RESEARCH REPORT
SWINE HEALTH

Title: Using genomic tools to link PRRSV quasispecies to disease severity in pigs infected with contemporary virulent isolates. **NPB #18-156**

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Industry summary

Porcine reproductive and respiratory syndrome (PRRS) continues to be the most devastating disease of swine in the USA. PRRSV is the biological viral agent responsible for this disease. Here we show that PRRSV exists as a cloud of genomic variants, especially in the infected tissue, e.g. lung, of the infected animal. A reduced cloud of PRRSV strains are released into the blood stream, highlighting that the infection found and routinely monitored from serum does not give the whole picture as to the true PRRSV infection dynamic occurring in the animal. We also show that orf5, the gene that encodes GP5, does not describe the true genetic variation and thus true origin and evolutionary potential of PRRSV. The greatest genetic variation exists in the non-structural region of the virus. A large deletion in the orf1 non-structural gene of one of our isolates had no impact on the replication of this virus. In fact, this deleted PRRSV strain had a more severe pathology that the standard wild-type PRRSV strain. In addition, even the genetic variation that exist across the rest of the genome does not translate into many changes in amino acid sequence and thus protein structure and function. The greatest changes in amino acids occurs in the PRRSV sequences found in the tissue samples. This was true for both PRRSV isolates screened, irrespective of whether they produced a chronic persistent or severe infection as indicated by the differing levels of viremia produced by the two isolates. Genetic variation within animal over a small window of time (up to 14 days post infection) was less when compared between sample type. This study once again re-emphasizes the wealth of new information that can be obtained from whole genome comparative analysis.

Scientific abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiological agent of PRRS. It is a member of the *Arteriviridae* family of positive single stranded rapidly evolving RNA viruses. Arteriviruses have the ability to establish either mild chronic or severe infections in domestic swine. Ever since the primary introduction, PRRSV continues to evolve and new genetic variants re-emerge routinely throughout the US and Europe. Here we compare two virulent PRRSV isolates, MN30100 (MN) and OK/2016 (OK), that were reported to have unique disease severity outcomes observed under field conditions. We describe the quasispecies nature of these two

infections, over time and from different cell types and sources, with both isolates showing increased heterogeneity in genomic variants in tissue over serum samples. Whole genome sequencing revealed a greater genetic variation in the 5' region of both genomes, notably in the non-structural protein coding region. For example, OK/2016 had a 1,151 bp deletion in the orf1a gene. Nonetheless, the majority of changesresulted in synonymous mutations. The genetic variation in Orf5 was atypical of the changes observed across the whole of the PRRSV genome. Due to the lack of sequence depth for MN30100, especially across the 5' non-structural protein encoded genes for all sources of material, we could not assign function to any differences that could be attributed to specific tissue type or time of sampling. This type of analysis is still feasible but it requires a larger financial and personnel investment. We nonetheless did observe genetic variation within animal over a small window of time (up to 14 days post infection), which was less when comparing sample type. This study emphasizes once again the potential whole genome comparative analysis can make towards a deeper understanding of the pathology associated with a rapidly evolving virus such as PRRSV.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) continues to be the most devastating disease of swine in the USA. The swine industry remains burdened with around $1 billion/year expenditure due to production losses and biosecurity measures associated with PRRS and other respiratory diseases of swine (https://www.pork.org/news/pork-checkoff-study-prrs-costs-industry-664-million-annually/). Although there has been significant advances in PRRS virus (PRRSV) control, PRRSV continues to be a challenge in part due to the increased disease severity and production disruption caused by certain contemporary strains and the extended duration it takes to reach herd stability[1].

PRRSV is a member of the *Arteriviridae* family and emerged almost simultaneously in North America and Europe in the late 1980s. Arteriviruses are highly species specific, but share many biological and molecular properties, including virion morphology, genome organization and replication strategy[2]. They also have the ability to establish prolonged or persistent infection in their natural host[3,4]. Ever since the primary introduction, PRRSV continues to evolve and new genetic variants re-emerge routinely throughout the US and Europe, irrespective of whether herds are vaccinated or not[5].

PRRSV shares a hallmark replication and transcription strategy with other nidoviruses. The PRRSV genome varies from 14.9 kb to 15.5 kb in length and expresses a range of nonstructural and structural proteins through two distinct transcription mechanisms[6]. Firstly, the genomic RNA is used as the template for the replication polyprotein translation. Replication of the genomic RNA leads to the creation of subgenomic, defective interfering and heteroclite RNAs[7]. The subgenomic RNAs are exclusive templates for the translation of structural proteins. Based on this fundamental and mechanistic understanding of PRRSV replication, transcription and translation, we know that PRRSV cellular RNA differs from virion encapsulated RNA. Cellular PRRSV RNA will contain multiple genomic copies of the structural region of the virus genome. Therefore, ORF5 & ORF7, genetic markers routinely used for monitoring and surveillance purposes[3,8], would provide misleading viral load values if it were to be used for monitoring cellular PRRSV. Moreover, given the underlying absence of a 3' proofreading mechanism of the PRRSV RNA replication enzyme, estimated at about $x10^3$ mutations per site per year[9], PRRSV genome evolution and selection can only be viewed at the cellular level. This is further supported by SNP variation observed across the whole genome of PRRSV over time within a breeding herd, i.e. variation is not restricted to ORF5 or ORF7[10]. Consequently, the only way to describe the re-emergence of PRRSV, and characterization of virulence, is to describe and monitor the quasispecies nature across the whole genome of PRRSV.

In the early 2000s, an isolate hereon in referred to as MN30100 or MN was recovered from a persistently infected sow on a commercial farm. It can be classified as having low pathogenicity by causing mild clinical signs[11]. It was also shown not to be readily transmitted via aerosols[12]. More recently, an isolate that originated from a farm in Oklahoma, hereon in referred to as OK/2016 or OK caused severe disease in weaned pigs. The infection was characterized by high mortality and neurologic and respiratory clinical signs (unpublished data).

