Virus Research 210 (2015) 34–41

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

# Emergence of a virulent porcine reproductive and respiratory syndrome virus in vaccinated herds in the United States

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ARTICLE INFO

*Article history:*
Received 27 May 2015
Received in revised form 2 July 2015
Accepted 3 July 2015
Available online 11 July 2015

*Keywords:*
Type 2 PRRSV
Virulent strain
Full genome
Phylogeny

ABSTRACT

In early 2014, a Minnesota sow farm with a solid vaccination history suffered a severe porcine reproductive and respiratory syndrome (PRRS) outbreak with unusually high morbidity and mortality in piglets and sows, as well as anorexia and secondary bacterial infections in nursery pigs. Due to the unusual clinical severity in a PRRS-immune herd, genetic characteristics of the virus were examined to determine if a new PRRSV genotype had emerged. Phylogenetic analysis indicated that the virulent strain (PRRSV2/USA/Minnesota414/2014) was related to virulent strains circulating in the mid-western United States in recent years, and that the nonstructural protein 2 (nsp2) gene of MN414 contained an insertion-deletion pattern typical of existing type 2 virulent strains. We conclude that the MN414 isolate is a recently evolved member of the virulent lineage 1 family of type 2 PRRSV.



## 1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an economically critical swine disease that causes reproductive failure in sows, weak-born piglets, and respiratory disease with secondary infections in growing pigs. PRRS is caused by PRRSV, which belongs to the *Arterivirus* genus with a positive-sense single-stranded RNA of approximately 15 kb (Cavanagh, 1997; Hanada et al., 2005; Murtaugh et al., 2010). PRRSV have an extremely rapid evolutionary rate and undergoes recombination, leading to increased genetic diversity, pathogenesis, and possible immune evasion.

PRRSV is comprised of two genetically distinct groups, type 1 and type 2, initially isolated in Europe and North America, respectively. Both groups are genetically divergent and have ≥25% nucleotide sequence variation in open reading frame (ORF) 5 or ORF7 (Stadejek et al., 2006, 2008; Batista et al., 2004; Shi et al., 2010; Brar et al., 2012; Key et al., 2001). Since the discovery of PRRS in the late 1980s, virulent type 2 strains have emerged periodically, including "atypical PRRS" in 1996, highly pathogenic Chinese PRRSV in 2006, and multiple offshoots of lineage 1 viruses starting in 2000 (Shi et al., 2010; Mengeling et al., 1998; Han et al., 2006; Yeske and Murtaugh, 2008; Tian et al., 2007). The novel virulence characteristics of PRRSV may be due to random chance, selective evolution, the identification of new isolates, or a combination of the previously listed characteristics.

Here, we report a genetic and phylogenetic analysis of a highly virulent PRRSV isolate, designated PRRS/USA/Minnesota414/2014 (MN414/2014), that appeared in June 2014, in a Minnesota pig farm that suffered a severe PRRS outbreak with high morbidity and mortality in sows and piglets, even though the herd was vaccinated quarterly. Initial ORF5 sequence analysis revealed only 93% nucleotide identity to existing reference sequences in the University of Minnesota Veterinary Diagnostic Laboratory (UMVDL) database and no closely similar virus in GenBank. The combination of severe clinical signs and low nucleotide percent similarity suggested the possible emergence of a new, virulent PRRSV strain. The whole genome was sequenced, and phylogenetic analysis indicated that the virus emerged from within lineage 1 of type 2 PRRSV.

## 2. Materials and methods

### 2.1. Clinical case

For 2 years prior to June 2014, a 5000-sow air-filtered herd in Minnesota was mass vaccinated four times per year with PRRS ATP (Boehringer Ingelheim, St. Joseph, MO). The herd was moved to an unfiltered farm following a fire and, 2 weeks later in June, 2014, a wild-type PRRSV, designated MN414/2014, was identified. Within

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http://dx.doi.org/10.1016/j.virusres.2015.07.004
0168-1702/

2 weeks of its detection, sows became inappetent and 200 died. Within 3 weeks, approximately 800 abortions occurred. Within 4 weeks, 20–25% of weaned pigs died, and a further 20% mortality occurred in the following 4 weeks. The piglets were unthrifty and viremic with severe secondary bacterial infections with *Streptococcus suis*, *Hemophilus parasuis*, and *Mycoplasma hyorhinis*.

### 2.2. Strain identification and whole genome sequencing

PRRSV infection was confirmed by RT-PCR testing the serum of affected sows (Alonso et al., 2013). Briefly, serum samples were extracted using MagMax™ Viral RNA Isolation Kit (Thermo) and screened with the TaqMan® NA and EU PRRSV assay (Thermo). The ORF5 of this PRRSV strain was Sanger sequenced at the University of Minnesota Genomics Center (UMGC) as previously described (Alonso et al., 2013). The ORF5 sequence restriction fragment length polymorphism (RFLP) pattern was inferred as described (Wesley et al., 1998).

For whole genome sequencing, extracted RNA was quantified with the fluorometric RiboGreen assay and RNA integrity was assessed by capillary electrophoresis on an Agilent BioAnalyzer 2100. A cDNA fragment library was created using oligo-dT purification of polyadenylated RNA, reverse transcription, fragmentation, polishing and blunt-ending the fragments, and ligation of indexed (barcoded) adaptors. Total cDNA was quantified by PicoGreen dye binding. The indexed library was clustered on a flow cell, and run on an Illumina MiSeq instrument with paired-end 250 base read sequencing. The sequencing sample was spiked with phiX174 (Illumina, Inc.) as a routine quality control.

