strain, has raised concerns due to their increased clinical severity and the limited understanding of their pathogenesis. Although immunohistochemistry is routinely used to assess viral distribution, its sensitivity is limited. In contrast, RNAscope® in situ hybridization (ISH) is a highly sensitive test to detect viral RNA in tissues. Therefore, this study aimed to characterize the tissue distribution and cellular tropism of a highly virulent PRRSV-1 strain using RNAscope®ISH technology.

Methods

Twenty-five 8-week-old PRRSV-naïve piglets were allocated into three groups. Groups 1 and 2 (n=10 pigs/group) were inoculated with the highly virulent PRRSV-1 Rosalia strain (Lleida 029_22; 2x10⁵ TCID₅₀) intranasally or intramuscularly, respectively, while group 3 (n=5) served as non-infected controls. Animals were monitored for 63 days post-infection (dpi) or euthanized earlier according to predefined human endpoint criteria. Lung, tonsil and lymph node samples were collected from all animals. Brain samples were collected from pigs euthanized during the acute stage of infection (14 dpi) that presented neurological clinical signs. Tissues were processed for routine histopathology and PRRSV RNA detection using RNAscope ISH.

Results

Disease severity differed markedly between inoculation routes. All intramuscularly inoculated pigs had to be euthanized by 14 dpi, whereas mortality in the intranasal group was 30% by 63 dpi. During the acute phase, histopathology revealed severe interstitial pneumonia, lymphoid depletion and lymphoid necrosis. In animals presenting neurological signs, the brain showed perivascular inflammatory infiltrates consistent with non-suppurative meningoencephalitis. In contrast, lesions were mild or absent at the end of the study. RNAscope ISH detected PRRSV RNA in all examined tissues during the acute phase, whereas no viral RNA was detected in tissues collected at the end of the study. During the acute phase, PRRSV RNA was detected within endothelial cells of the tonsils and lymph nodes in some animals (31,6%; 6/19 infected pigs). In the lungs, viral RNA was mainly located within necrotic alveolar debris and macrophages. In the brain, PRRSV RNA was detected primarily within vascular endothelial cells and, to a lesser extent, in adjacent neural tissue in animals with non-suppurative meningoencephalitis (100%; 3/3 infected pigs).

Discussion and conclusions

High-resolution ISH revealed distinct tissue-specific patterns of PRRSV-1 distribution associated with lesion severity and stage of infection. The detection of viral RNA within endothelial cells of lymphoid tissues and the brain indicates that highly virulent PRRSV-1 strains may exhibit an expanded cellular tropism compared with conventional PRRSV-1 strains. These findings provide new insights into the pathogenesis of highly virulent PRRSV-1 infection and support further investigations into the potential role of endothelial cell infection in disease progression. Additional immunohistochemistry using endothelial cell markers and digital pathology analyses are underway to confirm the endothelial localization of viral RNA and quantify its extent and spatial distribution.

[CONTINUE >](#)

Conflicts of interest

The authors declare no conflict of interest.

Ethics statements

All experiments were approved by the ethical committee of the University of Lleida and the government of Catalunya with project License 12028.

Financial support and Acknowledgements

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Keywords

Porcine Reproductive and Respiratory Syndrome • High-virulence strain • RNAscope in situ hybridization

F.11 • ID124

COMPARISON OF INFECTION OF PAM- AND MARC-145-PASSAGED PRRSV ISOLATES IN PK15CD163+ AND PK15SIALOADHESIN+CD163+ CELL LINES

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Introduction and objectives

PRRSV infection depends on host receptors, particularly Siglec1 (=Sialoadhesin) and CD163. However, whether virus propagation in different cell types affects receptor usage remains unclear. In this study, we compared the infection characteristics of PAM- and MARC-145-grown PRRSV isolates in two PK15-derived cell lines, PK15CD163+ and PK15Sialoadhesin+CD163+, to provide an *in vitro* model for investigating PRRSV receptor usage of PRRSV, grown in PAM and MARC-145.

Methods

Using a previously established PK15 cell line overexpressing both CD163 and Siglec1, Siglec1 was knocked out using CRISPR/Cas9, followed by cloning to generate a stable PK15CD163+ cell line. Both cell lines were infected with PRRSV-1 isolates (LV and 07V063) propagated either in PAM or adapted to MARC-145 cells at a M.O.I. of 0.5. Infection was assessed by immunofluorescence assay (IFA) at 0, 24, and 48 h post-infection (hpi), and infection rates were determined by the percentage of PRRSV-positive cells.

Results

PAM-grown virus LV replicated much better in PK15Sialoadhesin+CD163+ (48 hpi: 4.91%) than in PK15CD163+ (48 hpi: 0.13%). A similar pattern was found for 07V063 at 48 hpi (in PK15Sialoadhesin+CD163+: 37.97%; in PK15CD163+: 6.57%). MARC-145-grown virus LV replicated much better in PK15Sialoadhesin+CD163+ (48 hpi: 51.14%) than in PK15CD163+ (48 hpi: 0.46%). A similar pattern was found for 07V063 at 48 hpi (in PK15Sialoadhesin+CD163+: 93.02%; in PK15CD163+: 0.61%).

Discussion and conclusions

These results demonstrate that the efficiency of PRRSV infection is much better by the presence of Sialoadhesin. Indeed, in PK15Sialoadhesin+CD163+ cells, MARC-145-derived LV and 07V063 reached infection rates of 51.14% and 93.02% at 48 hpi, respectively, whereas infection in PK15CD163+ cells remained much lower. The effect of adapting and passaging the virus on MARC-145 was different for the two viruses. In PK15Sialoadhesin+CD163+ cells, MARC-145-derived LV and 07V063 infected much more efficiently than their PAM-derived counterparts at 48 hpi. In contrast, in PK15CD163+ cells, PAM-derived 07V063 infected more efficiently than MARC-145-derived 07V063, whereas LV remained at low infection levels irrespective of virus origin. More PRRSV isolates, both PRRSV-1 and PRRSV-2, are currently being tested and will be presented at the congress.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statements

This study used only *in vitro* cell culture models and did not involve animals or human samples; therefore, ethical approval was not required.

Financial support and Acknowledgements

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Keywords

PRRSV; CD163; Sialoadhesin; MARC-145; PAM

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F.12 • ID38

LIPID NANOPARTICLE ENCAPSULATION ENHANCES DNA VACCINE EFFICACY AGAINST HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS H5N1 CLADE 2.3.4.4B

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Introduction and objectives

Although highly pathogenic avian influenza virus (HPAIV) infection in pigs remains rare, the widespread circulation of H5N1 clade 2.3.4.4b viruses in poultry and multiple mammalian species raises concern that the virus could acquire mutations enabling sustained transmission in swine. This study aimed to develop and evaluate a vaccine for potential deployment if sustained pig-to-pig transmission emerges.

Methods

The HA gene from an H5N1 virus isolate currently affecting dairy cattle was cloned into a plasmid and encapsulated in lipid nanoparticles. The resulting LNP-pDNA vaccine were tested in mice and pigs. In mice, three groups were immunized with LNP-pDNA, naked pDNA, or left nonvaccinated (NV). The vaccines were administered intramuscularly on days 0 and 28. On day 49, mice were challenged with the H5N1 virus and monitored for 14 days. In pigs, four groups were immunized intramuscularly with 500 µg, 250 µg, or 100 µg of LNP-pDNA, or left unvaccinated. Blood samples were collected biweekly to assess hemagglutination inhibition (HI) antibodies and IFN-γ-secreting cells.

Results

In mice, LNP-pDNA induced high titers of virus-neutralizing antibodies, whereas naked pDNA did not do so until day 42. However, both vaccines induced comparable frequencies of IFN-γ-secreting cells. Following challenge, NV mice exhibited significant weight loss and died by day 6. Mice immunized with the LNP-pDNA vaccine showed no weight loss and all survived. In group that was immunized with the naked pDNA, one mouse died on day 7; one mouse experienced weight loss then recovered its initial body weight by day 10, and survived to the end of the study. In pigs, HI antibody responses showed a dose-dependent pattern. In the 500-µg group, HI antibodies were detected in 3 of 5 pigs by day 14 and in all pigs by day 28, with titers increasing to a geometric mean titer (GMT) of 1:485 by day 42. In the 250-µg group, low titers were detected in 2 pigs on day 14 and 4 pigs on day 28; all pigs were positive by day 42 (GMT 1:279). In the 100-µg group, HI antibodies were detected in 2 pigs on day 28 and all pigs at day 42 (GMT 1:135). HI titers in the 100-µg group were significantly lower than those in the 500-µg group on days 28 and 42, with no significant differences between the 100 µg and 250 µg groups. All vaccinated groups developed IFN-γ responses that were significantly higher than those of NV controls.

Discussion and conclusions

The LNP-pDNA vaccine provided complete protection against lethal H5N1 challenge in mice and induced dose-dependent humoral and cellular immune responses in pigs, with 100 µg sufficient to elicit detectable immunity. These findings support the potential of this LNP-pDNA vaccine as a preparedness strategy against H5N1 emergence in swine.

Conflicts of interest

H.L.X.V., T.N.N., and S.S. are inventors on a pending international patent application entitled “Methods and Compositions for Vaccine Development and Delivery”. The other authors declare no conflicts of interest.

Ethics statements

The animal experiments were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee under protocols number 2667 and 2744.

Financial support and Acknowledgements

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Keywords

Highly pathogenic avian influenza • Influenza A virus • H5N1 • Clade 2.3.4.4b • Lipid Nanoparticles • DNA vaccines

F.13 • ID114

LOCAL VIRAL LOAD SHAPES T-CELL COMPOSITION AND DIFFERENTIATION AT THE MATERNAL–FETAL INTERFACE DURING PRRSV INFECTION

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Introduction and objectives

Immune cells in the reproductive tract perform essential functions during pregnancy and T cells are involved in coordinating adaptive immune responses at the maternal–fetal interface (MFI). Transplacental transmission of porcine reproductive and respiratory syndrome virus (PRRSV) during late gestation has been well documented and is responsible for reproductive losses through infection of the MFI and local inflammatory responses that may contribute to the immunopathogenesis of the disease. The interplay of cell populations at the MFI following viral infection is yet to be characterized. In pigs, the maternal endometrium (ME) and fetal placenta (FP) represent distinct immunological compartments; therefore, we compared T cell composition and differentiation in relation to tissue viral load following experimental PRRSV infection of pregnant gilts.

Methods

Thirty-one pregnant gilts were experimentally infected with PRRSV on gestation day 85 and euthanized 3 weeks post-infection. Two to three fetuses per gilt with their corresponding ME and FP were analyzed. Mononuclear cells were isolated from each tissue and phenotyped by flow cytometry. PRRSV RNA was quantified by RT-qPCR in the ME and FP. Based on gilt and tissue infection status, samples were stratified into four groups: (I) non-infected gilts; infected gilts with (II) not detectable, (III) low and (IV) high viral load in tissue, based on tissue-specific mean viral load cutoffs calculated separately for the ME and FP. PRRSV-specific T cell responses, following *in vitro* restimulation, were assessed.

Results

CD8b+ T cells showed the greatest response to infection. Median CD8b+ proportions increased from 17.9% in non-infected to 36.5–39.9% in infected ME, and from 7.3% in non-infected to 25.0% in high viral load FP. Phenotypic analysis demonstrated a marked expansion of proliferating Ki67+ CD8b+ T cells with an early effector perforin+CD27+ phenotype, particularly in high viral load tissues. The proportion of total CD4+ and total γδ+ T cells was lower in infected groups in both ME and FP. The vast majority of γδ T cells expressed CD2, consistent with more functionally differentiated effector-like phenotype. No differences were found with regards to Ki-67 expression in CD4+ and γδ+ T cells. However, within Ki67+CD4+ T cells and Ki67+CD2+ γδ+ T cells an increase in central memory-like CD8a+CD27+ T cells could be observed at the MFI with high viral load. Following restimulation with PRRSV AUT15-33 peptide pools, PRRSV-specific CD4+ and CD8b+ T cells were identified by intracellular staining for IFN-γ and TNF.

Discussion and conclusions

These findings suggest that ME and FP tissues with higher viral load mount a strong antiviral response, reflected most strongly in the increase of effector Ki67+perforin+ CD8b+ T cells. Yet, the local increase of these activated T cells may contribute to immunopathology. Analyses are underway to finalize statistical modeling and extend immunological characterization to include B cell subsets and cytokine responses following PRRSV restimulation at the MFI.

Conflicts of interest

The authors declare that they have no competing interests.

Ethics statements

The experiment was approved by the institutional ethics and animal welfare committee (Vetmeduni Vienna) and the Austrian national authority according to 26ff. of Animal Experiments Act, Tierversuchsgesetz 2012-TVG 2012 (accession number: 2021-0.117.108).

Keywords

PRRSV • immunopathology • porcine maternal-fetal interface • T cells • CD8beta

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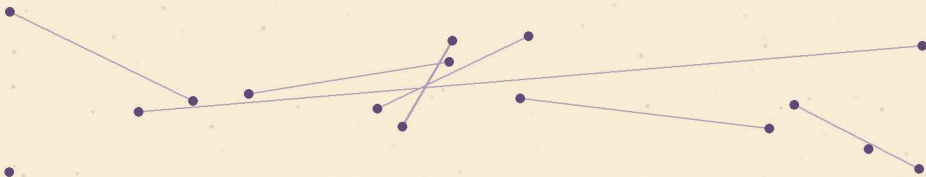
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P.01 • ID39

COMPARATIVE ASSESSMENT OF ORF5 AND ORF7 FOR EPIDEMIOLOGICAL INFERENCE IN PRRSV-1

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Introduction and objectives

PRRSV genome is composed by at least 10 Open Reading Frames (ORFs), encoding for non-structural and structural proteins. Historically, ORF5 and ORF7 were selected to investigate phylogenetic relationship between PRRSV strains, which can lead to mismatch between tree topologies. Here, we apply phylodynamics and discrete phylogeography to quantify the differences between these two fragments and how these differences affect downstream analysis.

Methods

Samples collected between 2021 to 2025 were analyzed as part of PRRSV monitoring activities in pig farms. Positive samples by qRT-PCR ($Cq \leq 30$) were subjected to Sanger sequencing targeting both ORF5 and ORF7. For both datasets, nucleotide sequences were translated into amino acid sequences and aligned with mafft algorithm. In total, 4 datasets were built: nucleotide and protein sequences of the ORF5 region (from now on, ORF5-n and ORF5-p), nucleotide and protein sequences of the ORF7 region (ORF7-n, ORF7-p). For each sample, collection date and province of origin were associated. Phylogenetic trees were built with iqtree3, while phylodynamic analysis on every dataset was performed with TreeTime. Statistical analysis on output data was performed with R 4.4.1. with dplyr and vegan packages.

Results

A total of 646 samples of PRRSV-1 positive samples were sequenced. Mutation rates (MRs) of ORF5-n and ORF5-p were almost identical, with values of $8.06 \cdot 10^{-3}$ and $8.09 \cdot 10^{-3}$ substitution/site/year, respectively. In contrast, ORF7-p MR was $4.17 \cdot 10^{-4}$ while ORF7-n MR was $9.79 \cdot 10^{-4}$. The Time to the Most Recent Common Ancestor (TMRCA) was inferred in 1943 for ORF7-n and 1964 for ORF7-p, while more recent origins were calculated for ORF5-n (2002) and ORF5-p (2004). Skyline plot revealed similar trend between amino acid and nucleotide datasets of the same ORF, but very different trends when comparing the same type of data from different genomic regions. Spearman correlation (ρ) was calculated within each ORF: between ORF5-n and ORF5-p ρ was 0.92 while for ORF7-n and ORF7-p, ρ was 0.77. Mantel's r for ORF5 datasets was 0.8989 while for ORF7 0.7586 ($p=0.0001$). Migration network analysis revealed that between ORF5 datasets there were 19 shared routes, while between ORF7 datasets were 18.

Discussion and conclusions

Phylodynamic and phylogeographic analysis play an important role in understanding the epidemiology of challenging diseases such as PRRS. Therefore, selecting the most appropriate genomic target is crucial. Among the two genomic regions, ORF5 appears to be the best candidate for inferring the spatial dynamics of PRRSV-1 population when whole-genome data are not available, by retaining both evolutionary rate estimates and phylogeographic inference when analyzing amino acid sequences. Conversely, ORF7 variability seems to be characterized by a higher amount of synonymous mutations, which contribute to a greater extent to the results obtained from nucleotide data. Although ORF5 emerges as the most informative marker, the next step toward higher-resolution epidemiological inference of PRRSV-1 will be enabled by the broader availability of whole-genome sequences, which remain underrepresented in public databases.

Conflicts of interest

None

Ethics statements

Not applicable

P.02 • ID48

ORF5 BASED PHYLOGENETIC ANALYSIS OF PRRSV-1 IN ITALY

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Introduction and objectives

ORF5 based phylogenetic analysis of PRRS-1 virus is a common tool for epidemiologic surveillance of strains circulating among farms. Recently, Yim-im *et al.*¹ defined a new PRRS-1 genetic classification system, statistically supported and more accurately considering geographical distribution and temporal dynamics. In this study, we present the results of an ORF5 phylogenetic analysis developed according to Yim-im *et al.*'s scheme with data collected during a period of 8 years of PRRS monitoring in Italy, and we compared the cluster stratification obtained with the previous one based on Balka *et al.*².

Methods

The present analysis include 332 PRRSV-1 ORF5 sequences collected during IZSVe routine monitoring between years 2019 and 2026, 150 reference sequences selected from those suggested by Yim-im *et al.* and 4 ORF5 sequences from vaccines strains. Sequences were aligned with MAFFT online service (version 7) and Maximum likelihood phylogenetic trees were calculated using IQ-TREE v.3.0.1.

Results

All sequences clustered in Lineage 1 and were further subdivided into sublineages L1.17, L1.18 and in L1-unclassified as defined by Yim-im *et al.* We also observed occasional clustering with Lelystad virus (L1.2 and L1.3 groups), suggesting the presence of vaccine derived strains. All the strains clustering in L1.17 and L1.18 are found, respectively, in Balka's old groups LIN3G and LIN3B (Subtype1), with some differences in the organization of sublineages. ORF5 sequences classified as L1-unclassified derived from Balka's LIN3E group with the exception of five sequences once attributed to LIN3G.

Discussion and conclusions

Phylogenetic analysis of Italian IZSVe PRRS strains confirms the distributions in sublineages L1.17, L1.18 and in L1-unclassified outlined by Yim-im *et al.* The stratification obtained with the new scheme is mainly consistent with the old classification by Balka *et al.*, with an interesting divergence. A group of five strains, once clustering apart in LIN3G, collected in different farms and times (2021-2026), with poor relative homology, was defined as L1-unclassified, clustering separately with respect to the main L1-unclassified bulk, underlining distinct features and uncertain origin. Considering that ORF5 gene covers only 5% of PRRS genome, sequencing of the entire genome by NGS could help exploring this type of events.

Conflicts of interest

The authors declare no competing interests.

Ethics statements

No ethical approval was required for the present study, as no animal study or experimental trial was performed. All sequences obtained were collected in the context of routine diagnostic activities.

Keywords

PRRS-1 • ORF5 • Phylogeny

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P.03 • ID81

PRRSV-1 ORF-5 GENETIC DIVERSITY IN SPAIN (2018-2025) WITHIN A MULTI-SITE INTENSIVE PIG PRODUCTION SYSTEM

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Introduction and objectives

PRRS virus is still one of the major infectious causes of economic losses in pig farms. In the case of Spain, the emergence in 2020 of a highly virulent PRRSV-1 strain, the so-called Rosalia, and its further spread across the country aggravated the impact of the infection. In large pig production systems, the spread of PRRSV is facilitated by the pig flow and the highly dense network of connections between farms. Understanding the epidemiology of the virus in these systems is key for control. The objective was to describe and analyse the PRRSV-1 genetic diversity and circulation patterns within a large intensive multi-site pig production system in Spain.

Methods

The study was conducted using a set of ORF-5 sequences from a pig production system in Spain encompassing data from 2018 to 2025. Sequences were first checked for quality, removing incomplete sequences, sequences with ambiguous values (N, ambiguous nucleotides), or excess private mutations. Then, the accepted sequences were assigned to a PRRSV-1 ORF5 clade using Nextclade, and then a Bayesian phylogenetic analysis was performed using MrBayes. Analysis of recombination was performed using RDP5. Then a spatial analysis was performed using the farm sequences, the geographical location (coordinates) of the farms, and the data related to the type of farm (multipliers, gilt-rearing units, breeding units, weaning units, and fattening units) and the known connections between them.

Results

After QC check, 173 sequences were included, corresponding to 82 farms. The set of examined sequences was assigned to 8 PRRSV-1 ORF5 clades (L1.1, L1.2, L1.3, L1.6, L1.8, L1.11, L1.17, L1-unclassified). After 2021, the dominating clade was L1.17, corresponding to the highly virulent “Rosalia” clade, which accounted for 98 (56.6%) detections in total. The phylogenetic analysis suggested that L1.17 strains were introduced in the system on several independent occasions between 2021 and 2025, with further diversification as the spread within the production system occurred. Eleven percent of the isolates could not be classified in any clade, and 4 strains were detected as being recombinant (3 L1.2 and 1 L1.6). Among the 28 breeding farms included in the analysis, in 22 cases the virus detected in the farm was closely related to at least one isolate from another breeding farm, suggesting lateral transmission between herds. Notably, two multiplier farms likely shared a common source of infection.

Discussion and conclusions

This study shows that PRRSV-1 circulation in large Spanish pyramidal multi-site pig production systems is genetically diverse and dynamic, with transmission that follows the pig flow but also lateral transmission. The predominance of the highly virulent L1.17 lineage emphasizes the need for improved monitoring and epidemiological analysis systems aimed at the identification of biosecurity breaches and potential ways of transmission. Further analysis should focus on animal movement connectivity networks to explain the epidemiological relationship of farms sharing closely related viral sequences. Overall, integrated molecular, temporal, and spatial surveillance is essential to identify introductions, trace spread, and guide PRRSV control in practice.

Conflicts of interest

None to declare

Ethics statements

Does not apply

Financial support and Acknowledgements

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Keywords

PRRSV-1 • Intensive pig production • Breeding pig farms • Phylogeny • Highly virulent strain Rosalia

P.04 • ID86

PERSISTENT REGIONAL LINEAGES DRIVE THE LONG-TERM EVOLUTIONARY DYNAMICS OF PRRSV-2 IN NORTH AMERICA

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Introduction and objectives

Porcine Reproductive and Respiratory Syndrome Virus 2 (PRRSV-2) is endemic in North American swine herds and causes substantial economic impact. Understanding the evolutionary dynamics and transmission patterns of contemporary lineages is essential for implementing region-specific surveillance and control strategies.

Methods

Twenty-six PRRSV-2 positive serum samples obtained from swine farms in eight U.S. states during 2012 to 2026. Virus isolation was performed primarily in ZMAC-4 cells, with two isolates (PC203, PC208) recovered in PAM cells. RNA was extracted using QIAamp Viral RNA Mini Kit and viral titers were determined by real-time quantitative RT-PCR (qRT-PCR). Whole genome sequencing was performed via Illumina MiSeq (2×300 bp), and viral genome sequences were generated for all 26 isolates using SPAdes (v4.2.0) assembly. BLAST screening identified 108 contextual genomes. Recombinant regions (RDP5 v.5.84) were excluded. Temporal signal was assessed with TempEst v1.5.3. Bayesian phylodynamic inference was performed using BEAST v1.10.4 parametrized with an uncorrelated relaxed molecular clock, GTR+G+I, SkyGrid coalescent prior, and a discrete phylogeographic model for the location trait. Convergence was assessed with Tracer v1.6, ensuring ESS ≥200.

Results

Virus isolation was successful in ZMAC-4 cells for 24 isolates and PAM cells for 2 isolates (PC203, PC208). High level of PRRSV RNA was detected by qRT-PCR in all 26 isolates with Ct values ranged from 15.56 – 21.24. All 26 isolates yielded complete genome sequences. These PRRSV sequences represent multiple geographically structured lineages rather than a single recent outbreak. The most recent common ancestor (MRCA) dates to approximately 1997 (USA-Illinois-Carthage), indicating sustained diversification over >25 years within North American swine populations, such as (1) a Minnesota/Iowa group including Minnesota-Sleepy Eye and Iowa-Humboldt sequences (n=6; June 2018 to December 2024); (2) an Illinois-Dieterich group (n=4; November 2019 to January 2023); (3) an Iowa-Garnavillo/USA-associated group (n=4; October 2017 to July 2021); (4) an Illinois/USA-associated group (n=4; approximately 2014 to 2020); (5) a Colorado-Burlington/Texas-related group (n=3; July 2007 to February 2022); and (6) a North Carolina/Iowa-Garnavillo-related group (n=3; August 2003 to November 2017). Two major national clades circulating had MRCAs around 2009 and 2013, respectively.

Discussion and conclusions

Bayesian phylodynamic analysis reveals that PRRSV-2 evolution in North America is characterized by persistent regional lineages that have circulated for >25 years. The Minnesota-Sleepy Eye cluster represents the most recent transmission activity, while deeper lineages indicate endemic establishment. These findings support implementation of region-specific molecular surveillance and targeted biosecurity measures tailored to dominant lineages in each production area and highlight how long-standing endemic lineages can silently sustain transmission and undermine control efforts if they are not explicitly targeted in regional disease-management plans.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

All experimental procedures were conducted according to the protocol approved by the Institutional Biosafety Committee (IBC).

P.05 • ID108

GENETIC DIVERSITY OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS 1 AND 2 IN DENMARK FROM 2020 TO 2025

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) are highly contagious viruses affecting pigs worldwide. Two distinct species, Betaarterivirus europensis (PRRSV-1) and Betaarterivirus americense (PRRSV-2), exist, and both are endemic in Danish pigs. PRRSV-1 is divided into four subtypes based on genetic variation, designated 1–4. In Western Europe, Asia, North and South America, and China, only viruses from subtype 1 have been detected, whereas subtypes 2–4 are common in certain European countries east of Poland. The genetic variation within PRRSV-2 is more limited in Europe, while more virulent variants from other lineages circulate in Asia and North America. The objectives of this study were to investigate the genetic diversity of PRRSV-1 and PRRSV-2 in Danish pigs from 2020 to 2025 and compare them to globally circulating strains.

Methods

PRRSV real-time RT-PCR-positive samples (serum, oral fluid, processing fluid, and tissue) were obtained from diagnostic submissions to veterinary diagnostic laboratories in Denmark. One sample per submission was selected for ORF2 (PRRSV-1-positive samples only) and ORF5 sequencing, and a subset of these samples was subsequently subjected to shot-gun full-genome sequencing. The obtained sequences were compared to each other, older Danish sequences, and all full genomes available in GenBank (2,748 sequences in total), and maximum likelihood phylogenetic trees were constructed.

Results

In total, 155 PRRSV-1 and 115 PRRSV-2 ORF5 sequences were obtained from viruses collected between 2020 and 2025. Among the Danish PRRSV-1 viruses, nucleotide differences in ORF5 of up to 20% were observed, whereas differences of up to 10% were observed for PRRSV-2. Phylogenetic analysis of PRRSV-1 ORF5 sequences showed that the Danish viruses belong to several clusters within subtype 1, while all PRRSV-2 ORF5 sequences belong to lineage 5.1. Local and global phylogenetic analyses revealed that the 33 Danish PRRSV-1 full genome sequences encompassed viruses from five major clades, a high variability (pairwise identities between clades: 81.3-93.0%), a likely novel variant (two sequences from 2023-2025 <86% identical any Danish or GenBank virus), and several recombinant viruses. Differently, 17 PRRSV-2 Danish genomes were much more genetically conserved (97.6-99.9% pairwise identity) and all included in the same clade. ORF5 and full-genome phylogenies were highly inconsistent.

Discussion and conclusions

Danish PRRSV-1 strains can be divided into five clades, most of which originating from the various modified live virus vaccines strains used in Denmark over the years. The genetic variability within PRRSV-2 in Denmark is more limited, as the detected viruses are all closely related to the vaccine strain used in Europe. Most sequenced viruses belong to known groups, indicating that apparently no new viruses have recently been introduced into the country. However, a divergent clade of unknown origin comprising exclusively Danish viruses was detected starting 2023. Sequencing of PRRSV from Danish herds provides information that can be applied in a range of contexts at the herd, national, and global levels. However, fully realizing this potential requires a substantial increase in full-genome sequencing efforts to overcome the limitations of ORF5-based classification and better resolve the origin of recombinant viruses.

Conflicts of interest

There are no conflicts of interest.

Ethics statements

Not applicable to this study.

P.06 · ID63

DISTINCT RECOMBINATION HOTSPOTS AND GENOMIC EXCHANGE PATTERNS IN PRRSV-1 FIELD/FIELD AND VACCINE-DERIVED RECOMBINANT STRAINS

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) is characterized by high genetic diversity partly due to recombination. Recombination can occur when different PRRSV strains co-infect the same host. As modified live virus (MLV) vaccines are routinely used for PRRSV control, they may also contribute to viral diversity through genetic exchange with circulating field strains. Data from the Belgian voluntary PRRS program suggest that recombination between vaccine and field strains is common and may be non-random. However, it remains unclear whether genomic exchange is region-specific and whether patterns differ between field/field and field/vaccine recombinants. Therefore, this study aimed to identify recombination hotspots and exchanged genomic regions in Belgian PRRSV-1 strains.

Methods

The initial dataset comprised 893 Belgian PRRSV-1 sequences spanning the NSP9-ORF7 region, collected from farms participating in the national PRRS program between June 2023 and January 2026. Vaccine strains were removed ($\geq 95\%$ nucleotide identity to a commercial MLV vaccine strain) and four commercial PRRSV-1 MLV reference strains available in Belgium (Ingelvac PRRSFLEX[®] EU, UNISTRAIN[®] PRRS, Porcilis[®] PRRS and Suvaxyn PRRS MLV) were added (n = 671). After alignment, dereplication and trimming to the NSP9-ORF7 region, 670 sequences remained. Recombination events were identified using Recombination Detection Program 5 (RDP5) and accepted when supported by at least four methods. Recombinants were classified as vaccine-derived when a commercial MLV vaccine strain was identified as a parent and the recombinant fragment met the $\geq 95\%$ identity threshold. Breakpoints and putative parental strains were subsequently determined. Breakpoint distributions were visualized using density plots, and permutation testing was used to identify recombination hot- and cold-spots. Recombination matrices assessed whether specific genomic regions were exchanged more or less frequently than expected by chance.

Results

Of the 670 sequences, 522 (78%) sequences were identified as recombinant strains. Among these, 269 (52%) were classified as vaccine-derived recombinants and 253 (48%) as field/field recombinants. RDP detected 167 unique recombination events, comprising 68 (41%) field/field and 99 (59%) vaccine/field events. Vaccine/field recombination events involved Ingelvac (n=48), Suvaxyn (n=19), Unistrain (n=17) and Porcilis (n=15). Breakpoint distribution analyses revealed that recombination was not randomly distributed across the genome. ORF5 stood out as a recurrent hotspot in PRRSFLEX-derived, Unistrain-derived, and field/field recombinants, while other hotspots varied among recombinant groups. Recombination region matrices further confirmed non-uniform genomic exchange. Frequent exchange was observed within the ORF5-ORF7 region for PRRSFLEX- and Unistrain-derived recombinants, between ORF2 and ORF7 for field/field recombinants, and within ORF1b and downstream regions for Suvaxyn- and Porcilis-derived recombinants.

Discussion and conclusions

Recombination between PRRSV-1 strains is common, with a substantial proportion involving vaccine-derived recombinants, indicating that recombination between field and MLV vaccine strains frequently occurs under field conditions. Vaccine-derived recombination events differed among the commercial MLV vaccines, suggesting that



some vaccines may recombine more readily than others. Differences in breakpoint distributions and exchanged genomic regions stress that recombination is not random. Recurrent hotspots, particularly within ORF5, suggest preferential exchange of specific genomic regions. Whether these hotspots result from mechanistic biases in the recombination process or from biological selection favoring specific recombination patterns remains unclear and should be investigated.

Conflicts of interest

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Ethics statements

/

Keywords

IPRRS • Modified Live Virus vaccines • Recombination

P.07 • ID43

ANALYSIS OF RECOMBINATION IN PRRSV-1 STRAINS

Stephanie Clive; Jean-Pierre Frossard

Animal and Plant Health Agency

Introduction and objectives

PRRSV-1 is one of the most diverse viruses of swine. The diversity is accumulated from both mutation and recombination between strains, but recombination in PRRSV-1 has not been investigated in any depth. No recombination hotspots have yet been identified and the frequency of recombination events has not been determined. By investigating available PRRSV-1 whole genome sequences in GenBank and in house generated sequences from Great Britain, the recombination potential across PRRSV-1 strains was analysed using RDP4. The analysis aimed to establish the frequency of recombination and to identify any hotspots of recombination within the different viral genes.

Methods

The analysis was conducted with 133 full length PRRSV-1 sequences, using SiScan, RDP, 3Seq, Bootscan, Geneconv, MaxChi and Chimaera algorithms. Predicted recombination events were only accepted if at least 5 of the 7 algorithms agreed that recombination was the likely cause of the signal.

Results

A total of 49 independent recombination events were thus identified across the selection, involving both vaccine and field strains. Recombination events appear to be focussed within nsp2 and nsp9 genes but did not seem to show any particular hotspots within these genes. However, the increased observation of recombination events within nsp2 is due, at least in part, to its size as the largest gene in the PRRSV-1 genome. Once recombination frequency is calculated according to the relative size of the genes, nsp1b demonstrates the most recombination events.

Discussion and conclusions

From this analysis it has been shown that there are some areas of the genome that contain more recombination events than others, consistent with the view that PRRSV-1 has some more conserved areas and some less conserved areas. The nsp2 gene is known to be one of the most variable sections of the genome and recombination has been identified here previously, whereas structural genes are generally more conserved and show less frequent recombination events. Vaccine strains of virus are identified as the major parent 22% of the time and the minor parent 12% of the time so have an overall role in recombination in approximately a third of strains identified. This analysis showed that nearly a quarter of PRRSV-1 strains in the dataset have likely experienced some form of recombination.

Conflicts of interest

No conflicts of interest.

Ethics statements

N/A

Financial support and Acknowledgements

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Keywords

Recombination • Hotspot • nsp2

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DIAGNOSTICS**

P.08 • ID5

MODELLING PRRS TRANSMISSION BETWEEN PIG HERDS IN DENMARK AND PREDICTION OF INTERVENTIONS IMPACT

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Introduction and objectives

Porcine reproductive and respiratory syndrome (PRRS) is an endemic viral disease in most pig-producing countries, including Denmark. In 2022, Denmark launched a national control program to reduce PRRS prevalence, followed by legislative changes in 2023 that made testing and status reporting mandatory. The program also enforces the loss of PRRS-free status for farms that purchase pigs from non-PRRS-free sources and implements region-specific control measures to coordinate PRRS elimination between herds. However, the effectiveness of these interventions remains uncertain and requires evaluation through transmission modelling and analysis of epidemiological data before and after the legislative changes.