In 2016, we carried out an infection trial to compare and contrast the infection dynamic of the two afore mentioned contemporary PRRSV isolates, MN30100 and OK/2016. Our experimental design was as follows:

1) Pigs were divided in three groups, each housed in a separate room with its dedicated ventilation system
2) Pigs in the negative control group (n=4) received an intramuscular injection of 1 ml of PBS at day 0 of the study.
3) Pigs in the MN group (n=8), were inoculated intramuscularly with 1 mL/5 x 10^3 tissue culture infective dose ($TCID_{50}$/ml) of the MN30100 PRRSV.
4) Pigs in group OK (n=8) were inoculated intramuscularly with 1 mL/5 x 10^3 $TCID_{50}$/ml of PRRSV OK/2016.
5) One day post-infection, four piglets were added to each PRRSV inoculated group to serve as contact sentinel pigs.
6) Body temperatures were recorded twice daily and blood samples and air samples collected on days 1, 3, 6, 9, 12, and 14 post-infection.
7) Serum samples were tested by RT-PCR (Figure 1). As expected, pigs infected with MN30100 shed less than OK/2016, however, both isolates ended up with the similar Cts at day 14DPI (Figure 1).
8) Results for the OK contact pigs can be seen in Table 1. After 6PDI all the four contact pigs were shedding viruses. A similar trend was observed for MN as well (data not shown)
9) Air samples were collected as previously described (Corzo et al., 2013) and also tested by RT-PCR. These were found to be positive for both isolates (data not shown).

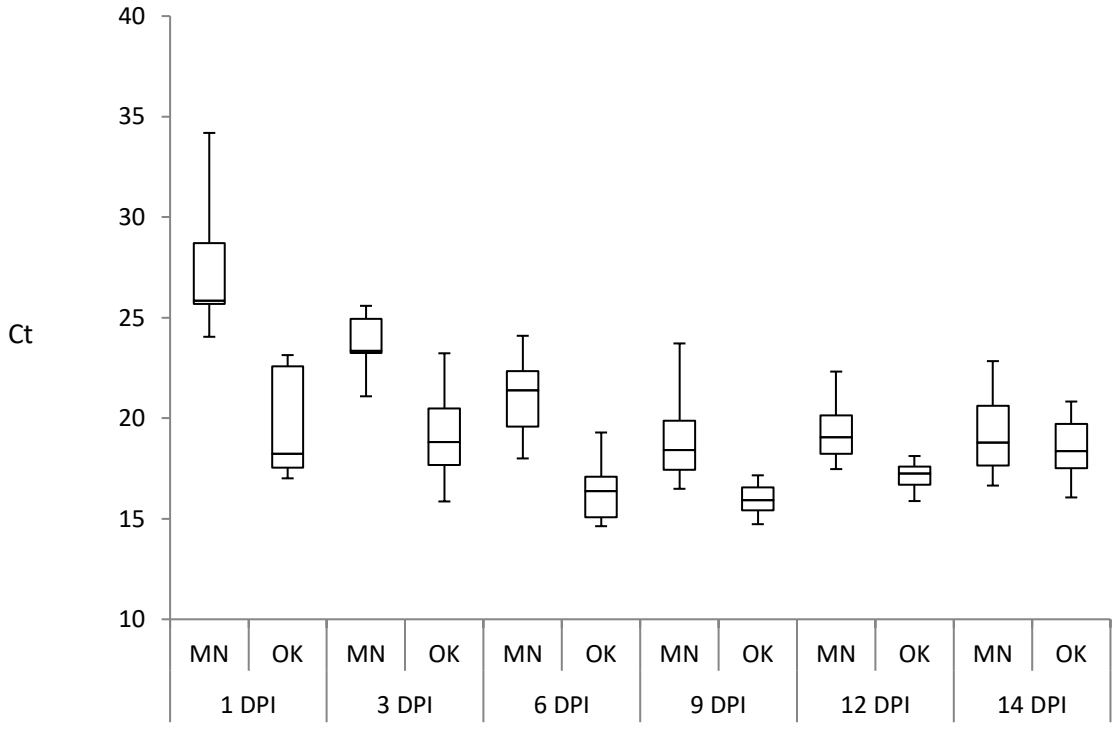

Figure 1. Box-plot of the distribution of PRRSV serum PCR results (inCt-value) in the inoculated groups depending on the strains (MN or OK) and on the day post-inoculation (DPI). Differences between MN and OK

where significant ($p<0.05$) for all timepoints except for 14DPI. The ends of the boxes represent the 25th and 75th percentiles, the ends of the whiskers represent the minimum and maximum values, and the central line represents the average.

Table 1. RT-PCR results for contact pigs introduced into the OK/2016 inoculated barn

Pig	-2DPI	1DPI	3DPI	6DPI	9DPI	12DPI	14DPI*
25	negative	negative	negative	19.57	17.39	17.93	17.65
26	negative	negative	28.52	18.09	17.12	17.26	18.27
27	negative	negative	negative	14.32	14.79	16.23	18.25
28	negative	negative	19.4	18.3	18.08	18.61	18.61

10) On day 14 post-inoculation, pigs were humanely euthanized and necropsied at the University of Minnesota, Veterinary Diagnostic Laboratory. Samples were collected from the brain and lung of each pig for histopathology investigation, virus detection and quantification. All samples collected came back PRRSV positive (data not shown).
11) We selected samples (Tables 2-4) for next generation sequencing using a bespoke PRRSV whole genome amplification protocol for Oxford Nanopore sequencing on the MinION platform (Provisional Patent filed, Schroeder Lab). Samples sequenced were the original isolates used to inoculate the pigs (positive and reference controls), the serum from a negative naive source pig (system negative control) and a technical RT-PCR negative control sample (Table 2).

