

Development of a Robust PRRSv Challenge Model in Pigs: *Paving the Way for Effective Control Solutions*

Porcine Reproductive and Respiratory Syndrome (PRRS) is considered one of the most relevant diseases in pig production worldwide and is caused by the PRRS virus (PRRSv). Two major PRRSv species are known, formerly defined as genotypes: PRRSv-1, defined as the European genotype and PRRSv-2, defined as North American (Wannarat *et al.*, 2025). Currently, most pig herds in major producing countries are endemically infected with PRRSv. Affected herds are impacted by reproductive failure in sows, respiratory signs in piglets and deprivation of the immune system. The disease does not only impact the health of swine herds but also leads to substantial economic losses and increased use of antibiotics due to secondary infections including those caused by influenza A virus, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* and *Streptococcus suis*.

PRRSv may spread via direct contact with infected pigs, semen, or indirectly via the air or contaminated fomites (e.g. clothes, equipment, vehicles). Control of PRRSv is often based on improvement measures at a farm site to limit the direct and indirect spread of the virus. Examples are redesign and reorganisation of the farm and farming processes, such as limiting contact between different animal age groups and implementation of adequate hygiene management measures. However, other aspects, such as proper monitoring programs, diagnostics and vaccination, are also key in the control of PRRSv. Since their introduction in the market, PRRSv vaccines are widely used to induce immunity and reduce virus circulation. Although their efficacy is supported by scientific publications and practical experience, efficacy is compromised by several factors such as flaws in farm management or biosecurity. Therefore, despite the extended use of PRRSv vaccines, there are still many outbreaks occurring in both vaccinated and unvaccinated pig farms around the world. Still, vaccines are considered essential to stabilise the situation on a farm in case of a PRRSv-outbreak and to minimise losses and as such the need for development of new vaccines is high.

Aim

The aim of the study was to set up a robust PRRSv challenge model to accurately reproduce PRRSv viraemia in weaned piglets. This model should provide a reliable method to test the efficacy of new vaccine candidates or other innovative control interventions.

Experimental Setup

- Selection and Housing of Animals:** Seven-week-old pigs (n=20) were obtained from a Dutch pig farm with high health status. The animals were free from PRRSv, as confirmed by absence of PRRSv-RNA and PRRSv antibodies by PCR and ELISA, respectively. At GD, pigs were housed in accordance with Directive 2010/63/EU. To accommodate natural behaviour, in addition to proper animal welfare, sawdust was offered daily on the solid floor and various playing materials were provided during the study.

- Selection of PRRS-virus:** A PRRSv-1 virus (GD strain 8147) was isolated from a pig diagnosed from PRRS in the Netherlands in 2021 by propagation of the virus in porcine alveolar macrophages (PAMs). Nanopore sequencing and analyses of open reading frames (ORFs) 2 to 7 identified this virus to be of the PRRSv lineage L1.8 (Wannarat *et al.*, 2025). Subsequent passage (n=5) and virus titration was performed on MARC-145 cells before challenge. A PRRSV-8147-specific in-house qPCR was developed for the detection of this strain during the study.
- Experimental Infection:** After an acclimatisation period of 7 days the animals were inoculated via the intra-nasal route (Figure 1). Subsequently, pigs were observed daily and rectal temperatures were measured prior and up to 15 days post inoculation (dpi). Blood and nasal swabs were collected for qPCR analyses at six time points after inoculation. Animals were euthanised at 10 (n=10) and 28 (n=10) dpi. Refinement opportunities for animal procedures were implemented. Post-mortem macroscopy and microscopy investigations of the lungs were performed for all animals. In addition, a representative sample of the lungs of every pig was tested by a PRRSV-8147-specific in-house qPCR. This study was approved by the Royal GD Animal Welfare Body and performed under license GDDAVD42600202317345.