Methods

To understand PRRS transmission prior to the legislative changes in 2023 and to predict the impact of control measures, we developed a between-herd stochastic compartmental model. The model includes compartments for susceptible (S), highly infectious (IH), lowly infectious (IL), and detected (D) pig herds, using Danish surveillance and movement data from 2020–2021. The model (i) quantifies the relative contributions of pig movements and local transmission to PRRS spread, (ii) generates herd-level maps of the basic reproduction number (R_0), and (iii) evaluates the effectiveness of targeted interventions for PRRS eradication in Denmark.

Results

Model results indicate that more than 50% of herds had an R_0 greater than 1, suggesting potential for sustained transmission in the absence of control measures implemented after 2022. Both local spread and movement-mediated transmission contribute to PRRS transmission, but local transmission largely drives the spatial heterogeneity in PRRS prevalence across Denmark. Although only 17% of infectious herds remain undetected under current surveillance, they are responsible for approximately 60% of total transmission. Local control measures, such as depopulation and repopulation, are the fastest way to reduce observed prevalence, but their impact on true transmission is limited due to hidden infections.

Discussion and conclusions

Achieving PRRS eradication in Denmark may therefore require a combination of more frequent testing, targeted within-herd PRRS elimination, and stricter risk-based trading. By identifying PRRS transmission routes and geographical hotspots, this study provides evidence-based insights to support the evaluation and improvement of Denmark's national PRRS control program.

Conflicts of interest

NA

Ethics statements

NA

Financial support and Acknowledgements

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Keywords

PRRS • Reduction plan • Transmission modelling • Intervention

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P.09 • ID107

DYNAMIC MODEL FOR THE PROBABILISTIC QUANTIFICATION OF PRRS TRANSMISSION THROUGH SWINE MOVEMENT NETWORKS IN COLOMBIA

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Introduction and objectives

PRRS is a disease with a substantial economic impact on swine production. This study aims to quantify the probability of PRRS transmission across Colombian municipalities using swine mobilization networks, identify likely origins and destinations of infection from a selected municipality, and estimate the potential number of infected farms using an interactive web platform.

Methods

The directional transmission model used the national registry of 5.181 georeferenced commercial swine farms; PRRS seroprevalence data; the municipal PRRS Health Risk Model; and the official 2024 dataset of swine mobilization, with 208.770 flows of 11.570.395 animals to slaughterhouses, farms, and swine trading hubs. Municipalities were modeled as nodes in a directed, weighted network, with weekly pig mobilizations as edges. For a selected municipality, two probabilities were estimated: the likelihood of infection sources (incoming flows) and the likelihood of infection destinations (outgoing flows). Transmission probability depended on movement intensity and destination risk score. Infection scenarios with potential outbreaks were defined from high-mobilization municipal clusters and simulated using Monte Carlo methods. Directional probabilities were computed as the proportion of simulations showing transmission and visualized in an interactive web viewer over 12 months.

Results

In Colombia, across 824 municipalities, both scenarios showed that 59.80% (seroprevalence 0.3-0.7) and 75.50% (seroprevalence >0.7) of farms became infected by the end of the study period. In both cases, Antioquia served as a major axis for virus dissemination. For example, for outgoing flow, if the outbreak begins in Medellín (Antioquia) and the focus is on transmission from Bello (Antioquia), 38 municipalities in six departments could be affected, with an 11.29% probability and 100% of farms affected.

Discussion and conclusions

At the end of the study, PRRS infection rates were high due to mobilization-related factors that need improvement, such as vehicle cleaning and disinfection, particularly in departments like Antioquia, which handles the majority of swine mobilizations in the country because of the high concentration of farms and pigs. This underscores the need to target biosecurity interventions in municipalities with high swine density, as these areas concentrate a large share of animal movements and are major axes for virus dissemination. PRRS dynamics highlight the need for quantitative epidemiological tools to inform intervention strategies based on connectivity within animal movement networks. The model and developed tool allow visualization, starting from a municipality of interest where a potential outbreak originates, of the municipalities and departments that would likely be affected, their probabilities, and the number of infected farms. It helps identify key municipalities to strengthen epidemiological surveillance efforts and to explore likely entry and exit routes of infection for a specific municipality, depending on seroprevalence contexts and development areas.

Conflicts of interest

The authors state there are no conflicts of interest.

Ethics statements

The authors declare that the work presented here was conducted with full transparency and integrity, in accordance with Colombian regulations. All individuals involved disclosed any conflicts of interest and safeguarded the confidentiality of private or sensitive data. Accordingly, the academic methodological approach adopted is grounded in honesty, accountability and respect. This yields a high-quality solution, acknowledges collaborators' work and addresses challenges objectively and fairly.

CONTINUE >

Financial support and Acknowledgements

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Keywords

Dynamic model • Transmission • Swine Mobilization Network

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P.10 • ID110

MODEL FOR PRIORITIZATION OF MUNICIPAL EPIDEMIOLOGICAL SURVEILLANCE OF THE HEALTH RISK FROM PRRS BASED ON SWINE MOVEMENT IN COLOMBIA

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Introduction and objectives

To concentrate resources in strategic areas, it is essential to transform a static annual risk calculation for each municipality into a system that reflects changes in risk over time. This system should enable the identification of critical locations in each period and support timely decision-making (prioritization) for the epidemiological surveillance based on Health Risk, as determined by swine mobilization.

Methods

A dynamic PRRS Health Risk prioritization model was developed to transform annual municipal risk estimates into a time-sensitive tool for surveillance and prevention. The model used a municipal PRRS Health Risk Index as a baseline and incorporated official 2024 datasets, commercial farm and the temporal variability of animal mobilization flows. Mobilization records were classified into origin-destination subfactors, and weekly indicators were calculated by destination municipality, swine production development area, animal volume and vehicle frequency. These indicators were normalized within comparable production areas and then integrated using expert-defined weights. Weekly mobilization profiles were grouped using unsupervised clustering; the elbow, silhouette, and hierarchical Ward criteria supported temporal partition. For each temporal group, the annual mobilization component of the risk index was replaced with its period-adjusted value. Empirical prediction intervals were used to estimate confidence and risk-confidence combinations were classified into five priority levels, from very high to very low.

Results

The model generated 3.296 municipality-period classifications, corresponding to 824 municipalities evaluated across four temporal groups. Overall, 715 outputs were classified as very high priority (P1) (21.70%), 128 as high priority (P2) (3.90%), 2.001 as medium priority (P3) (60.70%), 115 as low priority (P4) (3.50%), and 337 as very low priority (P5) (10.20%). The number of P1 municipalities remained relatively stable across periods, ranging from 175 to 182 per temporal group; however, 289 municipalities (35.10%) changed priority category over the year. A total of 129 municipalities remained classified as P1 in all four temporal groups, identifying persistent high-priority territories. High-priority outputs (P1-P2) were concentrated mainly in six departments. Temporal clustering showed that P3 represented the highest mobilization period, while Group 4 represented the lowest mobilization intensity.

Discussion and conclusions

The 289 municipalities (35.10%) in the P1 group that changed priority category over the year demonstrate that municipal prioritization varies dynamically with the intensity and structure of mobilizations over time, offering a more precise and operational tool for health management. Likewise, four departments have active trade flows and a high number of contacts within a given timeframe because they are areas where a large share of commercial farms is concentrated. The model provides an operational basis for distinguishing persistent high-risk municipalities from period-specific alerts, thereby supporting more timely and geographically targeted PRRS surveillance and control actions.

Conflicts of interest

The authors state there are no conflicts of interest.

Ethics statements

The authors declare that the work presented here was conducted with full transparency and integrity, in accordance with Colombian regulations. All individuals involved disclosed any conflicts of interest and safeguarded the confidentiality of private or sensitive data. Accordingly, the academic methodological approach

adopted is grounded in honesty, accountability and respect. This yields a high-quality solution, acknowledges collaborators’ work and addresses challenges objectively and fairly.

Financial support and Acknowledgements

The authors would like to express their sincere gratitude to DataLab-UNAL (Andres Daza, Johanna Avila an Andres Torres) for their significant contribution to this project. Project funded by Porkcolombia-FNP and DataLab-UNAL.

Keywords

Epidemiological Surveillance • PRRS Health Risk

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P:11 • ID105

DATA ANALYTICS APPLICATIONS FOR HEALTH RISK MANAGEMENT BASED ON RESPONSE AND ADAPTATION CAPACITIES OF PRRS IN COLOMBIA

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Introduction and objectives

Traditional disease surveillance approaches are characterized as reactive and static, which present limitations for efficient health management. Transitioning from reactive to proactive surveillance is essential to better inform decisions on managing and mitigating the negative impact of a disease. The objective of this study was to quantify Health Risk Management for PRRS based on municipalities response and adaptation capacities.

Methods

The Health Risk Management model was developed to translate municipal Health Risk estimates into an operational tool for prevention, mitigation, and control. That integration included six repositories with 210 files, including research articles, review articles, interviews, and chapter podcasts. Risk Factors, Categories, and Components were used to derive a Risk level per municipality, which was adjusted based on the effective Mitigation Capacity, which includes Response Capacity and Adaptation Capacity for each Risk Factor whose epidemiological influence has been recognized as relevant by Expert Criteria. This adjustment yielded a Risk value adjusted for specific capacities: $FR(m)=\max(FR(m)-Mit(m),0)$. The model is used $FR(m)$ to group, weight and combine them into categories. These are then regrouped into the Hazard and Vulnerability Components to generate territorial layers for Risk Factor, Category, and Component. Data analytics techniques were applied for normalization, weighting, and spatial pattern identification.

Results

Fifteen Mitigation Capacities were identified. For Response Capacity, five capacities were recognized and categorized under Economic analysis of disease, Knowledge management, and Training. For Adaptation Capacity, ten capacities were identified and grouped into Biosecurity and Veterinary Diagnosis. Of the 824 municipalities included in the study, the Mitigation Capacity is 2.50% for those with 0.80-1.00 risk levels, 2.20% for those with 0.60-0.80 risk levels and 2.40% for those with 0.20-0.60 risk levels.

Discussion and conclusions

The model integrates Health Risk with Mitigation Capacity for each municipality, developing indicators that reflect each municipality's Response Capacity, Adaptation Capacity, and the territory's actual Risk level, including its capacity. The model identifies municipalities where current capacities have a high impact per Risk Factor and territories where limited Mitigation Capacity leaves the Risk profile for each factor virtually unchanged. However, when grouping the fifteen capacities by category and component, mitigation is very low, reflecting the importance of adjusting pre-established action plans, implementing biosecurity programs, modifying transportation routes, altering the frequency of supply introductions in response to increased regional risk, and carrying out permanent structural changes in production systems to reduce the incidence of outbreaks in high-density swine regions. The tool set synthesizes PRRS Health Risk and the territory's relative capacity to mitigate its effects, supporting decisions on prevention, mitigation, and control by identifying territories where Hazard and Vulnerability, constrained by response capacity, generate scenarios of greater health fragility.

Conflicts of interest

The authors state there are no conflicts of interest.

Ethics statements

The authors declare that the work presented here was conducted with full transparency and integrity, in accordance with Colombian regulations. All individuals involved disclosed any conflicts of interest and safeguarded the confidentiality of private or sensitive data. Accordingly, the academic methodological approach

adopted is grounded in honesty, accountability and respect. This yields a high-quality solution, acknowledges collaborators’ work and addresses challenges objectively and fairly.

Financial support and Acknowledgements

The authors would like to express their sincere gratitude to DataLab-UNAL (Andres Daza Johanna Avila and Andres Torres) and Porkcolombia-FNP (Adriana Deluque) for their significant contribution to this project. Project funded by Porkcolombia-FNP and DataLab-UNAL.

Keywords

PRRS Health Risk Management • PRRS Response Capacity • PRRS Adaptation Capacity

P.12 · ID45

QUANTIFYING PRRSV INTRODUCTION RISK AND BIOSECURITY EFFECTIVENESS IN PIG FARMS

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Introduction and objectives

Farm-level biosecurity is important for preventing porcine reproductive and respiratory syndrome virus (PRRSV) in pig farms. However, the relative contribution of different transmission pathways varies among farms and production systems. FarmRISK is a stochastic, quantitative risk assessment model that was originally developed at the Universitat Autònoma de Barcelona for cattle diseases (Infectious Bovine Rinotracheitis, Bovine Viral Diarrhea, and Tuberculosis). It is used to estimate the risk of pathogen introduction in livestock farms and to evaluate the effectiveness of biosecurity measures. The objective of this study was to adapt this model to assess the risk of PRRSV introduction on pig farms by incorporating PRRSV-specific transmission pathways and biosecurity measures.

Methods

The model was adapted within the existing framework of the B-SAFE project under the European Partnership on Animal Health and Welfare. Three online meetings were held by the working group, involving specialists in pig health, epidemiology, and biosecurity. The meeting’s goals were to determine how to adapt the model from cattle to pigs. Existing farm-level questionnaires, model structure, and parameters were reviewed and modified to reflect PRRSV epidemiology. The stochastic structure of the original model was maintained to account for uncertainty in input parameters.

Results

Several modifications were required to represent PRRSV transmission dynamics. The animal introduction pathway was expanded to include the health status of the source farm, the probability of incoming animals being disease-free, the quarantine management practices, and the gilt acclimation procedures. Visitor-related pathways were refined by expanding the categories of farm visitors and by adding sub-pathways related to visitors. These additional sub-pathways accounted for different biosecurity measures depending on the virus being present on truck wheels, drivers’ boots, visitors’ equipment, and visitors’ bodies. The biosecurity measures added were using boot covers, showering, handwashing, clothing changing, and for the fomite, additional equipment and tools were considered, and disinfection methods such as ultraviolet light and heat treatment were incorporated. New pathways addressing feed biosecurity, pest control, and aerosol transmission from neighbouring farms were also included.

Discussion and conclusions

FarmRISK is being adapted into a PRRSV-specific quantitative risk assessment tool through expert-driven refinement. The updated framework incorporates key PRRSV introduction routes. Future work will focus on model parametrization, validation, and application in commercial swine farms, with subsequent adaptation to other pig diseases such as African swine fever and porcine epidemic diarrhea.

Conflicts of interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this abstract.

Ethics statements

This study is ongoing and focuses strictly on the implementation and development of risk assessment models. At this stage, the research is entirely methodological and does not involve direct animal experimentation, farm visits, or surveys with human participants.

CONTINUE >

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Keywords

Risk assessment • FarmR!SK • PRRSV introduction

P:13 · ID54

PRRSV MONITORING: FROM DETECTION TO DECISION-MAKING

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Introduction and objectives

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) prevalence varies markedly between outbreaks, over time, and between farms. This highlights the limitations of relying solely on detection and underscores the need for tools that can reflect the herd’s epidemiological status. This study aimed to evaluate the effect of sampling design on virus detection capacity under different prevalence scenarios, and to determine whether pooled RT-PCR results can provide epidemiological information to improve management decisions, including piglet vaccination strategies, epidemiological disease behaviour at weaning, defining gilt introduction timing, or optimizing piglet movements to nursery facilities.

Methods

A longitudinal follow-up of 10 PRRSV outbreaks in breeding herds was conducted in weaning-age piglets, 1,350 individual serum samples grouped into 270 pools of 5. Data were aggregated at the sampling event level to derive pooled metrics, including the proportion of positive pools and Ct distribution. A binomial generalized linear model (logit link) was used to estimate sample-level prevalence from pooled results. The model integrates pooling intensity and viral load information (CT_max_pool and CT_min_pool). Predicted prevalence curves across 0–100% positive pools were generated, with 95% confidence intervals derived from the model. Herd Sensitivity (HSe) estimates (calculated according to Christensen & Gardner, 2000) from a previously published experimental study (De Mergelina *et al.*, 2026).

Results

The longitudinal follow-up showed that more than 50% of sampling events before 100 days post-outbreak showed over 25% positive animals, whereas all observations after 150 days were below that threshold. The binomial model estimates sample-level prevalence from pooled RT-PCR results. The proportion of positive pools was the main predictor ($p < 2 \times 10^{-16}$), while the presence of at least one positive pool also contributed significantly ($p = 0.00182$). Among Ct-related variables, CT_max_pool was negatively associated with prevalence ($p < 0.001$), whereas CT_min_pool was not significant. Model fit improved substantially (deviance: 555.46 to 79.48). Model estimates that prevalence increases from near zero at low proportions of positive pools to >50% when more than 80% of pools were positive. Herd-level sensitivity was influenced by prevalence and sampling design. At 10% prevalence, HSe ranged from 94.8% (30 animals) to 99.9% (120 animals). but decreased significantly at lower prevalences, reaching 46.5–83.7% at 2% and 29.0–61.7% at 1%.

Discussion and conclusions

Pooled RT-PCR results provide quantitative information, as the proportion of positive pools reflects not only the presence of infection but also its magnitude. The modelling approach confirmed that pooled RT-PCR results, together with Ct distribution, explain a relevant proportion of variability, indicating that pooling outputs contain more epidemiological value than a simple positive/negative classification. The longitudinal follow-up revealed that early stages present high proportions of positive animals followed by a progressive decline over time. Under these conditions, HSe becomes highly dependent on prevalence, declining markedly at lower prevalence levels, reaching values as lower than 50%. These findings highlight that pooling may underestimate infection in later stages, while the modelling approach supports interpretation of prevalence trends, helping to differentiate between decreasing and persistent infection scenarios.

Conflicts of interest

Some authors are HIPRA employees. The remaining authors declare no competing interests

Ethics statements

Samples were obtained from routine diagnostic submissions.

Financial support and Acknowledgements

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Keywords

PRRSV • Prevalence estimation • Herd Sensitivity • Sampling strategies • PRRSV Monitoring

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P.14 • ID83

INTEGRATED INDICATORS TO QUANTIFY PRRSV DYNAMICS AND OUTBREAK IMPACT IN A LARGE SPANISH SWINE PRODUCTION SYSTEM

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) remain a major challenge for large swine production systems due to its persistence, genetic variability and impact on reproductive performance. Effective PRRSV control requires tools that quantify not only virus detection, but also infection dynamics, diagnostic stability, clinical recovery and productive losses. The objective of this study was to evaluate PRRSV epidemiological and clinical dynamics in a large Spanish swine production system using indicators derived from structured diagnostic monitoring, production performance data and sequencing.

Methods

A longitudinal PRRSV monitoring programme was implemented in a big Spanish company breeding herds between 2024 and 2026. Diagnostic monitoring included repeated RT-qPCR testing samples collected at birth and 30 serum samples at weaning. Herd status was classified over time according to RT-qPCR results. Prevalence was used to quantify the proportion of PRRSV-positive periods, while incidence identified new positive episodes after previous negative status. Minimum Ct values were evaluated as an indicator of viral circulation intensity. Diagnostic recovery time was defined as the time required to achieve stability after PRRSV detection, based on consecutive negative monitoring results. Production data were analysed to estimate time to baseline production after clinical outbreaks, using weaned piglets/litter and other reproductive indicators. Sequencing of selected samples was used to assess strain circulation, persistence and potential epidemiological links between farms.

Results

The programme included diagnostic data from 108 farms, of which 59 had more than 70% diagnostic coverage, and production data from 18 farms. In 2025–2026, 45.1% of monitored weeks classified as PRRSV-positive, compared with 46.2% in the previous campaign. Using stability criteria, 46.3% of monitored time was classified as unstable, 31.3% as in process of stabilisation and 22.4% as stable. Mean time to achieve diagnostic stability after PRRSV detection was approximately 9 months, while stability at weaning after achieving negative status at birth was reached after approximately 2 months. PRRSV-positive status was associated with poorer reproductive performance. In 2025–2026, PRRSV-negative periods showed 12.5 weaned piglets/litter versus with 12.1 during positive periods. Based on a weaned piglet value of €38.1, this represents an estimated economic impact of approximately €35,000 per 1,000 sows/year. When the Rosalía strain was involved, the impact was greater, with a reduction of 0.7 weaned piglets/litter and an estimated loss of approximately €61,000 per 1,000 sows/year. Clinical outbreak analyses showed marked variability between farms, with time to baseline production ranging from 5 to more than 8 months and estimated losses from €50,000 to €123,000 per 1,000 sows. Sequencing supported the identification of related strains and helped differentiate persistent circulation from potential new introductions.

Discussion and conclusions

Integrating diagnostic, epidemiological, productive and sequencing indicators provides a practical framework to quantify PRRSV status at system level. Prevalence and incidence describe infection burden and new instability events, Ct trends help interpret viral circulation intensity, diagnostic recovery time measures return to stability, and time to baseline production captures clinical and economic impact. This approach supports benchmarking, prioritisation of interventions and objective evaluation of PRRSV control progress.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

This study was based on routine diagnostic monitoring and production data collection; no experimental procedures were performed on animals.

Keywords

PRRSV • Longitudinal monitoring • Infection dynamics • Disease surveillance

P:15 • ID117

RELATIONSHIP BETWEEN BIOSECURITY AND PRODUCTIVE PERFORMANCE IN IRISH PIG FARMS: IMPLICATIONS FOR SWINE VIRAL DISEASE CONTROL

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Introduction and objectives

Biosecurity is essential for reducing the introduction and spread of infectious diseases in pig farms, including PRRS and other swine viral diseases. However, its relationship with farm productivity remains complex, as production performance is also influenced by year, farm structure, management and other external factors. Understanding this relationship may help support practical biosecurity improvement, which is an important part of viral disease prevention and control. This study aimed to explore whether biosecurity was associated with production performance in Irish pig farms based on a farm-year level.

Methods

Biosecurity assessment data based on Biocheck.UGent were combined with farm-year production records from Irish pig farms between 2020 and 2024. The final dataset included 187 farm-year observations. Biosecurity was represented by 12 section-level scores, covering six external and six internal biosecurity areas. Production outcomes included output, growth, feed efficiency and mortality indicators. Mixed-effects models were used to assess associations between individual biosecurity sections and each production outcome, with year and herd size included as fixed effects and farm code as a random intercept. In addition, principal component analysis (PCA) followed by clustering was used to identify broader biosecurity profiles and compare production outcomes between profiles.

Results

Most production outcomes were associated with at least one biosecurity section in the mixed-effects models. Some sections appeared more than once and showed consistent results, such as nursery unit. Higher scores in nursery unit were associated with higher meat produced per sow per year ($p = 0.038$) and higher number of pigs produced per sow per year ($p = 0.042$), as well as lower weaner and finisher mortality ($p = 0.047$ and 0.019 , respectively). However, in some cases, better biosecurity was linked to poorer performance, which should therefore be interpreted cautiously. The PCA and clustering analysis identified farm groups with distinct biosecurity profiles. Differences were found in most outcomes, with farms of the lowest biosecurity scores generally showing poorer production performance.

Discussion and conclusions

These findings highlight the importance of biosecurity, particularly in the nursery unit, for improving productivity and reducing mortality in general. Although this study did not include PRRSV or other viral diagnostic data, the results may still be relevant to swine viral disease control, as biosecurity is a key measure for reducing the introduction and spread of viral infections in pig farms. These findings may also provide guidance for lower-performing farms to prioritise management practices focusing on internal biosecurity for improvement. However, as associations do not prove causality, future work should integrate herd health, viral surveillance, management and economic data to clarify mechanisms, support practical recommendations and provide economic evidence to motivate farmers to improve biosecurity.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

This study used farm-level biosecurity and production data and did not involve experimental procedures on animals.

Keywords

Biosecurity • Ireland • Productive performance • Swine viral disease control

P:16 • ID133

DYNAMICS OF PRRSV IN THE NURSERY AND AT THE BEGINNING OF FATTENING IN FARMS IN COLOMBIA

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Introduction and objectives

PRRSV significantly impacts swine production worldwide and remains endemic in Colombia. The virus can circulate among pigs, both clinically and subclinically, with its effects on productivity influenced by the animals' immune status and the specific viral strain. Given these challenges, a thorough understanding of PRRSV dynamics is essential for establishing effective control strategies. In this study, we investigated the distribution and dynamics of PRRSV across tissues and age groups during the nursery phase and identified circulating viral strains on Colombian pig farms, where vaccination against PRRSV is not currently practiced.

Methods

A longitudinal study was carried out on 23 pig farms located in the five principal pig-producing regions of Colombia. Samples of serum, tonsil, and lung tissue were obtained from 130 pigs, categorized into three age groups (3–5, 6–8, and 9–12 weeks) spanning the nursery phase through the beginning of the fattening period, resulting in a total of 390 samples. Detection of PRRSV was performed using RT-qPCR ($Ct \leq 40$), and specimens with higher viral loads ($Ct \leq 30$) were selected for sequencing of the ORF5 gene. Statistical analyses included chi-square tests, linear trend analysis, and analysis of variance (ANOVA), with significance set at $p \leq 0.05$.

Results

PRRSV was detected across all studied regions and in 78.2% of the examined farms, with an overall individual prevalence of 44.6%. Prevalence increased with age, from 33.3% in piglets aged 3–5 weeks to 53.3% in those aged 9–12 weeks, although this upward trend did not reach statistical significance. Lung, tonsil, and serum samples exhibited similar rates of PRRSV positivity. Ct values indicated moderate to high viral loads, with the highest loads observed in lung tissue from pigs aged 6–8 weeks. Among 58 PRRSV-positive animals, 28 ORF5 gene sequences revealed high nucleotide identity (96.5%–99.8%), indicating a substantial degree of relatedness among circulating strains. Phylogenetic analysis classified 19 sequences as lineage 1A, 7 as 1B, and 2 as 1C. Notably, lineage 1B was found exclusively in one region, suggesting limited geographic distribution for this lineage.

Discussion and conclusions

The findings demonstrate that PRRSV circulates endemically throughout Colombia's primary swine-producing regions. The high prevalence and age-related increase in infection rates highlight ongoing transmission within farms. Moderate to high viral loads were observed, and lineage analyses suggest multiple regional introductions and sustained viral presence. These results underscore the need for targeted PRRSV control strategies in Colombia, where licensed vaccines are currently unavailable.

Conflicts of interest

The authors declare no conflicts of interest. This study was sponsored by Zoetis de Colombia; however, PRRSV vaccines are not authorized for sale in Colombia.

Ethics statements

This study received approval from the Bioethics Committee of the Faculty of Veterinary Medicine, National University of Colombia.

Financial support and Acknowledgements

This study was funded by Zoetis de Colombia and the National University of Colombia, Bogotá campus.

Keywords

Colombia • Infection dynamics • Lineage 1 • PRRSV

P:17 • ID135

ASSESSING VIRAL ENVIRONMENTAL CONTAMINATION IN AND AROUND MORTALITY HANDLING STRUCTURES IN WEAN-TO-FINISH FARMS

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Introduction and objectives

Mortality due to infectious diseases requires biosecure disposal of carcasses to prevent pathogen spread. Limited attention has been given to dead animal disposal methods as sources of environmental contamination and pathogen dissemination. Areas surrounding mortality management structures are frequently accessed by personnel, vehicles, and equipment, creating opportunities for fomite contamination. The objective of this study was to assess viral environmental contamination around dead animal disposal structures, determine whether contamination differs by mortality management method, evaluate pathogen movement between farms and evaluate one decontamination strategy for these structures.

Methods

Four related objectives were evaluated. First, environmental contamination was characterized on 40 Midwestern US wean-to-market farms reported as porcine reproductive and respiratory syndrome virus 2 (PRRSV-2) or porcine epidemic diarrhea virus (PEDV) positive. Environmental samples were collected from rendering (dead box) and composting sites (n=20 each) and tested by RT-PCR for PRRSV-2, PEDV, porcine deltacoronavirus (PDCoV), and transmissible gastroenteritis virus (TGEV). The proportion of positive farms and samples, and Ct values, were compared between methods using chi-square and t-tests. Second, GloGerm fluorescent powder was applied to roads leading to mortality structures on eight farms to evaluate movement of contaminated material. Vehicle transfer was assessed on six farms by driving through treated areas and visiting neighboring farms (~2–5 km). Third, personnel transfer was evaluated on two farms by having a researcher wearing disposable boot covers walk through treated areas toward the farm office, followed by UV inspection of boots and walking paths. Finally, on the fourth objective, ten farms were enrolled to evaluate hydrated lime whitewash application around dead box areas as a decontamination method. A pre-post treatment design was used, with post-treatment samples collected 2 days after treatment application. All samples were tested by RT-PCR.

Results

Out of 40 farms tested in the first objective, 22 (55%) had at least one positive environmental sample. Rendering sites (80%, 16/20) tested positive for at least one virus more frequently than composting sites (30%, 6/20) (p-value=0.001). The average Ct value for PRRSV-2 was lower at rendering sites (Ct 30.4) than at composting sites (Ct 33.83) (p=0.012). Leachate and carcass samples had the lowest Ct values for all tested pathogens. No viral isolates were obtained. GloGerm was detected on vehicle tires, mudguards, and undercarriages in all six vehicle experiments and remained detectable after travel to neighboring farm. In the personnel transfer test, GloGerm was detected on the disposable boots and along pathways leading toward farm office entrances. In the decontamination evaluation, PRRSV was detected in 13% (13/100) of pre-treatment samples and 3% (3/100) post-treatment samples, with similar Ct values (34.6 pre and post).

Discussion and conclusions

The environment around swine mortality management structures is frequently contaminated, with higher environmental contamination observed at rendering sites. Our study demonstrated that potentially contaminated material can be mechanically transported by vehicles and personnel, while a whitewash protocol reduced environmental contamination. Together, these findings suggest that mortality structures could potentially play a role in pathogen transmission in swine farms.

Conflicts of interest

The authors did not have any conflict of interest.

Ethics statements

N/A

Financial support and Acknowledgements

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Keywords

Biosecurity • Mortality management • Pathogen movement • Decontamination • PRRSV

P:18 • ID115

MOLECULAR SURVEILLANCE AND PATHOGENICITY ASSESSMENT OF SADS-COV AND PORCINE SAPELOVIRUS IN SOUTH KOREA AND VIETNAM

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Introduction and objectives

Swine acute diarrhea syndrome coronavirus (SADS-CoV) and porcine sapelovirus (PSV) are emerging swine diarrhea-associated viruses with potential impacts on pig health and production. This study aimed to investigate the genetic characteristics and pathogenicity of SADS-CoV and PSV through molecular surveillance, virus isolation, and experimental infection using diarrheic pig samples collected in South Korea and Vietnam.

Methods

A total of 211 diarrheic fecal samples from Korean pig farms and 503 diarrheic pig samples from Vietnamese pig farms were examined. Multiplex qRT-PCR assays were used to screen for swine diarrhea-associated coronaviruses, including SADS-CoV, porcine epidemic diarrhea virus (PEDV), and porcine deltacoronavirus (PDCoV), and picornaviruses, including PSV, enterovirus G (EV-G), and porcine teschovirus (PTV). Confirmatory PCR assays targeting SADS-CoV N and S genes and PSV 5'-UTR and 3B-3D regions were performed. The VP1 gene of PSV-positive samples was sequenced for phylogenetic analysis. A Vietnamese PSV isolate, VN20_231105.5, was used for pathogenicity assessment in 5-day-old suckling piglets.

Results

In Korean samples, SADS-CoV and PDCoV were not detected, whereas PEDV was detected in 58 of 211 samples (27.5%). Additional PCR assays confirmed that all Korean samples were negative for SADS-CoV. PSV, EV-G, and PTV were detected in 161 (76.3%), 188 (89.1%), and 170 (80.6%) samples, respectively. Confirmatory PCR identified 158 PSV-positive samples (74.9%). Complete VP1 gene analysis of 100 Korean PSV-positive samples showed that all belonged to PSV-1, with marked amino acid sequence variation and classification into three genetic clusters. In Vietnam, 290 of 503 samples (57.7%) were positive for one or more swine diarrhea-associated viruses. Although 20 samples were initially positive for SADS-CoV, all were negative by confirmatory PCR in both Korea and Vietnam. PSV was confirmed in 60 Vietnamese samples. VP1-based phylogenetic analysis showed that 46 Vietnamese PSV strains belonged to PSV-1 and were divided into up to five clusters; 29 strains (63.0%) were closely related to Chinese PSV strains. In the pathogenicity study, VN20_231105.5 caused no mortality and only mild transient diarrhea, but viral shedding was detected from 2 days post-inoculation, and high viral loads were found in intestinal tissues.

Discussion and conclusions

No confirmed evidence of SADS-CoV infection was identified in Korean or Vietnamese pig samples in this study. In contrast, PSV was widely detected and genetically diverse in both countries. The Vietnamese PSV isolate efficiently infected the intestinal tract of suckling piglets but showed limited clinical pathogenicity. These findings support continued molecular surveillance of SADS-CoV, PSV, and other emerging swine diarrhea-associated viruses in East and Southeast Asia.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

All animal experiments were conducted in accordance with institutional guidelines for animal care and use and were approved by the Institutional Animal Care and Use Committee of the Animal and Plant Quarantine Agency, Republic of Korea (IACUC 2026-2139).

Keywords

SADS-CoV • Porcine sapelovirus • Swine diarrheal virus • Molecular surveillance • Pathogenicity

P.19 · ID116

PHYLOGENETIC DIVERSITY AND TRANSMISSION PATHWAYS OF PRRSV IN UPPER AUSTRIAN PIG FARMS

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Introduction and objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) remains one of the most economically important viral diseases in the swine industry and it is endemic in Austria. Austria’s position within interconnected European swine production and trade systems facilitates frequent cross-border animal movements, posing a major challenge for disease control and eradication. In response, national PRRS Stabilization Program have been implemented to limit viral spread. Although PRRS virus (PRRSV) monitoring is not mandatory, diagnostic and surveillance samples have been collected since 2012. This decade-long dataset has not yet been analysed longitudinally, leaving gaps in understanding of endemic circulation, outbreak dynamics, and introductions from neighbouring countries. Our objectives: (i) characterize PRRSV genetic diversity in Upper Austria and identify regional clusters within the European phylogenetic context using ORF5 sequences, and (ii) assess the role of trade in viral spread by integrating genetic and pig movement data. Findings will provide insights to optimize national stabilization and control strategies.

Methods

A total of 457 PRRSV ORF5 gene sequences (606 bp) were collected from 96 swine farms in Upper Austria, 2012-2025. Amplification and sequencing followed Stadejek *et al.*,(2002) and Balka *et al.*,(2008) using standard RT-PCR and nested PCR protocol. PCR products were purified, and ORF5 fragments were sequenced bidirectionally. Sequences were edited manually, aligned using MAFFT, and a maximum-likelihood phylogenetic tree was inferred using RAxML-NG (GTR+G+I nucleotide substitution model; 1000 bootstrap replicates). The ancestral genotype was estimated via midpoint rooting. Additional PRRSV ORF5 sequences from European countries available in GenBank were retrieved and included for comparative analysis. Clustering analysis was performed using ClusterPicker (clade support >90%) with a mean Austrian pairwise distance threshold. Temporal signal was assessed in TempEst, and time-scaled phylogenies were reconstructed in BEAST using a 10-million MCMC chain, relaxed molecular clock, and Bayesian skyride model (10% burn-in). MCC trees were used in TransPhylo to infer transmission networks, which were linked with pig movement data from the Consumer Health Information System (Verbrauchergesundheitsinformationssystem; VIS).

