applied biosystems

Monitoring of European PRRSV strains using sequencing technologies

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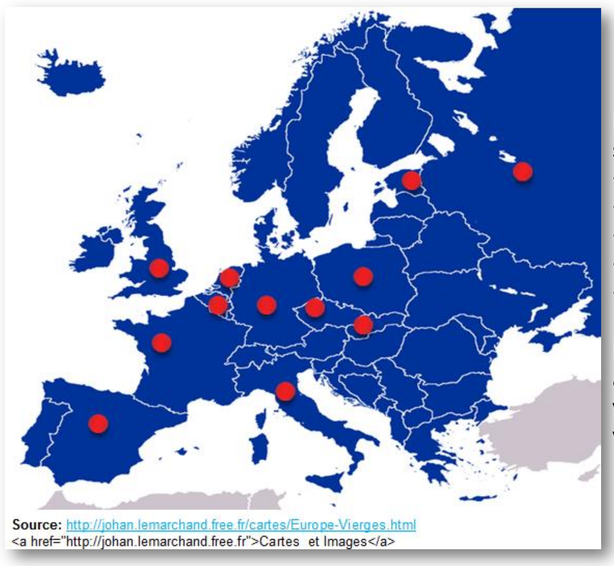
INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is considered one of the most economically important infectious diseases of swine. PRRS is caused by a RNA virus with a high mutation rate.
Thermo Fisher Scientific has improved sequencing workflows over the years, resulting in a larger percentage of field samples which can be sequenced (either whole genome sequencing or targeted). The quality of the sample impacts the options for sequencing. With the optimized workflows, even samples with different viral load can be sequenced. Sequencing positive samples give additional information about the origin of the sample and if it could be related to used vaccines or new field infections. Having this information helps the veterinarians and farm manager to evaluate the PRRS management and bio security in farms.

MATERIALS AND METHODS

Thermo Fisher Scientific established different partnerships to collect more than 100 PRRSV positive samples in more than 10 different countries (Figure 1).
Sequencing strategy applied depends on PRRS viral load and quality of the sampling process: sample collection, storage, shipment (Figure 2).
For 82 samples containing a high/medium PRRS viral load with a high quality sampling, RNA-Seq or Long Range protocols on PGM instrument were applied in order to obtain whole PRRS genome sequences.
For 20 samples containing a weak viral load or with a poor quality, capillary electrophoresis protocol on Genetic Analyzer was performed in order to obtain a specific target sequence of PRRS genome (ORF7 sequence).

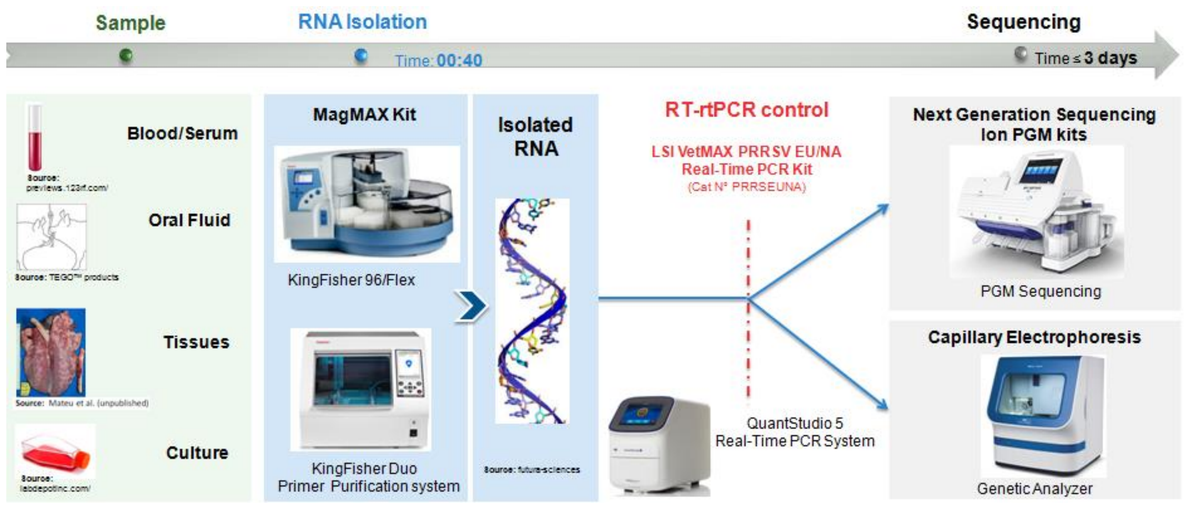
Figure 1. Sample origin coming from more than 10 countries



More than 100 PRRSV positive samples were sequenced :
- Serum/Blood samples
- Cultures
- Oral fluids
- Tissues
- RNA from various sample type