### 2.3. Bioinformatics analysis

Raw genome sequencing reads were analyzed by FastQC and Trimmomatic for quality analysis and quality control (Andrews, 2010; Bolger et al., 2014). Reads of low quality (quality score < 30) or missing Illumina barcodes were removed. De novo assembly was performed with VICUNA, which was designed to assemble RNA viruses (Yang et al., 2012; Langmead and Salzberg, 2012). The de novo PRRSV sequence was remapped using Bowtie 2 and compared to the output of a de novo assembly to confirm sequence authenticity (Langmead and Salzberg, 2012).

### 2.4. Phylogenetic analysis

A PRRSV whole genome database containing type 1 PRRSV from North America ($n = 1$), Asia ($n = 8$) and Europe ($n = 7$) and type 2 genomes from North America ($n = 75$), Europe ($n = 9$) and Asia ($n = 115$) was constructed from GenBank accessions. A database of 134 independent ORF5 type 2 PRRSV sequences with $\leq$97% nucleotide pairwise identity was used as a reference for phylogenetic analysis. Highly similar ORF5 type 2 strains were eliminated from the database for clarity while maintaining high resolution of phylogeny. PRRSV whole genome and ORF5 sequences were aligned using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm (Edgar, 2004; Tamura and Nei, 1993) in Geneious Pro 6.1.7 with default settings. Phylogenetic trees were constructed using the maximum likelihood algorithm with the Tamura-Nei nucleotide substitution model and bootstrap tests of 500 replicates in MEGA6 (Tamura et al., 2013).

### 2.5. Recombination and antigenic regions (ARs) analyses

Recombination analysis was performed using Recombination Analysis Tools (RAT), with a window size of 500 and increment size of 30. AR analysis was performed by the alignment of MN414/2014, VR2332, JXA1, ATP vaccine, and recently emerging virulent PRRSV strains from the midwest US in recent years, including MN184A, K9, K10, K11, MN9A, MN14, MN16, and MN17B (Etherington et al., 2005), and ARs as identified (de Lima et al., 2006; Wang et al., 2014; Zhou et al., 2006).

## 3. Results

### 3.1. Temporal and regional emergence of MN414/2014

The 603 nucleotide ORF5 sequence of MN414/2014 was compared to 869 PRRSV sequences obtained in 2014 at the University of Minnesota Veterinary Diagnostic Laboratory. It matched 25 sequences with >98% sequence identity and had a predicted RFLP pattern of 1-3-4. Comparison to PRRS sequences in GenBank confirmed the absence of close relatives (>95% identity), but indicated the virus belonged to lineage 1, which contains MN184, an early 2001 Minnesota virulent strain (GenBank accession number: DQ176019) and other virulent viruses with a 1-4-4 RFLP pattern that first appeared in 2010 in the mid-western US (Fig. 1) (unpublished data).

The first case submission was received on 5/6/14 from a sow herd in Martin County, Minnesota. MN414/2014 was later detected in Jackson (5/22/14), Cottonwood (6/9/14), Rice (7/14/14), Brown (8/5/14), Dakota (9/18/14), Filmore (10/30/14) and Blue Earth (12/10/14) Counties in Minnesota (Fig. 2). This strain was also found in La Crosse County, Wisconsin (9/17/14). Between May and October 2014, 17 of the 385 (4%) sequenced PRRS cases showed the same RFLP pattern and >98% sequence identity. Farms with this virus had similarly high morbidity and mortality as described here.

### 3.2. Whole genome recombination analysis

Whole genome phylogenetic analysis indicated that MN414/2014 was most closely related to a genetic cluster in type 2 PRRSV lineage 1 (Fig. 3). Lineage 1 is a large and diverse family of viruses circulating primarily in the mid-western United States since 2000, and may have originated in eastern Canada (Murtaugh et al., 2010; Shi et al., 2010; Brar et al., 2012).

Since the PRRS outbreak occurred in a well-vaccinated herd, it was of interest to determine if MN414/2014 resulted from recombination between a field strain and the Ingelvac ATP vaccine. Therefore, genetic recombination analysis was performed using MN184, a possible ancestral strain, MN14, MN17B, and the Ingelvac ATP vaccine. Recombination between MN414/2014 and Ingelvac ATP did not occur since the sequences here highly dissimilar across the entire genome (data not shown). The relationship of MN414/2014 with recent and historical lineage 1 strains was more complex. Generally, MN414/2014 was >90% similar to MN17B/2013 across the entire genome (Fig. 4, green line). Strain MN17B was closely related to MN14/2012 in the region of ORF1b and downstream, but not in ORF1a, suggesting a recombination event occurred with a parental strain of MN17B. MN14/2012 was similar to MN184 in the ORF1a region, suggesting an ancestral relationship to the earliest known lineage 1 introduction into Minnesota (Fig. 4, compare purple and blue lines).

### 3.3. NSP2 insertion-deletions (indels)

MN414 had three discontinuous deletions within hypervariable region II (HV-II) of nsp2 (aa 147–847) compared to prototype VR2332, but the deletion pattern in MN414 was consistent with strain MN184 (Fig. 5). MN414 has a 111 amino acid (aa) deletion from aa 323 to aa 433 (corresponding to nucleotides 967–1299), a single aa deletion at position 483 (nucleotide position 1448–1450), and a 19 aa deletion from position 502 to 520 (nucleotide position 1514–1570) compared to VR2332. No insertion was observed