Table 2: Positive (PRRSV isolates) and negative (NTC -no template RT-PCR control & negative source pigs) samples used in this study. N/A: not applicable.

Group	Room #	Sample ID	Pig ID	Exposure	Sample Type	Day in Study	PRRSV RT-PCR Results*	NGS data obtained
Negative	B1	1	1	No	Serum	-2 DPI	Negative	Negative
N/A	N/A	MN30100	N/A	N/A	N/A	N/A	9.66	Positive
N/A	N/A	OK/2016	N/A	N/A	N/A	N/A	13.66	Positive
N/A	N/A	NTC	N/A	N/A	N/A	N/A	Negative	Negative

12) Samples from the exposed barns, MN (B3, Table 3) and OK (B5, Table 4) included:
    a. serum from animals before and after intramuscular PRRSV inoculation,
    b. serum collected on different days post infection,
    c. tissue samples taken at necropsy on 14DPI from brain, lungs and a homogenate of both,
    d. serum collected from the contact pigs, and
    e. the respective barn air samples

13) We also downloaded the whole genome of MN30100 (accession number EF536000), previously sequenced using Illumina Mi-seq by the Murtaugh Lab, from the NCBI online database. This served as our reference genome in our whole genome comparative analysis. OK/2016 was not sequenced previously.
14) Offer reference genomes were the ATCC VR2332 and Ingelvac ATP vaccine stains, also available on the NCBI online database.

Table 3: Samples selected from MN30100 experiment. Tissue: Tissue homogenate; DPI: days post infection

Group	Room #	Sample ID	Pig ID	Exposure	Sample Type	Day in Study	NGS data obtained
MN	B3	5	5	No	Serum	-2 DPI	Negative
		33		Yes		1 DPI	Positive
		89				6 DPI	Positive
		173				14 DPI	Positive
		12 BRNX			Brain		Positive
		12 LUNG			Lung		Positive
		12 TSHM			Tissue		Positive
		34	6	Yes	Serum	1 DPI	Positive
		90				6 DPI	Positive
		174				14 DPI	Positive
		10 BRNX			Brain		Positive
		10 LUNG			Lung		Positive
		10 TSHM			Tissue		Positive
		35	7	Yes	Serum	1 DPI	Positive
		91				6 DPI	Positive
		175				14 DPI	Positive
		14 BRNX			Brain		Positive
		14 LUNG			Lung		Positive
		14 TSHM			Tissue		Positive
		36	8	Yes	Serum	1 DPI	Positive
		92				6 DPI	Positive
		176				14 DPI	Positive
		15 BRNX			Brain		Positive
		15 LUNG			Lung		Positive
		15 TSHM			Tissue		Positive
		37	9	Yes	Serum	1 DPI	Positive
		93				6 DPI	Positive
		177				14 DPI	Positive
		09 BRNX			Brain		Positive
		09 LUNG			Lung		Positive
		09 TSHM			Tissue		Positive
		38	10	Yes	Serum	1 DPI	Positive
		94				6 DPI	Positive
		178				14 DPI	Positive
		16 BRNX			Brain		Positive
		16 LUNG			Lung		Positive
		16 TSHM			Tissue		Positive
		39	11	Yes	Serum	1 DPI	Positive
		95				6 DPI	Positive
		179				14 DPI	Positive
		08 BRNX			Brain		Positive
		08 LUNG			Lung		Positive
		08 TSHM			Tissue		Positive
		40	12	Yes	Serum	1 DPI	Positive
		96				6 DPI	Positive
		180				14 DPI	Positive
		07 BRNX			Brain		Positive
		07 LUNG			Lung		Positive
		07 TSHM			Tissue		Positive
		97	13	Contact	Serum	6 DPI	Positive
		181				14 DPI	Positive
		11 BRNX			Brain		Positive
		11 LUNG			Lung		Positive
		11 TSHM			Tissue		Positive
		154	14	Contact	Serum	12 DPI	Positive
		182				14 DPI	Positive
		71	15	Contact	Serum	3 DPI	Positive
		99				6 DPI	Positive
		183			Serum	14 DPI	Positive
		13 BRNX			Brain		Positive
		13 LUNG			Lung		Positive
		13 TSHM			Tissue		Positive
		1021	N/A	N/A	Air	6 DPI	Positive
		1027				9 DPI	Positive
		1033				12 DPI	Positive

Table 4: Samples selected from OK/2016 experiment. Tissue: Tissue homogenate; DPI: days post infection