Results

Austrian sequences were widely distributed across the tree and showed a mean pairwise genetic distance of 14.2%. Within-municipality genetic diversity was heterogeneous, with mean genetic distances of 39.4% (SD = 18.9%) despite limited sample sizes (median: 4). Cluster analysis combining Upper Austrian and GenBank sequences identified 42 clusters. Cluster 23 was the largest (113 Austrian and two GenBank sequences), while Cluster 25 showed the lowest genetic distance (1.7%; 30 Austrian and three GenBank sequences). Both PRRSV-1 field strains and vaccine-(like) sequences were present in the dataset.

Discussion and conclusions

The results indicate that PRRSV endemicity in Upper Austria is maintained by multiple independent introduction events. The large (Cluster 23) indicates that a single highly adapted variant is primarily driving regional transmission. In addition, the widespread use of PRRSV-1 modified live vaccines was also reflected in the data. The distinct viral variants within the municipality indicate that pig movement is driving the persistence of PRRS. These preliminary findings highlight the need for farm-to-farm transmission tree reconstructions (TransPhylo) integrated with VIS data to inform biosecurity and strategic interventions within specific trade corridors.

CONTINUE >



Conflicts of interest

No conflict of interest.

Ethics statements

Not applicable.

Keywords

transmission network • phylogenetics • surveillance

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P.20 • ID20

DOSE DETERMINATION STUDY OF A HIGHLY VIRULENT PRRSV-1 CHALLENGE STRAIN IN PREGNANT SOWS

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Introduction and objectives

Determining the minimum infectious dose is a key aspect of veterinary infectious disease research. Highly virulent PRRSV strains exhibit expanded tissue tropism, which may reduce the dose required to establish infection and cause disease. This study aimed to evaluate the effects of four challenge doses of a highly virulent PRRSV-1 Rosalía strain in pregnant sows under experimental conditions.

Methods

Sixteen PRRSV-naïve pregnant sows at 90±2 days of gestation were allocated to four groups and intranasally challenged with 2 mL of the highly virulent Rosalía strain (Lleida 029_22) at the following doses: 10² (n=3), 10³ (n=3), 10⁴ (n=2), or 10⁵ (n=8) TCID₅₀/mL. The highest-dose group included a higher number of animals because it also included non-vaccinated infected sows from other efficacy studies following the same experimental design. Each group was housed separately. Sows were monitored for clinical signs, pyrexia, and abortions. Samples (blood, nasal, oral, and rectal swabs) were collected pre-challenge, at 7 days-post-challenge (DPC), farrowing (~24DPC), and weaning (~45DPC). Farrowing was assisted to evaluate litter outcomes (live-born piglets and non-viable fetuses). Vertical transmission was assessed using blood samples from live-born piglets and tissues from non-viable fetuses. Piglets were monitored for 21 days-post-partum (DPP), with weekly blood sampling. At weaning (21DPP), sows were euthanized and subjected to necropsy to assess gross lesions and collect tissue samples for virus detection.

Results

Sows challenged with the three highest doses presented mild and mostly transient clinical signs. Cyanosis was the most common finding, whereas altered demeanor, dyspnea, nasal discharge, and fever were observed less frequently. No clinical signs or fever were detected in sows infected with the lowest dose. Only one abortion was recorded, occurring in a sow infected with the highest dose. Between 30% to 50% of sows in each group required induction of farrowing due to delayed delivery (>117 days). Perinatal mortality reached 70% in sows challenged with the lowest dose and was >80% in those challenged with the three highest doses (p>0.05). Additionally, the proportion of live-born piglets was low (ranging from 3% to 30%), and none survived until weaning, with all deaths occurring within the first two weeks of life. All live-born piglets were viremic at birth, and viremia persisted until death. Moreover, the virus was also detected in all the samples coming from non-viable fetuses. All sows became viremic following challenge, and PRRSV shedding was detected via nasal, oral, and rectal routes, with no significant differences between groups (p>0.05). Macroscopic interstitial pneumonia was observed in all sows, except one challenged with 10³ TCID₅₀/mL.

Discussion and conclusions

The preliminary data from this study suggest that the minimum infectious dose of the highly virulent PRRSV-1 Rosalía strain is extremely low (<or=10² TCID₅₀/mL). Further studies are needed to increase the sample size and, consequently, to more precisely determine the minimum infectious dose of this highly virulent PRRSV-1 strain. Overall, these results highlight how easily this virus can be disseminated within a sow population.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statements

The study was approved by the Ethics Committee of A.M. Animalia Bianya, S.L. (CEEA 19/24).

Financial support and Acknowledgements

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Keywords

minimum infective dose • PRRSV • reproductive form • highly virulent • sow

P.21 • ID129

FIELD INVESTIGATION OF INFERTILITY AFTER ROSALÍA-LIKE PRRSV-1 OUTBREAKS IN COMMERCIAL SOW FARMS

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Introduction and objectives

Classical outbreaks of PRRSV in breeding herds are primarily associated with late-gestation failure but not with significant increases in returns to oestrus or persistent infertility. Experimental studies have confirmed limited direct PRRSV effects on early-stage embryos and increases in viral receptor expression throughout gestation^{1,2,3}. However, persistent infertility has frequently been reported following outbreaks of the highly-virulent Rosalía-like PRRSV-1 isolates (R-PRRSV-1).

The objective of this study was to compare reproductive failure, lesions and PRRSV-1 detection profile in sows culled following outbreaks of classical PRRSV-1 isolates (C-PRRSV-1) or R-PRRSV-1.

Methods

One hundred and ten sows (78 from two farms with outbreaks caused by R-PRRSV-1 in the previous 4 months and 32 from two farms with similar outbreaks but caused by C-PRRSV-1) were selected. Culling causes were recorded and classified as infertility, abortion, abortion followed by infertility, ovarian anoestrus or vaginal discharge-associated infertility. At slaughter, blood, tonsil, uterine lymph node (ULN) and the entire reproductive tract were collected. The reproductive tract was examined macroscopically, and representative samples of uterus, ovary, oviduct and ULN were fixed in formalin for histopathology. Microscopic lesions were recorded and scored. Tissue homogenates from tonsil, ULN and endometrium, together with serum, were tested by RT-qPCR for PRRSV RNA detection.

Proportions were compared using chi-square exact test, Ct values using ANOVA, and lesion severity scores using non-parametric tests.

Results

The distribution of causes of reproductive culling differed significantly between groups. Infertility was the predominant cause in R-PRRSV-1 sows (58/78; 74.4%) compared to C-PRRSV-1 sows (11/32; 34.4%) ($p=0.00016$). Abortion alone was more frequent in C-PRRSV-1 sows (13/32; 40.6%) than in R-PRRSV-1 sows (16/78; 20.5%) ($p=0.035$), but the differences were no significant when aborted sows with subsequent infertility were also considered. Notably, the abortion-infertility pattern was recorded only in R-PRRSV-1 sows (4/78; 5.1%) and vaginal discharge-associated infertility in C-PRRSV-1 sows. Post-mortem ovarian anoestrus was numerically, but not significantly, higher in R-PRRSV-1 sows (14/76; 18.4%) than in C-PRRSV-1 sows (4/29; 13.8%). However, on farms infected with R-PRRSV-1, ovarian anestrus affected sows of all parities, whereas on farms with C-PRRSV-1, it primarily affected gilts. Uterine and ovarian macroscopic or microscopic lesions did not differ significantly between groups. However, lymphoid depletion in ULN was more frequent in R-PRRSV-1 sows (24/78; 30.8%) than in C-PRRSV-1 sows (3/32; 9.4%) ($p=0.026$). PRRSV was mainly detected in tonsils and, less frequently, in ULN, while detection in serum and endometrium was rare. No significant differences in PRRSV detection or Ct values were observed between groups.

Discussion and conclusions

Infertility or abortion followed by infertility were the main causes of culling in farms affected by R-PRRSV-1. This pattern cannot be explained by PRRSV persistence in endometrium or by more severe reproductive tract lesions. The higher frequency of lymphoid depletion in ULN suggests that systemic or immune-mediated mechanisms⁴, potentially involving hormonal dysregulation, post-outbreak catabolic status or systemic inflammation, might contribute to the observed infertility. However, these findings should be interpreted cautiously and must be confirmed experimentally.

[CONTINUE >](#)

Conflicts of interest

Ruth Menjón work for MSD Animal Health

Ethics statements

Samples were obtained from sows culled under routine commercial procedures at slaughter.

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P.22 • ID41

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS HIJACKS THE NON-CANONICAL ENZYMATIC FUNCTION OF PHGDH TO ARREST AUTOPHAGIC FLUX FOR VIRAL REPLICATION

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Introduction and objectives

As obligate intracellular pathogens, viruses depend on host metabolism and frequently hijack cellular enzymes to fuel progeny production. Increasingly, such enzymes act beyond their catalytic roles as scaffolds or signaling hubs; yet how viruses exploit these non-canonical functions remains poorly understood. Porcine reproductive and respiratory syndrome virus (PRRSV), a Nidovirales member that causes devastating reproductive and respiratory disease and whose genetic diversity has frustrated vaccine development, manipulates multiple host pathways, including autophagy, to establish infection. Phosphoglycerate dehydrogenase (PHGDH), the rate-limiting enzyme of de novo serine synthesis within serine-glycine-one-carbon (SGOC) metabolism, is a candidate node exploited during infection. Using PRRSV as a model, we examined how it exploits PHGDH and whether this depends on its canonical enzymatic activity.

Methods

The crosstalk between PRRSV and host SGOC metabolism was delineated by integrating transcriptomic and metabolomic profiling with an unbiased RNA-interference screen, pinpointing PHGDH as a key enzyme governing viral replication. Viral replication and host-factor expression were quantified by complementary virological and molecular readouts, and stage-resolved assays localized the affected step. Transcriptional control of PHGDH was dissected by combining promoter-truncation luciferase reporters with DNA affinity-isolation coupled to quantitative mass spectrometry. To uncouple antiviral activity from catalytic function, chemical-genetic and structure-function strategies were applied, including metabolite rescue, catalytically dead point mutants, and selective inhibitors. Autophagy and autophagic flux were interrogated by tandem mCherry-EGFP-LC3 reporters, electron microscopy, and co-immunoprecipitation mapping of the STX17-SNAP29-VAMP8 SNARE complex; restriction was further tested against PEDV and PRV.

Results

PRRSV infection disturbed SGOC metabolism, reducing serine and S-adenosylmethionine levels and transcriptionally repressing PHGDH in a time- and dose-dependent manner. Mechanistically, infection induced the transcription factor ZNF143 and promoted its nuclear translocation, where it bound the PHGDH promoter to suppress expression. PHGDH acted as a host restriction factor: its knockdown increased viral titers nearly tenfold, whereas overexpression suppressed replication; PHGDH was involved in viral genome replication and release but not in attachment or internalization. This restriction was uncoupled from serine biosynthesis, as serine/glycine supplementation, enzyme inhibitors, and catalytically inactive mutants all failed to abolish it. PHGDH depletion activated autophagy initiation through the AMPK-ULK1 axis yet arrested autophagic flux at the autophagosome-lysosome fusion step. PHGDH further served as a scaffold for the STX17-SNAP29-VAMP8 SNARE complex, whose loss disrupted the STX17-SNAP29 interaction and produced incomplete autophagy. This restriction extended to PEDV and PRV.

Discussion and conclusions

These findings establish PHGDH as a moonlighting, non-enzymatic host restriction factor that PRRSV antagonizes via ZNF143-mediated transcriptional repression, coupling serine metabolism to autophagic membrane trafficking. Its loss yields a cytosolic niche permissive for replication, while its activity against PEDV and PRV indicates broad-spectrum potential. Because the proviral state reflects loss of PHGDH protein rather than catalytic activity, classical enzyme inhibitors are unlikely to be effective; host-directed strategies that restore PHGDH or reactivate autophagic flux represent more promising antiviral approaches.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statements

This study used established cell lines and previously isolated viral strains only; no human participants or live animal experiments were involved, and no ethical approval was required.

Financial support and Acknowledgements

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Keywords

PRRSV • PHGDH • non-canonical enzymatic function • Autophagic flux • SNARE • viral replication

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P.23 • ID24

A COINFECTION MODEL FOR PRRSV AND PCV3

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Introduction and objectives

Porcine productive and respiratory disease syndrome virus (PRRSV) continues to remain a long-standing and leading cause of economic losses to the pork industry. Porcine circovirus type 3 (PCV3) is a more recently discovered small DNA virus which is also epidemiologically associated with reproductive failure, respiratory disease and the porcine dermatitis and nephropathy syndrome. PCV3 and PRRSV are frequently co-detected in reproductive failure cases, confounding clinical diagnosis and case etiologies. Further, PCV3 is difficult to culture in the laboratory, limiting the experimental exploration of its pathogenesis. The primary objective of this study was to develop a PCV3 infectious clone to enable the rescue of recombinant PCV3 and evaluate whether coinfection of pigs with PRRSV and PCV3 resulted in exacerbation of clinical manifestations under experimental settings.

Methods

Fifteen 10-day old piglets which were negative for PRRSV, PCV3 and PCV2 by PCR and ELISA were used for the study. Ten piglets were administered PCV3 strain US MN 2016 at 1x10⁶ TCID₅₀/ml (2ml intranasal, 2ml intramuscular), while 5 pigs remained uninfected. Ten days after PCV3 infection, five of the PCV3 infected pigs and 3 uninfected pigs were infected with PRRSV, JA 142 at 1x10⁴ TCID₅₀/ml (2ml intranasal). The pigs were observed daily for clinical signs and euthanized 8 days after PRRSV infection. Tissues from major organs such as lungs, liver, tonsils, spleen, heart, intestines, lymph nodes and kidneys were collected for histology. Serum was collected weekly for ELISA and qPCR evaluation. PBMC's were collected from the PCV3 infected piglets to evaluate recall responses and infection status.

Results

Sero-conversion to PCV3, correlating with the detection of PCV2 DNA in serum and viral antigen in lymphocytes by flow cytometry occurred by fifteen days post-infection with the recombinant PCV3 rescued from the infectious clone. PCV3 virus was re-isolated from the serum of infected pigs and was viable during serial passages. Significant gross pathological lesions were not detected in pigs that were infected with PCV3 alone, except for mild lymphoid hyperplasia. While pigs infected with PRRSV alone developed significant lung lesions correlating with viremia, surprisingly, coinfection with PCV3 did not enhance PRRSV replication or lung disease in the experimental model of infection.

Discussion and conclusions

Therefore, PCV3 rescued from the infectious clone was infective in pigs and is a viable solution to difficulties associated with PCV3 laboratory culture. However, field observations that PCV3 and PRRSV coinfection may worsen clinical manifestations were not supported in the tested experimental infection model, which could be influenced by several factors including the lack of environmental stressors, timing or sequence of infection and the selection of viral strains.

Conflicts of interest

The authors declare that they do not have any competing interests.

Ethics statements

All research described in this abstract was carried out in compliance with institutional IACUC (Institutional Animal Care and Use Committee) and IBC (Institutional Biosafety Committee) policies.

Financial support and Acknowledgements

This project was supported by an Agriculture & Food Research Initiative Competitive Grant 2021-67015-34419 from the USDA National Institute of Food and Agriculture.

Keywords

PRRSV • PCV3 • Coinfection • replication • pathology • ELISA • qPCR

P.24 • ID21

PORCINE ENTEROIDS AS AN EX VIVO PLATFORM FOR ASSESSING PORCINE DELTACORONAVIRUS INFECTIVITY

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Introduction and objectives

Porcine enteric coronaviruses, including porcine deltacoronavirus (PDCoV) and porcine epidemic diarrhea virus (PEDV), continue to cause substantial economic losses to the swine industry. Rigorous disinfection of potentially contaminated surfaces (e.g., trucks, fomites, and pig-contact equipment) is critical for preventing transmission via the fecal-oral route. Although molecular assays are highly analytical sensitive, detection of viral RNA does not confirm infectivity. Bioassays can be used for viability assessment but are often impractical due to cost, logistics, and ethical constraints. Porcine enteroids provide greater physiological relevance than traditional *in vitro* systems and may serve as an *ex vivo* alternative for assessing viral infectivity. This study evaluated the feasibility of porcine enteroids cultured on transwell inserts (PETCs) as a diagnostic platform for detecting viable PDCoV. The objectives were to (1) determine the minimum infectious dose of PDCoV required to establish infection in PETC compared to swine testicular (ST) cells, and (2) assess the infectivity of PCR-positive field samples using *ex vivo* (PETC), *in vitro* (ST cells), and *in vivo* (neonatal piglet) models.

Methods

In the first phase, a PDCoV strain (Michigan/8977/2014; 3.16×10^5 TCID₅₀/mL) was two-fold serially diluted (2^{-1} to 2^{-14}) and inoculated into PETC derived from duodenum, jejunum, and ileum of 7-day-old CDCD piglets ($n = 3$), as well as into ST cells. Infection was assessed by cytopathic effect (CPE), immunofluorescence assay (IFA) targeting the N protein, and RT-qPCR detecting the N gene. In the second phase, environmental samples (feeders, rubber teats, pen floor, and sewage) collected from a PDCoV-affected farm after thorough cleaning and disinfection were tested. These samples were inoculated into jejunum-derived PETCs, ST cells, and neonatal piglets. ST cells and PETC were evaluated by CPE, IFA, and RT-qPCR, while piglets were monitored for clinical signs, histopathology, and viral shedding.

Results

ST cells displayed more pronounced CPE and IFA signals across all dilutions. In PETCs, CPE and IFA were minimal or absent, whereas RT-qPCR detected PDCoV RNA at dilutions ranging from 2^{-1} to 2^{-10} (Cq 24-35). Jejunum-derived PETCs were more susceptible to PDCoV than duodenal and ileal cultures, supporting their selection for field sample testing. All field samples, except the pen floor and rubber teats samples, were PCR-positive (Cq 30-34). Following inoculation with PCR-positive field samples, no evidence of productive infection was detected in PETCs, ST cells, or neonatal piglets.

Discussion and conclusions

Unlike transformed *in vitro* cell lines, PETCs preserve epithelial polarity, differentiated intestinal cell populations, and native host-factor expression, enabling biologically relevant assessment of viral entry and replication. Although ST cells demonstrated higher analytical sensitivity, and further optimization of PETC culture conditions is required, PETCs represent a promising *ex vivo* platform aligned with 3R principles. This system bridges conventional cell culture and animal bioassays and supports infectivity assessment of enteric viruses relevant to swine health.

Conflicts of interest

Jesús Horacio Lara Puente is an employee of Laboratorio Avi-Mex. The remaining authors declare no conflicts of interest.

Ethics statements

PETCs were collected from donor piglets euthanized following animal care guidelines of the Iowa State University Institutional Animal Care and Use Committee (IACUC-24-153).

Keywords

Enteroids • Porcine enteric coronavirus • Porcine deltacoronavirus • *Ex vivo* culture • Viability

P.25 • ID55

INTESTINAL ORGANOIDS FROM EPTESICUS FUSCUS SUPPORT REPLICATION OF SELECT SWINE ENTERIC CORONAVIRUSES: INSIGHTS INTO THE BAT-SWINE VIRAL INTERFACE

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Iowa State University

Introduction and objectives

Bats are recognized as reservoirs for zoonotic coronaviruses (CoVs), including SARS-CoV and MERS-CoV. Understanding their susceptibility to other CoVs is crucial. Swine CoVs, such as porcine epidemic diarrhea virus (PEDV) and swine acute diarrhea syndrome coronavirus (SADS-CoV), may originate from bats. Furthermore, recent survey studies have detected PEDV in bat samples. However, studies on bats are limited due to ethical, ecological, and technical challenges. This study aimed to develop an *in vitro* model using intestinal organoids (enteroids) from the big brown bat (*Eptesicus fuscus*) and assess susceptibility to PEDV, transmissible gastroenteritis virus (TGEV), and porcine deltacoronavirus (PDCoV).

Methods

Small intestine tissues from three bats were used to isolate crypt cells, which were cultured in Matrigel to form differentiated enteroids (BEMCs). BEMCs were dissociated and seeded into transwell inserts (BETCs) to create polarized epithelial monolayers. Cell phenotypes of bat tissue, BEMCs, and BETCs were characterized using immunohistochemistry (IHC) and gene expression analysis, including CoV receptors. BETCs were inoculated with PEDV (USA/Colorado/2013), TGEV (Purdue and Miller strains), and PDCoV (Michigan/8977/2014), with or without trypsin, and monitored for 48 hours post-inoculation (hpi). Susceptibility was evaluated through cytopathic effects (CPE), detection of viral nucleocapsid protein (NP) via immunofluorescence (IFA), and quantification of viral RNA using RT-qPCR.

Results

Crypt cells successfully differentiated into enteroids and were capable of being subcultured over 10 passages. BEMCs exhibited crypt-villus structures and central lumens, while BETCs formed confluent monolayers within 5-7 days. Morphology and cell phenotypes of BEMCs and BETCs matched native bat intestinal epithelium, including CoV receptor expression. Trypsin-supplemented PEDV/TGEV/PDCoV-inoculated BETCs showed moderate to severe CPE from 24-48 hpi, while non-trypsin groups exhibited mild or no CPE. Viral NP was detected in infected BETCs by IFA, with stronger fluorescence in trypsin-treated groups. RT-qPCR confirmed the presence of viral RNA across all samples.

Discussion and conclusions

This study successfully established bat enteroid cultures and demonstrated their susceptibility to swine enteric CoVs, regardless of the presence of trypsin. The model offers a valuable tool for studying viral pathogenesis and potential interspecies transmission involving bats.

Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics statements

Three big brown bats from Iowa, submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) for rabies testing between August 2021 and June 2023, were used in this study. The bats were euthanized with carbon dioxide (CO2) following American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, and all tested negative for rabies by direct fluorescent antibody assay.

Keywords

Bat • Intestinal organoids • Porcine epidemic diarrhea virus • Porcine deltacoronavirus • Transmissible gastroenteritis virus

P.26 • ID99

REPLICATION KINETICS ANALYSIS OF SWINE IAV IN A PORCINE MACROPHAGE MODEL

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Universitat Autònoma de Barcelona

Introduction and objectives

Influenza A virus (IAV) is one of the major causes of respiratory disease in pigs. IAV primarily targets the respiratory epithelium and replicates in nasal, tracheal, and bronchial epithelial cells. However, the respiratory tract also contains other cell types, including macrophages, which play an essential role in host defense, but at the same time, they have been implicated in inflammatory responses associated with severe influenza infections. Most current knowledge on macrophage susceptibility to IAV comes from human and murine models and suggests that macrophages generally show limited susceptibility to IAV infection, although this may depend strongly on the virulence of the viral strain. However, little is known about the susceptibility of porcine macrophages to swIAV, despite anecdotal evidence suggesting that these cells may be more susceptible to IAV infection. The aim of this study is to establish an *in vitro* infection model for swIAV in porcine macrophages and to analyze the interaction between contemporary swIAV strains and these cells.

Methods

Porcine alveolar macrophages (PAMs) were used as *in vitro* models. The contemporary swIAV used in this study was selected from laboratory collection. PAMs were used to infect swIAV with different multiplicities of infection (MOIs) or different concentrations of TPCK-treated trypsin. The supernatant was collected at different time points. A viral infection model was established by assessing swIAV replication kinetics and optimal infection conditions. Under suitable infection models, susceptibility to different subtypes of swIAV was assessed, and swIAV replication kinetics were assessed using anti-IAV nucleoprotein antibody detection and plaque assays. Statistical analysis was performed using nonparametric tests.

Results

In the presence of TPCK-treated trypsin, PAMs infection at different MOIs showed that PAMs supported H1avN2s replication, as the infectious viral titer released from PAMs gradually increased between 2, 24, 48, and 72 hours post-infection(hpi). When different concentrations of TPCK-treated trypsin were compared in H1avN2s infection of PAMs at an MOI of 0.1, 1 µg/ml and 5 µg/ml TPCK-treated trypsin showed comparable effects on viral replication. Finally, an infection model of different swIAV subtypes was established at an MOI of 0.01 and 1 µg/ml TPCK-treated trypsin. Results showed that in addition to H1avN2s, H3MaIN1av and H3MaIN2s also replicated effectively in PAMs. However, H1avN1av replication was poor in PAMs, as evidenced by the undetectable expression of nucleoprotein over time and the detection of low concentrations of released infectious virions at 72 hpi.

Discussion and conclusions

This study established an *in vitro* model to assess infection of PAMs by contemporary swine influenza A viruses. PAMs supported productive replication of H1avN2s, H3MaIN1av, and H3MaIN2s when TPCK-treated trypsin was provided, while H1avN1av replicated poorly. These findings suggest strain-dependent susceptibility and support the use of porcine macrophage models rather than extrapolation from other species. The model provides a useful platform to study viral tropism, innate responses, and macrophage involvement in swine influenza pathogenesis more broadly.

Conflicts of interest

None to declare.

Ethics statements

The present study only performed *in vitro* work.

Financial support and Acknowledgements

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Keywords

Swine influenza A virus • Macrophages • Replication

P.27 • ID42

HOST PHOSPHOLIPASE PLA1A ORCHESTRATES VIRUS-INDUCED MEMBRANE REMODELING AND STRUCTURAL PROTEIN STABILIZATION TO PROMOTE ENVELOPED VIRUS ASSEMBLY AND EGRESS

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Introduction and objectives

Enveloped viruses, such as Porcine reproductive and respiratory syndrome virus (PRRSV), rely on host membrane remodeling for efficient virion assembly, budding, and release. However, whether viruses actively exploit host lipid metabolic pathways to regulate membrane biophysical properties during viral assembly and egress remains poorly understood. Phospholipase A1 member A (PLA1A) selectively hydrolyzes phosphatidylserine (PS) to generate lyso-phosphatidylserine (Lyso-PS) and free fatty acids, altering lipid molecular geometry and redistributing lateral pressure within the phospholipid bilayer. The objective of this study was to systematically investigate the biological role of PLA1A in enveloped virus infection using PRRSV as a model, focusing on its impact on host membrane fluidity, viral structural protein stability, and its potential as a broad-spectrum antiviral target.

Methods

To investigate host factors regulating PRRSV infection, transcriptomic profiling and siRNA screening were conducted. The proviral roles of c-FOS and PLA1A were characterized by using plasmid overexpression, CRISPR/Cas9 knockout, and RNA interference, assessing viral kinetics via RT-qPCR, TCID₅₀, and immunofluorescence. Specific viral life-cycle assays (attachment, internalization, replication, release) pinpointed PLA1A's functional stages. To elucidate upstream regulation, dual-luciferase assays, ChIP-PCR, and EMSA were utilized to verify the c-FOS/JUND complex binding to the PLA1A promoter. For downstream mechanisms, Co-IP, IP-MS, and confocal microscopy mapped PLA1A's interaction with the viral glycoprotein GP5. Host membrane fluidity was quantified using TMA-DPH and Laurdan fluorescent probes, while GP5 stability and degradation pathways were analyzed through cycloheximide chase and ubiquitination assays. Finally, molecular docking and differential scanning fluorimetry (DSF) were employed to identify and validate small-molecule PLA1A inhibitors.

Results

PRRSV infection activated the TLR4/p38-MAPK signaling pathway, upregulating c-FOS, which formed an AP-1 complex with JUND to promote PLA1A transcription. PLA1A facilitated viral release through dual mechanisms. First, it hydrolyzed PS to generate Lyso-PS, enhancing membrane fluidity and reducing the energetic barrier for viral budding. Second, PLA1A directly interacted with GP5 (via PLA1A-Asn148 and GP5-Gln196), inhibiting its K48-linked ubiquitination and proteasomal degradation to maintain GP5 stability. Crucially, this interaction with GP5 also recruited PLA1A to the cell membrane, enabling its localized function to further increase membrane fluidity, reduce the resistance of viral fusion and budding, and facilitate virion release. Additionally, PLA1A was passively incorporated into progeny virions. Virtual screening identified Bromocriptine, an FDA-approved drug, as a PLA1A-targeting inhibitor exhibiting potent anti-PRRSV activity.

Discussion and conclusions

PRRSV hijacks host lipid metabolism to orchestrate membrane remodeling and structural protein homeostasis. The TLR4/p38-MAPK/c-FOS-PLA1A signaling axis is exploited to generate a biophysically favorable membrane microenvironment and stabilize the GP5 envelope protein, thereby driving efficient progeny virion assembly and release. Pharmacological targeting of PLA1A using Bromocriptine effectively disrupts membrane fluidity and simultaneously impairs viral replication and egress. These findings highlight host phospholipid metabolism as a promising target for broad-spectrum antiviral intervention.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethics statements

The protocol for primary PAM preparation was approved by the Laboratory Animal Ethical Committee of CAU, with the approval No. AW11606202-2-04.



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Passion for Innovation

P.28 • ID6

INGELVAC® PRRS MLV: AN EFFECTIVE TOOL FOR REDUCING PRRS MORTALITY, INDEPENDENT OF HOMOLOGY WITH THE FIELD VIRUS

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) infects pig populations, causing reproductive disorders and systemic diseases¹. It is estimated that 55% of this impact occurs during the growing phase of pigs². The objective of this study was to evaluate the effectiveness of different vaccination protocols by analyzing the on-site mortality parameter and the respective homology of biological agents and the field PRRSV variant.

Methods

The trial was conducted on a farm at Site 2 in central Mexico between August and September 2025. The groups were composed as follows: Group 1 (G1), which had been affected by an enteric problem in the farrowing ward, had a total of 450 pigs that received 2 ml intramuscularly of the Ingelvac® PRRS MLV vaccine with a 2_5_2 L5A RFLP (Boehringer Ingelheim Animal Health) at an average age of 14 days. Group 2 (G2) consisted of 381 pigs that received a 1 ml intramuscular dose of a biological product aimed at protecting against PRRSV with a 1_4_4 L7 variant at 14 days of age. Both groups were weaned at an average of 26 days, and their stay at Site 2 lasted 8 weeks. During their stay, real-time PCR monitoring of PRRSV in blood serum was performed at weeks 3, 5, and 7 of the pigs' stay. Subsequently, the pigs were sequenced, and we found that the field variant found at site 2, to which the groups were exposed on the farm, was 1_6_3 LND. Statistical analysis was based on the Chi-square test using Statistics V.27 software.

Results

Under this analysis, a decrease in the number of pig deaths per week has been demonstrated, yielding a p-value of 0.01. It has also been shown that the 5A lineage vaccine, despite having less homology with respect to the field variant it challenges, provides efficient cross-protection.

Discussion and conclusions

Based on this field study, we can demonstrate that the group vaccinated with the Ingelvac® PRRS MLV vaccine, despite the enteric problem, had a better outcome with fewer pig deaths per week compared to group 2. It also demonstrates that the issue of homology does not apply to providing greater protection in the field, since group 1 performed better with respect to the evaluated parameter.

Conflicts of interest

During this field test, there were no issues of influence of interest.

Ethics statements

During this field trial, the protocols and measures were established and implemented at each stage of production of the vaccinated groups, with the consent of each participant.

Keywords

Vaccination • PRRS • mortality

Bibliography

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P.29 • ID17

EFFICACY OF A COMMERCIAL PRRS MLV VACCINE IN REDUCING THE IMPACT OF A VIRULENT PRRSV-1 ROSALIA CHALLENGE

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1. IRTA-CReSA; 2. Boehringer Ingelheim

Introduction and objectives

In 2020, a highly virulent Porcine Reproductive and Respiratory Syndrome Virus type 1 (PRRSV-1) strain emerged in Spain and has become predominant across the northeastern region. The disease caused severe reproductive losses, increased sow mortality, and nursery pig mortality exceeding 20%. This study aimed to assess the efficacy of a modified-live virus (MLV) PRRS vaccine against experimental challenge with one of these highly virulent isolates.

Methods

Fifty four-weeks-old PRRSV-naïve pigs were assigned to three groups: T1 (vaccinated and challenged, n=20), T2 (non-vaccinated and challenged, n=20), and T3 (non-challenged controls, n=10). After one week of acclimation, group T1 was vaccinated intramuscularly with Ingelvac PRRSFLEX® EU (Boehringer Ingelheim), while T2 and T3 received PBS. Four weeks later, groups T1 and T2 were intranasally challenged with a highly virulent PRRSV-1 (strain UC Lleida N5) ($\geq 10^{4.8}$ TCID₅₀/ml, 1 ml per nostril). Animals were monitored for clinical signs, rectal temperature, and growth performance. Blood, nasal swabs, and air samples were collected to assess viremia, viral shedding, and air transmission. The lung weight:body weight ratio was calculated at necropsy as an indicator for lung inflammation.

Results

All challenged pigs became infected. Non-vaccinated animals had, on average, more prolonged rectal temperatures ($>40^{\circ}\text{C}$) (25 days vs 14.15 days). At 14 days post-challenge, vaccinated pigs had significantly less lung inflammation (LW/ BW = 1.31 ± 0.24 % vs 1.69 ± 0.49 %, T-test; $p<0.05$) and an five-fold lower viremia Area Under the Curve from the first 35 days after infection (6.39 ± 0.41 vs 7.14 ± 0.37 log RNA copies/ml, T-test; $p<0.05$). Vaccination also reduced nasal shedding and improved average daily weight gain (505.7 ± 126.9 g vs 334.6 ± 140.6 g, T-test; $p<0.05$).

Discussion and conclusions

Intramuscular administration of the MLV PRRS vaccine provided significant clinical, pathological, virological, and productive protection against the challenge with the highly virulent PRRSV-1 Rosalia strain.

Conflicts of interest

This study is funded by Boehringer Ingelheim.

Ethics statements

The experimental protocol was approved by the Ethics Committee for Animal Experimentation of IRTA-CReSA and authorized by the competent authority of the Government of Catalonia. All procedures complied with Directive 2010/63/EU. Experimental challenges were conducted in BSL-2 facilities under strict biosafety and animal welfare conditions.

Financial support and Acknowledgements

This study is funded by Boehringer Ingelheim.

P.30 • ID19

EVALUATION OF A MODIFIED LIVE PRRSV VACCINE IN PREGNANT SOWS CHALLENGED WITH A HIGHLY VIRULENT PRRSV-1 STRAIN

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Introduction and objectives

In Spain, vaccination against porcine reproductive and respiratory syndrome virus (PRRSV) is widely implemented and is considered one of the most effective strategies for disease control. The emergence of highly virulent PRRSV strains has raised concerns regarding the ability of existing vaccination strategies to provide sufficient protection in this epidemiological context. This study evaluated the efficacy of a commercial modified live virus (MLV) vaccine in pregnant sows following intranasal challenge with a highly virulent PRRSV-1 Rosalía strain.

