

# Mapping the Geographical Spread of PRRSv in the Netherlands

The Porcine Reproductive and Respiratory Syndrome virus (PRRSv) presents a major economic and welfare challenge to the global swine industry. In one retrospective study from the Netherlands, the economic losses during an outbreak varied between €59 and €379 per sow for an 18-week period, depending on the type of farm.<sup>1</sup> Due to the prolonged nature and high prevalence within a farm, PRRSv has a significant impact on animal welfare.<sup>2</sup> The risk of introducing a new PRRSv strain into a farm is not limited to PRRSv-negative farms; the impact of PRRSv introductions on both PRRSv-negative and PRRSv-positive farms can vary significantly, ranging from mild, asymptomatic cases to severe disease outbreaks, depending on the virulence of the strain and the cross-reactivity of the existing immunity.<sup>3</sup>

Currently, there is a lack of data on the relative contribution of direct and indirect routes to the transmission of PRRSv onto farms. Whereas direct transmission is mostly attributed to trade and transport of infected pigs, indirect routes include people, semen, manure, domestic/feral animals, rodents, insects, aerosol, animal feed, water and fomites.<sup>4</sup> In our analysis, it was aimed to compare the prevalence of Dutch PRRSv-positive farms in areas with high and low pig densities, respectively. Furthermore, the relative contribution of trade contacts to PRRSv introduction on farms was investigated, contrasting with other possible other risk factors for PRRSv introduction such as proximity between farms and airborne transmission of the virus.

## Materials and Methods

Between 2022 and 2023, a total of 49 farms were included: 38 farms, evenly distributed across four areas (two with high pig density and two with low pig density, as shown in Figure 1), along with 11 gilt- and piglet-producing farms that supplied animals to at least one of the participating farms. A biosecurity questionnaire was conducted on each of the farms. The collected data encompassed, for example, location, management of animal flow, and animal contacts between herds.

Blood samples from 30 piglets at the end of the nursery phase in pools of five, or, in case only fatteners were present, six oral fluid samples were analysed via PRRSv RT-qPCR and ORF5 sequencing from one sample with the highest viral load. Phylogenetic analysis of ORF5 aimed to identify close matches ( $\geq 98\%$  nucleotide identity) and trace them back to potential direct or indirect contacts between farms. Chi-squared tests were applied to detect differences between areas using contingency tables.

## Results

The farm-level prevalence of PRRS virus detection (RT-qPCR positive) in the two pig-sparse areas was 44% and 75%, while in pig-dense areas, it was 80% and 91% (Table. 1). The prevalence of field virus detection ( $< 98\%$  nucleotide identity in ORF5 with a licenced PRRSv MLV strain) ranged from 30% to 38% across all four areas, with no significant differences in the detection rates of field strains between the regions. 55% of the supplying farms were RT-qPCR-positive, and in 5/6 cases field virus could be detected (Table. 1). Farms with external

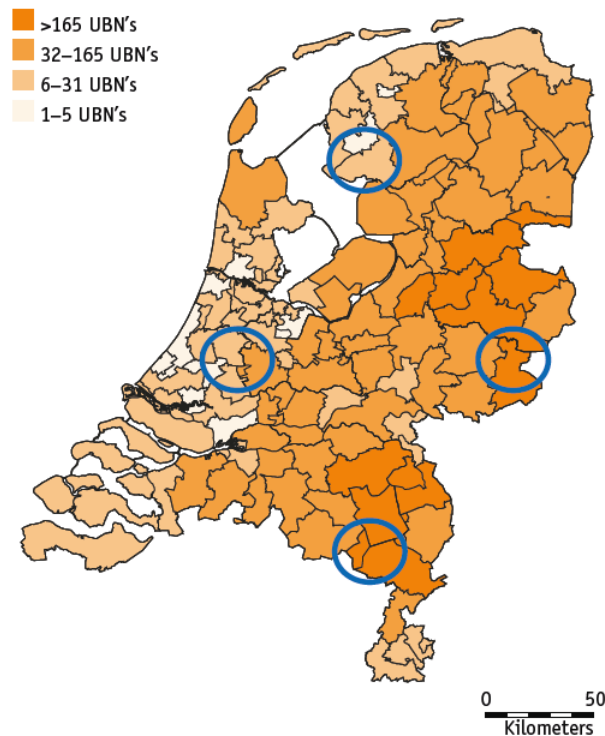


Figure 1. Illustration showing the density of pig farms across 2-digit postal code regions in the Netherlands in 2023 and the localisation of the four areas (blue circles; two pig-dense and two pig-sparse areas). Each UBN (=unique business/farm number) is specific to a particular farm or business location.

animal supply showed higher PRRSv farm-level prevalence (84%) compared to those without (63%). Field virus was less frequently detected on farms without animal supply (5%) in contrast to those with animal supply (63%;  $\chi^2=9.2$ ,  $df=1$ ,  $p=0.002$ , Table. 2). Analysis of ORF5 sequences identified four close matches, three linked to direct contact between suppliers and supplying farms (99.0 to 99.2% nucleotide identities). A match was detected in two farms located close to each other in a pig-sparse area. However, these farms also had the same supplier, who declined to participate in this project.