Group	Room #	Sample ID	Pig ID	Exposure	Sample Type	Day in Study	NGS data obtained
OK	B5	17	17	No	Serum	-2 DPI	Negative
		45		Yes		1 DPI	Positive
		101				6 DPI	Positive
		185				14 DPI	Positive
		26 BRNX			Brain		Positive
		26 LUNG			Lung		Positive
		26 TSHM			Tissue		Positive
		46	18	Yes	Serum	1 DPI	Positive
		102				6 DPI	Positive
		186				14 DPI	Positive
		17 BRNX			Brain		Positive
		17 LUNG			Lung		Positive
		17 TSHM			Tissue		Positive
		47	19	Yes	Serum	1 DPI	Positive
		103				6 DPI	Positive
		187				14 DPI	Positive
		25 BRNX			Brain		Positive
		25 LUNG			Lung		Positive
		25 TSHM			Tissue		Positive
		48	20	Yes	Serum	1 DPI	Positive
		104				6 DPI	Positive
		188				14 DPI	Positive
		19 BRNX			Brain		Positive
		19 LUNG			Lung		Positive
		19 TSHM			Tissue		Positive
		49	21	Yes	Serum	1 DPI	Positive
		105			Serum	6 DPI	Positive
		189			Serum	14 DPI	Positive
		20 BRNX			Brain		Positive
		20 LUNG			Lung		Positive
		20 TSHM			Tissue		Positive
		50	22	Yes	Serum	1 DPI	Positive
		106				6 DPI	Positive
		190				14 DPI	Positive
		22 BRNX			Brain		Positive
		22 LUNG			Lung		Positive
		22 TSHM			Tissue		Positive
		51	23	Yes	Serum	1 DPI	Positive
		107			Serum	6 DPI	Positive
		191			Serum	14 DPI	Positive
		21 BRNX			Brain		Positive
		21 LUNG			Lung		Positive
		21 TSHM			Tissue		Positive
		52	24	Yes	Serum	1 DPI	Positive
		108				6 DPI	Positive
		192				14 DPI	Positive
		27 BRNX			Brain		Positive
		27 LUNG			Lung		Positive
		27 TSHM			Tissue		Positive
		81	25	Contact	Serum	3 DPI	Positive
		109				6 DPI	N/D
		193				14 DPI	Positive
		24 BRNX			Brain		Positive
		24 LUNG			Lung		Positive
		24 TSHM			Tissue		Positive
		54	26	Contact	Serum	1 DPI	Positive
		110				6 DPI	N/D
		194				14 DPI	Positive
		18 BRNX			Brain		Positive
		18 LUNG			Lung		Positive
		18 TSHM			Tissue		Positive
		83	27	Contact	Serum	3 DPI	Positive
		111				6 DPI	Positive

		195				14 DPI	Positive
		28 BRNX			Brain		Positive
		28 LUNG			Lung		Positive
		28 TSHM			Tissue		Positive
		56	28	Contact	Serum	1 DPI	Positive
		112				6 DPI	Positive
		196				14 DPI	Positive
		23 BRNX			Brain		Positive
		23 LUNG			Lung		Positive
		23 TSHM			TissueMix		Positive
		1023	N/A	N/A	Air	6 DPI	Positive
		1029				9 DPI	Positive
		1035				12 DPI	Positive

Objectives

The overall goal of this proposal is to advance our understanding of PRRSV epidemiology by investigating PRRSV quasispecies diversity and evolution of contemporary variants that cause significant disease and production disruption in infected pigs. More specifically, our objectives are:

Objective 1: Map the genomic variation within the PRRSV quasispecies in experimentally infected pigs at the level of an individual over time during an active PRRSV infection.

Objective 2: Relate SNPs (single nucleotide polymorphisms), indels (insertions and deletions) or genomic variances within PRRSV quasispecies to disease severity outcomes.

Results & Discussion

1. We were able to generate near complete genomes for both MN & OK isolates (Tables 5-6, Figure 2). The individual reads generated were large, some covering 1/3 of the genome (Table 5), therefore making it relatively easier to assemble whole genomes. That said, we lacked sufficient sequence depth (at least 20x coverage) for us to assemble the complete 5' UTR region. We therefore focused our analysis on the coding genes for both isolates.
2. Isolate OK/2016 was the most unique of all the four PRRSV genomes compared, with between 3,259 to 3,310 nucleotide or 22.55 to 22.82% divergence from reference strains, respectively (Table 6). OK differed from MN by a similar degree of variance.
3. MN30100 published by the Murtaugh Lab differed by 26 nucleotides to our MN30100 isolate. This difference was not due to the different sequencing technology used to sequence these isolates. We were able to re-sequence VR2332 using both sequencing methods, showing 100% identity (Schroeder D, personal communication). Differences observed here was likely due to genetic drift from several *in vitro* passages through PAM cell lines. **This result highlights the importance of sequencing the PRRSV isolate prior to starting any new infection experiment.**
4. Given that MN30100 was originally recovered from a sow farm in the early 2000s (Bierck et al. 2001), it was expected that it would be genetically closer to VR2332 (90%) than OK/2016 (78%) (Table 6). VR2332 was the first North American PRRSV isolate to be described in the early 1990s (Benfield et al 1992) and fully sequenced in 2003 (Nielsen et al 2003). The vaccine (Ingelvac ATP) differed from VR2332 by 10% or 1,414 nucleotides. This is a similar distance between MN30100 and VR2332, yet

MN30100 only differed from Ingelvac ATP by 6.2% or ~930 nucleotides (Table 6). **This suggests that certain mutations are more important than others for maintaining virulence.**

5. OK/2016 was recovered as a highly virulent form of PRRSV in 2016. Nearly 1/3 of the nucleotide differences observed when compared to all isolates came from a 1,151bp deletion in the non-structural orf1a (Figure 2)

Table 5. Next generation sequence results obtained from PRRSV isolates and from source material

Sample	# reads	Max read length
MN30100_isolate	3,149	4,500
OK/2016_isolate	576	5,365
MN_serum	161,350	8,421
OK_serum	1,901,410	8,755
MN_tissue	394	2,193
OK_tissue	13,543	5,671

Table 6 Distance matrix for full genomes of PRRSV isolates used in this study compared to reference strains. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_this study	ATCC VR2332	Ingelvac ATP	MN30100-_EF536000	MN30100_this study
OK/2016_this study		3,259	3,310	3,295	3,301
ATCC VR2332	78.45%		1,414	1,493	1,512
Ingelvac ATP	78.11%	90.62%		923	941
MN30100-EF536000	78.21%	90.09%	93.88%		26
MN30100_this study	78.18%	89.98%	93.77%	99.84%	