Methods

Seventeen PRRSV-naïve sows were assigned to vaccinated-and-challenged (V/Ch; n=9) or non-vaccinated-and-challenged (NV/Ch; n=8) groups. V/Ch sows were vaccinated with Porcilis PRRS at 62±2 days of gestation, while NV/Ch sows were mock vaccinated with a placebo. Both groups were challenged intranasally four weeks later (90±2 days of gestation) with the highly virulent Rosalía strain (Lleida 029_22; 2x10⁵ TCID₅₀). Sows were monitored for clinical signs, pyrexia, and abortions. Samples (blood, nasal, oral, and rectal swabs) were collected pre-challenge, at 7 days post-challenge (DPC), farrowing (~24DPC), and weaning (~45DPC). Farrowing was assisted to evaluate litter outcomes, and offspring were classified as born alive or non-viable. Vertical transmission was assessed using blood samples from live-born piglets and tissues from non-viable fetuses. Piglets were monitored for 21 days post-partum (DPP), with weekly blood sampling to also evaluate horizontal transmission. At weaning (21DPP), sows and piglets were euthanized and subjected to necropsy to assess gross lesions and collect samples for analytical testing.

Results

Most clinical signs were mild in both groups, except for one V/Ch sow, which suffered severe signs. Pregnancy length was comparable between groups; one sow per group aborted, and approximately 35% of sows required induction of farrowing due to delayed delivery (>117 days of gestation). Offspring viability was low overall, with 60% and 85% of fetuses classified as autolytic in the V/Ch and NV/Ch groups, respectively. Although the proportion of live-born piglets was limited (30% in V/Ch and 11% in NV/Ch), pre-weaning mortality was significantly reduced in the V/Ch group (p<0.05). Consistently, piglets in the V/Ch group remained in generally better condition, whereas those in the NV/Ch group showed rapid clinical deterioration during the first days of life (p<0.05). At birth, live-born piglets in the V/Ch group had significantly lower viremia levels than piglets belonging to the NV/Ch group and the virus was also detected in the samples coming from non-viable fetuses in NV/Ch and V/Ch groups. V/Ch sows tended to have lower viremia, although this difference did not reach statistical significance (p<0.10). In contrast, vaccination significantly reduced nasal PRRSV shedding (p<0.05). Regarding lung lesions, interstitial pneumonia in V/Ch sows was classified as mild to moderate, whereas two cases (25%) in the NV/Ch group were classified as severe.

Discussion and conclusions

Although vaccination with a modified live PRRSV vaccine provided partial protection against a heterologous highly virulent PRRSV-1 strain, the impact of the Rosalía strain remained high, both in the reproductive and respiratory forms of the disease.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statements

The study protocol was approved by the Ethics Committee of A.M. Animalia Bianya, S.L. (CEEA 32/24).

Financial support and Acknowledgements

This work was supported by funds from A.M. Animalia Bianya, S.L. and CDTI (IDI-20230887). Additional funding was provided by the European Union's Horizon Europe research and innovation program (REPRODIVAC project; HORIZON-CL6-2021-FARM2FORK-01). Ariadna Pey is the recipient of an Industrial Doctorate grant from the Government of Catalonia (2025 DI 00021).

Keywords

efficacy • modified live virus vaccine • PRRSV • reproductive form • sow

P.31 • ID26

VACCINATION WITH A PRRS MODIFIED LIVE VACCINE TO PIGLETS LESS THAN ONE WEEK OF AGE REDUCES FEVER AND CLINICAL SCORE IN A ROSALIA PRRSV CHALLENGE

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Zoetis

Introduction and objectives

Suvaxyn® PRRS MLV (Suvaxyn PRRS) can be administered IM from 1 day of age onwards and nasal (IN) from 3 days onwards, which is ideal for antibody production prior to weaning. Recently, we have demonstrated that Suvaxyn PRRS is efficacious to PRRSV-1 Rosalia strain (Rosalia). Our objective was to determine if Suvaxyn PRRS administered at processing would reduce fever and clinical observations in piglets challenged with Rosalia at weaning.

Methods

Naïve-PRRSV piglets were randomly assigned to treatment prior to vaccination (5-7 days of age, Day -28): T01: Saline (IM; n=14); T02: Suvaxyn PRRS (IM; n=14), and T03: Suvaxyn PRRS (IN; n=14). At weaning (Day 0), pigs were weighed, co-mingled, and challenged with Rosalia. Daily rectal temperatures were recorded until day 15 (study end). Clinical scores (the greater the number, the worse the condition) for: General condition (0 to 3); Depression (0 to 3); Sneezing/coughing (0 to 1); Respiratory distress (0 to 3); and other (such as hyperthermia; injuries, anorexia, etc; 0 to 3) were recorded daily and summed. Variables were analyzed with the fixed effects of treatment, and when appropriate, day, and treatment-by-day as well as all random effects.

Results

There was a significant effect ($P = 0.0136$) of treatment for temperature on day 0 (prior to challenge) and therefore it was used as a covariate (Saline: 40.25 ± 0.07 , IM: 40.46 ± 0.07 ; IN: $40.17 \pm 0.07^{\circ}\text{C}$). There was a temperature $\times$ day interaction ($P = 0.0001$). There was no effect of treatment on temperature from days 1 to 9. By day 10, while temperatures began to decline in vaccinated pigs, they remained elevated in saline pigs. On days 10 and 11, saline pigs had a greater temperature (0.2 to 0.5°C) than IM pigs, with IN piglets being intermediate. From day 12 until 15, saline pigs had an elevated temperature compared to both IM and IN pigs, which did not differ. The cumulative clinical scores were greater ($P = 0.0015$) in saline (13.18 ± 2.41) vs IM (3.95 ± 2.42) and IN (4.64 ± 2.42) piglets that did not differ.

Discussion and conclusions

Suvaxyn PRRS MLV administered by IN or IM route at 5 to 7 days of age reduced the occurrence of an extended fever and reduces clinical signs of illness in a PRRSV-1 Rosalia strain challenge.

Conflicts of interest

All authors are employees from Zoetis and have read and agreed to the published version of the abstract

Ethics statements

All the animal experiment was conducted in accordance with the local regulations and with the approval of the Animal Care and Ethics Committee of Zoetis Manufacturing & Research, Spain.

Keywords

Clinical observations • PRRS • Rosalia

P.32 • ID58

FIELD EXPERIENCE WITH THE NEXT-GENERATION PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS TYPE 2 (PRRSV2) MODIFIED LIVE VIRUS (MLV) VACCINE IN PRRSV-ENDEMIC COMMERCIAL FARMS IN THAILAND

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) remain a major constraint for swine production in Thailand, where PRRSV2 and mixed PRRSV1+2 circulation complicate control. The next-generation, PRRSV2 modified live virus vaccine, attenuated by codon-pair-deoptimization, was evaluated under Thai field conditions. This overview summarizes field experiences from randomized, parallel, comparative trials assessing health, production, and economic outcomes versus conventional PRRSV MLV vaccines.

Methods

Four randomized, parallel, field evaluations according to the same protocol were conducted in four Thai commercial production systems with documented PRRSV challenge verified by PCR. Piglets were allocated to NEXT-GEN, Persoporc® (Ceva, France) or, farm-wise, a group vaccinated by one of three conventional PRRSV MLV and housed in separate barns under same management. 625-2136 pigs/group/farm. Trials included two PRRSV2-endemic farms and two farms with concurrent PRRSV1 and PRRSV2 circulation. Vaccination occurred between two and five weeks of age depending on farm practice. Across studies, mortality, growth (ADG) and/or feed efficiency (FCR), and economic returns were recorded through nursery and finishing phases. Economic outcomes were calculated using Gompertz standardization on full farm datasets, and current local prices; mortality comparisons were analysed where applicable using Fisher's exact test. Significance level set $p < 0.05$.

Results

Across field conditions, NEXT-GEN was consistently associated with favourable production and economic outcomes. In a PRRSV2-endemic 2000-sow, wean-to-finish farm, compared to a conventional PRRSV2 L7 MLV, the NEXT-GEN improved mortality from 1.74% to 1.44%, and ADG from 676 to 709 g/day, hence improved net profit of €10.05/pig. In a PRRSV1+2-infected 2,000-sow system, compared to a conventional PRRSV1 MLV, the NEXT-GEN improved nursery mortality from 1.08% to 0.22%, and finishing mortality from 2.79% to 1.80%, with an improved net profit of €8.3/pig. Compared with a conventional PRRSV2 L1 MLV: in a 1500-sow farm with concurrent PRRSV1+2 circulation, NEXT-GEN improved mortality from 4.28% to 3.55%, and FCR from 2.72 to 2.57 kg/kg, improving net profits by €2.45/pig, and in a 2000-sow PRRSV2-endemic herd, NEXT-GEN reduced nursery mortality from 1.32% to 1.12% and ADG from 280 to 309 g/day; finishing mortality from 3.08% to 3.00%, and ADG from 862 to 896 g/day, all in all improving net profits by €5.39/pig.

Discussion and conclusions

These Thai field experiences indicate that the next-generation PRRSV2 MLV can be integrated into PRRS control programs under both PRRSV2 and mixed PRRSV1+2 field challenge. Across diverse commercial settings, its use was associated with reduced losses, improved growth or feed efficiency, and positive economic returns compared with conventional MLV vaccination. The findings support the use of the next generation PRRSV vaccine as a practical tool for improving herd health, animal welfare, productivity, and profitability in PRRSV-endemic swine production.

Conflicts of interest

The studies were conducted and/or authored by representatives of Ceva Animal Health and Ceva Corporate Swine; Persoporc® is a Ceva product.

Ethics statements

The studies were conducted as commercial field evaluations under routine farm conditions. Animals were vaccinated and managed according to farm veterinary health programs and applicable welfare practices.

Keywords

next-generation • MLV • PRRSV2 • Benefits • Field

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P.33 • ID59

NEXT GENERATION PRRSV-2 VACCINE IMPROVES INTERFERON RESPONSE AND REDUCES CHALLENGE VIRUS TRANSMISSION OPPORTUNITIES COMPARED TO CONVENTIONAL MLV

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) remain a major threat to the global pork industry. A next-generation PRRSV2 MLV vaccine has been developed using Synthetic Attenuated Virus Engineering¹. Through targeted codon pair deoptimization, NSP1 translation is significantly slowed which facilitates cross-protective immunity, vaccine safety and efficacy². This study aimed to confirm the increased type-1 interferon (IFN) induction and protective capacity of this vaccine compared to a traditional PRRSV2 MLV when challenged with a PRRSV (sub)type prevalent in Southeast Asia.

Methods

Seventy-three weeks old piglets were allocated into four groups as follows: G1: Persoporc[®] (Ceva), G2: Ingelvac PRRS[®] MLV (Boehringer Ingelheim), G3 and G4: sham vaccinated (PBS). Four weeks post-vaccination (wpv) vaccinates and G3 were challenged intranasally with a type-2 PRRSV strain. Clinical signs, rectal temperature, and nasal virus shedding were recorded daily during the first week post-challenge (wpch), then weekly until slaughter at 3 wpch. Main efficacy parameters included viremia, nasal shedding, lung lesion scoring, and viral load in lungs and lymph nodes (qPCR). IDEXX PRRS X3 ELISA was used to measure antibody production weekly from pre-vaccination. Serum IFN α , IFN γ , IL-6, and IL-10 were quantified at 2wpv and 2wpch by Luminex.

Results

No clinical signs occurred after challenge. Antibody responses appeared from 2 wpv in vaccinates and 2 wpch in naïve animals. Viral nucleic acid persisted in vaccinates up to challenge without increase, while controls peaked at 1 wpch. Both vaccines significantly reduced nasal shedding and lung lesion scores versus controls, with no significant differences between them. However, the next-generation vaccine showed significantly lower viral loads in lungs and tonsils versus the conventional MLV; no significant differences were seen in lymph nodes. Post- vaccination IFN- γ suppression was observed only in the serum of traditional MLV. At 2 wpch, a strong IFN- α response was detected only in G1 pigs, along with significantly elevated IL-10 and IL-6 compared to controls.

Discussion and conclusions

While both vaccines similarly reduced lung lesions, Persoporc[®] achieved significantly greater reductions in viral load in lungs and tonsils, suggesting improved control of transmission. The post-challenge IL-10 response indicated balanced immune modulation rather than suppression of pro-inflammatory cytokines. Induction of IFN- α post-challenge supports vaccine design effectivity in overcoming NSP1-mediated interferon suppression and promoting adaptive immunity³. Overall, this study provides initial insights into the mechanisms underlying the protective efficacy of the Persoporc[®] against a highly relevant Southeast Asian PRRSV strain.

Conflicts of interest

Study conducted by Ceva representatives; Persoporc[®] is a Ceva product.

Ethics statements

Conducted in compliance with Directive 2010/63/EU and Hungarian regulations; approved under BA02/2000-39/2022.

Keywords

Next-generation MLV • Type 1 Interferons • PRRSV2 • Pro-inflammatory cytokines • Lung viral load • Tonsillar viral load

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P.34 • ID62

BENEFICIAL EFFECT OF THE ADMINISTRATION OF AN ADJUVANTED MLV VACCINE CANDIDATE TO BREEDING SOWS FACING A SEVERE PRRSV CHALLENGE

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Introduction and objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) remains a major challenge and an important cause of economic losses in the swine production system. Reproductive failure in sows, is characterized by abortions and delivery of stillborns, mummified foetuses or weak piglets, as well as a high preweaning mortality. Modified-live virus (MLV) vaccination is the main strategy used in the field to control the impact of the infection, although available efficacy data are limited. The aim of this study was to assess the beneficial impact of an adjuvanted MLV vaccine candidate in the reproductive disorders of breeding sows exposed to a severe PRRSV-1 challenge.

Methods

Ten PRRSV naïve breeding sows were intramuscularly vaccinated at the 9th week of pregnancy with the vaccine at a potency of $10^{3.7}$ CCID₅₀/dose and subsequently challenged on approximately day 90 of pregnancy by the intranasal route with a virulent PRRSV-1 strain (PRRSV-8147, provided by Royal GD, Deventer, The Netherlands) at a target dose of $10^{5.3}$ CCID₅₀/animal. A control non-vaccinated group of 10 sows was also included and challenged in parallel. Animals were monitored for reproductive performance until the weaning of their offspring.

Results

Challenge induced a severe negative impact on the reproductive performance in the non-vaccinated breeding sows, that was significantly reduced in the vaccinated group. Thus, 59 % of total born piglets from the control group were born dead (stillborn + mummified foetuses) whereas in the vaccinated group, this proportion was decreased to almost half (31%). Significantly, a lower number of mummified foetuses was observed in the vaccinated compared to the control group (median per litter 2.0 vs. 6.5). Besides, the median number of healthy piglets born per litter was significantly higher in the vaccinated compared to the control group (10.5 vs 2.5). During lactation, piglet mortality was also significantly reduced in the vaccinated group (32% vs 63%), resulting in a significantly higher proportion of weaned piglets. Indeed, vaccinated sows weaned more than threefold the number of piglets compared with the control group (median per litter 9.0 vs 2.5).

Discussion and conclusions

The present study provided evidence of the beneficial effect of the candidate vaccine in pregnant sows challenged on day 90 of gestation. In the non-vaccinated group, this challenge resulted in a severe reproductive failure comparable to the findings reported by Martín-Valls *et al.* during the peak of a field outbreak caused by a highly virulent strain in Spain, with similar proportions of born dead piglets (59% vs 49%) and piglet mortality rate during lactation (63% vs 38%). In these conditions, vaccination of breeding sows reduced the negative impact of the severe PRRSV challenge on the reproductive outcomes and on the litter performance (preweaning mortality), demonstrating efficacy.

Conflicts of interest

None

Ethics statements

The experimental procedures of this study were reviewed and approved by the ethics committee of the corresponding contract research organization in accordance with applicable legislation.

Keywords

MLV Vaccine • PRRSV • Breeding sows

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P.35 • ID66

IMPACT OF A PRRSV-1 VACCINATION PROGRAMME ON NURSERY MORTALITY IN A COMMERCIAL SWINE FARM

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) remains one of the most important pathogens affecting swine production worldwide, causing substantial economic losses driven by increased mortality, reduced productivity and predisposition to secondary infections. In Europe, PRRSV-1 predominates and shows marked genetic and pathogenic diversity, including the emergence of highly virulent field strains in Spain in recent years. Despite widespread use of modified-live vaccines, field evidence under commercial conditions remains limited regarding the longitudinal impact of vaccination on nursery mortality and mortality causes in herds exposed to genetically diverse PRRSV-1 strains. The objective of this study was to evaluate the impact of implementing a PRRSV-1 modified-live vaccination programme in piglets on nursery mortality and mortality causes under commercial farm conditions.

Methods

A longitudinal observational study was conducted on a commercial pig farm in southeastern Spain. Active PRRSV circulation in suckling piglets was confirmed by PCR in May–June 2022. A modified-live PRRSV-1 vaccine (UNISTRAIN® PRRS, strain VP-046 BIS) was subsequently introduced, with piglets vaccinated at 17–18 days of age. The vaccinated cohort was followed during July–August 2022, and a subsequent post-vaccination cohort was evaluated during September–October 2022. Sequential production cohorts were evaluated during pre-vaccination (PRE-VAC), vaccination (VAC), and post-vaccination (POST-VAC) periods. Nursery mortality, age at death and cause of mortality were recorded at individual level. Mortality incidence was analysed using a negative binomial regression model with log link and population offset, estimating incidence rate ratios (IRR) for vaccinated periods relative to pre-vaccination. Differences in age at death among periods were assessed using the Kruskal–Wallis test, and differences in the distribution of mortality causes between periods were assessed using pairwise chi-square tests, with the number of dead piglets in each category used as frequency weights.

Results

A total of 13,103 piglets were evaluated. Nursery mortality decreased significantly over time, from 14.95% in the pre-vaccination period to 5.29% during vaccination and 3.59% post-vaccination. Negative binomial modelling confirmed significant reductions in mortality incidence in vaccinated (IRR 0.39; 95% CI 0.26–0.58; $p < 0.001$) and post-vaccination cohorts (IRR 0.27; 95% CI 0.18–0.41; $p < 0.001$) compared with pre-vaccination, corresponding to reductions of approximately 61% and 73%, respectively. Age at death was significantly lower in the post-vaccination period ($p < 0.001$), while body weight at death did not differ among periods. The distribution of mortality causes also changed significantly between periods, with a marked reduction in epidermitis-associated deaths and a relative increase in neurological causes over time.

Discussion and conclusions

Implementation of a PRRSV-1 modified-live vaccination programme was temporally associated with a substantial reduction in nursery mortality and with changes in mortality patterns under commercial conditions. Although the observational design precludes definitive causal attribution, the consistency of these findings across successive cohorts supports a strong epidemiological association between vaccination and improved piglet survival.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

Animal procedures and sample collection were conducted in accordance with applicable European and national regulations.

Keywords

PRRSV-1 • vaccination • commercial conditions

P.36 • ID71

EFFICACY OF A COMMERCIALY AVAILABLE MLV (PYRSVAC-183) AGAINST THE HIGHLY VIRULENT PRRSV-1 ROSALÍA STRAIN

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Introduction and objectives

The emergence of highly virulent PRRSV-1 strains such as 'Rosalía', is of particular concern. The genetic distance with the existing vaccines leads to question about the level of protection these vaccines can offer. Therefore, assessing this efficacy is key. The objective of the present study was to assess the efficacy of a currently licensed vaccine, PYRSVAC-183, against the challenge with the highly virulent PRRSV-1 strain R1.

Methods

Thirty 4-week-old PRRSV-naïve piglets were allocated to three groups (n = 10/group). Group 1 (G1) received a single intramuscular (IM) dose of the PYRSVAC-183 modified-live virus (MLV) vaccine while groups 2 and 3 (G2 and G3) remained as unvaccinated controls. Forty-nine days after the initial treatment, G1 and G2 were challenged intranasally with the PRRSV-1 Rosalía R1 strain (Genbank accession OM893828, 105.5 TCID₅₀/mL), whereas G3 remained unchallenged. Clinical signs and weight gains were monitored after challenge. Serum and nasal swabs were collected to assess viremia, viral shedding and antibody responses. PRRSV-specific IFN-γ responses were evaluated by ELISPOT. Half of the animals were euthanized at 7 days post-challenge (dpc) and the remainder at 28 dpc for pathological examination.

Results

After the challenge, unvaccinated G2 pigs developed fever from 3 to 7 dpc, with up to 50% febrile at peak, together with reduced feed intake and impaired growth. Additionally, significant differences were observed between vaccinated and unvaccinated animals, when the scores corresponding to the clinical records of each individual were summed to construct a cumulative clinical score. In G1 pigs, clinical signs were generally mild, with little evidence of lethargy or respiratory disease, although fever developed. Average daily gain during the challenge period was 867 ± 165 g in G1, 763 ± 46 g in G2, and 1,160 ± 320 g in unchallenged G3 pigs, which remained clinically normal. On the day of challenge, 9/10 vaccinated animals had neutralizing antibodies against the vaccine strain (1:8–1:64), but these did not neutralize the challenge strain. Neutralizing antibodies against the challenge strain were not detected until 27 dpc. Significant cell-mediated responses were detected after vaccination (>400 IFN-γ-secreting cells/106 PBMC at 28 dpv) and were clearly boosted after challenge (>800 cells/106 PBMC at 7 dpc). However, vaccinated animals still developed viremia and significant lung lesions.

Discussion and conclusions

Challenge with the highly virulent Rosalía strain induced severe clinical disease, growth retardation, lung lesions, and viremia in non-vaccinated piglets. A single dose of PYRSVAC-183 alleviated clinical disease, with vaccinated animals showing reduced lethargy and improved post-challenge growth. Although complete protection against infection and virological outcomes was not achieved, as commonly observed with current PRRSV MLV vaccines under heterologous challenge conditions, the clinical and productive impact of disease was mitigated. Overall, these results indicate that a single dose of PYRSVAC-183 may help reduce the clinical and productive consequences of Rosalía infection, while highlighting the need to further optimize vaccines against emerging highly virulent PRRSV variants.

Conflicts of interest

Laeticia Delgado, Hans de Smit and Luisa de Arriba are employees of Laboratorios SYVA.

Ethics statements

Experiments were conducted under permission no. 6176 of the CEEAH of UAB.

Financial support and Acknowledgements

The study was funded by Laboratorios SYVA.

Keywords

PRRSV • vaccination • highly virulent PRRSV-1

P.37 • ID84

IMMUNE RESPONSES OF US COMMERCIAL PRRSV-2 VACCINES IN WEANED PIGS CHALLENGED WITH A NORTH CAROLINA PRRSV-2 STRAIN (L1A.60)

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Introduction and objectives

The emergence of porcine reproductive and respiratory syndrome virus (PRRSV) variants, such as PRRSV-2 Lineage 1A.60 (L1A.60) in North Carolina (NC), challenges disease control. This study evaluated PRRSV-specific cellular and humoral cross-protection elicited by US commercial vaccines against an NC L1A.60 strain to guide regional vaccine selection.

Methods

Forty-eight PRRSV-negative, weaned pigs were divided into six groups (n=8/group). At 0 days post-vaccination (dpv), Group 1 (control) received PBS, while Groups 2–6 received Foster, Ingelvac, PrimePac, Prevacent, or PRRSGard, respectively. At 35 dpv, pigs were challenged intranasally/intramuscularly with NC PRRSV-2 L1A.60 (106 TCID₅₀/pig) and monitored until euthanasia at 45 dpv (10 days post-challenge). Anti-PRRSV antibodies and IFN- γ -secreting cells were assessed at 14, 28, 35, 38, 42, and 45 dpv; neutralizing antibodies (NAbs) were measured from 35 to 45 dpv.

Results

Vaccinated groups seroconverted at 14 dpv, maintaining significantly higher mean anti-PRRSV antibodies levels than the control group from 14 to 38 dpv. Controls remained seronegative until challenge, after which all pigs seroconverted by 42 dpv. PrimePac and Prevacent consistently maintained significantly higher antibody levels than the control group. Notably, no pigs developed detectable NAbs against NC L1A.60. Regarding cell-mediated immunity, Foster induced significantly higher IFN- γ -secreting cell counts than control, Ingelvac, and PrimePac groups at 35 dpv. Post-challenge, IFN- γ responses in the PrimePac group (at 38, 42, and 45 dpv) and the Prevacent group (at 42 and 45 dpv) significantly exceeded those of the control group.

Discussion and conclusions

While all five US commercial vaccines successfully induced robust systemic anti-PRRSV antibodies, the complete absence of cross-neutralizing antibodies highlights clear limitations against the NC L1A.60 variant. Conversely, cell-mediated immunity exhibited delayed pre-challenge kinetics but showed a marked, rapid expansion of PRRSV-specific IFN- γ -secreting cells post-challenge across all vaccinated groups. This demonstrates that these vaccines successfully primed host T-cells for a robust anamnestic response upon heterologous exposure, especially in the PrimePac and Prevacent groups. Ultimately, given the complete lack of neutralizing humoral protection, PRRSV-specific IFN- γ -secreting cells constitute the primary defense against the L1A.60 variant. These divergent immunological profiles provide critical data and actionable insights to optimize strategic regional vaccine selection in NC endemic areas.

Conflicts of interest

Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statements

All methods and animal studies were conducted under the approval of North Carolina State University Animal Care and Use Committee (IACUC) ID# 23-369: approval date on 25 October 2023.

Financial support and Acknowledgements

This study was funded by the Inter-Disciplinary Engagement in Animal Systems, project award no. 2022-68014-37266, from the U.S. Department of Agriculture's National Institute of Food and Agriculture. Dr Sirisereewan is supported by the Inter-Disciplinary Engagement in Animal Systems, project award no. 2022-68014-37266, from the U.S. Department of Agriculture's National Institute of Food and Agriculture.

Keywords

Immune responses • PRRSV-2 • Vaccines • Pigs

P.38 • ID112

EFFICACY OF UNISTRAIN® PRRS AGAINST EXPERIMENTAL CHALLENGE WITH THE HIGHLY VIRULENT SPANISH PRRSV-1.1 ROSALIA STRAIN IN PIGLETS

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Introduction and objectives

In 2020, atypical outbreaks of porcine reproductive and respiratory syndrome virus type 1 (PRRSV-1) were reported in northeastern Spain, characterized by high abortion rates, severe respiratory signs, and increased mortality in affected nurseries (Martín-Valls *et al.*, 2023). Sequencing analyses identified a novel highly virulent PRRSV-1.1 strain, commonly referred to as Rosalia. This study evaluated the efficacy of a commercial modified-live PRRS vaccine against experimental infection with a PRRSV-1.1 Rosalia field isolate from 2020.

Methods

Seventy PRRS-naïve, 3-week-old piglets were randomly allocated to vaccinated and non-vaccinated groups. Vaccinated piglets received UNISTRAIN® PRRS intradermally, whereas controls received placebo. Five weeks later, all piglets were intranasally challenged with a PRRSV-1.1 Rosalia field strain obtained during the initial outbreaks in northeastern Spain in 2020, at a dose of $1 \times 10^{3.5}$ CCID₅₀ per animal. The ORF5 of the challenge strain shared 84.2 % nucleotide identity with the vaccine strain. Clinical signs, mortality, body temperature, weight gain, viraemia, nasal shedding, tissue viral loads, lung lesions, and serological response were evaluated. Statistical analyses included Mann–Whitney U or Student’s t-tests for group comparisons, Fisher’s exact or chi-square tests for proportions, and Kaplan–Meier log-rank analysis for survival; $p < 0.05$ was considered significant.

Results

Following challenge, 80 % of non-vaccinated piglets died within 22 days, with peak mortality between days 10 and 14, whereas mortality in vaccinated animals was 8.8 % ($p < 0.0001$), corresponding to an 89 % reduction. Vaccinated piglets also showed significantly lower global clinical sign scores than controls (32.5 vs 55.0; $p = 0.0002$), representing a 40.9 % reduction in clinical disease. Vaccinated animals showed greater weight gain and reduced lung lesions, with decreases of 57.7 % in interstitial pneumonia and 77.7 % in bronchopneumonia-affected lung area. Viral loads in serum on days 3, 7, 14, and 17 post-challenge and in nasal secretions on days 10 and 14 post-challenge were significantly lower in vaccinated animals. Vaccinated piglets also showed fewer PRRSV-positive serum and nasal samples, with reductions of 44.0 % and 42.7 %, respectively. Lung and tonsil viral loads were also significantly reduced. All vaccinated piglets seroconverted by ELISA before challenge. Following challenge, all vaccinated piglets developed detectable neutralising antibody titres, which were significantly higher than those of non-vaccinated animals ($\log_2 5$ vs $\log_2 3$, $p = 0.0009$).

Discussion and conclusions

UNISTRAIN® PRRS vaccination provided significant clinical and virological protection against the highly virulent Spanish PRRSV-1.1 Rosalia strain, despite genetic divergence from the vaccine strain.

Conflicts of interest

H. Pla, M. Simon-Grifé, H. Clilverd and M. Fenech are employees of HIPRA. The study was sponsored and conducted by HIPRA Scientific S.L.U.

Ethics statements

The protocol of this study was reviewed and approved by the Ethical Committee of HIPRA Scientific S.L.U. and the Department of Climate Action, Food, and Rural Agenda of the Government of Catalonia, under approval code 11600, approved on 12 January 2022.

Keywords

highly virulent PRRSV-1 • modified-live vaccine • vaccine efficacy • piglets • experimental challenge

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P.39 • ID118

ENHANCED PRODUCTIVITY ASSOCIATED WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VIRUS VACCINATION STRATEGIES COMBINING MODIFIED LIVE VACCINES AND AN INACTIVATED VACCINE: A COMPILATION OF EVIDENCE

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Ceva Animal Health

Introduction and objectives

Since its emergence in the late 1980s, PRRS has remained one of the most economically significant diseases in the global swine industry, causing an estimated \$1.2 billion annual loss in the United States¹. Control strategies commonly include biosecurity, management practices, and vaccination of sows and piglets. Commercial PRRS vaccines include modified live vaccines (MLV) and inactivated formulations derived from both genotype 1 and 2 strains². Evidence shows that pigs previously exposed to PRRSV—through MLV vaccination or natural infection—develop an enhanced humoral response when subsequently vaccinated with an inactivated vaccine, resulting in increased levels of virus-neutralizing antibodies³. This document summarizes findings from multiple European studies evaluating a complementary PRRSV prophylaxis approach, the Complementary Vaccination Scheme (CVS), which combines MLV priming with a booster using a specific inactivated vaccine, INV-P (Progressis®, Ceva, France).

Methods

Seven published studies were systematically reviewed to synthesize their relevant findings on the implementation of the INV-P CVS across diverse pig production systems from Europe^{4–10}.

Results

Implementing INV-P CVS—especially as a booster before farrowing and with gilt priming—consistently improved disease control and production outcomes when applied in multiple swine farms (300–2,800 sows) with persistent PRRS issues despite routine MLV vaccination. Key findings: Improved PRRSV control: Reduced viral circulation in breeders and piglets, often delaying or preventing infection until ≥8–14 weeks of age. Stronger piglet immunity: Increased maternally derived antibodies (MDA), leading to better early-life protection compared to MLV-only programs. Production recovery: Marked improvements in reproduction, piglet survival, nursery performance, and overall herd productivity. Stabilization of herds: Reduced clinical signs (especially respiratory disease) and more predictable health status. Enhanced outcomes with biosecurity: Combining INV-P CVS with good biosecurity enabled the production of PRRSV-negative pigs in nurseries and even control of highly virulent strains.

Discussion and conclusions

This review compiles evidence from a diverse set of studies supporting the robust scientific basis of the Complementary Vaccination Scheme (CVS) for PRRSV control. Progressis® has demonstrated efficacy both as a standalone intervention and in combination with PRRSV MLV vaccines. With appropriate scheduling, the two vaccination approaches can be co-administered without immunological interference or additional safety risks beyond those already characterized for each product. Under these conditions, integrating the CVS into existing vaccination protocols provides superior PRRSV control compared with MLV-only approaches, leading to improved herd stability and productive performance.

Conflicts of interest

The studies were conducted and/or authored by representatives of Ceva Animal Health and Ceva Corporate Swine; Progressis® is a Ceva product.

Ethics statements

The studies were conducted as commercial field evaluations under routine farm conditions. Animals were vaccinated and managed according to farm veterinary health programs and applicable welfare practices.

Keywords

Complementary vaccination schemes • CVS • PRRSV MLV • Progressis • Control

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P.40 • ID7

ANALYSIS OF CLINICAL BEHAVIOR AT A PIG PRODUCTION SITE 2 AFTER PRRSV VACCINATION IN MEXICO

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1. Boehringer Ingelheim Animal Health Mexico; 2. Boehringer Ingelheim animal health Mexico; 3. Production manager; 4. Grupac; 5. Statistical analyst

Introduction and objectives

Vaccines based on specific strains of PRRSV depend on the induction of cross-immunity for their efficacy¹. Vaccination with a full dose of modified live PRRSV virus (MLV) has been shown to significantly reduce the consequences of PRRSV exposure compared to unvaccinated pigs². The objective of this study was to evaluate the behavior of a PRRSV-vaccinated population exposed to the 1-10-3 LND, 1-6-3 LND, 1-6-4 LND, and 1-7-3 LND PRRSV variants in an area challenged with the virus.

Methods

The piglet farm at Site 1, where the piglets were vaccinated, is positive for PCV2, Mhyo, IAV-S, PRRSV, and PEDV. A total of 32,875 pigs were received from Site 1 to Site 2 at 28 days of age from December 2024 to June 2025. These pigs were receiving a PRRSV vaccination schedule (2 ml of Ingelvac® PRRS MLV from Boehringer Ingelheim Animal Health, administered intramuscularly at an average age of 14 days). The pigs were housed at Site 2 for 7 weeks. Monthly mortality rates were assessed, and serum samples were taken and sent to the Boehringer Ingelheim Animal Health HMC laboratory for PRRSV sequencing and PCR Ct values measured at 3, 5, and 7 weeks of housing. The analysis of mortality dynamics was performed in the first and last vaccinated groups using the Student's t-test for independent samples Statistics V.27.

Results

PCR results from site 2 showed a progressive stabilization of Ct values across the monitoring period (December to May), following the introduction of vaccinated populations. Ct values became more stable across all evaluated weeks, generally remaining above 29 in weeks 5 and 7, which is indicative of lower viral shedding and increased herd stability. Analysis of the first vaccinated group versus the last vaccinated group. (P=0.03).

Discussion and conclusions

Under field conditions, we can observe in this study that the administration of the Ingelvac® PRRS MLV vaccine helped improve the farm's productive performance against different virus variants present in the area.

Conflicts of interest

Under this test, there was no conflict of interest between those involved

Ethics statements

This test was carried out in accordance with the protocols established with the production unit and always respecting each of the guidelines.