Results

Inoculation of pigs with PRRSV-8147 led to an increase in rectal temperature in the challenged pigs after inoculation (average rectal temperature at the peak (2 dpi) was 40.3 °C). All challenged pigs became viremic (20/20) with the challenge strain 4 dpi and RNA of the challenge strain was also detected in the lungs of all pigs euthanised 10 dpi (10/10) by RT-qPCR. At 28 dpi, 7 of 10 remaining pigs had a RT-qPCR positive lung, with a lower average viral load. Nasal excretion of PRRSv after inoculation was lower than viremia, both in terms of proportion of positive pigs (11/20 at the peak of excretion) as well as viral load. Mild respiratory clinical signs characterised by mild dyspnoea sometimes accompanied by coughing or sneezing were observed (Table 1 and 2 and Figure 2).



Figure 1. A picture of the intra-nasal inoculation of the challenge strain using a syringe placed directly in the nostril of the nose.

Model Parameter	Model Outcome
PRRSv-1 Strain	PRRSV-8147
Age of Pigs at Inoculation	7 weeks old
Inoculation Route	Intra-nasal
Model results (post-inoculation)	Outcome
PRRSv Viremia	100% at 4 days post-inoculation
PRRSv Nasal Shedding	Peak at 7 days post-inoculation (55% of the pigs)
PRRSv qPCR Positive Lungs	100% at 10 days post-inoculation
Respiratory Clinical Signs	Mild dyspnoea sometimes accompanied by coughing or sneezing and peak of fever at 2 days post-inoculation (average 40.3°C)

Table 1. Summary of the PRRSv challenge model set up and results at Royal GD.

Dpi	Mean	sd	# positive pigs/ total
4	24.06	2.07	20/20
7	22.21	1.54	20/20
10	22.08	1.21	20/20
14	23.82	1.75	10/10*
21	27.9	2.12	10/10*
28	32.93	2.39	9/10*

*At 10 dpi half of the pigs were euthanised, and therefore the total number of pigs afterwards was ten.

Table 2. Mean and standard deviation (sd) of the Ct (Cycle threshold) values from positive serum samples and number of positive pigs (#) from the total number of tested pigs tested with PRRSv-8147-specific in-house qPCR at different days post inoculation (dpi).

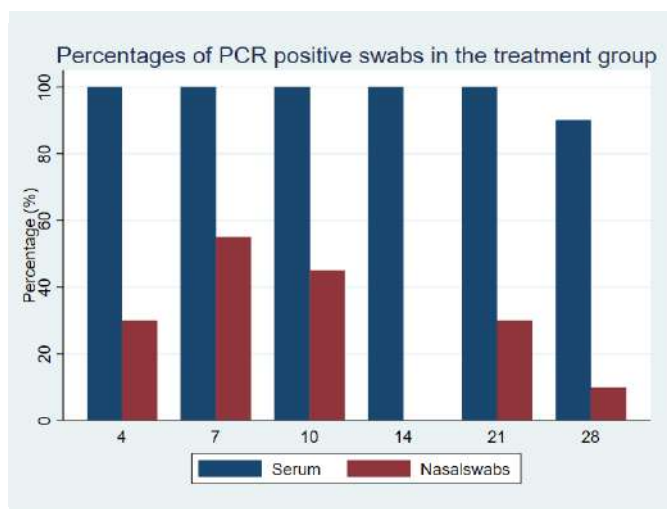


Figure 2. Percentage of positive serum and nasal swab samples tested with PRRSv-8147-specific in-house qPCR at 4, 7, 10, 14, 21 and 28 dpi. From 14 to 28 dpi the total number of pigs tested was 10 since at 10 dpi half of the pigs were euthanised

Conclusion

This PRRSv-1 challenge study shows to be a suitable model to test new vaccine candidates or alternative interventions

based on prevention or reduction of PRRSv viremia in infected pigs. By mimicking the infection process, researchers can assess the effectiveness of interventions under controlled conditions.

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