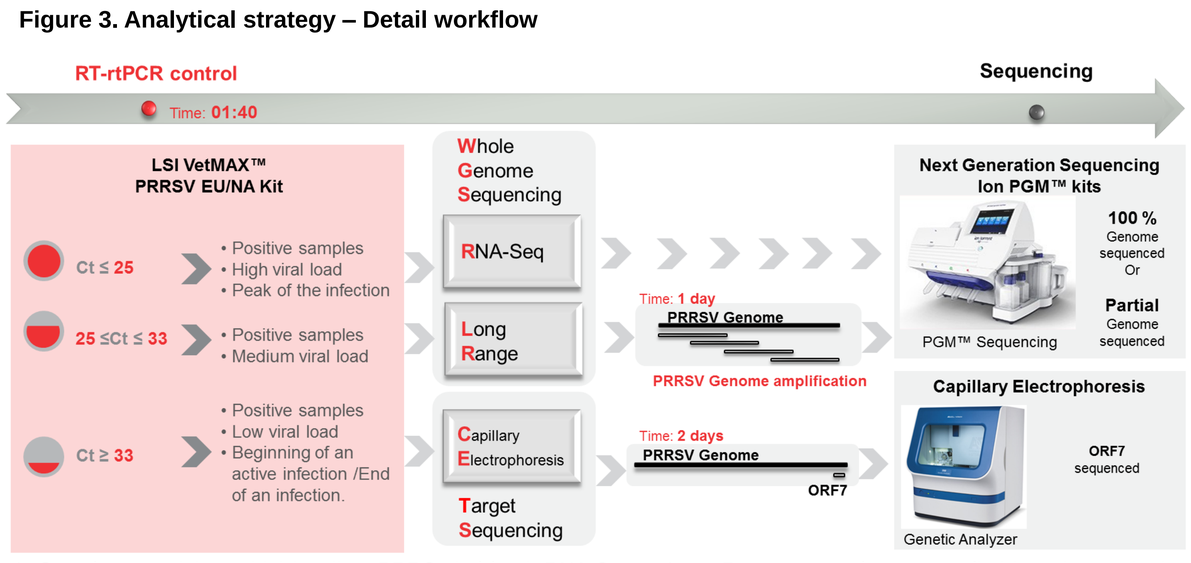
Different viral load were obtained for all samples:
a majority of sample containing high/medium PRRS viral load and some samples containing a weak PRRS viral.

Figure 2. Analytical strategy – Global workflow



The analytical strategy is divided into different steps: Viral RNAs are isolated using the MagMAX Pathogen RNA/DNA Kit on KingFisher machines. Isolated RNA is amplified using LSI VetMAX PRRSV EU/NA Kit on QuantStudio 5 real-time PCR system. Depending on the PRRS viral load estimation into samples, two sequencing strategies were applied: Next Generation Sequencing (NGS) or Capillary Electrophoresis.

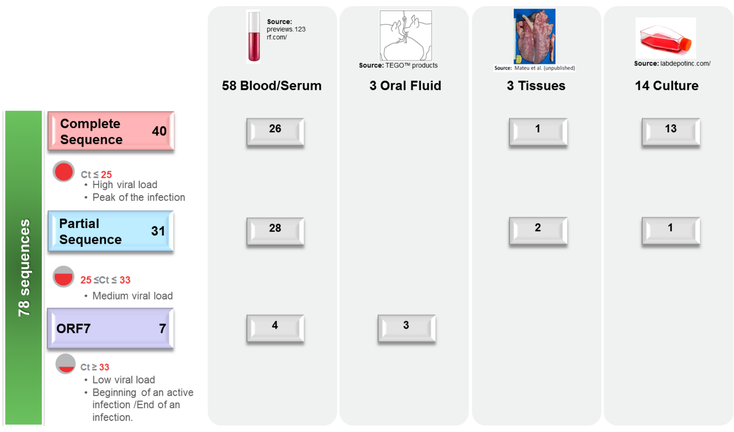
Figure 3. Analytical strategy – Detail workflow



- Samples containing a high/medium PRRS viral load, RNA-Seq or Long Range protocols were applied in order to obtain complete PRRS genome sequences.
  - Using the RNA-Seq protocol, no additional step is needed between isolated RNA and Sequencing step.
  - Using the Long Range protocol, 2 additional steps are required before the sequencing: Step 1, full-length cDNA synthesis. Step 2, cDNA amplification (4 fragments of 4Kb). Each fragment is used as a template for the sequencing.
- Samples containing a weak viral load, capillary electrophoresis protocol was performed in order to obtain a specific target sequence of PRRS genome (ORF7 sequence).