Keywords

Vaccination • stability

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P.41 • ID28

EFFICACY EVALUATION OF A BIVALENT READY-TO-USE PROTEIN SUBUNIT VACCINE AGAINST PRRSV AND MYCOPLASMA HYORHINIS CHALLENGE

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Introduction and objectives

Porcine respiratory and reproductive syndrome virus (PRRSV) and *Mycoplasma hyorhinis* (Mhr) are pathogens contributing to porcine respiratory disease complex (PRDC). A strong correlation between Mhr and PRRSV infection was reported in Korea and this correlation has also been observed in Taiwan and other Asian countries. In the present study, the efficacy of a bivalent ready-to-use protein subunit vaccine composed of antigens derived from Mhr and PRRSV was evaluated in pigs.

Methods

Specific-pathogen-free pigs were vaccinated with one dose of PRRS/Mhr bivalent subunit vaccine or normal saline at 3 and 6 weeks old. At 9 weeks of age, pigs were challenged with either *Mycoplasma hyorhinis* (Mhr) or a virulent NA PRRSV strain (TSYM) isolated from field sample in Taiwan. For Mhr challenge, pigs were observed daily for clinical symptoms including anorexia and joint swelling. At 21 days post challenge, animals were sacrificed and examined for polyserositis (peritonitis, pleurisy, pericarditis) and arthritis and scores were assigned according to the severity of lesion. For PRRSV challenge, blood samples were collected for viremia and antibody analysis. At 21 days post challenge, animals were sacrificed and examined for lung lesions.

Results

After Mhr challenge, vaccinated pigs had lower clinical scores and fewer pigs exhibited swollen joints than the unvaccinated pigs. The average lesion score in peritonitis, pleurisy, pericarditis, and arthritis in vaccinated pigs was also lower than in unvaccinated pigs. Vaccinated pigs were PRRSV antibody positive on the day of PRRSV challenge and had lower PRRSV viremia 10 days after challenge. In addition, vaccinated pigs had lower lung lesion scores in apical lobes, cardiac lobes, and diaphragmatic lobes.

Discussion and conclusions

Results from this efficacy study demonstrated that vaccinating pigs with one dose of a bivalent ready-to-use PRRS/Mhr subunit vaccine at 3 and 6 weeks old can reduce clinical symptoms and tissue lesions associated with PRRSV and Mhr, suggesting that it can be an efficient method to control *Mycoplasma hyorhinis* and PRRSV infection in swine population and reduce the economic loss.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statements

Animals used in this study were cared for following the “Guideline for the Care and Use of Laboratory Animals” published by Council of Agriculture, Executive Yuan, Taiwan. Study protocol was reviewed and approved by the institutional Animal Care and Use Committee (IACUC) at Reber Genetics Co., Ltd.

Keywords

Bivalent subunit vaccine • Efficacy • PRRSV • *Mycoplasma hyorhinis*

P.42 • ID79

GENOTYPE-PHENOTYPE RELATIONSHIP IN PRRSV: COMPARISON OF VIRULENCE BETWEEN RECOMBINANT STRAINS, WILD TYPE AND VACCINE STRAIN

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Introduction and objectives

PRRSV evolves rapidly due to high mutation rates and frequent recombination. Recombination has been documented between two field strains, field and vaccine strains, and two vaccine strains. For many of these recombinants, genotype-phenotype relationships remain poorly understood. Addressing these gaps requires direct comparisons between recombinant viruses and their parental strains. In this study we compared two recombinant strains (AUT22-97, AUT22-1552) with the parental strains - the AUT15-33 field strain and the PRRSFlex vaccine strain (Boehringer Ingelheim) - with respect to clinical signs, histopathology, viral load and the immune response by evaluating cytokine production after PRRSV stimulation.

Methods

68 PRRSV-naïve piglets were infected intranasally on day 0 (1.5 mL/nostril; 5×10^5 TCID₅₀/animal) with the respective strain: AUT22-97, AUT22-1552, AUT15-33. The backbones of AUT22-97 and AUT22-1552 have a similarity of ~95% to AUT15-33. In AUT22-97 the region ORF5-3' is replaced by PRRSFlex, while in AUT22-1552 ORF5-ORF6 is replaced. Controls received mock inoculum. The vaccine strain PRRSFlex was applied intramuscularly (1×10^6 TCID₅₀/mL). Clinical signs, viremia, viral load in lung, lymph node and tonsil as well as pathohistological lesions were evaluated. PBMCs were isolated and restimulated with the respective homologous PRRSV strains at an MOI of 0.5. An 18-color intracellular cytokine staining panel was used to phenotype T-cell subsets and multifunctional cytokine responses by flow cytometry.

Results

One recombinant (AUT22-97) behaved like the parental field strain (AUT15-33) with severe disease in infected animals within the first week (fever up to 40.5°C, reduced activity), whereas piglets infected with AUT22-1552 showed similar signs about one week later. This clinical pattern was mirrored by virological readouts, with viremia and high viral loads in lung, lymph nodes, and tonsils. No clinical signs were observed in vaccinated pigs. Intracellular cytokine staining revealed higher frequencies of multifunctional (IFN γ +TNF α +) cells within CD8+ than within CD4+ T cells at 10 and 28 dpi. At 10 dpi, among CD8+ T cells, the frequency of multifunctional IFN γ +TNF α + cells was highest in AUT22-97 (median 0.06%), whereas AUT15-33 showed the highest frequency of TNF α + cells within CD8+ T cells (median 0.28%). By 28 dpi, AUT22-97 stood out, showing the highest frequencies of CD8+TNF α + (median 0.32%), CD8+IFN γ +TNF α + (median 0.31%), and CD8+IFN γ + cells (median 0.39%). In contrast, AUT22-1552 displayed the lowest frequencies at 28 dpi for CD8+TNF α + (median 0.10%), CD8+IFN γ +TNF α + (median 0.04%), and CD8+IFN γ + cells (median 0.05%). In head-to-head comparison, AUT22-97 elicited earlier and stronger CD8 multifunctional responses than both parental strains, whereas AUT22-1552 showed lower, vaccine-like responses; AUT15-33 induced a weaker CD8 response than the vaccine at 10 dpi but a stronger one by 28 dpi.

Discussion and conclusions

Recombinant, field, and vaccine strains showed different disease courses, but all induced mainly CD8 T cell responses. At both 10 dpi and 28 dpi, the recombinant strain AUT22-97 showed the highest levels across CD8+IFN γ +TNF α + cells, indicating stronger and more multifunctional CD8 responses over time. These results suggest that recombination can change viral replication, clinical and pathological signs, and the timing and strength of antiviral T cell immunity.

Conflicts of interest

no conflicts

Ethics statements

approved according to the Austrian Tierversuchsgesetz (BMFW-2021-0.117.108) approval code 2025-0.052.808

P.43 • ID121

INFLUENCE OF PRRSV GENETIC DIVERGENCE AND VIRULENCE ON THE PROTECTIVE EFFICACY OF A MODIFIED LIVE VACCINE IN AN EXPERIMENTAL CHALLENGE MODEL

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Introduction and objectives

Porcine reproductive and respiratory syndrome (PRRS) continues to be one of the most important infectious diseases affecting swine production worldwide. The extensive genetic diversity of PRRS virus (PRRSV) limits the cross-protective efficacy of modified live virus (MLV) vaccines, particularly against highly divergent field strains.

Methods

The present study compared the outcome of vaccination following experimental challenge with two PRRSV-1 isolates differing in virulence. Two groups of vaccinated pigs ($n = 6$ per group) received a commercial MLV vaccine and were subsequently challenged with either the classical strain CReSA-70 or the highly virulent strain Rosalía (CReSA-JP5). Equivalent non-vaccinated challenged groups were included for comparison.

Results

Vaccinated animals challenged with CReSA-70 developed only mild clinical signs, lower levels of viremia and virus shedding, and limited pulmonary lesions. In contrast, vaccination provided substantially lower protection against the highly virulent JP5 isolate, as evidenced by persistent fever, increased viral loads in serum and nasal swabs, and more extensive lung pathology. Although vaccinated animals showed seroconversion before challenge, which is not necessarily associated with protective immunity against PRRSV, they were not effectively protected against disease caused by the virulent strain. Protection appeared to correlate with the genetic relatedness between vaccine and challenge viruses, which shared 91.20% and 82.28% whole-genome nucleotide identity for CReSA-70 and CReSA-JP5, respectively.

Discussion and conclusions

As expected, disease severity was greater in non-vaccinated animals regardless of the challenge strain, confirming a partial protective effect of vaccination. However, the marked differences observed between vaccinated groups indicate that vaccine performance is strongly influenced by viral virulence and genetic divergence. Ongoing next-generation sequencing (NGS) analyses of viruses recovered from infected animals will characterize viral population diversity and quasispecies dynamics, providing insight into genomic changes associated with vaccine-driven selection, viral adaptation, and potential immune escape mechanisms.

Conflicts of interest

None

Ethics statements

Approved by the Ethics Committee from the Generalitat of Catalonia, according to Spanish and European regulations.

Financial support and Acknowledgements

Acknowledgements to personal from CReSA facilities for the support provided in the animal experiment

Keywords

PRRSV • VACCINE • EVOLUTION

P.44 • ID127

GUT MICROBIOTA DYNAMICS FOLLOWING PRRSV VACCINATION AND INFECTION: EVIDENCE FOR ENHANCED MICROBIAL STABILITY IN VACCINATED PIGLETS

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Introduction and objectives

The vaccination of piglets with modified live virus (MLV) is a common practice for the control of porcine reproductive and respiratory syndrome (PRRS). PRRS virus (PRRSV) modulates immunity response in the host, which can be also balanced by intestinal microbiota at systemic level. Here, we aimed to analyse the impact of PRRS vaccination and further PRRS infection in MLV vaccinated (n=7) and non-vaccinated control (n=6) piglets in the gut microbiota using shot-gun metagenomics.

Methods

Besides microbiota analyses, the infection outcome was evaluated by monitoring the disease, the viremia and antibody production and evaluating gross pathology and histopathology of the lungs as well as lung viral load.

Results

The MLV vaccination defined the prokaryotic community with compositional (beta-diversity) differences between vaccinated and non-vaccinated pigs, in which putative species of genus "Candidatus Cryptobacteroides" presented a differential abundance in vaccinated animals. Furthermore, this species strongly correlated with clinical signs and PRRSV antibodies after challenge. The impact of PRRSV infection in the microbiota was evidenced by compositional analyses and associated both to the infection and antibody response. The infection had a lower impact in the community diversity of MLV animals than in the samples from non-vaccinated pigs which was reflected in a more stable Bacillota/Bacteroidota ratio in the former along the study.

Discussion and conclusions

This study provides the first shotgun metagenomic assessment of the effects of MLV PRRS vaccination and PRRSV challenge on gut microbiota composition. Although no major differences in species or functional diversity were detected, the MLV vaccine altered the microbiota composition and after PRRSV challenge the absence of vaccination was associated with greater microbiota divergence in parallel with PRRS antibody responses and clinical signs. These findings suggest that MLV PRRS vaccination may influence gut microbiota composition and contribute to maintaining microbial stability during PRRSV infection.

Conflicts of interest

The authors declare no conflict of interest regarding the results of this research.

Ethics statements

This experiment was performed according to the guidelines of the European Union (Directive 2010/63/EU) and approved by the Regional Autonomous Government (Project 12055; FUE-2023-03053650).

Keywords

PRRS • modified live virus vaccine • gut microbiota

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P.45 • ID8

NANOENCAPSULATION INCREASED THE SOLUBILITY AND EFFICACY OF AN ANTI-PRRSV SMALL MOLECULE

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Introduction and objectives

While the Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most economically devastating diseases in the global porcine industry, current mitigating strategies of vaccines and genetic engineering are either only partially effective or slow in integration. Using artificial intelligence, we identified a small molecule, B7 (4-fluoro-2-methyl-N-(3-([3-(morpholine-4-sulfonyl)phenyl]amino)quinoxalin-2-yl)benzene-1-sulfonamide), which blocks the interaction of CD163 with the PRRS virus (PRRSV). We also experimentally showed that B7 effectively inhibits the amplification of both Types 1 and 2 PRRSV at 75–95% *in vitro*. Our objectives were to 1) overcome the insolubility of B7 by nanoencapsulation (Nano-B7); 2) determine the *in vitro* release of B7 from nanoparticles, 3) Nano-B7's cytotoxicity and 4) *in vitro* anti-PRRSV efficacy.

Methods

B7 was encapsulated with 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol. Physicochemical stability of Nano-B7 was evaluated at 0 and 6 months after manufacture for particle size, polydispersity index (PDI), and zeta potential using Malvern Zetasizer Nano ZS. The cytotoxicity of Nano-B7 was compared to B7 dissolved in DMSO (DMSO-B7) in the range of 1.25–100 µM using porcine alveolar macrophages (PAMs). B7's *in vitro* release was determined using simulated lung fluid, blood plasma, and lysosomal environment using a Shimadzu UV-1900i UV-Vis Spectrophotometer. Additionally, the anti-PRRSV efficacy of Nano-B7 was assessed for amplification in PAMs by qRT-PCR and for viral replication by tissue culture infectious dose assay (TCID₅₀) using CD163-transfected MARC145 cells.

Results

B7's solubility after nanoencapsulation was up to 30 mg/ml and Nano-B7 was stable after 6 months of storage at 4°C because all physicochemical parameters remained in acceptable ranges. B7 was released from nanoencapsulation at 67% in 12 hours in simulated lung fluid, suggesting a favorable delivery rate to respiratory tissues. For Type 1 PRRSV (Lelystad strain), Nano-B7 achieved 86% and 92% amplification inhibition at 15 µM and 20 µM, respectively, corresponding to 2.4 and 3.4 log₁₀ reductions in viral replication. In comparison, DMSO-B7 reached 75% inhibition and a one-log₁₀ reduction at 20 µM. For the Type 2 PRRSV (VR2332 strain), Nano-B7 produced 92% and 98% amplification inhibition at 10 µM and 15 µM, with 1.1 and 2 log₁₀ decreases in viral replication, respectively. Under the same conditions, DMSO-B7 achieved 85% inhibition and a 1.3 log₁₀ reduction at 20 µM. Similarly, for a highly pathogenic Type 2 PRRSV (1-4-4 L1C.5), Nano-B7 generated a 95% inhibition at 10 µM, accompanied by a 1.4 log₁₀ reduction in viral replication. In contrast, DMSO-B7 produced a 70% inhibition at 10 µM, and a 1.3 log₁₀ reduction at 20 µM.

Discussion and conclusions

The hydrophobic tails in the self-assembled nanoparticles encapsulate poorly soluble drugs, while their hydrophilic heads interact with water. Liposome nanoparticles thereby improve drug solubility and dispersion. Encapsulation also protects B7 from degradation and enhances cellular uptake, increasing its bioavailability and therapeutic effectiveness. Nano-B7 represents a promising antiviral therapeutic for the control of PRRSV infection and mitigating the substantial economic burden of PRRSV in the swine industry.

Conflicts of interest

The authors declare no competing interests.

Ethics statements

Not applicable, no live animals were used directly.

Keywords

artificial intelligence • nanoencapsulation • small molecule therapeutics

P.46 · ID15

PRRSV-GP5-DERIVED SYNTHETIC PEPTIDES MODULATE EPIGENETIC AND ANTIVIRAL RESPONSES IN 3D4/31 PORCINE MACROPHAGES

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) remains a major challenge for swine health, and rational peptide-based strategies may help refine protective immunity. Previous work from our group identified GP5-derived peptides associated with differential immune profiles in swine. However, beyond inducing immunogenicity, it is important to understand mechanisms that may shape the magnitude, duration, and functional impact of the response. In this context, profiling epigenetic regulators and global DNA methylation can provide mechanistic insight into how peptide formulations may refine the quality and persistence of the induced response. Here, we evaluated whether individual GP5-derived peptides and multi-epitope peptide mixtures modulate innate cytokine responses and epigenetic regulation in porcine alveolar macrophages.

Methods

3D4/31 porcine alveolar macrophages were stimulated with individual GP5-derived peptides (GP5T, GP5T3, GP5B, and GP5B3; 5 µg/mL each), a three-peptide mixture (Mix 3: GP5T+GP5T3+GP5B3), or a four-peptide mixture (Mix 4: GP5T+GP5T3+GP5B+GP5B3). LPS was used as positive control. Cells were collected at 8, 24, 48, and 72 h post-stimulation. Cytokine and epigenetic regulator transcripts were quantified by RT-qPCR, and global DNA methylation (%5-mC) was evaluated using an ELISA-like assay based on antibodies specifically recognizing 5-methylcytosine (5-mC).

Results

GP5-derived peptides induced an early antiviral transcriptional signature dominated by type I interferons, with the strongest responses observed at 8 h, particularly for GP5T, GP5T3, and Mix 3. In contrast, pro-inflammatory cytokines, including IL-1β, TNF-α, and IL-12, showed limited and mostly transient induction, whereas IL-10 remained undetectable under the conditions tested. Epigenetic regulators were modulated selectively and in a time-dependent manner, with greater dynamics in repression-associated genes than in acetylation-related genes. DNMT3b emerged as the most responsive node, while DNMT1 showed subtler changes. The most pronounced effects were observed at the level of global DNA methylation. Mix 3 induced sustained increases in %5-mC from 8 to 72 h, whereas Mix 4 displayed a biphasic pattern, with an early decrease at 8 h followed by robust increases from 24 to 72 h. Overall, peptide mixtures showed stronger and more sustained epigenetic remodeling than individual peptides.

Discussion and conclusions

These findings indicate that PRRSV GP5-derived multi-epitope peptide mixtures promote an early antiviral response together with selective epigenetic reprogramming in porcine macrophages. Compared with individual peptides, mixtures induced the most consistent changes in global DNA methylation, supporting the idea that formulation composition influences both the magnitude and kinetics of innate and epigenetic responses. Together, these results support the potential of GP5-derived synthetic immunogenic peptides as rational peptide-based immunogens for PRRSV.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

No animal experiments were conducted for this study. The work was performed using an *in vitro* porcine macrophage cell model and synthetic PRRSV-GP5-derived peptides. Therefore, ethical approval for animal experimentation was not required, in accordance with institutional guidelines.

Financial support and Acknowledgements

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Keywords

PRRSV • GP5-derived peptides • Porcine macrophages • Epigenetic regulation • Antiviral response • 3D4 cells

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P.47 • ID65

UNDERSTANDING THE MECHANISMS OF IMMUNOSUPPRESSION INDUCED BY PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Introduction and objectives

Porcine reproductive and respiratory syndrome viruses (PRRSV) are known for evading and manipulating host immune responses resulting in poor neutralising antibody responses and subsequent prolonged infection. However, virulent PRRSV strains have emerged which induce stronger immune responses, but also severe pathology and increased mortality rates. Activation of the cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2) pathway is a canonical promotor of inflammatory responses and has been shown to induce pathology during virulent PRRSV infection. However, this pathway may also be responsible for the immunoregulatory phenotypes seen during infection with classical PRRSV strains, through induction of anti-inflammatory cytokines and M2-like polarisation of macrophages. Therefore, we aimed to assess whether the COX-2/PGE2 signalling pathway is involved in the induction of immunoregulatory phenotypes observed during classical PRRSV infection and whether a modified live virus (MLV) could be engineered to be unable to directly activate this pathway, resulting in a safer and more immunogenic vaccine.

Methods

Using porcine induced pluripotent stem cell-derived macrophages (iPSCdM) as a model system, we evaluated the induction of COX-2 expression and the modulation of immunoregulatory cytokines by classical and virulent PRRSV-1 and -2 strains. Through inhibition of COX-2, we assessed the direct effects of this signalling pathway on macrophage responses to PRRSV infection. Ongoing experiments are investigating the specific role of the non-structural protein 2 (NSP2) in the modulation of COX-2 signalling by measuring immunoregulatory responses following transfection of plasmids encoding PRRSV NSP2 sequences. Finally, we are also investigating how modification of NSP2 sequence could induce more favourable immune responses to aid in the design of a more efficacious MLV vaccine.

Results

Our data demonstrate that all the PRRSV strains tested induce the activation of COX-2/PGE2 signalling and immunoregulatory responses *in vitro*. COX-2 was significantly upregulated by both classical and virulent PRRSV-1 and -2, as was the expression of immunoregulatory cytokines and M2 macrophage markers. COX-2 was found to be directly related to the expression of these immunoregulatory responses as biochemical inhibition of the COX-2/PGE2 pathway resulted in significantly decreased expression of markers such as interleukin-10.

Discussion and conclusions

This study has shown that both classical and virulent PRRSV-1 and -2 strains induce the activation of the COX-2/PGE2 signalling pathway and how this modulates the characteristic immunoregulatory host phenotypes seen during classical PRRSV infection. We are now confirming the specific role for NSP2 in the activation of COX-2/PGE2 signalling and assessing how differences in NSP2 sequence can differentially modulate immunoregulatory responses. Identification of modifications to NSP2 that can downregulate immunoregulatory responses, will be exploited to construct and test a novel MLV vaccine that may be more immunogenic and efficacious than the current array of PRRSV vaccines on the market.

Conflicts of interest

Nothing to declare.

Ethics statements

Nothing to declare.

P.48 • ID68

TISSUE-SPECIFIC INDUCIBLE CYTOKINE MRNA PROFILES IN PIGS INFECTED WITH WILD AND MODIFIED-LIVE VACCINE PRRSV STRAINS

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) induces persistent infection and complex dysregulation of adaptive immunity. Previous studies showed that PRRSV affects thymic T-cell development, alters the TCR repertoire and impairs the generation of effective antiviral immunity. Modified-live vaccine (MLV) strains may induce similar immune alterations in young piglets. The aim of this study was to characterize infection-associated changes in inducible cytokine mRNA expression in cells isolated from tissues of pigs infected with two wild PRRSV strains and a modified-live vaccine inoculum.

Methods

PRRSV-negative five-week-old piglets were inoculated with the wild strains VR2332 and Lelystad, or with a modified-live vaccine inoculum designated VACC. Two animals per group were examined at 10, 14 and 17 days post infection. Cells were isolated from blood, spleen, mesenteric lymph nodes, bronchoalveolar lymph nodes and thymus, stimulated *ex vivo* with PMA and ionomycin, and analysed for cytokine and immune-response-related mRNA expression by qPCR. Data were evaluated descriptively with respect to viral strain, tissue compartment and time after infection.

Results

PMA/ionomycin-inducible cytokine mRNA expression showed marked temporal and tissue-related variability. Cells isolated during the early phase of infection displayed altered inducible expression of inflammatory, antiviral and regulatory mediators, including CXCL10, IL-6, TNF- α and IL-10. Later after infection, the inducible cytokine profile shifted towards broader cellular, regulatory and mucosal immune activation, involving IL-2, IFN- γ , IL-17, IL-22 and IL-10. The most pronounced patterns were observed in lymphoid tissues, particularly thymus and spleen, whereas blood showed a less distinct profile. The VACC group showed active cytokine modulation after stimulation and should not be interpreted as immunologically inert; rather, its response was broadly consistent with the previously described dysregulatory potential of MLV PRRSV in young piglets.

Discussion and conclusions

These findings indicate that PRRSV infection is associated with dynamic changes in the inducible cytokine-producing capacity of tissue-derived cells. Both wild and MLV-derived PRRSV were linked to complex, tissue-specific cytokine patterns, most evident in lymphoid organs. Cytokine profiling after PMA/ionomycin stimulation therefore provides an additional functional layer to previously described changes in thymic development, T-cell repertoire and humoral immunity. Because this approach reflects inducible cytokine capacity rather than spontaneous *in vivo* cytokine expression, and due to the limited number of animals per group and time point, the results should be interpreted as descriptive and hypothesis-generating.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statements

All animal procedures were approved by the Expert Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic and were performed in accordance with national legislation and institutional requirements.

Financial support and Acknowledgements

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Keywords

PRRSV • Immunomodulation

P.49 • ID74

ANALYSIS OF LINEAR EPITOPES ON PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS 1 GLYCOPROTEINS AND IMPLICATIONS FOR ANTIBODY CROSS-REACTIVITY AND NEUTRALISATION

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Introduction and objectives

Porcine reproductive and respiratory syndrome viruses (PRRSV) are rapidly mutating resulting in genetic and antigenic diversity between strains of the same species and subtype. This diversity limits the cross-protectivity provided by current vaccines. Of these vaccines, modified live virus (MLV) vaccines are widely used, however they are limited in their capacity to elicit broadly neutralising antibody responses. The prime target antigens for future vaccine development are the surface glycoproteins (GP2-5), which bear neutralising epitopes. However, this is complicated by the presence of immunodominant non-neutralising epitopes, glycan shielding of neutralising epitopes, and the potential for non-neutralising epitopes to mediate antibody-dependent enhancement of infection. The elucidation of both neutralising and non-neutralising epitopes is therefore critical to the design of protective PRRSV glycoprotein-based immunogens.

Methods

Using serum from piglets vaccinated with experimental PRRSV-1 Olot/91-based MLV vaccines, we determined antibody responses by testing sera against overlapping peptide libraries representing PRRSV-1 Olot/91 GP2-5. Bioinformatics was then performed to determine the predicted topology and conservation of the peptide encoding sequences amongst PRRSV-1 strains. Since a previously described linear neutralising epitope on GP4 stood out as a variable but immunodominant epitope, sera from piglets vaccinated with experimental and licensed PRRSV-1 MLV vaccines were tested against peptides representing the corresponding sequence from 31 PRRSV-1 strains to determine the cross-reactivity of responses.

Results

Serum from experimentally immunised piglets responded predominantly to peptides likely corresponding to a single immunodominant linear epitope in GP3, GP4 and GP5. Due to previous our research indicating GP3 being a poor inducer of neutralising antibodies, and the GP5 epitope being predicted bioinformatically as being located within the virion envelope, GP4 was focussed on. GP4-specific antibody responses targeted a previously determined immunodominant neutralising epitope located within a hypervariable region, in which amino acid conservation is low between PRRSV-1 strains. Consequently, serum from animals experimentally vaccinated by MLV showed limited cross-reactivity to peptides derived from heterologous PRRSV-1 strains, suggestive of this region mutating to facilitate immune escape.

Discussion and conclusions

The immunodominant linear epitope of GP4 coincides within a hypervariable region of this protein and is likely a mechanism of immune escape by PRRSV-1. Thus, this neutralising epitope may function as a decoy; directing the immune system away from epitopes the virus cannot so easily mutate. Thus, future vaccine design should consider strategies to avoid eliciting this response and focus on elucidating responses to conserved conformational epitopes.

Conflicts of interest

Nothing to declare

Ethics statements

Nothing to declare

P.50 • ID77

PRRSV MAY PROMOTE M2 MACROPHAGE POLARISATION VIA IL-13 SIGNALLING AND MIR-192-MEDIATED SUPPRESSION OF TYPE I IFN RESPONSES

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Introduction and objectives

Macrophages play a vital role in regulating immune responses and mediating crosstalk between innate and adaptive immunity. Macrophage phenotype is typically an equilibrium between the M1 (classically activated, proinflammatory phenotype) and M2 (alternatively activated, anti-inflammatory/tissue repair phenotype) states, regulated by cytokines such as the pro-M2 interleukin-13 (IL-13). Porcine reproductive and respiratory syndrome viruses (PRRSV) infect macrophages and may modulate their responses so as prolong infection. Previous studies have shown that PRRSV infection upregulates IL-13 via NSP9-mediated modulation of host transcriptional pathways and skews macrophages toward a M2 phenotype. However, the molecular mechanisms underlying this process remain poorly understood. Canonically, M2 polarisation involves activation a signal transducer and activator of transcription 6 (STAT6) and interferon regulatory factor 4 (IRF4), dependent on the histone demethylase, jumonji domain-containing protein 3 (JMJD3). The JMJD3-IRF4 axis upregulates transcription of M2-associated genes. We hypothesised that PRRSV upregulates IL-13 in macrophages, leading to the activation of STAT6 and subsequent JMJD3-IRF4-mediated transcription of M2-specific genes, while suppressing type I interferon (IFN) responses, thereby promoting its own survival.

Methods

Primary porcine alveolar macrophages (PAMs) and immortalised porcine macrophages (PLTA-58 cells) were used for *in vitro* assays. Expression of IL-13, JMJD3, IRF4, ARG-1, IL-10, and microRNA-192 (miR-192) was evaluated by RT-qPCR following PRRSV-1 infection. Type I IFN expression was assessed at both the mRNA and protein level. Ongoing experiments are investigating the role of IL-13 by inhibiting STAT6 phosphorylation and assessing its effect on the JMJD3-IRF4 axis in PAMs and PLTA-58 cells. Additionally, we are investigating whether PRRSV-1 nucleocapsid contributes to macrophage polarisation.

Results

PRRSV-1 infection of both PAMs and PLTA-58 cells activates the IL-13 pathway and the JMJD3-IRF4 axis. mRNA levels of IL-13, JMJD3 and IRF4 were increased at 12 and 24 hours post-infection, along with IL-10 and ARG-1 expression at 24 hours post-infection, indicative of M2 macrophage polarisation. PRRSV also upregulated the pro-M2 microRNA miR-192. In contrast, type I IFN responses were reduced at the protein, but not mRNA level, suggesting post-transcriptional regulation.

Discussion and conclusions

Our findings suggest that PRRSV-1 promotes macrophage polarisation toward an M2 phenotype through activation of the IL-13 signalling pathway and JMJD3-IRF4 axis. PRRSV also modulates type I IFN responses, potentially via miR-192-mediated suppression, miR-192 may act in parallel with or downstream of IL-13 signalling, currently under investigation. Importantly, miR-192 is known to target IRF3 and IRF7 via a canonical microRNA-mediated post-transcriptional repression mechanism, thereby suppressing type I IFN responses. Ongoing work using IL-13 neutralisation, STAT6 inhibition and JMJD3/IRF4 silencing aims to confirm these mechanisms. We further seek to link these effects to the PRRSV nucleocapsid protein, as a candidate mediator of M2 polarisation. These results will provide insight into molecular mechanisms of immune evasion by PRRSV.

Conflicts of interest

Nothing to declare

Ethics statements

Nothing to declare

Keywords

PRRSV • Macrophage polarisation • IL-13 signalling

P.51 • ID49

ORAL IMMUNIZATION WITH MAIZE-EXPRESSED PEDV SPIKE PROTEIN-DERIVED ANTIGEN MODULATES MUCOSAL CELL-MEDIATED IMMUNITY AND CLINICAL OUTCOMES IN WEANED PIGS

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Introduction and objectives

Porcine epidemic diarrhea virus (PEDV) is a major enteric pathogen of swine, causing severe diarrhea, high mortality in neonatal piglets, and impaired growth performance. Oral vaccines offer the potential to stimulate gut-associated immunity; however, the mucosal and systemic immune responses induced by oral PEDV vaccination remain under investigated. This study evaluated a maize-expressed PEDV-S1 oral vaccine by assessing clinical outcomes, viral shedding, and lymphocyte responses following PEDV challenge.

Methods

Sixty 3-week-old PEDV-negative pigs were assigned to four groups (n=15/group): Control, Vaccine-1 (S1), Vaccine-2 (S1-LTB), and Challenge (2x). Vaccinated pigs received a prime-boost regimen of maize-expressed PEDV-S1 incorporated into feed and were challenged orally with a non-S INDEL PEDV strain on day 35. The Challenge (2x) group received two live PEDV inoculations. Clinical signs, body weight, antibody responses, and fecal viral shedding were monitored. Spleen and mesenteric lymph nodes (MLNs) were collected at serial necropsies for flow-cytometric characterization of T cells, B cells, $\gamma\delta$ T cells, and NK cells, including IFN- γ and TNF- α expression.

Results

Vaccine-1 pigs showed shorter diarrhea duration following challenge, whereas Vaccine-2 pigs achieved greater body weight by day 45 than Control (p<0.01) and Challenge (2x) pigs (p<0.05). Viral shedding was highest in Controls, intermediate in vaccinated groups, and lowest in Challenge (2x) pigs after re-exposure. Vaccinated and Challenge (2x) pigs showed increased frequencies of B cells and CD4⁺CD8⁺ T helper cells in MLNs following challenge, indicating activation of the mucosal immune compartment. IFN- γ -producing lymphocytes were more abundant in MLNs than in the spleen, particularly during the early post-challenge period, supporting a localized mucosal immune response. In contrast, $\gamma\delta$ T cells and NK cells were more prevalent in the spleen, while TNF- α responses remained generally limited across groups. However, TNF- α ⁺ $\gamma\delta$ -T cells exhibited tissue- and treatment-specific mucosal responses following challenge.

Discussion and conclusions

Oral vaccination modified disease progression and altered immune-cell dynamics following challenge. Vaccine-1 was associated with faster clinical recovery, whereas Vaccine-2 was associated with enhanced growth performance. Immune responses were characterized by expansion of mucosal B-cell and CD4⁺CD8⁺ T-cell populations and localized IFN- γ production within MLNs. Maize-expressed oral PEDV-S1 oral vaccination elicited measurable mucosal and systemic immune responses, reduced clinical disease severity, and decreased viral shedding following PEDV challenge. These findings support further development of feed-based oral vaccination strategies to improve PEDV control in swine populations.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statements

Pigs from this study were euthanized according to AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. All procedures complied with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (4th edition, FASS, 2020) and were approved by the VRI Animal Care and Use Committee (IACUC no. VRI 1593 approved 19 Apr 2024).

Financial support and Acknowledgements

The authors thank the VRI team (Ames, Iowa) for the support in providing animal facilities and performing the *in vivo* portion of this study. This project was partially funded through an Iowa State University VPR Seed Grant.

P.52 • ID87

DEVELOPMENT OF A FOOT-AND-MOUTH DISEASE VIRUS VACCINE CANDIDATE: UTILIZING NON-TOXIC ENTEROTOXIN AND E. COLI AS AN ADJUVANT-DELIVERY SYSTEM

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Introduction and objectives

Foot-and-Mouth Disease Virus (FMDV) induces vesicular lesions in cloven-hoofed animals, causing significant economic losses in livestock industry worldwide. Among all FMDV serotypes, serotype O is highly prevalent globally, causing annual economic losses of \$6 billion to \$21 billion in livestock industries due to reduced productivity, increased control costs, and trade restrictions. Current available vaccines face challenges with safety concerns, lack of cross-protection, thermal instability, and complications in differentiating infected from vaccinated animals (DIVA). In this study, we adapted a novel Multi-Epitope Fusion Antigen (MEFA) platform for FMDV vaccine development. It integrates consensus B- and T-cell epitopes from all four structural proteins (VP1, VP2, VP3, VP4) and the non-structural protein 3A, which was expressed using detoxified E. coli heat-labile toxin as the backbone.

Methods

Three vaccine constructs were generated (VC1, VC2, and VC3), each contains different epitope compositions. VC1 contains B-cell epitopes from the VP1 region, and VC2 comprises five T-cell epitopes from VC1 and 3A regions, while VC3 includes eight T-cell epitopes from VP1, VP2, VP3, and VP4 regions. For oral immunization, these constructs were cloned into pBR322 and transformed into nonpathogenic E. coli. For intramuscular immunization, the vaccine constructs were cloned into pET28a, and recombinant proteins were expressed and purified. For all immunizations, the three vaccine constructs were combined and administered together. A total of thirty-six pigs of 3-4 weeks old were obtained and randomly divided into 6 groups. Group 1 pigs were immunized orally; group 2 pigs were primarily immunized orally and boosted after 2 weeks with recombinant protein and dmLT adjuvant; group 3 pigs were immunized and boosted intramuscularly along with dmLT. Group 4-6 pigs were used as controls, which were immunized with dmLT, non-pathogenic E.coli, or remained as negative, respectively. At 28dpi, all pigs were euthanized. Serum, peripheral blood mononuclear cells (PBMCs), and jejunal wash samples were collected to measure the immune responses.