## Discussion and Conclusion

This pilot study revealed that farms with external animal supply exhibited a significantly higher farm-level prevalence of field virus positive samples. Whereas it is important to acknowledge the study's limitation due to the small number of suppliers included, the identification of three closely matched sequences from connected farms highlights the effectiveness of our approach. Furthermore, the findings are consistent with an U.S. study, which reported that the median number of case farms (farms with a specific PRRSv lineage) connected through animal movements was approximately 4.1 times higher than random expectations. In contrast, for farms linked by spatial proximity, this number was only 2.7 times higher than random expectations.<sup>5</sup> This knowledge should be given particular consideration in regional and national PRRSv stabilisation and eradication measures.

In the study group, no statistically significant difference in field virus prevalence at the farm level was observed between

Farm	No PCR available	Negative <sup>1</sup>	Positive, no sequence <sup>2</sup>	Positive, vaccine strain <sup>3</sup>	Positive, field strain <sup>4</sup>	All
Sparse 1	0	2	2	1	3	8
Sparse 2	0	5	1	0	3	9
Dense 1	0	2	0	5	3	10
Dense 2	0	1	3	3	4	11
Supplier herd	2	3	0	1	5	11
All	2	13	6	10	18	49

<sup>1</sup> Negative indicates that all samples were below the detection limit of the PRRSv RT-qPCR  
<sup>2</sup> PRRSv ORF5 sequencing was not possible due to a low viral load  
<sup>3</sup> Vaccine strain was defined as >98% nucleotide identity in ORF5 with a licenced PRRSv MLV strain  
<sup>4</sup> Field strain was defined as <98% nucleotide identity in ORF5 with a licenced PRRSv MLV strain

Table 1. Detection of PRRSv by RT-qPCR at participating farms and typing by ORF5 sequencing, stratified by area.

Farm type	Negative <sup>1</sup>	Positive, no sequence <sup>2</sup>	Positive, vaccine strain <sup>3</sup>	Positive, field strain <sup>4</sup>	All
Without external animal supply	7	5	6	1	19
With external animal supply	3	1	3	12	19
All	10	6	9	13	38

<sup>1</sup> Negative indicates that all samples were below the detection limit of the PRRSv RT-qPCR  
<sup>2</sup> PRRSv ORF5 sequencing was not possible due to a low viral load  
<sup>3</sup> Vaccine strain was defined as >98% nucleotide identity in ORF5 with a licenced PRRSv MLV strain  
<sup>4</sup> Field strain was defined as <98% nucleotide identity in ORF5 with a licenced PRRSv MLV strain

Table 2. Detection of PRRSv by RT-qPCR at participating farms and typing by ORF5 sequencing, stratified by farms with and without external animal supply. Supplier herds were omitted.

pig-dense and pig-sparse areas. Due to the sample size and limited sequencing results per farm, definitive conclusions cannot be drawn, and there is potential for false negatives inherent to the cross-sectional study design. Additionally, the analysis was limited to the ORF5 gene, covering only approximately 4% of the genome. It is recognised that relying solely on ORF5 analysis can lead to misclassification, such as mistaking field strains for vaccine strains.<sup>6</sup> Nevertheless, it is well documented in the literature that external biosecurity plays a crucial role, particularly in the introduction of new strains.<sup>4</sup> For instance, factors related to animal movements, specifically each additional farm contact through outward animal shipments, incur a 3.0% increased likelihood of a specific strain occurrence on the farm.<sup>7</sup> This suggests potential deficiencies in external biosecurity practices on these farms during loading and transportation of pigs.

In conclusion, this study highlights the importance of considering the PRRS status of supplying herds in operational, regional, and national PRRS stabilisation and eradication programmes, as farms with external animal supply showed a higher farm-level prevalence of field virus detection. Notably, no significantly higher prevalence of field virus was detected in pig-dense regions, emphasising the need to focus on improving external biosecurity practices, particularly during animal movements, to prevent the introduction of new strains.

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