EUROPRRS2012

Understanding and combating PRRS in Europe

10-12 October 2012, Budapest, Hungary

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TÁMOP 4.2.2.B-10/1-2010-0011

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The projects are supported by the European Union.

EuroPRRS2012 Symposium

„Understanding and combating PRRS in Europe"

COST Action FA902

PROCEEDINGS

10th – 12th October 2012

Grand Hotel Margaret Island
Budapest, Hungary

EuroPRRS2012 Symposium

Publishers

Szent István University Faculty of Veterinary Science, Budapest
The Roslin Institute of the University of Edinburg

COST FA0902 Action

„Understanding and combating porcine reproductive and respiratory syndrome in Europe”

General sponsors

European Cooperation in he field of Scientific and Technical Research (COST Office)

Boehringer Ingelheim

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ISBN 978-963-269-312-5

Printed by

A/3 Nyomdaipari és Kiadói Szolgáltató Kft.
150 copies

Budapest, Hungary
2012

EuroPRRS2012 Symposium

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

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Keywords: PRRSV, ORF5, phylogeny, variability

Objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) belongs to two genotypes: 1 (formerly European) and 2 (formerly American). Pevious 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 easterm 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 includedResults

In total we obtained 27 ORF5 sequences from Poland, 18 from Hungary, 13 from Romania, 12 from Serbia and 8 from Croatia. The analys 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 countyries can be explained by different history and scale of import of live pigs. Further studies are needed. The finding of Romanian ORF7 sequece with 3 nt insertion indicated that ORF7 is prone to this kind of mutations as it was preaviously 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 suprising as this is common feature of modiefied 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 asses 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.

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Acknowledgements

The study was supported by the János Bolyai fellowship of the Hungarian Academy of Sciences and by the grants TÁMOP-4.2.2.B-10/1, and TÁMOP-4.2.1.B-11/2/KMR-2011-0003.