Results

We observed a significant increase in serum IgG titers in all pigs vaccinated with FMDV vaccine constructs compared to the control groups. Additionally, immunized pigs exhibited elevated jejunal IgA responses compared to control groups. Flow cytometry analysis of PBMCs isolated from FMDV vaccinated pigs revealed a significant increase in IFN gamma-producing CD4+ and CD8+T cell response, while control animals exhibited minimal CD4+ and CD8+ response, demonstrating the vaccine's ability to induce a specific immune response. Neutralizing B-cell response is yet to be measured in BSL3 laboratory.

Discussion and conclusions

The MEFA-based FMDV vaccine induced robust systemic, mucosal, and cellular immune responses in pigs, as evidenced by increased serum IgG, jejunal IgA, and IFN- γ -producing CD4+ and CD8+ T-cell responses. These findings demonstrate the potential of combining multiple FMDV epitopes with dmLT to enhance immunogenicity and provide systemic immune activation. Future studies will evaluate neutralizing antibody responses and protective efficacy to identify the optimal vaccine construct for further development.

Conflicts of interest

The authors declare no conflict of interest

Ethics statements

All experimental procedures were conducted according to the protocol approved by the Institutional Biosafety Committee (IBC) and Institutional Animal Care and Use Committee (IACUC).

P.53 • ID69

DIETARY SUPPLEMENTATION WITH GLYCERIDES OF LAURIC ACID IMPROVES PIGLES' RESILIENCE THROUGH IMMUNE MODULATIONAmine Mellouk¹; Olga Lemâle¹; Tom Meyns¹; Jessika Consuegra

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus PRRSV remain a severe challenge, and nutritional strategies that support immune competence become of increasing interest. Glycerides of lauric acid (GLA) have been reported to exhibit antimicrobial and antiviral properties, potentially through both direct effects on viral integrity and on the modulation of host's immunity. The present study aims to evaluate the impact of GLA-supplementation on immune responses and viral dynamics during PRRSV infection using complementary *ex vivo* and *in vivo* models.

Methods

In the *ex vivo* study, PAMs were isolated from healthy 4-week-old piglets. To assess the direct effect of GLA, they were infected with PRRSV (strain 13V091) preincubated for 1 hour with increasing concentrations of GLA (0, 0.022, 0.044, and 0.088%). To evaluate indirect effects of GLA, PAMs were exposed to the increasing concentrations of GLA for 24 hours before the viral infection. Viral replication was assessed by viral titration, while cytokine production (IL-8, TNF- α) was measured to characterize innate immune responses. *In vivo*, weaned piglets (~24 days old) were assigned to three groups: non-challenged control (Ctrl), PRRSV-challenged (Chal), and PRRSV-challenged and GLA-supplemented (Chal GLA; 3–4 kg/T feed). Animals were challenged at 60 days of age and monitored for 14 days post-infection (DPI). Viremia, nasal shedding, and cytokine profiles in serum and bronchoalveolar lavage fluids (BALFs) were evaluated. Statistical analysis was performed using one-way ANOVA, Tukey's Honestly significant differences and Pearson tests ($P < 0.05$).

Results

Ex vivo, GLA-treated PAMs, but not GLA-treated PRRSV, induced a dose-dependent reduction in PRRSV replication, reaching a ~3.5 log decrease compared with untreated controls ($P < 0.001$). This antiviral effect was associated with enhanced macrophage activation, shown by increased IL-8 secretion ($P = 0.046$) and a positive correlation between GLA concentrations and TNF- α production ($P = 0.019$). These results suggest that GLA improves macrophage resilience to PRRSV infections. *In vivo*, GLA supplementation reduced both viremia and nasal shedding following PRRSV challenge. GLA-supplemented piglets showed numerically slower increase in viremia after challenge, having a ~14-fold lower viral load at 3 DPI, while nasal shedding was significantly reduced with GLA supplementation, showing an ~20-fold reduction compared to non-supplemented group at 7 DPI ($P = 0.030$), indicating decreased viral dissemination. This effect was accompanied by increased pro-inflammatory and Th1-associated cytokines, suggesting mitigated PRRSV-induced immunosuppression. Serum TNF- α , at 7 DPI, reached 148 pg/mL in GLA-supplemented group compared to 16 pg/mL ($P < 0.001$) and 103 pg/mL ($P = 0.016$) in Ctrl and Chal groups, respectively. Similar trends were observed locally at 14 DPI, with increased TNF- α and IFN- γ levels in BALFs ($P = 0.056$ and 0.089, respectively), supporting enhanced pulmonary anti-viral immune response.

Discussion and conclusions

GLA-supplementation enhanced both innate and Th1-type immune responses, mitigating PRRSV-induced immunosuppression resulting in reduced viral replication and shedding. These findings support an indirect antiviral mechanism driven by improved host immunity. GLA may therefore represent a promising nutritional strategy to increase resilience to PRRSV infection.

Conflicts of interest

Adisseo commercialise GLA as feed-additive.

Ethics statements

This trial was approved by the Ethical Committee Poulpharm according to EU and belgian laws: Directive 2010/63/EU, ETS123 and L197.

Keywords

Immune modulation • Glycerides of lauric acid • Nutrition



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P.54 • ID9

A NATIONAL CONTROL PROGRAMME FOR PORCINE RESPIRATORY AND REPRODUCTIVE SYNDROME VIRUS IN PIG HERDS ON THE ISLAND OF IRELAND

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Introduction and objectives

PRRSv-1 is an endemic disease in the UK and Ireland pig herds causing significant economic loss to the swine industry. PRRS is characterised by sow reproductive disease, immunosuppression and respiratory diseases leading to secondary disease infections and porcine respiratory disease complex (PRDC). The PRRSv RNA genome is highly divergent with high mutation rates and recombination events permitting the continuous global emergence of new virulent strains. The PRRS NCP incorporates synchronised vaccination, biosecurity measures with serological and molecular testing for PRRSv alongside parallel testing for influenza A&D virus and porcine circovirus (PCV2-4). The primary goal to reduce PRRSv prevalence will have beneficial effects on animal health, antibiotics usage and production parameters.

Methods

A two-stage approach was implemented, involving pre-screening at slaughter for a subset of farms, with only seropositive farms retained for further analysis. Farms that were not screened at slaughter were also included in the study totalling N.Ireland at n=95 farms and ROI n=17 from a pig dense region. 10-12wks aged pigs were sampled for verification PRRSv qPCR assays, inclusive of PRDC pathogens PCV2-4 (n=30 sera) and influenza A&D virus (n=4 oral fluid) per farm (12 of 95 NI farms omitted oral fluids). Positive PRRSv-1 samples ORF5 gene and occasionally ORF7 and NSP2 genes were sequenced, phylogenetically analysed determining wild-type or vaccine strains. Method development for PRRSv WGS by 3'-poly-A-tails transcriptomics, targeted amplification and target enrichment capture probes are in development for complete genome recombination detection and phylogenomics.

Results

Analysis to date found N.Ireland PRRSv at 68% positive (n=65 positive farms), with sequencing determining n=39 wild-type, n=21 vaccine-like virus and n=5 untyped. The ROI PRRSv positivity of farms tested was 76.5% of which 41% were wild-type positive. The wild-type strains were phylogenetically designated as PRRSv-1 lineages L1.1-L.1.3. swIAV in N.Ireland farms were 36% positive (n=30) with ROI 24% (n=4) and WGS determined as H1N1, H1N2 & H3N2 strains. A high prevalence of PCV3 was detected in N.Ireland at 66.7% from a subsection of farms tested.

Discussion and conclusions

The prevalence and molecular characterisation of the PRRSv strains will be established with data generated incorporated into other NCP measures and metadata to help reduce PRRSv-1 prevalence. The WGS data from PRRSv and other PRDC causing viruses, combined with metadata analysis from production data, pleurisy scoring and biosecurity information will provide a framework for understanding PRRSv lineage circulation and virulence in relation to PRDC outcomes. This approach will also improve understanding of PRRSv transmission, virulence, and strategies for reducing PRRSv-1 prevalence delivering benefits in animal health, PRDC reduction, antimicrobial usage, and production performance.

Conflicts of interest

No conflits

Ethics statements

Samples were sourced from routine PRRSv surveillance, including diagnostics, herd-health programmes, and statutory disease monitoring. No procedures beyond standard care were performed, and no additional samples were collected for research. Sampling required no ethical approval under the UK Animals Act 1986. Sampling was conducted by trained personnel in line with animal welfare regulations. Owner consent was obtained where appropriate, and all data were anonymised before analysis.

Financial support and Acknowledgements

The PRRS National Surveillance Control Programme in NI is funded by the PigRegen Levy Body and DAERA Evidence and Innovation Research funding. ROI scheme is funded by European Innovation Partnership.

Keywords

PRRSv • PRDC • Co-infection • NCP • phylogenetics

P.55 • ID82

A COORDINATED REGIONAL APPROACH TO PRRS CONTROL: BASELINE FINDINGS FROM THE IRISH BORDER REGION

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Introduction and objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) remains one of the most economically important diseases affecting the pig industry, causing reproductive failure, respiratory disease, reduced performance, and substantial production losses. Approximately 58% of Irish pig herds are PRRS-positive. To address this, a three-year European Innovation Partnership (EIP)-funded regional PRRS control programme was established across the border counties of Cavan, Donegal, and Monaghan. The programme integrates routine surveillance, herd-status mapping, synchronised vaccination, and targeted biosecurity improvements. This paper describes the project design and presents baseline findings from the first year.

Methods

Pig herds within the target region were surveyed and classified as PRRS-positive, PRRS-negative, or unknown. PRRS-positive and unknown-status herds underwent pooled PCR testing of blood samples from 10–12-week-old pigs, with ORF5 sequencing performed on positive samples to characterise circulating strains. Where required, virus recirculation was further investigated using processing fluids, blood, or oral-fluid samples. PRRS-negative herds underwent ELISA testing on 30 blood samples collected at or near slaughter. All participating herds completed annual BioCheck.UGent biosecurity assessments. Influenza surveillance was conducted using oral-fluid rope samples, and slaughterhouse monitoring recorded respiratory and systemic lesions. Herd disease maps were updated following baseline testing. All herds will be resampled after one and two years.

Results

A total of 92 herds completed the initial survey. Of these, 66.3% (61/92) were classified as PRRS-positive or suspected positive, 28.3% (26/92) as PRRS-negative, and 5.4% (5/92) as unknown. The project commenced in May 2025 with 17 herds in two regional pods (Cavan and Donegal). Baseline sampling and biosecurity assessments were completed on all herds. PRRS was detected in 75.5% of farms, with wild-type virus identified in 53.8% of positive herds by ORF5 sequencing. Influenza A and Influenza D viruses were each detected in 23.5% of farms. Slaughterhouse assessments showed mean batch prevalences of pneumonia (16.0%), pleurisy (15.6%), liver white spots (16.5%), and pericarditis (4.0%). Second-year sampling is ongoing. In 2026, the programme expanded to include approximately 60 additional herds across Cavan and Monaghan, with baseline sampling currently underway.

Discussion and conclusions

Baseline data confirm a high prevalence of PRRS in pig herds across the border region. This is the first coordinated regional PRRS control initiative in Ireland, integrating surveillance, molecular diagnostics, mapping, vaccination coordination, biosecurity assessment, and longitudinal health monitoring. Continued evaluation over the three-year programme will determine its effectiveness in reducing PRRS circulation and improving herd health, productivity, and biosecurity, while informing future national control and eradication strategies.

Conflicts of interest

No conflicts.

Ethics statements

Samples were sourced from routine PRRSv and influenza surveillance, including diagnostics and herd-health programmes. No procedures beyond standard care were performed, and no additional samples were collected for research. Sampling required no ethical approval under the S.I. No. 543 of 2012. Sampling was conducted by trained personnel in line with animal welfare regulations. Owner consent was obtained where appropriate, and all data were anonymised before aggregated analysis.

Financial support and Acknowledgements

The study is part of the TAILWARDS project that is funded by European Innovation Partnership (CSP2001). We would like to acknowledge all the farmers and veterinarians who are part of this study.

Keywords

PRRS • control • biosecurity

P.56 • ID113

ORF5 SEQUENCING INFORMS A PRRSV LOCAL AREA CONTROL PROGRAMME IN ENGLAND

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Introduction and objectives

A group of seven pig producers with a total of ten farms in an area of England and three farms supplying pigs into two of local farms, have formed a consortium with the aim of better controlling diseases, including PRRS. A variety of control strategies are being considered, with a desire to prioritise these according to cost and effectiveness. An evidence-based approach would benefit these choices, so information regarding the spread of PRRSV-1 in the area would be useful.

Methods

Pooled serum samples from the various sites have been periodically tested over a two-year period by ELISA and PCR for PRRSV. PCR-positive samples have then been followed up by ORF5 sequencing and analysis. Pairwise comparisons of the sequences were produced detailing the distance between pairs and the percentage similarity. The evolutionary history of the sequences was inferred using the neighbour-joining method. The evolutionary distances were computed using the Kimura 2-parameter method. The rate variation among sites was modelled with a gamma distribution (shape parameter = 0.66). Evolutionary analyses were conducted in MEGA7.

Results

Seven PRRSV-1 ORF5 sequences from the participating farms were obtained and analysed. All sequences were of wild-type virus, not vaccine-like. The percentage similarity between these ranged from 96.2% to 100%. Phylogenetic analysis showed that the sequences could be grouped into three distinct clades.

Discussion and conclusions

Genetic sequence data for PRRSV-1 strains circulating within farms in a local area control programme for PRRS have been analysed and compared to suggest potential links between farm sites. This approach is limited by the availability of samples from each location at regular time intervals. The possible links between sites could then be investigated according to geographic proximity, pig movements, and any staff or management links based on Biocheck.Ugent results as well as historic and local knowledge. These preliminary results have served as an encouragement for producers to establish more regular testing for PRRS, in order to better understand potential sources of infection, thereby enabling more targeted control measures.

Conflicts of interest

None

Ethics statements

None

Keywords

Control • Sequencing

P.57 · ID125

TEMPORAL TRENDS AND SPATIAL CONSIDERATIONS OF PRRS HERD STATUS IN SCOTLAND

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Introduction and objectives

Assessing Porcine Reproductive and Respiratory Syndrome (PRRS) herd status over time is essential for developing effective regional and national control strategies. In Scotland, previous abattoir serological surveys have demonstrated relatively stable herd level prevalence of 45-48%. This study aimed to determine individual herd PRRS status using data from a 2024–2025 survey, integrating PCR, serological results and vaccination data, and to evaluate regional variations in the proportion of PRRS-free Scottish herds.

Methods

A national, voluntary, PRRS Control and Elimination Scotland (PCES) programme was launched in 2024 and offered to all pig producers. The programme includes annual PRRS testing -PCR or serology- for all pig holdings moving more than 200 pigs annually. PRRS status was initially characterised as positive, negative, or untested. In addition, an adapted classification based on the model proposed by Holtkamp *et al.* (2021) was used for a more granular categorisation of PRRS status to be included in a national mapping tool. Regional variations were evaluated using the Chi-squared test, with statistical significance (α) set at 0.05.

Results

The 2024–2025 results revealed a national herd-level PRRS prevalence of 48.6% of those tested (66.2% of all herds). Of the herds with breeding pigs, 40.9 % were PRRS negative with the remainder all vaccinating using modified live virus vaccines. Of the nursery and finishing farms, 58.9% were PRRS negative, with only 16.4% of positive herds not vaccinating. Regional variation in prevalence was observed, with lower PRRS positivity in Southern Scotland (4.2%) compared to the other regions (48.8 – 66.7%; $P<0.001$), although testing rate was low in this area (39.3% of herds).

Discussion and conclusions

The figure of 48.6% herd PRRS prevalence is likely an overestimate, since a large proportion of the untested herds belong to presumed PRRS negative integrator flows, mostly based in Southern Scotland. Compared to previous surveys, which looked at finisher seroprevalence only, this figure is slightly higher than the figure of 44% obtained in 2017-2018. However, the results are not directly comparable since there have been substantial changes in the industry's structure and the present survey involved not only finisher herds, but also breeding herds and nurseries. Combining this temporal analysis with spatial mapping has highlighted geographic clusters of high-health zones. These findings suggest that regional elimination programmes are technically and epidemiologically feasible within the relatively low density Scottish pig industry. However, the operational success of these depends heavily on highly-coordinated, collective biosecurity actions and synchronised management strategies among participating local producers. To bridge this gap between data and collective action, a dedicated PRRS mapping tool has been developed and is currently being launched for farmers and veterinarians. This platform allows all signatories of the Scottish Pig Disease Charter to securely visualise the real-time PRRS status of pig herds, thereby facilitating regional risk identification and synchronisation of area-wide PRRS management initiatives (such as coordination of vaccination and herd health upgrade programmes).

Conflicts of interest

None to declare.

Ethics statements

This study was approved by the Animal and Social Ethics Committees at SRUC. Formal research licenses were unnecessary as data was collected by veterinarians during routine clinical practice.

Financial support and Acknowledgements

This study was funded by the PRRS Control and Elimination Scotland programme, funded by Scottish Government.

Keywords

PRRS • herd prevalence • Control • mapping

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P.58 • ID27

MONITORING PRRSV CIRCULATION IN NURSERY UNITS OF BELGIAN PIG HERDS PARTICIPATING IN THE BELGIAN PRRS PROGRAM

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Introduction and objectives

The Belgian PRRS Program was launched in 2015 as a national initiative to support the control of Porcine Reproductive and Respiratory Syndrome virus (PRRSV). The voluntary program is structured around three pillars: artificial insemination centers, conventional pig farms, and communication. Within the conventional farm pillar, four unique PRRSV monitoring systems enable farms to regularly assess PRRSV circulation within their herds. This study aimed to evaluate monitoring results and longitudinal changes in PRRSV status among participating Belgian pig herds.

Methods

The PRRS Piglet Monitor is the most used monitor within the Belgian PRRS Program. At each monitoring event, blood samples are collected from 20 piglets: 10 at the start and 10 at the end of the nursery period. Samples are pooled in groups of five animals and subsequently tested by PCR for PRRSV, resulting in four PCR results per herd per monitoring event. Positive pool samples are sequenced at Sciensano. Based on repeated monitoring results, herds are classified as consistently negative (all pools PCR-negative), consistently positive (at least one positive pool in every monitoring event), or variable (alternating positive and negative monitoring results over time). Long-term changes in herd classification and the geographical distribution of herds with negative results are also evaluated.

Results

In 2025, 162 Belgian pig farms (approximately 9% of all eligible farms) participated in the PRRS Piglet Monitor. More than half of these farms had participated regularly in the PRRS program in previous years. Among herds with repeated monitoring results in 2025, 17% remained consistently negative, 49% remained consistently positive for a PRRSV field strain, and 34% showed variable results over time. In contrast, 83% of farms monitored only once tested positive for a PRRSV field strain. Across the 195 herds monitored during 2024–2025, 11% remained PCR-negative in the nursery across all monitoring rounds, with several herds achieving more than three consecutive negative results. An additional 14% evolved from a positive to a negative status during this period. Herds with exclusively negative PCR monitoring results in the nursery were identified in all Belgian pig-producing provinces, including regions with high pig density.

Discussion and conclusions

These findings demonstrate substantial variation in PRRSV circulation and emphasize the importance of repeated monitoring. The high proportion of persistently positive herds, together with herds showing variable results over time, highlights the continued challenge posed by PRRSV. Regular monitoring enables early detection of virus circulation and supports evidence-based control strategies. At the same time, the repeated identification of PCR-negative herds, as well as herds transitioning from positive to negative status, indicates that sustained control of PRRSV circulation in the nursery is achievable under field conditions. The presence of such herds across all Belgian pig-producing provinces, including high-density regions, further suggests that effective control at this production stage is possible even in areas with intense pig production.

Conflicts of interest

To the best of the authors' knowledge, there are no conflicts of interest.

Ethics statements

Data were collected during routine veterinary practice and herd monitoring. No specific ethical approval was required.

Financial support and Acknowledgements

The Belgian PRRS Programme is partially funded by the Belgian Sanitary Fund.

Keywords

PRRS-Program • Nursery pigs • Monitoring

P.59 • ID34

INTENSIVE VETERINARY SECOND LINE GUIDANCE AND MONITORING SUPPORT PRRSV CONTROL IN A BELGIAN FARROW-TO-NURSERY HERD: A CASE STUDY

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Introduction and objectives

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) remains a major cause of reduced productivity and health problems in pig herds. This case study describes the diagnostic investigation, intervention strategy, and follow-up monitoring of a Belgian sow herd affected by poor piglet performance, increased mortality, and persistent health issues. The objective was to identify underlying causes and evaluate the impact of implemented control measures.

Methods

A 280-sow herd operating a three-week batch production system and selling piglets at approximately 25 kg body weight was investigated. The herd experienced poor growth, recurrent diarrhoea, skin lesions, and mortality exceeding 15%. A comprehensive diagnostic investigation was performed, including post-mortem examinations, PRRSV PCR testing and sequencing of processing fluids and blood samples from piglets of different ages. Specialised second-line veterinary consultancy was provided by DGZ throughout the process, covering risk assessment, interpretation of diagnostic results, implementation of control measures, and follow-up through the Belgian PRRS Program. Monitoring was used to evaluate the effectiveness of the interventions.

Results

Post-mortem examination identified *Staphylococcus hyicus* as the cause of the observed skin lesions. However, PRRSV was considered a major underlying contributor to the herd health problems. Initial monitoring showed negative processing fluids, suggesting no detectable PRRSV transmission at birth. In contrast, PRRSV detection was observed in piglets aged 3, 6, and 9 weeks, indicating virus circulation later in life. Based on these results, the herd implemented a revised PRRSV control strategy. Replacement gilts, which had previously not been vaccinated during quarantine, received a PRRSV booster vaccination upon arrival. Teaser boars, previously left out of the vaccination program, were also included. In addition, sows received a one-time whole-herd vaccination with an inactivated PRRSV vaccine. Key biosecurity improvements included more frequent needle replacement, the use of barn-specific boots, and segregation of slow growing piglets. Following implementation of vaccination and biosecurity measures, clinical performance improved, with healthier piglets, reduced mortality, and improved sow performance. During follow-up monitoring, however, processing fluids tested positive for PRRSV, accompanied by clinical signs and reproductive problems in young sows. Investigation of potential risk factors revealed that, on one occasion, a group of incoming replacement gilts had been introduced into the herd without undergoing the mandatory quarantine period. This biosecurity breach was considered the most likely source of renewed virus introduction and circulation.

Discussion and conclusions

This case illustrates the multifactorial nature of PRRSV-associated herd problems and the importance of combining diagnostics, vaccination, biosecurity, veterinary support, and monitoring. These findings demonstrate how targeted monitoring can help identify critical points of virus introduction and support evaluation of implemented control measures. Furthermore, the case highlights the importance of strict adherence to biosecurity measures, as a single deviation from established protocols was associated with renewed PRRSV circulation. Continuous monitoring combined with structured veterinary guidance proved essential for evaluating control measures, supporting timely management decisions, and achieving sustained improvement in herd health.

Conflicts of interest

To the best of the authors' knowledge, there are no conflicts of interest.

Ethics statements

Data were collected during routine veterinary practice and herd monitoring. No specific ethical approval was required.

Financial support and Acknowledgements

The Belgian PRRS Programme is partially funded by the Belgian Sanitary Fund.

P.60 • ID40

COMPARISON OF HERD CHARACTERISTICS FOR SUCCESSFUL AND UNSUCCESSFUL PRRSV ELIMINATIONS IN DANISH SOW HERDS

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Introduction and objectives

Although herd elimination of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) has been widely studied, less attention has been given to direct comparisons between successful and unsuccessful elimination efforts in sow herds. Such comparisons might provide valuable insight into which herd characteristics and management conditions are associated with successful PRRSV eliminations in sow herds. This study aimed to compare the characteristics of Danish sow herds after successful and unsuccessful PRRS elimination programmes for PRRSV in 2024 to 2025. Focus was placed on differences in PRRSV type, herd size, age-group structure, and use of PRRSV vaccines to identify variables associated with elimination outcome.

Methods

A retrospective observational study included Danish sow herds that initiated the process of obtaining PRRS-negative status in 2024 or 2025. In Denmark, official PRRS status is based on serological monitoring, and PRRS-positive herds may obtain negative status after two sets of antibody-negative serum samplings six months apart. The first set of samples is submitted when the herd is considered PRRSV-free, typically after LCH or a modified LCH (Load-Close-Homogenize procedure. Herds were included at the first antibody-negative sampling. Elimination was classified as successful if the second sampling was also antibody-negative, and as unsuccessful, if antibody- or virus-positive samples were detected during or at the end of the six-month period. Outdoor herds were excluded. Herd-level data included PRRSV type, SPF status, number of sows, nursery pigs on the sow site, integrated production, and PRRS MLV vaccine prescription in the last 6 months before submission of the first set of samples. Successful and unsuccessful PRRSV herd eliminations were compared using t-test and Fisher's exact tests.

Results

In total, 88 sow herds were included, of which 74 achieved successful PRRSV elimination and 14 were unsuccessful and thus remained PRRSV-positive, corresponding to an overall success rate of 84%. Herds with unsuccessful elimination had a higher mean number of sows than successful herds (1,294 vs. 924; $p = 0.0327$), but the prevalence of herds with more than 1000 sows was not significantly different in the two groups. Prevalence of herds with SPF status and the proportion of herds with integrated production, weaners on the sow site, and prescription of PRRS MLV vaccine for sows in the period before the first sampling did not differ significantly between groups.

Discussion and conclusions

PRRSV elimination was achieved in most included herds. Apart from a higher mean number of sows in unsuccessful herds, none of the recorded herd characteristics differed significantly between successful and unsuccessful elimination attempts. This suggests that herd size may increase the complexity of elimination, while other important determinants are likely related to herd-specific management and biosecurity practices.

Conflicts of interest

No conflicts of interests

Ethics statements

Data for the study was collected from an already existing database

P.61 • ID67

THE CLASSYFARM PRRS DASHBOARD: INTEGRATING GENOMIC AND EPIDEMIOLOGICAL DATA FOR DISEASE SURVEILLANCE IN ITALY

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Introduction and objectives

Sharing surveillance outputs with stakeholders and policy-makers is a key component of disease surveillance activities. In recent years, web-based technologies and business intelligence applications have emerged as powerful tools to facilitate output dissemination and improve accessibility for relevant users. Ad hoc platforms and interactive dashboards can aggregate data from multiple sources, visualise spatio-temporal disease dynamics, and deliver actionable insights to end users in a timely manner, thereby supporting evidence-based decision-making. In the context of PRRS, timely access to sequencing information is of particular relevance, as its extensive genetic diversity and the limited cross-protective immunity between circulating strains make genomic data essential for outbreak investigation and response. Here we describe the development of an interactive genomic surveillance dashboard for PRRS implemented within ClassyFarm, the Italian national livestock information system.

Methods

Swine samples submitted to our laboratories (IZSLER, Brescia, Italy) are screened for PRRSV using commercial RT-qPCR kits, with positive samples subjected to Sanger sequencing of the open reading frame 7 (ORF7) region. On a weekly basis, sequence files are uploaded to a cloud environment and processed through an automated pipeline built on Microsoft Azure services. Briefly, FASTA files are first combined with farm- and sample-level metadata, including animal category, geographic coordinates of the farm and vaccination status. Multiple sequence alignment (MSA) and pairwise distance estimation are then performed across the full sequence database. A dedicated clustering step is applied to identify vaccine-like strains. Finally, the generated outputs and analytical results are rendered in an interactive Microsoft Power BI dashboard, accessible through the ClassyFarm web platform.

Results

The dashboard, currently in its testing phase with restricted access, includes nearly 7,500 PRRSV ORF7 sequences generated between 2005 and 2025, originating from over 1,100 pig farms. It combines sequence similarity analysis with epidemiological and spatial information in an interactive environment. Users can explore pairwise sequence similarity matrices, visualise the geographic distribution of related strains, and analyse temporal and spatial patterns of vaccine-like sequences. Flexible filtering options enable direct comparison of outbreak strains against the historical sequence database, with queries definable by farm, production company, sampling window, and similarity threshold.

Discussion and conclusions

At farm level, by enabling rapid discrimination between endemic strain recirculation and the introduction of new variants, the dashboard supports targeted disease management and the identification of potential biosecurity breaches. At a regional scale, it may also facilitate early detection and tracking of emerging highly pathogenic PRRSV strains. By combining long-term genomic, epidemiological, and spatial data within a nationally coordinated platform, the ClassyFarm PRRS dashboard provides a scalable framework for genomic surveillance and decision support in disease control, with potential extension to other swine pathogens.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statements

This work describes the development of a digital surveillance tool and does not involve animal experiments, clinical interventions, or the collection of personal data. As such, no ethical approval was required.

P.62 • ID10

DEVELOPING A U.S. PRRSV ELIMINATION FRAMEWORK INTEGRATING INDUSTRY PRIORITIES AND SYSTEMATIC REVIEW EVIDENCE

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Introduction and objectives

Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) is estimated to cost the U.S. \$1.2 billion in production costs annually. A recent U.S. producer survey conducted to inform the National Swine Health Strategy (NSHS) engaged 822 stakeholders, including 564 producers across multiple industry segments, and 45 listening sessions reached approximately 1,000 participants. Across both efforts, PRRSV emerged as the top health challenge, cited 134% more often than any other issue. These findings established PRRSV elimination as the long-term goal for the U.S. swine industry. The objective of this study was to synthesize stakeholder-driven priorities with current scientific evidence to inform a U.S.-applicable framework for the elimination of PRRSV.

Methods

The NSHS survey prioritized industry goals through a qualitative analysis of individual surveys and stakeholder listening sessions. Two systematic reviews were conducted, including peer-reviewed, evidence-based methods for PRRSV elimination (sow farm and regional levels) and implementable PRRSV biosecurity interventions (for both breeding and grow-finish farms). The extracted data included elimination strategies, time to stability or negative outcomes, regional program characteristics, and evidence-based PRRSV biosecurity interventions. All findings were synthesized into U.S.-applicable and adaptable components.

Results

The NSHS survey ranked PRRSV as the dominant health concern for the U.S. pork industry. The elimination systematic review included 25 peer-reviewed studies from 456 records, and the biosecurity systematic review included 48 peer-reviewed studies from 727 records. Across elimination studies, herd closure, test and removal (TR), and depopulation-repopulation (DRP) achieved PRRSV-negative farm status. Herd closure with live-virus inoculation demonstrated a shorter time to stability, while DRP provided the highest probability of virus elimination with the highest economic cost. Successful regional eliminations required PRRSV surveillance, restrictions on animal movements, and transparency of farm disease status. Voluntary regional programs demonstrated reductions in PRRSV prevalence, but have difficulty in maintaining the high participation and data sharing needed to sustain progress. High-impact interventions from the biosecurity review target major transmission routes, including transport sanitation, air filtration, and the implementation of a comprehensive, multi-component biosecurity program. Additional scalable interventions addressed animal sourcing, personnel flow, and fomites to reduce PRRSV risk.

Discussion and conclusions

This study uniquely integrates U.S. stakeholder prioritization with systematic evidence to provide a technically grounded pathway for U.S. PRRSV elimination. Findings indicate that elimination is not limited by a lack of effective tools but rather by variability in implementation, coordination, and industry-level alignment. A U.S. elimination program requires three core components: deployment of farm-level elimination strategies tailored to site-specific constraints, implementation of a comprehensive, validated biosecurity program, and regional coordination that incorporates surveillance, data transparency, and controlled animal movements. The alignment of industry priorities with evidence-based interventions supports the feasibility of a U.S. PRRSV elimination and the adaptation of interventions to site-specific factors, including herd size, pig flow, biosecurity infrastructure, and regional disease pressure.

Conflicts of interest

No conflicts of interest

Ethics statements

No animal experimentation was used in this study. All stakeholder input was provided voluntarily with consent, and all data was analyzed in aggregate.

Keywords

PRRSV • Biosecurity • Elimination

P.63 • ID29

ANALYSIS OF PRRSV BEHAVIOR, INCORPORATING INTESTINAL HEALTH PROGRAM, NON-VACCINATION AND ANTIBIOTICS PROGRAM

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Amvecaj

Introduction and objectives

From the late 1980s to the present, the PRRS virus has been present on almost all farms with its different serotypes, causing reproductive and production problems and affecting the profitability of the operation. Some farms in Mexico, with gut health programs, have improved their health status. PRRS increases the presence of secondary diseases of bacterial origin, which often necessitates the use of antimicrobials and gut health programs. PRRS is characterized by its high mutation and recombination rate, which leads to its perpetual expansion and facilitates the emergence of new strains. Batista (2024) comments that controlling or eliminating PRRS requires a comprehensive strategy that demands the implementation of all available strategies. Some of these strategies include vaccines, management practices, biosecurity, antibiotics, and gut health. Medium-chain fatty acids destroy enveloped viruses. By causing disintegration of the viral envelope, they can inhibit the late maturation stage in the viral replication cycle, preventing viral proteins from binding to the host cell membrane. Gut integrity and alpha diversity maintain a good immune response for overall health. The objective is to understand how gut health contributes to the control of PRRSV.

Methods

Location: Jalisco, Mexico Population: 2000 females (complete cycle) The gut health program includes medium- and short-chain fatty acids, phytobiotics, and probiotics. It is administered in gestation feed, pre-starter feed, and starter feed. The breeding herd and production line do not use antibiotics in any form or PRRSV vaccine.

Results

Table 1 shows that in October 2023, piglets were born positive and remained so until week 7 with a CTS of 19.22. In November 2024, the sampled weeks of the piglets tested positive. In December 2024, weeks 7 and 8 were positive with an average CTS of 25. In February 2025, week 9 was positive with a CTS of 26.2. In March 2025, only week 7 was positive with a CTS of 23.8. In July 2025, week 9 was positive with a CTS of 34.6, and one month later all were negative. Without vaccination, some animals tested positive for several weeks, but with high CTS (concentration-to-test) levels. Table 2 shows PRRS PCR sampling of females. In December 2024, the herd had gone 13 months without vaccination. PRRS PCR tests were performed, all of which were negative. Vaccination and antibiotic use during gestation and lactation continued. Samples were taken again in April, May, and July 2025, and January and June 2026, with all PCR tests coming back negative. Tables 3 and 4 show villi and goblet cellulase levels. Image 1 shows villous histology, and Image 2 represents the microbiome.