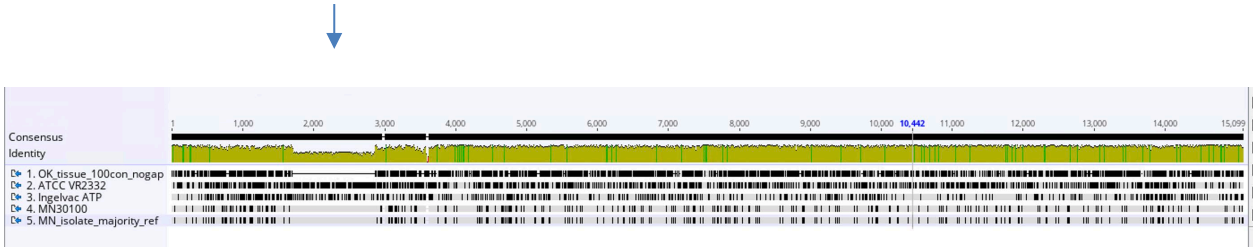

Figure 2. Sequence alignment view of full genomes of PRRSV isolates used in this study compared to reference strains. The arrow showing the location of the large 1,151bp deletion in the OK/2016 genome. Vertical black lines showing the location of the SNPs across all the genomes.

6. As expected, we were able to generate genomes from all of the samples that were found to be positive for PRRSV via RT-PCR (Tables 3-4). However, many of the higher Ct days (<6DPI, Figure 1) did not produce full length genomes (data not shown). We could more reliably recover the last 2 to 3 orfs from the 3' region of the genome from these higher Cts. This therefore made it difficult to compare the earlier PRRSV variants to those produced later on in the infection. Given the original budget requested, we could not repeat the sequencing effort. These earlier DPI samples could be sequenced in the future with a marginally larger sequencing budget.
7. Interestingly, some negative PRRSV RT-PCR samples, for example the serum collected from 1DPI from the contact pigs (Table 1), did produce partial 3' genomes (e.g. Figure 3A). The example provided in Figure 3A shows a section of genome of the OK/2016 isolate 1DPI. This matched the original OK/2016 isolate that was used to inoculate pigs. However, in this pig a mutation was observed 5 days later (6DPI) and again at 14DPI (Figure 3B-C). This clearly demonstrates how quickly mutations do occur during PRRSV infections. **We were also able to detect this mutation in the lung tissue taken at 14DPI (data not shown).**

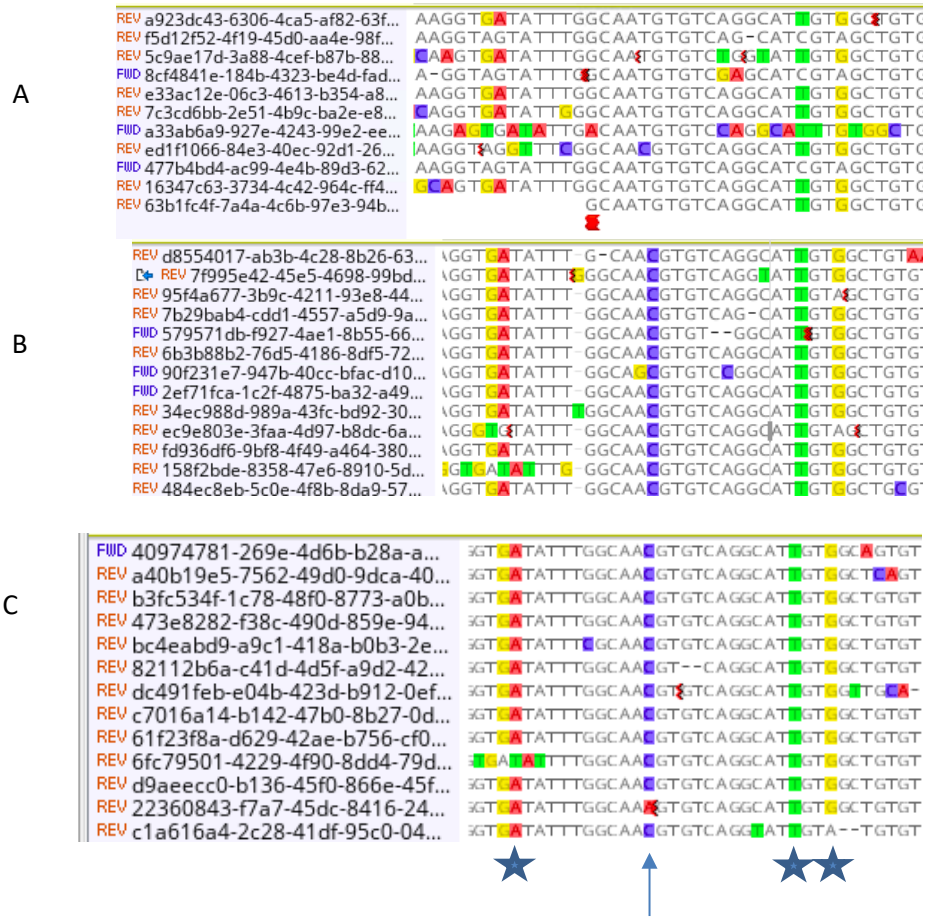

Figure 3. A random but representative subsample of Oxford Nanopore OK/2016 reads aligned to MN30100 PRRSV reference genome (EF536000) generated from serum collected after A) 1DPI, B) 6DPI and C) 14DPI from a contact pig exposed to OK/2016 intramuscular injected pigs. Arrow: SNP location showing new T to C mutation Stars: showing the mutations that separate MN30100 from OK/2016

