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Genetic variation of the prevailing porcine respiratory and reproductive syndrome viruses occurring on a pig farm upon vaccination

Brief Report

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Summary. Recurrence of porcine respiratory and reproductive syndrome (PRRS) was observed on a pig farm after introducing two PRRS live virus vaccines to combat preceding outbreaks. The phylogenetic analysis of the nucleotide sequence encoding the GP5 envelope glycoprotein and the nucleocapsid protein coding sequences (ORF5 and ORF7, respectively) showed a close genetic relationship between the new outbreak-related and one of the vaccine viruses, while the prevailing PRRS virus genetic variants disappeared from the farm. These findings, supported by the epidemiological data, indicate that the new variant PRRS viruses might originate from a vaccine virus and demonstrate the limited efficacy of modified live vaccines against heterologous PRRS virus strains.

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Porcine respiratory and reproductive syndrome virus (PRRSV) is responsible for severe economic losses in infected pig herds by causing respiratory problems including mild coughing, sneezing, or even pneumonia in piglets, poor growth rates and increased mortality in young pigs, and abortion in late-term sows or gilts [2, 13]. The PRRSV is a member of the genus *Arterivirus*, family *Arteriviridae*, order *Nidovirales* [11]. The single-stranded positive-sense viral RNA is approximately 15 kb long, 5′-capped and 3′-polyadenylated. There are two large (ORF1a and ORF1b) and seven smaller (ORF2-ORF7) open reading frames on the genome,

Note: Nucleotide sequence data reported are available in the GenBank databases under the accession numbers listed in Table 1.

Table 1. The PRRSV strains investigated during the study. Each virus strain of clinical origin was collected from different pigs

Strain	GenBank accession no.	Origin	Date of sample collection
HUN01	DQ384979	lungs of aborted foetus	20.06.2003
HUN02	DQ384980	lungs of aborted foetus	11.09.2003
HUN03	DQ384981	lungs of aborted foetus	11.09.2003
HUN04	DQ384982	lungs of aborted foetus	18.09.2003
HUN17	DQ384983	lungs of a dead weaned pig	08.02.2005
HUN18	DQ384984	Vaccine "A" (EU type virus)	16.02.2005
HUN19	DQ384985	Vaccine "B" (EU type virus)	16.02.2005
HUN22	DQ384986	lungs of aborted foetus	17.02.2005
HUN23	DQ384987	lungs of a dead weaned pig	24.02.2005
HUN25	DQ384988	lungs of a dead fattening pig	23.03.2005
HUN26	DQ384989	lungs of a dead fattening pig	23.03.2005

encoding the non-structural and structural polyproteins, respectively [12, 14]. Two genetically, clinically, and pathologically diverse types of the virus exist, the European (type 1) and the North American (type 2) PRRSV [5, 11]. Due to the continuous mutations affecting the viral genome, the PRRSV has the ability to persist in herds for long periods of time [1, 4]. This persistence and variability poses serious challenges for the diagnosis and control of PRRS that might be further complicated by reversion of live vaccine viruses into the ancestor wild-type virus and recombination of viruses in the field [3, 8, 12, 15].

Here we report a series of PRRS outbreaks on a pig farm commencing in 2003 where the genetic composition of the outbreak-causing PRRS viruses substantially changed after the use of live vaccines (the summarized case history is shown in Fig. 1). The 3200-sow farrow-to-finish farm was free of Aujeszky's disease, brucellosis, leptospirosis (*L. pomona* and *hyos*), and PRRS based on the compulsory serological investigation of 10% of the breeding stock carried out regularly in half-year intervals. The farm had been infected with porcine circovirus type 2 (PCV2), *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae*, and porcine parvovirus, and vaccination against the latter two pathogens using Respisure-One (Pfizer) and Parvosuin-MR (Hipra) vaccines, respectively, was used according to the manufacturer's instructions.