Discussion and conclusions

After implementing intestinal health programs, a decrease in PRRS virus signs was observed in both the breeding herd and the production line, with 31 months of vaccination-free females and 12 months of vaccination-free piglets. Intestinal integrity and bacterial balance are related to the reduction of PRRS viremia and associated clinical signs, and intestinal health programs represent an excellent tool.

Conflicts of interest

There was no conflict

Ethics statements

Everything was done in compliance with official Mexican standards: • NOM-033-SAG/ZOO-2014, Methods for euthanizing domestic and wild animals. • NOM-056-ZOO-1995, Technical specifications for diagnostic tests performed by approved animal health testing laboratories.

Financial support and Acknowledgements

A Entera Animal health

Keywords

gut health

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P.64 • ID35

USE OF PRRSV-POSITIVE ORAL FLUID ROPES FOR ACCLIMATION OF REPLACEMENT GILTS

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Introduction and objectives

Porcine reproductive and respiratory syndrome (PRRS) remains one of the most important viral diseases affecting pig health and production worldwide (Zimmerman *et al.*, 2019; Kedkovid *et al.*, 2020). Successful acclimation of replacement gilts is essential for PRRS control, but natural exposure provides limited control over infection timing and immune response. Oral fluid (OF) sampling has been described as a practical tool for PRRSV monitoring and acclimation programmes under field conditions (Woonwong *et al.*, 2018; Henao-Diaz *et al.*, 2021). This study evaluated whether PRRS virus (PRRSV)-positive OF ropes could be used as an additional or sole exposure tool during gilt acclimation and assessed the usefulness of serum and OF samples for monitoring PRRSV infection and antibody responses.

Methods

Ninety-six PRRSV-naïve replacement gilts were allocated to six groups of 16 gilts each. Gilts were exposed to PRRSV through natural contact with infected pigs, natural contact combined with PRRSV-positive OF ropes, or PRRSV-positive OF ropes alone. PRRSV RNA and anti-PRRSV antibodies were monitored weekly for 9 weeks using RT-PCR and ELISA in individual serum samples and group OF samples.

Results

PRRSV infection and seroconversion occurred in all gilts exposed through natural contact, whereas exposure to PRRSV-positive OF ropes alone did not result in infection or reliable acclimation. Compared with natural exposure alone, the addition of PRRSV-positive OF ropes resulted in significantly earlier detection of viremia and earlier seroconversion. A higher proportion of gilts in this group also became non-viremic sooner. Detection of PRRSV RNA in OF generally reflected serum results, supporting its value as a practical group-level monitoring sample. However, discrepancies between serum and OF results were observed, and antibody detection in OF showed some variability.

Discussion and conclusions

PRRSV-positive OF ropes can support gilt acclimation when used as a supplement to natural exposure, as they accelerate early infection and enhance antibody responses. However, they are insufficient as a standalone exposure method and do not ensure complete control of viremia. Oral fluid sampling is a useful and practical tool for monitoring PRRSV acclimation under field conditions, but OF-based results, particularly serological results, should be interpreted with caution due to differences in sensitivity between matrices and challenges related to antibody detection in OF.

Conflicts of interest

The authors declare no commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statements

The animal study was approved by the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection, Ministry of Agriculture, Forestry and Food, Republic of Slovenia; No. U34401-36/2017/5, issued on 11 January 2018. The study was conducted in accordance with local legislation and institutional requirements.

Financial support and Acknowledgements

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Keywords

PRRS virus • gilt acclimatization • disease control

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P.65 · ID136

GENERATION OF CD163/TMPRSS2 DOUBLE-KNOCKOUT PIGS BY CRISPR/CAS9 REVEALS THE IMPORTANCE OF CD163 MUTATION DESIGN FOR PRRSV RESISTANCE AND ESTABLISHES A PLATFORM FOR FUTURE SWINE INFLUENZA STUDIES

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Introduction and objectives

Porcine reproductive and respiratory syndrome (PRRS) remains one of the most important infectious diseases affecting the global swine industry. Genome editing offers an alternative strategy to improve disease resilience by disrupting host factors required for viral infection. The CD163 receptor is essential for PRRSV entry into macrophages, whereas TMPRSS2 encodes a transmembrane serine protease involved in the infection process of several respiratory viruses, including swine influenza virus. The objective of this study was to generate pigs carrying simultaneous knockouts of CD163 and TMPRSS2 using CRISPR/Cas9 technology, to evaluate their preliminary response to PRRSV vaccination and to establish a genetic platform for future studies on resistance to swine influenza virus.

Methods

Guide RNAs were designed to induce loss-of-function mutations in exon 7 of the CD163 gene and exon 3 of the TMPRSS2 gene. CRISPR/Cas9 components were microinjected into 93 *in vivo*-derived zygotes, which were transferred into nine synchronized recipient sows. Five recipients became pregnant; three aborted during gestation and two completed pregnancy to term. A total of 17 fetuses and 10 live-born piglets were recovered and genotyped. Preliminary PRRSV resistance was evaluated in five edited piglets vaccinated at 56 days of age with a commercial modified-live PRRSV vaccine (Porcilis® PRRS, MSD Animal Health). Blood samples were collected at 85, 99, 115, 129 and 140 days of age to detect PRRSV RNA by RT-qPCR and anti-PRRSV antibodies by ELISA. A non-edited control group was included. Body weight, hematological parameters and serum biochemistry were monitored throughout the study.

Results

Genotyping of the 27 recovered fetuses and piglets identified eight biallelic CD163/TMPRSS2 double-knockout animals, nine non-edited animals, two animals carrying biallelic TMPRSS2 mutations only, four animals carrying monoallelic CD163 and biallelic TMPRSS2 mutations, one animal carrying a biallelic CD163 and monoallelic TMPRSS2 mutation, and three samples that could not be genotyped. Four of the five edited piglets remained negative for PRRSV RNA by RT-qPCR and showed no seroconversion throughout the study, whereas non-edited control animals developed detectable viral replication and antibody responses following vaccination. In contrast, one edited pig became RT-qPCR- and ELISA-positive during the first two sampling points. No significant differences in body weight, hematological parameters or serum biochemistry were observed between edited and control animals.

Discussion and conclusions

These results demonstrate the feasibility of generating CD163/TMPRSS2 double-edited pigs while maintaining normal health and growth. The absence of detectable viral RNA and serological response in most edited animals following vaccination suggests a potential impairment of PRRSV infection, although further challenge studies are required to confirm resistance. The additional disruption of TMPRSS2 provides a basis for future studies on susceptibility to other respiratory viruses

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

Protocols approved by the Ethics Committee of the University of Murcia and the Regional Government of Murcia (PHENOCRISPR No. CARM-A13221206).

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Keywords

gene editing

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P.66 • ID61

PROTECTING AGAINST AFRICAN SWINE FEVER: TAILORING BIOSECURITY FOR OUTDOOR PIG FARMING SYSTEMS IN SPAIN

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Introduction and objectives

Outdoor pig farming systems are at higher risk of African Swine Fever (ASF) infection due to greater interaction with wild boars and greater exposure to the external environment. These types of farms face specific challenges in implementing biosecurity practices, which can be the cornerstone in protecting them against ASF and other important pathogens. This study aims to develop a comprehensive and adaptable biosecurity framework that suits non-intensive pig farms in Spain, while empowering farmers and advisory services to co-create solutions tailored to their specific needs.

Methods

A mixed methods approach will be used to achieve our objective. The first part involves developing a biosecurity improvement framework, using information gathered through interviews and a legislative review. Overall, 4-5 interviews with outdoor pig farmers and 3-4 interviews with outdoor pig vets have been planned. So far, 2 interviews with farmers and 2 with veterinarians have been conducted. A qualitative analysis will be conducted using transcripts of these interviews to identify barriers and needs related to the assessment or implementation of biosecurity. The second part will consist of developing an economic analysis for which a Biosecurity Cost Calculator from a previous project on intensive dairy cattle will be adapted to these types of farms. The third will be the pilot studies to test and refine the framework with outdoor pig farming systems. We will select 3 outdoor pig farms that will be followed for a year, during which we will implement and refine the biosecurity framework. These processes will consider each farm’s specific context, including biosecurity challenges, available resources, animal species, production type, and potential animal welfare and economic impact.

Results

Since the project is still ongoing, we have no results yet.

Discussion and conclusions

By the time of the conference, we will be able to present the biosecurity framework, the finalised protocol for the pilot studies, and descriptions of the selected pilot farms. This methodology is being followed in another 4 European countries.

Conflicts of interest

The authors have no conflict of interest to declare.

Ethics statements

Ethical approval of the project by the Ethical Research Committee of the Autonomous University of Barcelona (Comitè d’Ètica en la Recerca (CERec)). Project number: UAB-CERec487

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Keywords

Biosecurity • Outdoor pigs • Framework • Pilot farms • Co-creation

P.67 • ID126

CLASSICAL SWINE FEVER IN BRAZIL: THE ALAGOAS PILOT VACCINATION PROJECT

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Introduction and objectives

Classical Swine Fever (CSF) is a highly contagious viral disease that exclusively affects swine (*Sus scrofa*) and does not impact humans or other species. It is a disease of significant economic impact and requires mandatory notification to the Official Veterinary Service, as mandated by the World Organisation for Animal Health (WOAH). Brazil is currently divided into a CSF-Free Zone (FZ), encompassing 16 states and four municipalities in the state of Amazonas, and a Non-Free Zone (NFZ), covering 11 states in the North and Northeast regions, where the CSF is still in circulation. The disease limits swine production development in these regions and poses a potential risk of incursion into the FZ, which could lead to the closure of international markets and affect the entire national swine sector. Over the past years, 87 disease outbreaks were confirmed, resulting in millions in compensation losses. Most outbreaks occurred in the states of Ceará, Piauí, and Alagoas, all resolved through prompt action by the Official Veterinary Service (OVS), involving the culling of affected animals and compensation to rural producers. In response to recurring outbreaks, the Ministry of Agriculture, Livestock, and Supply (MAPA), in partnership with the private sector, established a working group to develop a Strategic Plan for CSF eradication in Brazil. This plan outlined measures to strengthen systematic surveillance and the need for a regionalized vaccination program in the NFZ.

Methods

The state of Alagoas was selected as the pilot project site due to local partner support, its geographic and herd characteristics, and the concern over a 2019 outbreak located near the border with the FZ. Implementation began in 2021 with the hiring of vaccination teams and distribution of vaccines (Pest-Vac, Zoetis) through partner agricultural retailers.

Results

The five stages of the CSF vaccination campaign in Alagoas mobilized teams across 102 municipalities, achieving high vaccination coverage throughout. In total, over 640,000 immunizations were carried out between 2021 and 2024, delivering free vaccination to all rural properties raising swine.

Discussion and conclusions

Beyond immunizing animals, the campaign generated significant socioeconomic benefits for producers, including: higher swine market prices; reduction in reproductive losses such as abortions; healthier piglets and lower mortality rates; improved commercialization of vaccinated animals; greater producer credibility in local markets; increased organization among producers through the formation of local associations; and overall development of swine farming in Alagoas. The occurrence of outbreaks in endemic areas of the NFZ continues to be a critical concern for Brazil's swine sector and OVS. Eradicating CSF from the 11 states that comprise the NFZ requires a united effort between public authorities and the private sector, ensuring the health and competitiveness of Brazilian swine production on both national and international levels.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

Does not applicable.

Financial support and Acknowledgements

The vaccination campaign was supported by a public-private partnership. The authors would like to thank all supporters, donors, pig farmers, vaccination teams, the Brazilian Association of Pig Farmers (ABCS), the Brazilian Animal Protein Association (ABPA), the Alagoas Agricultural and Livestock Defense and Inspection Agency (ADEAL), the Brazilian Confederation of Agriculture and Livestock (CNA), the National Rural Learning Service (SENAR), the Inter-American Institute for Cooperation on Agriculture (IICA), and the MAPA for their joint efforts to advance the swine industry in Brazil.

Keywords

CSF • Disease Surveillance • Non-Free Zone • Swine Production • Socioeconomic Impact

P.68 • ID132

CLASSICAL SWINE FEVER CONTROL IN BRAZIL’S NON-FREE ZONE: VACCINATION CAMPAIGN PERFORMANCE AND PRODUCER PERCEPTION IN ALAGOAS

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Introduction and objectives

Classical Swine Fever (CSF) or Hog Cholera is a highly contagious viral disease of domestic and wild swine, caused by a *Pestivirus* from the Flaviviridae family. In Brazil, the first record of the disease dates back to 1899, and after years of vaccination, CSF has been eradicated in most Brazilian states. Currently, Brazil is divided into two zones: the Free Zone (FZ), where 95% of industrial swine production is carried out, and the Non-Free Zone (NFZ) of CSF — both recognized by the World Organization for Animal Health (WOAH). In the NFZ, a Vaccination Campaign against CSF was implemented in accordance with the objectives of the Brazil Free of CSF Plan, through a public-private partnership, which selected the state of Alagoas to host the Experimental Project. The eradication of the disease is fundamental for international recognition, demonstrating the country’s good sanitary conditions, as well as strengthening the quality of products offered, keeping the country competitive and generating additional benefits such as the opening of new markets. Thus, the objective of this study was to present and analyze the data collected from the 2nd to the 5th stage of the vaccination campaign, in addition to assessing the acceptability of swine farmers regarding this initiative.

Methods

A satisfaction questionnaire was applied to a sample of producers regarding the execution of the vaccination campaign, following the 2nd, 3rd, 4th, and 5th phases. A total of 136 producers were interviewed, selected by lottery, covering municipalities from the three regions of Alagoas (Leste, Sertão, and Agreste), over the period between 2022 and 2024.

Results

According to data from the 2nd and 3rd phases, 98.11% of producers rated the free vaccination initiative with a score of 10, and 86.79% expressed high confidence in the vaccine. Furthermore, all respondents answered affirmatively when asked about the importance of vaccination and whether they would regularly vaccinate their herds. In the questionnaire applied during the 3rd, 4th, and 5th phases, 71.82% of producers reported greater confidence in expanding their swine operations due to the vaccination program, 70% noted a reduction in mortality losses, and 67.27% observed improvements in local commercialization. When asked whether they would purchase the vaccine if it were no longer free of charge, 96.33% of the swine farmers participating in the 2nd, 4th, and 5th phases answered yes.

Discussion and conclusions

These results demonstrate that the campaign brought meaningful improvements to local pig farming, such as maintaining herd health and updating the registry of rural properties engaged in swine breeding — which, in turn, facilitates the implementation of other public policies directed at the sector. Therefore, the continuous improvement of the vaccination campaign is essential, as its practice is critical for maintaining the health of the swine herd, supporting the development of rural properties in Alagoas, and fulfilling the objectives of the Brazil Free of CSF Plan.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

Does not applicable.

CONTINUE >

Financial support and Acknowledgements

The vaccination campaign was supported by a public-private partnership. The authors would like to thank all supporters, donors, pig farmers, vaccination teams, the Brazilian Association of Pig Farmers (ABCS), the Brazilian Animal Protein Association (ABPA), the Alagoas Agricultural and Livestock Defense and Inspection Agency (ADEAL), the Brazilian Confederation of Agriculture and Livestock (CNA), the National Rural Learning Service (SENAR), the Inter-American Institute for Cooperation on Agriculture (IICA), and the Ministry of Agriculture, Livestock, and Supply (MAPA) for their joint efforts to advance the swine industry in Brazil.

Keywords

Hog Cholera • Disease Eradication • Public-Private Partnership • Immunization Coverage • Swine Health



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P.69 • ID4

EVALUATION OF LESS INVASIVE SAMPLING METHODS FOR PRSSV DETECTION IN ADULT PIGS: FILTER PAPER AND TONSIL ORAL SCRUBBING

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Introduction and objectives

Effective surveillance is essential for controlling and eliminating porcine reproductive and respiratory syndrome (PRRS). Blood-soaked filter papers dry blood spots (DBS) and tonsil oral scrubbing (TOSC) can detect pathogens in recently infected swine, but their accuracy when applied to real outbreak is still unclear.

Methods

Sows from a sow farm with an ongoing PRRS outbreak were sampled twice: prior to live virus inoculation (LVI), approximately 5 weeks after the outbreak was detected, and four months later. At the first sampling, ear-prickling DBS, serum (collected by jugular venipuncture) and TOSC were collected from 213 animals selected using systematic spatial sampling (gestation stalls) and randomly (farrowing stalls). On the second sampling, DBS, serum, TOSC, and tonsil scrapings (TS) were collected from 126 animals from the initial cohort of 213 animals.

Results

At the first sampling, 38 serum (17.8%), 184 TOSC (86.4%) and 12 DBS (5.6%) samples were RT-PCR positive, and all serum and three (1.4%) DBS samples were ELISA positive. Compared to serum, TOSC and DBS samples had a sensitivity of 89.5% and 13.2%; and a specificity of 14.3% and 96.0%, respectively. No clear spatial diagnostic clustering was observed. At the second sampling, one serum (0.8%), 6 TOSC (4.7%), 8 TS (6.3%), and no DBS (0%) samples were RT-PCR positive. Compared to TS, TOSC had 50% sensitivity and 98.3% specificity. Compared to serum, DBS had 0% sensitivity and 100% specificity. After 4 months, 119 (94.4%) of the animals remained ELISA positive on serum. No animal tested RT-PCR positive on serum at both sampling points. However, one animal was negative on serum and positive on TOSC at the first sampling but was found to be viremic at the second sampling. Of the 107 TOSC positive samples from the first sampling, six remained TOSC positive on the second sampling.

Discussion and conclusions

DBS low sensitivity in sampling one is likely due to the lower viral load detected in most viremic sows. Also, TOSC samples had higher sensitivity than DBS but lower specificity when using serum as a reference. However, because virus is found on tonsils at a different stage of infection than viremia, the two specimens might not be directly comparable. At sampling two, TOSC had a higher specificity but a lower sensitivity when using TS as reference. However due to the small number of positive animals in sampling two and subsequent low statistical power, comparisons between sample types is limited. Alternative sample types showed superficial accuracy compared to standard samples, despite being easier to collect. Their performance may be influenced by the stage of infection at sampling. However, they may still be valuable for herd-level surveillance and screening programs, where ease of collection can outweigh lower diagnostic accuracy.

Conflicts of interest

NA

Ethics statements

Procedures involving animals were approved by the University of Minnesota Institutional Animal Care and Use Committee protocol #2304-40960A

Financial support and Acknowledgements

University of Minnesota Rapid Agricultural Response Fund (RARF)

Keywords

PRRS • Surveillance

P.70 • ID22

EVALUATION OF ORAL FLUID PRRS SEROLOGY AGAINST SERUM SAMPLES UNDER FIELD CONDITIONS USING A LATENT CLASS APPROACH WITHOUT ASSUMING A PERFECT REFERENCE TEST

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Introduction and objectives

In Denmark, official pig herd PRRSV status is currently based on testing 20 individual serum samples for PRRSV antibodies. Oral fluid sampling is less invasive, enables sampling of a larger share of animals in a herd, and a commercial PRRSV antibody kit is available. This study compared the diagnostic performance of a herd-level oral fluid procedure with that of 20 individual serum samples from the same epidemiological unit. The study was restricted to finisher-only herds, because reports have shown that a common feeding regime for young pigs in Denmark can cause false-positive PRRSV antibody results in oral fluid. We applied a latent class approach in which neither test was assumed to be perfect.

Methods

A Bayesian latent class analysis was used to estimate herd-level sensitivity (Se) and specificity (Sp) for the two test procedures. Herds were selected according to their most recent official PRRS status (positive/negative) for sampling of two populations with expected different true prevalences. A total of 107 herds were included. From each herd, 20 individual serum samples and four oral fluid samples were collected from the same epidemiological unit. For both procedures, herds were classified as positive if at least one sample tested positive. Oral fluid results were evaluated using an S/P value of 1.0 as cut-off based on recommendations from the literature.

Results

Median herd-level sensitivity (Se) was higher for serum sampling (97.8%) than for oral fluid sampling (88.4%), while specificity (Sp) was comparable between methods (serum: 97.2%; oral fluids: 97.7%). Across posterior simulations, serum sampling achieved higher Se than oral fluids in 93% of iterations, indicating a high posterior probability that serum had higher sensitivity, although not at a conventional 5% significance threshold. No meaningful difference in Sp was supported by the posterior distributions. Estimates of true prevalence in the two latent populations aligned with expectations for finisher herds based on previous classification.

Discussion and conclusions

Under field conditions in finisher herds, a procedure based on four oral fluid ropes showed slightly lower herd-level sensitivity than the current Danish standard of 20 serum samples, while specificity was comparable. The lower sensitivity of oral fluid is expected given fewer effective individual contributions and the aggregated sample. This may be offset by greater feasibility, less labor, and broader within-pen coverage. Oral fluid sampling enables less invasive monitoring and may support more frequent surveillance and earlier detection. Although serum sampling remains the more sensitive herd-level approach, the relatively small difference suggests that oral fluid sampling is a robust and scalable alternative under field conditions. The latent class framework strengthens this conclusion by avoiding bias from assuming a perfect reference test. Overall, oral fluid-based surveillance has considerable potential as a supplement or, in some contexts, an alternative to serum-based PRRS monitoring in finisher herds.

Conflicts of interest

None declared

Ethics statements

Samples were collected as part of field surveillance under routine herd conditions.

Financial support and Acknowledgements

Funded by the Pig Levy Fund (Denmark), PRRS reduction program. Thanks to the two veterinary pig practices that helped with the sample collection.

Keywords

Diagnostic test evalution • oral fluids • serum samples • latent class analysis • serology

P.71 • ID25

PRRSV ANTIBODIES IN ORAL FLUID: THE IMPACT OF PROCESSED PORCINE PLASMA IN FEED

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Introduction and objectives

In 2019, porcine reproductive and respiratory syndrome virus (PRRSV) was made notifiable in Denmark and since 2023 a national eradication program has been implemented. Oral fluid (OF) has been proposed as a non-invasive and cheap alternative to serum, to detect PRRSV-antibodies. However, previous reports showed that piglets receiving feed with plasma protein (PP) tested positive in Enzyme-Linked Immunosorbent Assay (ELISA) for OF. Feed containing PP is widely used in Danish herds. This study investigated the presence and dynamics of antibodies against PRRSV in OF from piglets fed with commercial batches with PP.

Methods

Fifteen 4-week-old Danish Landrace crossbreed pigs (body weight 7.0 kg) originating from a Danish conventional PRRSV negative herd were obtained. The pigs were equally divided into three groups and fed a postweaning PP-free diet. After acclimatization on day 0 feed with PP was introduced to Groups 2 (3% PP) and 3 (5% PP). Group 1 continued with a PP-free diet. On day 7 the pens and equipment were changed or cleaned, and all three groups received a diet unintentionally containing PP. On day 14, the cleaning procedure was repeated, and all groups were fed a PP-free diet. During the experiment blood and OF were collected. OF was sampled by the grouped rope method and by individual buccal swabs. Feed, serum and OF were tested by IDEXX ELISA-kit (PPRS X3 Ab Test and OF Ab Test).

Results

Diets with PP had S/P values of 6.0 and 7.3, whereas diets with traces of PP ranged from 1.6-5.7. In week 1, Groups 2 and 3 (PP diet), were positive for PRRSV-antibodies in buccal swabs (S/P values: 0.4-5.2), whereas all pigs in Group 1 were negative. In week 2, 13/15 pigs in all groups were positive for PRRSV-antibodies in buccal swabs after being fed a diet with traces of (S/P values: 0.40-3.7). When changed to a PP-free diet, pigs were negative the day after, but three pigs were positive once each, up to seven days after the diet-change. In week 1, OF from Group 1 was negative, and positive in Groups 2 and 3 (S/P values: 6.9 and 6.4). All OF samples were positive after introduction of contaminated diet except Group 2 on day 10 (S/P values: 0.4-2.9). No OF samples were positive after changing to PP-free diet. No serum tested positive for PRRSV-antibodies.

Discussion and conclusions

The results confirmed that PRRSV-antibodies or fractions of these from PP interferes with the PRRSV ELISA. OF samples had S/P values at the same level as the values measured in the feed, however, a relatively short wash out period was registered, indicating that OF can be used for PRRSV surveillance few days after change to a PP-free diet. Environmental contamination is possible if thorough cleaning is not applied as illustrated by the pigs testing positive several days after the feed change. The study also illustrated that PP-free feed still can contain traces of PP, which should be considered in the future.

Conflicts of interest

None.

Ethics statements

The pig experiment (February to April 2026) was approved by the Danish Animal Experimentation council.

Financial support and Acknowledgements

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Keywords

PRRSV • Oral fluid • Surveillance • Plasma protein • ELISA • Antibodies

P.72 • ID56

TONGUE TIP SAMPLING: UNVEILING PRESERVATION AND DILUTION EFFECTS IN PRRSV DETECTION

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Introduction and objectives

Recently, alternative matrices such as postmortem tongue tip fluid (TTF) have emerged as reliable tools for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) monitoring. However, differences in pooling tissue-based samples, where individually contributes differently to the final output, compared to homogeneous pools such as serum, may influence the dilution effect. This study aimed to evaluate the effect of sample preservation and quantify the impact of dilution on RT-PCR sensitivity.

Methods

The first part of the study assessed the effect of sample preservation on TTF production. A total of 207 fresh and 54 frozen-thawed tongue tips were stored by groups of 10-15 tongues in filter-bags, recording tongue weight and fluid volume recovered from each bag to calculate and compare fluid production (mL/g). In the study's second part, the effect of pooling-induced dilution on RT-PCR detection was evaluated. Fifteen PRRSV-positive tongue fluid samples were categorized into different Ct range groups: 18-25, 25-30, 30-35, and 35-39. Samples were serially diluted simulating pools of 10, 20, 30, 40 and 50 tip tongues, by adding the equivalent fluid contribution of one positive sample into 10 PRRSV-negative tongue tips bags (previously frozen and thawed). At each step, 0.4 mL of homogenized fluid was collected for RT-PCR analysis. Sensitivity was calculated for each Ct group and dilution level. In addition, a linear mixed-effects model was fitted to quantify the effect of dilution.

Results

Frozen-thawed samples showed a significant ($p < 0.05$) higher liquid production per gram of tissue compared to fresh samples, 0.07 ± 0.021 ml/g versus 0.029 ± 0.022 ml/g, respectively. Samples with initial Ct values between 20–25 showed 100% sensitivity across all dilution levels (up to 1:50). Similarly, samples with Ct values between 25–30 maintained 100% sensitivity up to a 1:30 dilution, decreasing to 50% at higher dilutions (1:40–1:50). In contrast, samples with Ct values above 30 showed a marked reduction in sensitivity. For Ct 30–35 samples, sensitivity decreased from 100% in the initial sample to 50% at 1:10 dilution and dropped to 0% at higher dilution levels. Samples with Ct values between 35–39 were only detected in undiluted conditions (100%) and were negative at all dilution levels. The linear mixed model:

- $Ct = 28.9041 + 4.8728 \times \log_{10}(\text{dilution})$.
- $R^2M = 0.24$
- $R^2C = 0.979$

Discussion and conclusions

Samples with initial $Ct < 30$ maintained high sensitivity across dilution levels, whereas samples $Ct > 30$ showed a marked decrease in detection as pool size increased, even at 1:10 dilution. Compared with other matrices, these results indicate greater increase in Ct values associated with dilution 4.8728 Ct per each $\log_{10}$ dilution, and a lower R^2M . This suggests that dilution has a greater impact, due to the heterogeneity of pooled samples and the potential presence of inhibitory factors, as reported for other sample matrices such as oral fluids, which may also contribute to this effect. Overall, pooling strategies based on tongue-tip fluid must consider both the expected viral load and pool size to avoid compromising detection, especially under low viremia or low prevalence scenarios. Further studies are required to better understand the stronger dilution effect observed.

Conflicts of interest

Some authors are HIPRA employees.

[CONTINUE >](#)

Ethics statements

Samples were obtained from routine submissions.

Financial support and Acknowledgements

This study was funded by HIPRA, and Fernando de Mergelina is also a holder of an Industrial PhD grant from the Catalan Government (Spain) The authors are very grateful to all the HIPRA Diagnos Lab staff for their contribution to the article.

Keywords

PRRSV • Tongue tip PRRSV monitoring • Sample preservation • Dilution effect • RT-PCR Sensitivity

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P.73 • ID88

NEW POSSIBILITIES IN PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS SURVEILLANCE IN DANISH SOW HERDS

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Introduction and objectives

The Danish national reduction plan for porcine reproductive and respiratory syndrome virus (PRRSV) was initiated in 2022 and since then the number of PRRSV infected herds have decreased. In 2025 the first geographical region was declared PRRSV negative and in 2026 73.8% of the Danish herds were PRRSV negative. Fast and feasible PRRSV detection at herd level is necessary to avoid transfer of PRRSV infected pigs into PRRSV negative regions. Alternative test materials are investigated in this study, and the objective is to evaluate if slurry and carcass container fluids (CCF) can strengthen the surveillance of PRRSV in Danish sow herds.

Methods

From 15 PRRSV infected Danish sow herds, four types of diagnostic test materials were collected: serum, processing fluids (PF), slurry and CCF. Among the 15 sow herds eight herds were sampled within four weeks after changing health status to PRRSV positive, and seven herds were sampled later than four weeks. In all sow herds two age groups of suckling piglets before weaning were sampled. PF, slurry and CCF were collected from suckling piglets from birth to seven days of age. Serum, slurry and CCF were collected from suckling piglets 21 days of age or older. PRRSV-1 and PRRSV-2 RT-qPCR analysis and a simple comparison of PRRSV detection were performed.

Results

In 11 of 15 sow herds (73%) at least one test material was confirmed PRRSV positive. Surgical castration was performed in 14 sow herds, and 57% of the PF samples were PRRSV positive. The agreement between PF and CCF was 93% and the agreement between PF and slurry was 71%. Serum was collected from all herds and in 53% of the herds PRRSV was detected. The agreement between serum and CCF was 73% and the agreement between serum and slurry was 60%. From all eight sow herds sampled within four weeks after changing status, PRRSV was detected in CCF samples. Where available, PF samples were also PRRSV positive.

Discussion and conclusions

In this study, no complete agreement in detection of PRRSV between the collected sample types was detected. One reason for this could be that sample materials such as serum are typically obtained from piglets close to weaning, whereas PF is collected from piglets at processing. Relying on PF as a sampling material in future PRRSV surveillance in Denmark may be problematic due to the increasing number of herds leaving out surgical castration. In contrast, CCF representing piglets from birth to 7 days of age as well as piglets close to weaning is a diagnostic material that could be collected regardless of castration method used on the herd.

Conflicts of interest

No conflicts of interest.

Ethics statements

The Animal Ethics Institutional Board (AEIRB) at Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen have approved the project.

Keywords

PRRSV • surveillance • slurry • CCF • sow herds

P.74 • ID91

LEARNINGS FROM PARITY BASED NEONATAL PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS MONITORING

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Introduction and objectives

In sow herds affected by Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), identifying the source of (re)infection is important to reduce virus circulation in neonatal piglets. This study evaluated whether parity-based monitoring, comparing PRRSV PCR results from first parity (P1) and older parity (P2+) sows, can help identify sources of herd instability.

Methods

A prospective cohort study was performed on 41 Dutch farms between 2023 and 2025 during periods of PRRSV instability. Neonatal samples, including processing fluids, tongue fluids, or umbilical cords, were collected by farm staff and pooled by parity: P1 versus P2+. Samples were tested for PRRSV by PCR at one of two commercial laboratories. Cases were included when at least 12 samples were available and PCR positivity was $\geq 25\%$. Positivity rates and cycle threshold (Ct) values were compared between P1 and P2+ using matched herd-week pairs.

Results

Thirteen cases from nine farms met the inclusion criteria. P1 samples showed $\geq 20\%$ higher PCR positivity than P2+ in 4/13 cases, while average Ct values were $\geq 20\%$ lower in P1 in 1/13 cases and minimum Ct values in 3/13 cases. In contrast, P2+ samples showed $\geq 20\%$ higher PCR positivity in 9/13 cases; Ct values were never $\geq 20\%$ lower than in P1. Overall, P2+ litters had 1.54 times higher odds of testing positive than P1 litters, although this was not statistically significant ($p = 0.18$). Estimated positivity was 37% for P1 and 48% for P2+, with substantial variation between pairs. Ct values did not differ significantly ($p = 0.87$).

Discussion and conclusions

Grouping results by quarter-year provided only an artificial snapshot, as PRRSV outbreaks may start at any time and relevant data may have been missed. In addition, parity-based sampling began at variable intervals after outbreak onset, potentially missing the initial phase when differences between P1 and P2+ may be most pronounced. Learnings:

- Processing fluids may contain mixed parity material due to cross-fostering; tongue fluids from stillborn piglets or umbilical cords reduce this risk.
- Strict hygiene during sampling is needed to prevent cross-contamination.
- In small farrowing batches (<50 farrowings), tongue fluid volume from P1 stillborn piglets may be insufficient; sampling piglets dying before cross-fostering or collecting more tongue tissue can help.
- Because outbreaks are unpredictable, continuous batch monitoring is advised. For batches with >60 farrowings, collecting two samples per batch allows consistent separation of P1 and P2+ material.
- Visualizing P1 versus P2+ results in scatterplots supported farm-level discussions.

In conclusion, parity-based PCR monitoring may help identify sources of PRRSV instability and inform vaccination and biosecurity strategies. Although not scientifically validated, the approach was useful for guiding field interventions. Continuous monitoring and strict sampling protocols are recommended to improve reliability and minimize contamination.

Conflicts of interest

Two of the authors are Boehringer Ingelheim employees. This study however does not involve or promote any of the products sold by Boehringer Ingelheim.

Ethics statements

Sampling methods for this study did not induce additional pain or stress to the piglets.

Keywords

parity based monitoring • PRRS PCR • neonatal monitoring

P.75 • ID122

PRELIMINARY ASSAY FOR THE DETECTION OF PRRSV ON ENVIRONMENTAL SURFACES IN NON-ANIMAL AREAS OF COMMERCIAL PIG FARMS WITH ACTIVE INFECTION

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Introduction and objectives

PRRSV can be shed in large quantities by infected animals, and part of this virus may become deposited in environmental matrices. Under field conditions, farms often include multiple high-traffic areas where biosecurity barriers may be compromised. In these contexts, contaminated surfaces may facilitate the mechanical dissemination of PRRSV through personnel movement, equipment and routine management practices. The possible presence of PRRSV on surfaces in different farm areas could compromise internal biosecurity and even biocontainment and, as consequence, may play a important role in the spread of the disease to other farms. The objective of this study was to evaluate the possible presence of PRRSV RNA in the environment of non-pig areas in commercial farms with active PRRSV infections.

Methods

This study included four farms infected with PRRSV, confirmed by viremia and PRRSV detection in tongue samples from dead piglets. On each farm, environmental samples were collected using swabs moistened with PBS from the following surfaces: warehouse floor, handling tools, office floor, office desk, clean-area locker room, dirty-area locker room, loading bay, carcass storage area, and parking area. Each swab was placed in a tube containing PBS and stored under refrigeration until laboratory analysis. Once in the laboratory, tubes containing the swabs were vortexed for 1 minute and allowed to stand for 15 minutes. Subsequently, RNA was extracted from the eluates and analyzed for PRRSV by qPCR.