8. We were also able to get partial PRRSV genomes for the air samples from each room. These matched the isolate used for inoculation in each room (data not shown). We could not get any sequences from the naïve pigs nor the negative RT-PCR RNA control used during the RNA extraction and sequencing protocol (Table 2).
9. Given the low sequence coverage for some of the samples, we grouped the reads into those generated from serum or tissue (Table 5). Sequence depth and total number of reads acquired correlated with the Ct values obtained. MN samples produced an order of magnitude fewer reads than OK, whether it came from serum or tissue (Table 5). In addition, our analysis hereon in focused on genes 2 to 7 for MN and OK. We could however look at comparing or1 for OK/2016 sequences generated from serum
10. When comparing the OK/2016 orf2a sequences from the isolate to those generated from the serum and tissue samples, 11 nucleotide differences were observed (Table 7). This was also observed in genes orf3 (14, Table 9), orf 5 (1 or 2, Table 11), orf 6 (11, Table 12) and orf1 (39 in serum, Table 14). No differences were observed in orf2a (Table 8), orf 4 (Table 10) and orf 7 (Table 13). These differences led to two non-synonymous mutations in the amino acid sequences in GP2a (Figure 4), and GP6 (Figure 9).
11. MN30100 isolate gene sequences also changed when recovered from serum and tissue. Differences were found in orf 2a (100, Table 7), orf 2b (11 tissue only, Table 8), orf 3 (17 tissue only, Table 9), orf 4 (47 tissue only, Table 10), orf 5 (3, Table 11) orf 6 (1 or 2, Table 12) and orf 7 (1 tissue only, Table 13). **The greatest variation occurred in the sequences generated from the tissue samples**
12. Comparing all the genes together, no one particular gene provided a good proxy for the others. Therefore, looking at orf5 or GP5 (Table 15) in isolation can provide misleading information on the rate of change occurring in the PRRSV genome. A closer look at the important structural and function amino acids in the 5' region of GP5 (Figure 11), reveals little on substantial changes occurring elsewhere in the genome.

Table 7. Distance matrix for orf2a of PRRSV isolates used in this study compared to reference strains. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_ isolate	OK/2016_ serum	OK/2016_ tissue	**MN30100_ tissue**	**MN30100_ serum**	**MN30100_ isolate**	**MN30100_ EF536000**
OK/2016_ isolate		11	11	123	95	95	96
OK/2016_ serum	98.96%		0	128	100	100	100
OK/2016_ tissue	98.96%	100%		128	100	100	100
MN30100_ tissue	85.41%	84.37%	84.37%		37	37	38
MN30100_ serum	88.07%	87.03%	87.03%	96.17%		0	1
MN30100_ isolate	88.07%	87.03%	87.03%	96.17%	100%		1
MN30100_ EF536000	87.94%	87.03%	87.03%	96.04%	99.87%	99.87%	

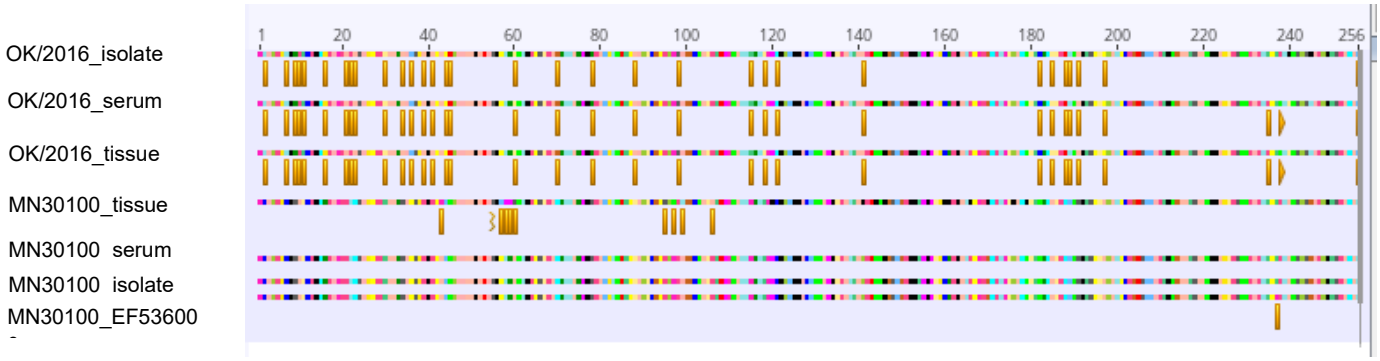

Figure 4. Sequence alignment view of GP2a of PRRSV isolates used in this study, comparing amino acid differences (orange annotations below sequence) between reference and source.

Table 8. Distance matrix for orf2b of PRRSV isolates used in this study compared to reference strains. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_ isolate	OK/2016_ serum	OK/2016_ tissue	MN30100_ tissue	MN30100_ serum	MN30100_ isolate	MN30100_ EF536000
OK/2016_ isolate		0	0	37	28	28	28
OK/2016_ serum	100%		0	37	28	28	28
OK/2016_ tissue	100%	100%		37	28	28	28
MN30100_ tissue	83.33%	83.33%	83.33%		11	11	11
MN30100_ serum	87.39%	87.39%	87.39%	95.05%		0	0
MN30100_ isolate	87.39%	87.39%	87.39%	95.05%	100		0
MN30100_ EF536000	87.39%	87.39%	87.39%	95.05%	100	100	

Figure 5. Sequence alignment view of GP2b of PRRSV isolates used in this study, comparing amino acid differences (orange annotations below sequence) between reference and source.

