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Editors
Dr Gyula Balka
Dr Tahar Ait-Ali


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PHYLOGENETIC ANALYSIS OF PRRSV STRAINS FROM SELECTED CENTRAL AND EASTERN EUROPEAN COUNTRIES

Gyula Balka¹, Daniel Cadar¹, Dinko Novosei², Tamas Petrovic³, Imre Biksi¹, Miklós Rusvai¹, Katarzyna Szymanek⁴, Tomasz Stadejek⁵

¹Szent István University, Faculty of Veterinary Science, Budapest, Hungary
²Croatian Veterinary Institute, Zagreb, Croatia
³Scientific Veterinary Institute „Novi Sad“, Novi Sad, Serbia
⁴National Veterinary Research Institute, Pulawy, Poland
⁵Warsaw University of Life Sciences, Faculty of Veterinary Medicine, Warsaw, Poland

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Objectives
Porcine reproductive and respiratory syndrome virus (PRRSV) belongs to two genotypes: 1 (formerly European) and 2 (formerly American). Previous investigations revealed extreme genetic diversity of genotype 1 PRRSV in countries located east from Poland (Belarus, Lithuania, Ukraine, Latvia and Russia). Four genetic subtypes have been defined within genotype 1 PRRSV. Subtypes 1–4 were found only in countries east from eastern Polish border while subtype 1 is common west from eastern Polish border. However, the number of PRRSV sequences available from other countries neighboring of central Europe is low and the true distribution of the diverse strains of genotype 1 PRRSV is unknown.

The aim of the study was to collect and characterize PRRSV strains from different Central European countries: Poland, Hungary, Croatia, Romania and Serbia.

Methods
Samples of serum, aborted fetuses or lungs were submitted for diagnosis to the authors’ laboratories. Total RNA was extracted, complete ORF5
sequences were amplified and sequenced. The sequences were assembled and analysed using the Lasergene and the ClustalW software. As a reference, a set of ORF5 sequences representing the full range of EU-PRRSV diversity (all four subtypes, and sequences of MLVs) was used. The reference Type 2 sequence (VR2332), the Canadian reference strain (“Quebec”), and the American MLV sequences were also included.

Results

In total we obtained 27 ORF5 sequences from Poland, 18 from Hungary, 13 from Romania, 12 from Serbia and 8 from Croatia. The analysis revealed, that they belonged to genotype 1 and genotype 2. All genotype 2 sequences were clustered in genetic subtype 1. No sequences of subtypes 2-4 were identified. Differences in within country diversity was observed. Sequences from Poland and Hungary belonged to several genetic clusters, Romanian sequences belonged to two genetic clusters and Croatian and Serbian sequences belonged to single clusters. In Poland, Romania, Hungary and Croatia sequences resembling genotype 1 vaccine strains were identified. In Poland, Hungary and Romania sequences having >95% identity to Amervac (HIPRA) vaccine were found while in Poland, Hungary and Croatia sequences having >95% to Porcilis PRRS (MSD) were found. All farms where Porcilis PRRS sequences were found applied Porcilis PRRS vaccine so it can be speculated that the local strains were originating from the vaccine strain which was described before. Interestingly, Amervac PRRS vaccine was not used in any of the farms where Amervac-like sequence was found. Genotype 2 strains were found in Hungary and Poland. Most of them was highly similar to Ingelvac PRRS MLV (Boehringer Ingelheim) strain (>95% nucleotide identity). In one Polish farm a strain sharing 93.7% was detected. These farms used Boehringer Ingelheim vaccine at the time of sampling, or in the past. In one Hungarian farm genotype 2 strain not related to Boehringer Ingelheim vaccine was identified. The nucleotide identity to Ingelvac strain was only 87.7% and this sequences clustered with Canadian Quebec and USA MN184 strains.

Additionall, ORF7 sequences were obtained from selected samples. Generally, the results of ORF7 sequence analysis recapitulated clustering obtained from ORF5. In one Romanian ORF7 sequence three nucleotide insertion between nucleotides 35 and 36 of Lelystad virus sequence was identified. ORF7 sequence of this strain was 390 nt long.
**Discussion**

It can be concluded that genotype 1 strains circulating in Central Europe exhibit level of genetic diversity similar to those from Western Europe. No indications were found of circulation of Eastern European variants in Central Europe. Differences in within the country diversity in different Central European countries can be explained by different history and scale of import of live pigs. Further studies are needed. The finding of Romanian ORF7 sequence with 3 nt insertion indicated that ORF7 is prone to this kind of mutations as it was previously found in Eastern European subtypes. Similar finding was described in Slovakia (Jackova et al. 2012) but in this case mutation in the stop codon caused generation of extremely long 399 nt ORF7. Unexpected variability on this ORF however might have an impact on the performance of ORF7 based in-house, or commercially available RT-PCR based molecular detection assays, as observed by Toplak et al. (2012).

Identification of sequences similar to vaccines is not surprising as this is common feature of modified live vaccines against PRRSV to shed and transmit between pigs. However, in case of Amervac-like sequences no direct link to the vaccination was established. Also, it is difficult to conclude on the true identity of these strains based on small fragment of the genome. Full genome sequencing is needed to fully assess this observation.

It is important to note that not all type 2 sequences found in Europe are coming from BI vaccine. In Hungary (and also in Slovakia) wild-type, Quebec-like type 2 strains have been detected, and isolated. Surprisingly the presence of this strain is limited to 2 farms in Hungary that belong to the same owner and are in direct contact with each other. From time to time we can identify the virus and the genetic drift seems to be very limited, as only minimal change was observed in the ORF5 gene from 2004 till 2012.

**References / suggested papers**


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