Results

PRRSV RNA was detected in surface samples from all farms: out of a total of 31 samples, 16 (51.61 %) were positive. In addition, considerable variability was observed between farms, with the proportion of positive samples ranging from 16.67 % to 77.78 %. Interestingly, in all these samples the amount of PRRSV was low, as all showed Cq values above 35. The surface where PRRSV was most frequently detected was the loading bay, which tested positive in 3 out of 4 farms (average Cq 38.81 ± 0.69). The warehouse floor, handling tools, office floor, clean-area locker room, and dirty-area locker room were positive in 2 of the farms, while the office desk, carcass storage area, and parking area were positive in only one farm.

Discussion and conclusions

PRRS RNA was detected on multiple environmental surfaces from non-pig-contact areas, particularly in the loading area and in the changing rooms. Although viral loads were low, these findings highlight the need to reinforce biocontainment measures.

Conflicts of interest

There is no conflict of interest in this study.

Ethics statements

No animal studies were carried out; only samples were taken from the surfaces of fenced-off farms, so this study has no bearing on animal welfare.

Financial support and Acknowledgements

We would like to thank Boehringer Ingelheim for funding this studyPRRSV.

Keywords

PRRS Surface Sampling

P.76 • ID57

COMPARATIVE EVALUATION OF TWO RT-QPCR KITS FOR PRRSV DETECTION

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Introduction and objectives

Reliable RT-qPCR detection and typing of Porcine reproductive and respiratory syndrome virus (PRRSV) are essential for outbreak investigation, surveillance and experimental studies. This study compared the performance of two commercial RT-qPCR systems for PRRSV detection.

Methods

Two datasets were analysed. The first consisted of 240 nasal swabs representing 60 farms in which respiratory disease outbreaks were investigated or respiratory disease monitoring was carried out. The second consisted of 30 samples collected at different days post-inoculation (DPI) from experimentally infected pigs inoculated with the PRRSV-1 Rosalía strain. RNA extraction was performed using the MagMAX CORE extraction kit. Each sample was tested simultaneously for the presence of PRRSV using the Gene Lyo PRRSV Triplex 3.0 kit (IDvet) and the LSI VetMAX PRRSV EU/NA 3.0 kit (Thermo Fisher), according to the manufacturers' instructions. Samples were classified as positive or negative using the recommended cut-offs. Agreement between kits was assessed using contingency tables, Cohen's kappa with bootstrap 95% confidence intervals, positive and negative agreement, and McNemar's test. Ct values were compared using linear regression, Deming regression and Bland–Altman analysis.

Results

At farm level, both kits agreed in the classification of 94.9% of farms. For nasal swabs, both kits agreed in 203 samples (84.6%), including 151 concordant positive and 52 concordant negative results. Thirty-seven samples yielded discordant results, resulting in a Cohen's kappa of 0.63 (95% CI: 0.52–0.73). 68% of the discrepant results were from samples that yielded Ct values greater than 35.0 in one of the two kits In experimental samples, overall agreement was 93.3%, and Ct correlation was high ($R^2 = 0.973$), with no significant differences between kits in terms of sample classification or Ct values.

Discussion and conclusions

Both kits showed high agreement for overall PRRSV detection and excellent concordance for PRRSV-1 detection. Ct values were generally comparable among samples detected by both assays, indicating that the main differences between kits concerned qualitative detection near the limit of detection rather than Ct quantification in clearly positive samples. These results support the use of either system for routine PRRSV detection.

Conflicts of interest

Innovative Diagnostics provided the Gene Lyo PRRSV Triplex 3.0 kit. Despois, Barbier, Méchin, Bononat and Pourquier are employees of Innovative Diagnostics.

Ethics statements

Field diagnostic samples were obtained as part of routine diagnostic or surveillance activities. The experimental infections were conducted after approval by the Ethics Committee for Animal and Human Experimentation (CEEAH) of the Autonomous University of Barcelona, reference number 6176.

Keywords

PRRS virus • RT-qPCR • Kappa value

P.77 • ID73

COMPARISON STUDY ON PERFORMANCE CHARACTERISTICS OF DIFFERENT PRRSV-QPCR-KITS WITH THE NOVEL KYLT® PRRSV NA/EU ON CURRENT EU FIELD STRAINS INCLUDING HIGH VIRULENT STRAINS FROM SPAIN

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) remains a major challenge in swine production due to its genetic diversity and continuous evolution. The KYLT® PRRSV NA/EU real-time RT-PCR assay was recently updated to improve reliable detection and differentiation of PRRSV-1 and PRRSV-2 across diverse diagnostic matrices. This study evaluated its performance on current European field strains, including highly virulent strains from Spain, and compared sensitivity, specificity and field performance with established commercial PRRSV qPCR assays.

Methods

Internal validation data, product documentation and comparative field data were reviewed. The triplex assay detects PRRSV-1 and PRRSV-2 in separate fluorescence channels and includes an exogenous internal control. Diagnostic performance was assessed using 159 field samples against alternative commercial real-time RT-PCR methods. Additional analyses included current Spanish field material tested by Eurofins Convet with RT-PCR. The comparator assays were VetMAX™ PRRSV EU & NA v3.0 Kit, Thermo Fischer Scientific Inc., USA, and RealPCR PRRSV Type 1 and Type 2 Multiplex RNA Mix, IDEXX Laboratories Inc., USA. Further analyses covered inclusivity and exclusivity testing, NCBI BLAST target-region analysis, GD Animal Health (NL) proficiency testing panels from 2023 and 2024, pooling experiments, and reproducibility/robustness studies.

Results

The updated KYLT® PRRSV NA/EU assay achieved a LOD of two copies per reaction, with PCR efficiencies for both PRRSV-1 and PRRSV-2 within the expected valid range. In 159 field samples, diagnostic sensitivity was 99.07% for PRRSV-1 and 100% for PRRSV-2, with 100% specificity for both targets. Agreement with the comparator method, assessed using Cohens’s kappa coefficient, was almost perfect for PRRSV-1 ($\kappa = 0.99$) and perfect for PRRSV-2 ($\kappa = 1.0$). In directly comparable Spanish positive field specimens, the KYLT assay generated lower Ct values than the comparator assay in most cases, consistent with a trend toward higher analytical sensitivity in this dataset. Further validation confirmed detection of vaccine strains and highly virulent PRRSV-1 reference material, absence of cross-reactivity with tested swine pathogens, broad in silico coverage of PRRSV-1 and PRRSV-2 target-region sequences, correct identification of external proficiency testing panels, pooling feasibility, reproducibility and robustness.

Discussion and conclusions

The data supports the updated KYLT® PRRSV NA/EU real-time RT-PCR assay as a reliable diagnostic option for PRRSV-1 and PRRSV-2 detection in routine veterinary diagnostics. Its strong diagnostic performance, broad sample compatibility and reliable detection of highly virulent PRRSV-1 strains support its use in surveillance, outbreak investigations and herd health monitoring.

Conflicts of interest

The study includes data generated with the KYLT® PRRSV NA/EU assay developed by SAN Group Biotech Germany GmbH. The current Spanish field material was evaluated by Eurofins Convet as part of the comparative evaluation.

Ethics statements

Field samples were obtained from routine diagnostic material; no experimental animal infection was performed for this abstract.

Financial support and Acknowledgements

This work was supported by SAN Group Biotech Germany GmbH. The authors thank Johannes Kramer from R&D for his scientific and technical contribution, Sonia Camon and her team from Eurofins Convet for providing and testing current Spanish PRRSV field material, and Jordi Serra as SAN Group representative in Spain for his support in the collaborative evaluation.

Keywords

PRRSV • Diagnostics • Update • qPCR • PRRSV-1 • PRRSV-2 • real-time RT-PCR • Molecular Diagnostics • Swine Diagnostics • Field strains • Highly virulent • european PRRSV strains • Matrix compatibility • Surveillance • outbreak investigation • Herd health monitoring • KYLT • SAN Group

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P.78 • ID12

A GP5 PEPTIDE-BASED ELECTROCHEMICAL IMMUNOSENSOR FOR SENSITIVE AND RAPID SEROLOGICAL DETECTION OF PRRSV

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Introduction and objectives

Porcine reproductive and respiratory syndrome (PRRS) remains one of the most economically devastating viral diseases affecting the global swine industry. In this scenario, timely serological surveillance is essential for effective health management and epidemiological control. The GP5 structural protein of the PRRS virus (PRRSV) contains major immunodominant epitopes that induce neutralizing antibody responses, representing an important and antigen-specific target for serological diagnostics. While conventional enzyme-linked immunosorbent assays (ELISAs) serve as the reference diagnostic method, their reliance on centralized laboratory infrastructure, specialized personnel, sample transportation, lengthy processing times, and limited connectivity limits their utility for real-time, on-site decision-making in commercial pig production. To address this gap, this study developed a peptide-based electrochemical immunosensor as a rapid, field-deployable platform for the quantitative detection of PRRSV-specific antibodies to support herd-level surveillance.

Methods

A synthetic GP5-derived peptide (GP5-B) was utilized as the specific biorecognition element and immobilized onto gold screen-printed electrodes (AuSPE). Stepwise surface modification and functionalization were systematically characterized using electrochemical impedance spectroscopy (EIS). The analytical and clinical performance of the immunosensor was evaluated using serum samples from Balb/c mice immunized with the GP5-B peptide, alongside field porcine sera from three distinct cohorts: PRRS-free non-vaccinated pigs, commercially vaccinated pigs, and naturally infected animals. The diagnostic efficacy was directly compared against conventional commercial ELISA assays.

Results

The electrochemical immunosensor achieved a limit of detection (LOD) of 6 ng mL⁻¹. It demonstrated high analytical precision and reproducibility, yielding intra-assay and inter-assay coefficients of variation of 5.05% and 4.08%, respectively. Crucially, validation with clinical porcine samples demonstrated a diagnostic sensitivity of 100% and a specificity of at least 93%.

Discussion and conclusions

The developed GP5-B peptide-based electrochemical immunosensor delivers diagnostic performance comparable to gold-standard laboratory ELISAs while offering the benefits of portability and rapid testing. By bypassing the need for complex laboratory logistics, this platform represents a robust tool for decentralized point-of-care testing (POCT), offering strong potential to enhance real-time veterinary diagnostics and swine health management.

Conflicts of interest

The authors declare that they have no conflict of interest regarding this study.

Ethics statements

All animal procedures were conducted in accordance with the Norma Oficial Mexicana NOM-062-ZOO 1999 (Technical Specifications for the Production, Care, and Use of Laboratory Animals, including mice and pigs). The study protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine and Zootechnics at the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH)

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(approval number: CICUMSNH-A103-FMVZ). Pig sera used in this study were obtained from previously collected and cryopreserved samples under approved experimental protocols, as reported in prior studies (Pérez-Durán *et al.*, 2024; Núñez-Anita *et al.*, 2023).

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Keywords

Electrochemical immunosensor • PRRSV • Rapid diagnostics • Serological surveillance • GP5 peptide antigen

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P.79 • ID44

PCR DIFFERENTIATION OF PRRSV-1 BETWEEN VACCINATED AND WILD TYPE INFECTED PIGS

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Introduction and objectives

Diagnosis of PRRSV-1 presents challenges due to the use of modified live vaccines (MLVs), with difficulty distinguishing between vaccinated animals or those carrying wild-type field strains. Presently, differentiation of field and vaccine strains requires genotyping, often based on the ORF5 sequence, which is costly and time consuming. Novel approaches to differentiate between field and vaccine strains by PCR include use of locked nucleic acids (LNAs) or high-resolution melt (HRM) curve analysis. Both techniques have the capacity to discriminate between as little as 1 or 2 base pair (bp) differences in a sequence. LNAs are modified nucleotides which enhance primer binding during PCR, thus increasing affinity for the intended target (1,2). Single nucleotide differences, when analysed with HRM generate different T_m and melt curve analyses. This study investigated a combined approach of LNA and HRM curve differences to differentiate between the four licensed PRRSV-1 MLVs in the UK and field strains

Methods

PRRSV-1 MLV ORF5 sequences were aligned in DNASTAR MegAlign Pro. The alignments were screened for differences with and between four MLV sequences: Porcilis® PRRS, PRRSFLEX® EU, Suvaxyn PRRS and Unistrain® PRRS. Several primers pairs were designed against sections complementary to each vaccine, with an amplicon length of 150-200bp and demonstrating at least two bp differences per primer when aligned with the other vaccines. Nucleotide differences that were unique for each vaccine sequence were selected as potential targets for LNAs. Selected primers pairs were further screened against sequence data from 1800 field samples to evaluate mismatches in sequence alignment. Primers without LNAs were initially evaluated using the iTaq Universal SYBR Green One-Step PCR kit. Primers pairs were assessed with their specific RNA template, against other vaccines, and field samples. Primers were subsequently tested using LNAs and two separate commercial HRM PCR kits (Agilent Loci and Microzone) to determine if this enhanced PCR sensitivity and specificity of SNP detection.

Results

SYBR Green data showed a consistent $\geq 0.5^\circ\text{C}$ difference when vaccine samples were evaluated against other templates. Discrimination between vaccines was observed between PRRSFLEX ($82.5^\circ\text{C } T_m$) and Unistrain ($85.5^\circ\text{C } T_m$) but less between Porcilis ($84.6^\circ\text{C } T_m$) and Suvaxyn ($84.8^\circ\text{C } T_m$). Results from the Microzone HRM assay showed greater differences ($\geq 0.4^\circ\text{C}$) between T_m values for vaccines and field samples. Similar testing using the Agilent Loci HRM kit showed differences in T_m values for all four vaccines, ranging from 0.2 - 0.4°C . Field strains evaluated with the Agilent Loci HRM kit returned T_m values with $\geq 0.5^\circ\text{C}$ difference, less one sample with Porcilis primers that was 0.4°C different. Use of LNA primers was partially successful, with PRRSFLEX LNA primers with Suvaxyn template varying from 81.5°C to $68.^\circ\text{C } T_m$. Specific vaccine amplification T_m consistency was calculated from three separate runs with average values for Porcilis, PRRSFLEX, Suvaxyn and Unistrain of 84.6°C (± 0.2), 82.5°C (± 0.14), 84.8°C (± 0.2) and 85.5°C (± 0) respectively.

Discussion and conclusions

The preliminary results indicate HRM curve analysis has potential for discrimination between PRRSV-1 vaccine and field strains. Further testing is required to determine sensitivity and specificity of the assay.

Conflicts of interest

No conflict of interest.

Ethics statements

None required.

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P.80 • ID53

DEVELOPMENT OF A COLORIMETRIC LAMP FOR PRRSV-1 AS A DIAGNOSTIC ASSAY

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Introduction and objectives

Time is a critical factor in diagnostics, as rapid delivery of results can determine the success or failure of control measures. One way to shorten turnaround time is to perform testing directly in the field, commonly referred to as point-of-care (POC) diagnostics. Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification method that uses specific primers and a DNA polymerase to amplify DNA at a constant temperature, without the need for a thermocycler. In recent years, colorimetric LAMP kits have become available, allowing results to be read visually. These characteristics make LAMP particularly suitable for adaptation into POC assays. The objective of this work was to establish a LAMP protocol for the detection and characterization of PRRSV-1 that could be adapted for in situ use with portable tools and devices.

Methods

Consensus sequences representing highly pathogenic Spanish “Rosalia” isolates and “classical” PRRSV-1 strains were generated from representative genomes retrieved from GenBank. These consensus sequences were used to design several LAMP primer sets throughout the whole genome, each consisting of eight primers, using the NEB LAMP Primer Design Tool. Subsequently, the number of mismatches between each primer and each sequence in the two datasets was calculated. The five primer sets, one located in nsp2, two located in nsp9 and two located in ORF6, showing the highest overall identity across both datasets were selected for further evaluation. A panel of seven PRRSV-1 isolates, including one commercial vaccine strain, the original highly pathogenic Rosalia R1 strain and five field isolates, was produced and titrated. Each isolate was subjected to serial ten-fold dilutions and quantified using a commercial RT-qPCR assay and was then examined in LAMP.

Results

After assaying the 7 strains in all the ten-fold dilutions available, the primer set that consistently amplified them all to a lower ten-fold dilution was P1-36, located in the ORF6 protein. The sample with the lowest positive ten-fold dilution had a RT-qPCR Ct value of 33, and a TCID50 concentration of 10 viral particles/ml. The worst ten-fold dilution among the 7 strains had a RT-qPCR value of 29.2, corresponding to a TCID50 concentration of 100 viral particles/ml The positive and negative samples were clearly distinguishable at naked eye.

Discussion and conclusions

The main objective of the project – to develop a LAMP protocol for PRRSV-1 – was achieved; however, the results, albeit positive, were not as good as expected. Several published LAMP assays are able to show sensitivity and specificity values as good as those of RT-qPCR assays, sometimes even better. In our case, we were not able to consistently detect for all the strains assayed positive results in the range of Cts values located between 30 and 36, thus lowering the sensitivity of the assay developed and limiting the applicability of the technique in field conditions. The application of LAMP in situ can shorten diagnostic and characterization times that will rely in greater chances to detect and control PRRS outbreaks, but also to bring the procedures closer to field veterinarians and farm staff at a competitive cost.

Conflicts of interest

None

Ethics statements

N/A

Financial support and Acknowledgements

This project was awarded a European PRRS Research Award and funded by Boehringer Ingelheim.

P.81 • ID95

RAPID PEN-SIDE MOLECULAR TESTING FOR SWINE RESPIRATORY DISEASES USING A POINT-OF-CARE LAMP PLATFORM

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Introduction and objectives

The control and regional eradication of devastating swine diseases including Influenza A virus (IAV) and Porcine Reproductive and Respiratory Syndrome virus (PRRSV) infections, relies heavily on rapid diagnosis and strict biosecurity protocols. Traditional laboratory diagnostics can offer excellent sensitivity but introduce significant turnaround delays and potential sample degradation during transport to centralised laboratories. In intensive production systems, delays can allow undetected lateral transmission before control measures can be implemented. This study evaluates the performance of ProtonDx's point-of-care, pen-side diagnostic platform, Dragonfly™, for swine viral disease surveillance. We present longitudinal swine influenza A virus data as a controlled demonstration of the value of rapid molecular testing during an active respiratory viral infection time course in pigs.

Methods

Dragonfly combines two ProtonDx proprietary components: SmartLid™ sample preparation and LyoLAMP™ molecular detection, delivering nucleic acid extraction and isothermal amplification without laboratory infrastructure, cold chain, or specialist training. We conducted a longitudinal swine influenza (H1N1) study in experimentally infected pigs over 7 days of infection. Serial samples were collected across early, peak and resolving infection and tested using Dragonfly LAMP alongside laboratory RT-qPCR comparator. In parallel, we are conducting preliminary field studies using real-world porcine samples collected during farm visits. These studies are designed to collect field performance data, assess usability in veterinary hands, and augment development of the IAV and PRRSV assays aiming to deploy future multiplexed testing for PRRSV and other major swine pathogens.

Results

Across the longitudinal swine influenza study, Dragonfly showed good agreement with PCR across the infection time course, detecting infection during the clinically relevant positive window and tracking the expected rise and fall in viral signal over the 7-day study. The system provided rapid sample-to-result testing in approximately 45 minutes, compared with the typical 48-hour delay associated with sample shipment and centralised PCR testing. The influenza data demonstrate that LAMP-based point-of-care testing can generate actionable results during active infections, rather than retrospectively after laboratory processing. Preliminary field studies have also demonstrated strong usability of the Dragonfly workflow in veterinary hands. Following 2-hour training sessions, field vets were able to run tests autonomously without ProtonDx supervision, supporting the suitability of the platform for real-world farm and veterinary use.

Discussion and conclusions

The swine influenza data provide a relevant demonstration of Dragonfly's value in porcine respiratory viral disease surveillance. A rapid, pen-side molecular test that performs well against the gold-standard PCR can reduce the diagnostic time gap between infection, sampling and intervention. This technology can serve as an actionable first-line defence, bridging the critical time-gap in outbreak scenarios, directly empowering field veterinarians to make immediate, data-driven treatment and containment decisions that safeguard herd health and regional biosecurity for IAV and multiplexed swine pathogen panels.

Conflicts of interest

Jesus Rodriguez-Manzano and several authors are employees of ProtonDx Ltd, developer of the Dragonfly™, SmartLid™ and LyoLAMP™ technologies.

Ethics statements

The experimental infection study was conducted under appropriate institutional and regulatory ethical approvals at The Pirbright Institute, UK.

Keywords

Point-of-care • Diagnostics • Respiratory Diseases

P.82 • ID85

ESTABLISHING A MONOCLONAL ANTIBODY CORE FACILITY FOR SWINE VIRAL PATHOGENS

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Introduction and objectives

An important aspect for the swine production system is animal health and biosecurity sustainability. Among the etiological agents causing swine diseases, viral pathogens are most devastating and difficult to control as they usually spread fast and rarely have cost-effective or safe antiviral drugs for the treatment. Vaccines are rarely available for emerging viral pathogens. Early effective disease control and prevention require the coordinated use of surveillance and biosecurity measures, including rapid diagnostics to identify and eliminate the infected animals and stop the spread of pathogens. A key reagent for viral detection is monoclonal antibody (mAb). In our work, we generated and characterized a panel of mAbs to detect major swine viral pathogens. These mAbs are categorized and made available to swine disease community.

Methods

For each targeting swine virus, we selected a conserved antigenic protein for mAb production. The genomic region encoding each (partial) of the selected viral protein was codon-optimized and synthesized commercially. The synthetic gene was cloned into a recombinant protein expression vector for antigen production. The purified viral antigen was used for mice immunization. BALB/c mice were immunized three times with 50-100 ug purified viral antigen mixed with Freund's incomplete adjuvant at 2-3 weeks intervals. Mouse splenocytes were fused with NS-1 myeloma cells. Specific mAbs were initially screened by immunofluorescent assay and hybridomas producing specific mAbs were subcloned and further characterized by various assays, including ELISA, Western blot, immunoprecipitation and immunohistochemistry. The isotype of each mAb were determined, and specific epitopes recognized by each of the mAbs were further identified. The characterized hybridoma cell lines and mAbs are maintained in a controlled, rapid- access storage facility. Information (database) of each material is recorded and maintained by using a custom-designed information management system. A website is being created to disseminate this panel of reagents through the Veterinary Diagnostic Laboratory of University of Illinois Urbana-Champaign.

Results

A panel of specific monoclonal antibodies has been developed and made available for major swine viral pathogens, including porcine reproductive and respiratory disease virus (PRRSV endemic and emerging variants), African swine fever Virus, classical swine fever virus, swine influenza virus, porcine epidemic diarrhea virus, porcine teschovirus, porcine sapelovirus, porcine circovirus (PCV type 2 and 3), porcine deltacoronavirus, porcine parainfluenza virus-1, atypical porcine pestivirus, and Seneca Valley virus.

Discussion and conclusions

This panel of mAbs will be valuable diagnostic tools in swine viral pathogen surveillance in aid of disease control and prevention. On the other hand, they will serve as important research tools to study the viral pathogenesis/ host immunity for development of vaccines and other antiviral measures.

Conflicts of interest

The authors declare no conflict of interest

Ethics statements

All experimental procedures were conducted according to the protocol approved by the Institutional Biosafety Committee (IBC) and Institutional Animal Care and Use Committee (IACUC).

P.83 • ID119

NEUROLOGICAL DISEASE IN GROWING PIGS ASSOCIATED WITH PRRSV AND PCV2 CO-INFECTION

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) are two of the most significant viral pathogens affecting modern swine production. Co-infections involving PRRSV and PCV2 are frequently reported in field outbreaks and are well known to exacerbate clinical severity, particularly within the respiratory tract of post-weaning and growing pigs. Reports describing the involvement of these two viruses in neurological disease, however, remain rare and poorly characterized. The objective of this case report is to describe a field outbreak of neurological disease in growing pigs from a commercial fattening herd in Italy, in which PRRSV and PCV2 co-infection was identified and associated with viral involvement of the central nervous system (CNS).

Methods

Neurological episodes occurred in a commercial fattening herd located in Northern Italy between February and March 2023. The farm introduced approximately 600 pigs per batch on a monthly basis, with animals weighing around 35 kg at arrival. During two consecutive batches, numerous pigs developed acute neurological signs starting about two weeks after placement. Affected animals exhibited sudden onset of ataxia, followed by paresis of either the hind or fore limbs, progressing to permanent recumbency and death. No significant respiratory signs were observed in these cases. A total of 11 clinically affected pigs were submitted to our laboratory for diagnostic investigation. Samples from the brain and spinal cord, were collected and tested using real-time PCR assays targeting several viruses (ADV, PRRSV, PCV2, PoAstV-3, PTV, PSV and APPV). Histopathological examination of CNS tissues was performed on all animals. In selected cases, in situ hybridization for PRRSV was carried out to assess viral localization within the CNS. Additionally, shotgun metagenomic sequencing was performed on CNS samples using the MinION Nanopore platform.

Results

Real-time PCR testing of CNS samples from all examined pigs revealed positivity for both PRRSV and PCV2. Quantitative PCR performed on brain tissue showed high PCV2 viral loads, exceeding 1×10⁸ genome copies/g. Histopathological examination of CNS tissues demonstrated lesions consistent with viral infection, characterized by non-suppurative meningoencephalitis with marked lymphohistiocytic perivascular cuffing. In situ hybridization for PRRSV, performed on brain and spinal cord sections from two pigs, confirmed the presence of viral nucleic acid within cells located in the observed microscopic lesions. Shotgun metagenomic sequencing performed on brain tissue from one pig detected PRRSV and PCV2 within the CNS and did not reveal additional viral pathogens, supporting the targeted diagnostic findings.

Discussion and conclusions

Systemic PCV2 infection is known to induce immunosuppression, increasing susceptibility to secondary viral pathogens such as PRRSV. This interaction can exacerbate clinical severity and contribute not only to respiratory disease, but also to neurological syndromes in growing and fattening pigs, as observed in the present outbreak. Effective control of PCV2 infection in breeding herds remains essential to reduce the downstream impact of co-infections in fattening units.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics statements

This study did not involve experimental procedures on live animals. All samples were collected from pigs submitted for routine diagnostic investigation, in accordance with national and EU animal welfare regulations.

Financial support and Acknowledgements

This study was funded by Italian Ministry of Health project. Grant number IZSLER 2022/012 RC.



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P.84 • ID51

ESTABLISHMENT OF A CIRCULAR POLYMERASE EXTENSION REACTION-BASED REVERSE GENETICS SYSTEM FOR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRS) is one of the most economically important pathogens affecting the global swine industry. Reverse genetics systems are powerful tools for studying PRRSV replication, pathogenicity, and genetic determinants of viral phenotypes. However, conventional infectious clone-based systems can be labor-intensive and technically demanding. Circular polymerase extension reaction (CPER) is a rapid and straightforward method for generating infectious viruses from overlapping DNA fragments and has been applied to various RNA viruses with long genomes, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In this study, we aimed to establish a CPER-based artificial generation system for PRRSV.

Methods

Eight cDNA fragments covering the full-length genome of a PRRS vaccine strain, together with a linker DNA fragment encoding essential transcriptional elements, including a promoter, poly(A) signal, and ribozyme sequence, were amplified by PCR. Each DNA fragment was designed to contain overlapping regions with adjacent fragments. The nine DNA fragments were assembled into a circular DNA molecule by CPER. The resulting circular DNA was transfected into HEK293T cells, which are suitable for efficient DNA transfection. Culture supernatants from the transfected cells were subsequently inoculated onto PAM-T43 cells, a porcine alveolar macrophage-derived cell line highly susceptible to PRRSV, to assess recovery of infectious virus. Viral protein expression was examined by indirect immunofluorescence assay. The growth kinetics of the CPER-derived PRRSV were compared with those of the parental vaccine strain in PAM-T43 cells. In addition, a recombinant virus carrying a site-directed mutation in the M1u1 recognition site within the ORF5 gene was generated, and the introduced mutation was confirmed by restriction enzyme digestion followed by agarose gel electrophoresis.

Results

Cytopathic effects were observed in PAM-T43 cells inoculated with culture supernatants from transfected HEK293T cells. PRRSV N protein expression was also detected in the inoculated PAM-T43 cells by indirect immunofluorescence assay. The growth kinetics of the CPER-derived PRRSV were comparable to those of the parental PRRS vaccine strain. Furthermore, the ORF5 amplicon derived from the virus carrying the mutation in the M1u1 recognition site was resistant to M1u1 digestion, resulting in an altered band pattern on agarose gel electrophoresis.

Discussion and conclusions

We successfully established a CPER-based reverse genetics system for PRRSV. This system enables the rapid generation and genetic modification of PRRSV without relying on conventional infectious clone construction. Therefore, it provides a useful platform for investigating the molecular basis of PRRSV replication and viral phenotypes.

Conflicts of interest

The authors declare no conflicts of interest relevant to this study. Makoto Ozawa and Kosuke Okuya have financial relationships with external organizations, but these did not influence the design, conduct, or interpretation of the present work.

Ethics statements

Not applicable.

Financial support and Acknowledgements

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Keywords

Circular polymerase extension reaction • Reverse genetics

P.85 • ID92

UNEXPECTED PCV2 CIRCULATION DYNAMICS? EARLY PRRSV INFECTION MAY PROVIDE THE ANSWER

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Introduction and objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus 2 (PCV2) are considered two of the most economically important viruses in the swine industry. Both agents target the host immune cells by disrupting their function and enhancing susceptibility to other pathogens. Moreover, the co-infection between PRRSV and PCV2 play a major role in the porcine respiratory disease complex as primary and immunomodulator agents. In virtually all farms, PCV2 vaccination is implemented as a routine practice around weaning regardless the continuously evolving PRRSV farm status. PRRSV infection at early ages can compromise the development of the cell-mediated immune response upon PCV2 vaccination, which is critical for long-term clinical protection. To assess the impact of early-life PRRSV infection on PCV2 humoral immune response and infection dynamics throughout the production cycle.

Methods

Eight Spanish farms were evaluated. Fifty piglets/farm were longitudinally bled at 2, 6, 12, 18 and 24 weeks of age [woa]. Sera were tested by ELISA and qPCR to assess antibodies against PCV2 and its load, respectively. PCV2 ELISA S/P values are presented as median (IQR). Additionally, PRRSV infection was determined at 2 and 6 woa by RT-qPCR. Differences between groups were assessed using Kruskal-Wallis test, whereas changes over time intra-group were evaluated using repeated-measures ANOVA.

Results

Four farms tested PRRSV-negative at early ages (2 and/or 6 woa), and no PCV2-infection was detected at these time points. During the growing period, PCV2 infection was absent or negligible (1 positive/50 animals) in three out of these four farms. In the remaining one, PCV2 qPCR positivity rates reached 44%, 26% and 8% at 12, 18 and 24 woa, respectively, with viral loads ranging from 102-109 PCV2 genome copies/mL. Interestingly, this farm registered low PCV2 ELISA S/P values at 2 woa (1.74; 0.75-2.32) ($p>0.05$). In contrast, four farms tested PRRSV-positive at early ages with percentages of viraemic pigs between 20%-100%. Only one of these farms also showed PCV2 infection at 6 woa. During the growing period, three of these farms experienced substantial PCV2 circulation, with the highest percentage of qPCR positive pigs peaking between 52%-92% and viral loads ranging 102-109 PCV2 genome copies/mL. Notably, the only farm that remained free of detectable PCV2 infection throughout the study period registered the highest PCV2 S/P value at 2 woa (3.26; 2.85-3.53) ($p<0.05$).

Discussion and conclusions

Early-life PRRSV circulation seems to jeopardize the effectiveness of PCV2 vaccination generally implement at weaning (3-4 woa) by interfering with the development of protective immune responses. Farms with PRRSV circulation during the first weeks of life showed a higher likelihood of subsequent PCV2 circulation during the growing period. In contrast, farms free from early PRRSV infection generally exhibited limited or absent

[CONTINUE >](#)

PCV2 replication after vaccination. Our results suggest that early PRRSV infection can compromise this long-term protection and promoting subsequent PCV2 replication. Early-life PRRSV infection was associated with increased PCV2 replication during the growing period. These findings highlight the importance of controlling PRRSV circulation to optimize PCV2 vaccination strategies.

Conflicts of interest

The authors declare no conflict of interest. B.Aznar and M.L.D.Arriba are employees of Laboratorios Syva, S.A.

Ethics statements

Not applicable

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Keywords

PRRSV • PCV2 • coinfection



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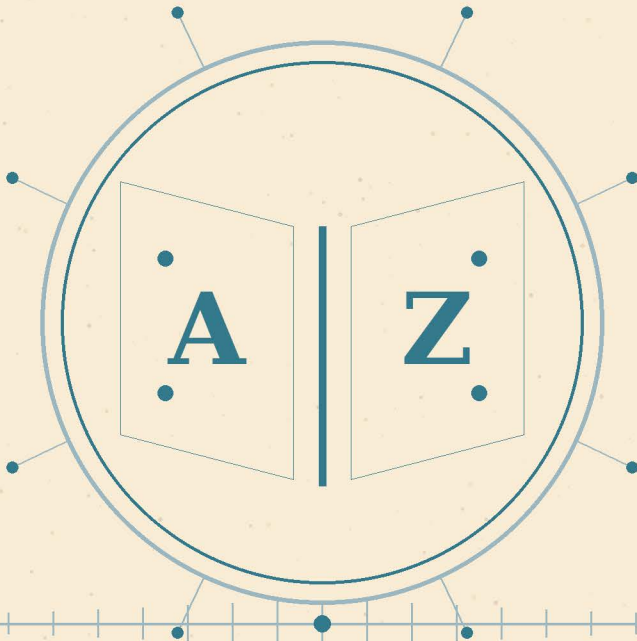
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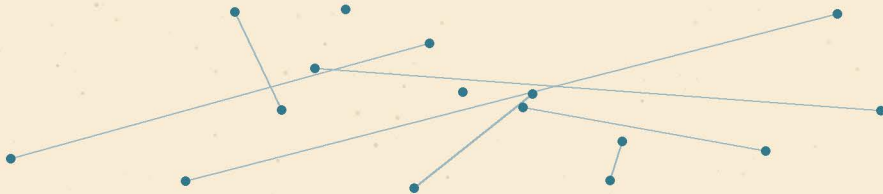
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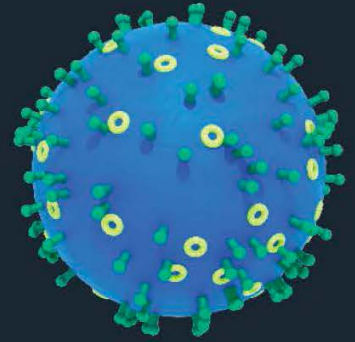
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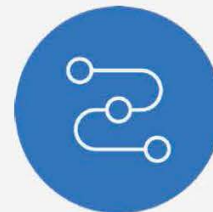
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