Table 9. Distance matrix for orf3 of PRRSV isolates used in this study compared to reference strains. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_ isolate	OK/2016_ serum	OK/2016_ tissue	MN30100_ tissue	MN30100_ serum	MN30100_ isolate	MN30100_ EF536000
OK/2016_ isolate		14	14	119	106	106	108
OK/2016_ serum	98.76%		0	122	109	109	110
OK/2016_ tissue	98.76%	100%		122	109	109	110
MN30100_ tissue	85.65%	84.67%	84.67%		17	17	19
MN30100_ serum	86.73%	85.75%	85.75%	98.46%		0	2
MN30100_ isolate	86.73%	85.75%	85.75%	98.46%	100%		2
MN30100_ EF536000	86.47%	85.62%	85.62%	98.20%	99.74%	99.74%	

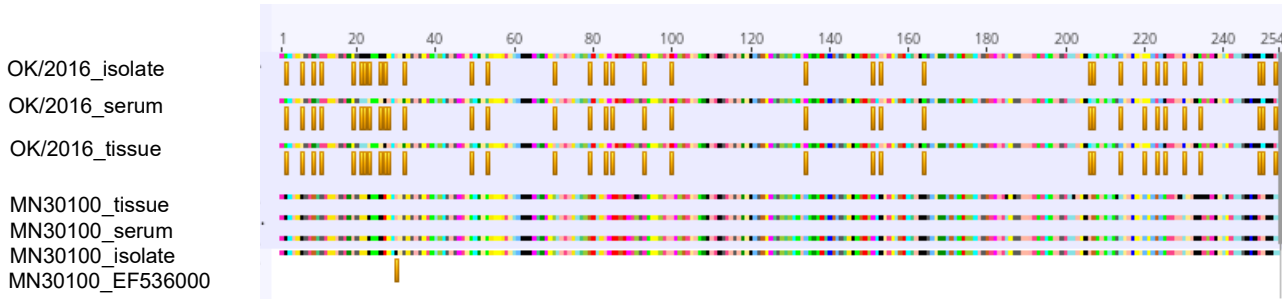

Figure 6. Sequence alignment view of GP3 of PRRSV isolates used in this study, comparing amino acid differences (orange annotations below sequence) between reference and source.

Table 10. Distance matrix for orf4 of PRRSV isolates used in this study compared to reference strains. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_ isolate	OK/2016_ serum	OK/2016_ tissue	MN30100_ tissue	MN30100_ serum	MN30100_ EF536000	MN30100_ isolate
OK/2016_ isolate		0	0	103	67	67	67
OK/2016_ serum	100%		0	103	67	67	67
OK/2016_ tissue	100%	100%		103	67	67	67
MN30100_ tissue	83.05%	83.05%			47	47	47
MN30100_ serum	87.52%	87.52%	87.52%	93.67%		0	0
MN30100_ EF53600	87.52%	87.52%	87.52%	93.67%	100%		0
MN30100_ isolate	87.52%	87.52%	87.52%	93.67%	100%	100%	

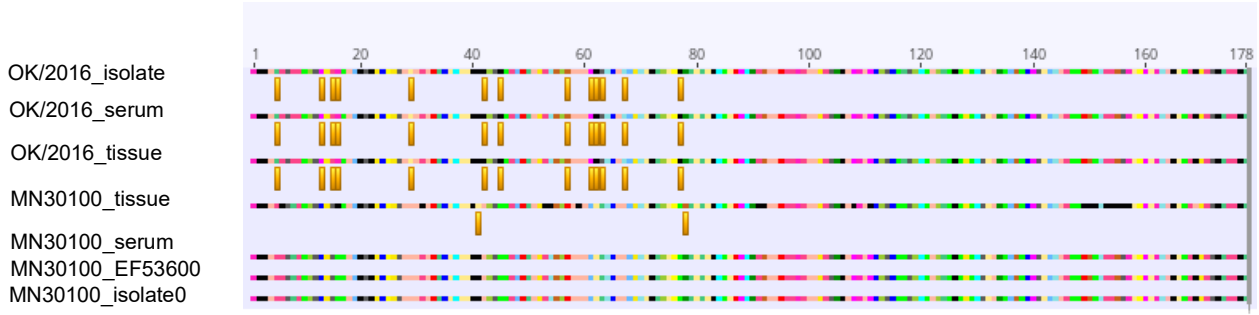

Figure 7. Sequence alignment view of GP4 of PRRSV isolates used in this study, comparing amino acid differences (orange annotations below sequence) between reference and source.

Table 11. Distance matrix for orf5 of PRRSV isolates used in this study compared to reference strains. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_ isolate	OK/2016_ serum	OK/2016_ tissue	MN30100_ tissue	MN30100_ serum	MN30100_ EF53600	MN30100_ isolate
OK/2016_ isolate		1	2	82	82	79	79
OK/2016_ serum	99.79%		1	81	81	79	78
OK/2016_ tissue	99.63%	99.75%		81	81	79	78
MN30100_ tissue	86.53%	86.65%	86.65%		6	4	3
MN30100_ serum	86.77%	86.90%	86.90%	99.25%		4	3
MN30100_ EF536000	87.02%	86.98%	86.98%	99.34%	99.59%		1
MN30100_ isolate	87.02%	87.15%	87.15%	99.50%	99.75%	99.83%	

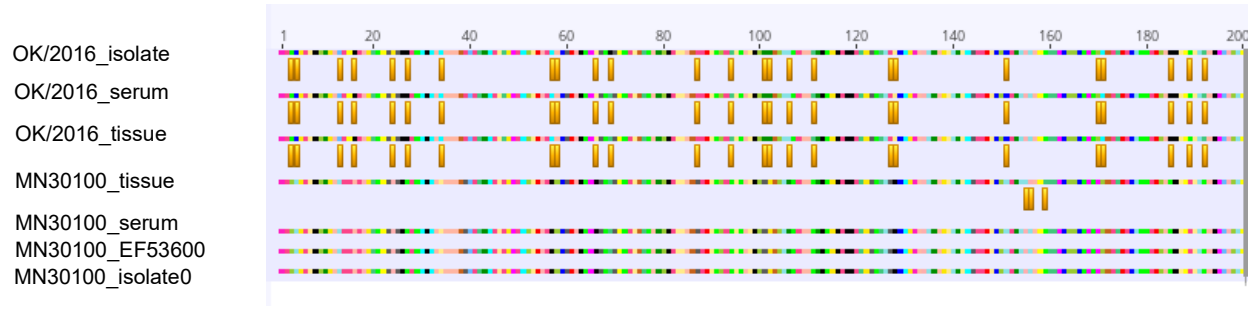

Figure 8. Sequence alignment view of GP5 of PRRSV isolates used in this study, comparing amino acid differences (orange annotations below sequence) between reference and source.