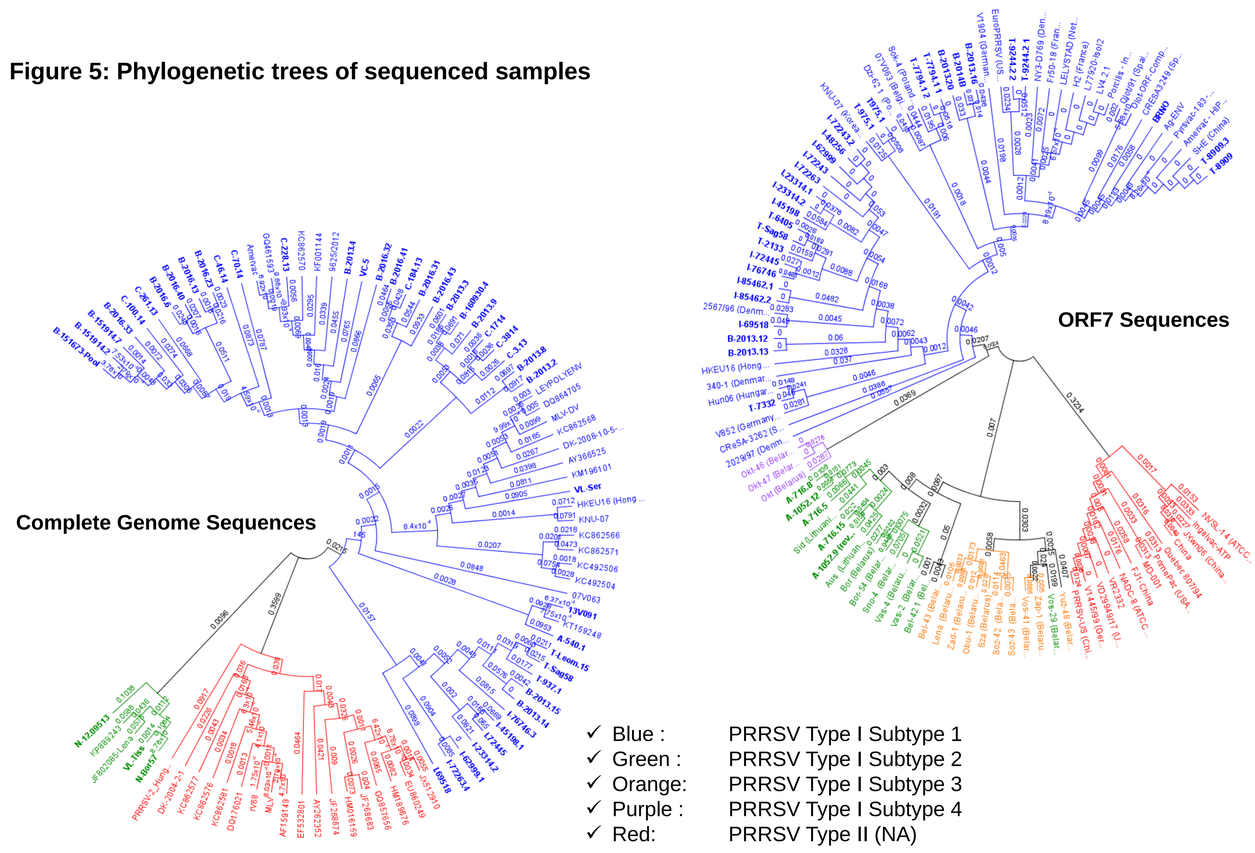
RESULTS

Figure 4: Sequenced samples



- Majority of obtained sequences are classified as Type I subtype 1. BLAST results highlight at least 85% of homology with known strains, like Olot/91 [KC862570], Cresa [JF276434; JF276435], Lelystad [M96262], German [KT344816] strains or vaccine strain as Amervac [GU067771].
- 5 strains coming from Russia and 1 strain coming from Poland were classified as Type I subtype 2. These strains shared 83% of homology with Belarus strains [KP889243].
- 1 addionnal strain coming from Poland was classified as Type I subtype 3 and shared more than 95% of homology with Lena strain [JF802085].

Figure 5: Phylogenetic trees of sequenced samples



- ✓ Blue : PRRSV Type I Subtype 1
- ✓ Green : PRRSV Type I Subtype 2
- ✓ Orange: PRRSV Type I Subtype 3
- ✓ Purple : PRRSV Type I Subtype 4
- ✓ Red: PRRSV Type II (NA)

CONCLUSIONS

Compared to a Real Time PCR assays that enables the pathogen presence/absence, sequencing approaches offer the possibility to identify new PRRSV strains.

The monitoring of circulating European PRRSV strains using sequencing technologies enables to sequence RNA, directly isolated from various field samples.

Thermo Fisher Scientific offers a range of adapted workflows from the sampling, extraction methods to the sequencing solutions.

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TRADEMARKS/LICENSING