Table 12. Distance matrix for orf6 of PRRSV isolates used in this study compared to reference strains. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_ isolate	OK/2016_ serum	OK/2016_ tissue	MN30100_ tissue	MN30100_ serum	MN30100_ isolate	MN30100_ EF536000
OK/2016_ isolate		11	11	55	54	55	54
OK/2016_ serum	98.48%		0	50	49	50	49
OK/2016_ tissue	98.48%	100%		50	49	50	49
MN30100_ tissue	90.10%	90.48%	90.48%		1	2	1
MN30100_ serum	90.29%	90.67%	90.67%	99.81%		1	0
MN30100_ isolate	90.14%	90.52%	90.52%	99.67%	99.86%		
MN30100_ EF536000	90.29%	90.67%	90.67%	99.81%	100%	99.86%	

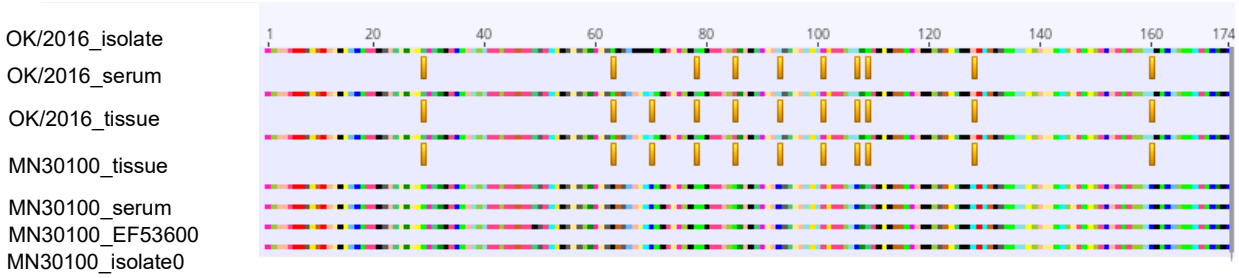

Figure 9. Sequence alignment view of GP6 of PRRSV isolates used in this study, comparing amino acid differences (orange annotations below sequence) between reference and source.

Table 13. Distance matrix for orf7 of PRRSV isolates used in this study compared to reference strains. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_ isolate	OK/2016_ serum	OK/2016_ tissue	MN30100_ tissue	MN30100_ serum	MN30100_ isolate	MN30100_ EF536000
OK/2016_ isolate		0	0	32	31	31	31
OK/2016_ serum	100%		0	32	31	31	31
OK/2016_ tissue	100%	100%		32	31	31	31
MN30100_ tissue	91.40%	91.40%	91.40%		1	1	3
MN30100_ serum	91.67%	91.67%	91.67%	99.73%		0	2
MN30100_ isolate	91.67%	91.67%	91.67%	99.73%	100%		2
MN30100_ EF536000	91.67%	91.67%	91.67%	99.19%	99.46%	99.46%	

Figure 10. Sequence alignment view of GP7 of PRRSV isolates used in this study, comparing amino acid differences (orange annotations below sequence) between reference and source.

Table 14. Distance matrix for orf1 for the OK/2016 isolate from isolate vs serum. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_ isolate	OK/2016_ serum
OK/2016_ isolate		39
OK/2016_ serum	99.68%	

#

Table 15. Distance matrix for Orf5 of PRRSV isolates used in this study compared to reference and vaccine strains. Upper section: nucleotide differences. Lower section: % identity

	OK/2016_ isolate	ATCC_ VR2332	Ingelvac ATP	MN30100_ EF536000	MN30100_ isolate
OK/2016_ isolate		89	86	78	77
ATCC_ VR2332	85.24%		57	63	64
Ingelvac ATP	85.74%	90.55%		55	56
MN30100_ EF536000	87.06%	89.55%	90.88%		1
MN30100_ isolate	87.23%	89.39%	90.71%	99.83%	

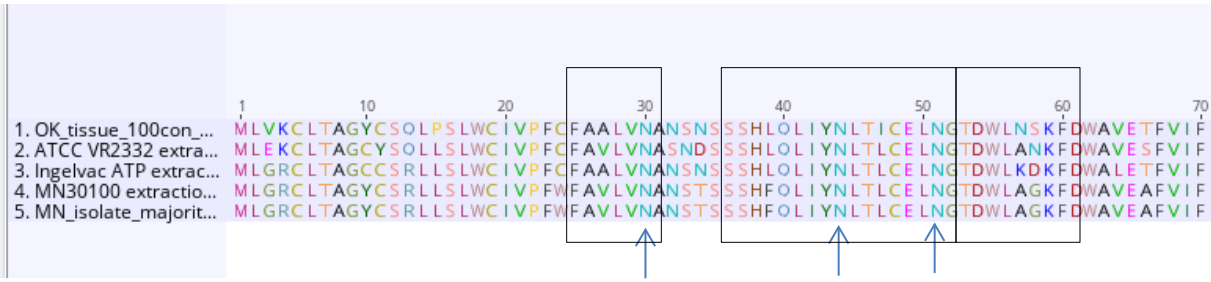

Figure 11. Sequence alignment view of GP5 of PRRSV isolates used in this study. Boxes show conserved epitopes thought to be important for infection. Arrows: N glycosylation sites.

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