The farm reported an increase in the number of abortions, premature births, and piglet mortality in mid-2003 (Fig. 1). This was followed by respiratory signs among weaned pigs and an increased mortality up to 10% from the average 4% in this age group. The routine laboratory diagnostic procedures (necropsy, histopathology, bacteriological investigations, virus isolation, and serology) included serological investigations for PRRSV antibodies using a commercially available enzyme-linked immunosorbent assay (HerdCheck PRRS ELISA, IDEXX), and a PRRSV-detecting PCR assay that was described by Stadejek et al. [14]. This PCR amplifies nucleotide sequences of open reading frame

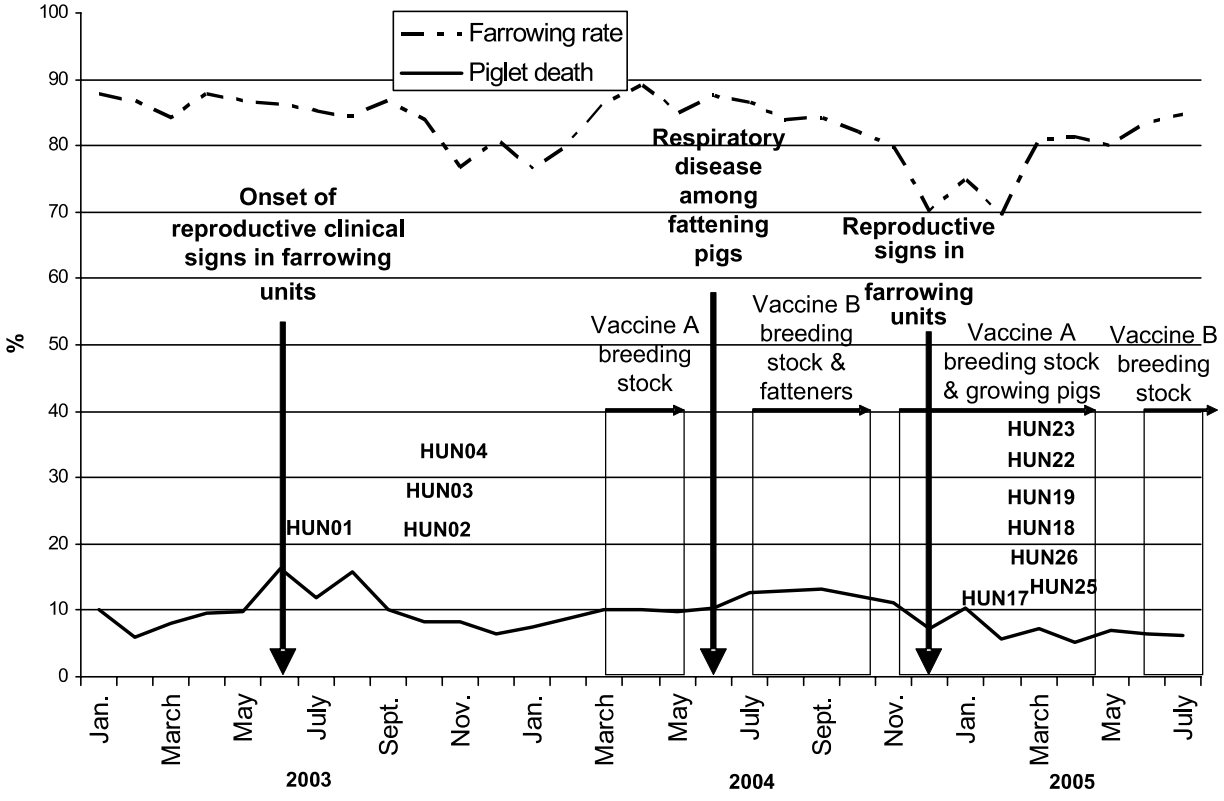


Fig. 1. Summary of the course of the PRRS outbreaks. The indices include the farrowing rate and the preweaning mortality. The time points of the collection of samples positive for PRRSV strains (HUN01-HUN26) are indicated as well as those of the courses of vaccinations (vaccines *A* and *B*)

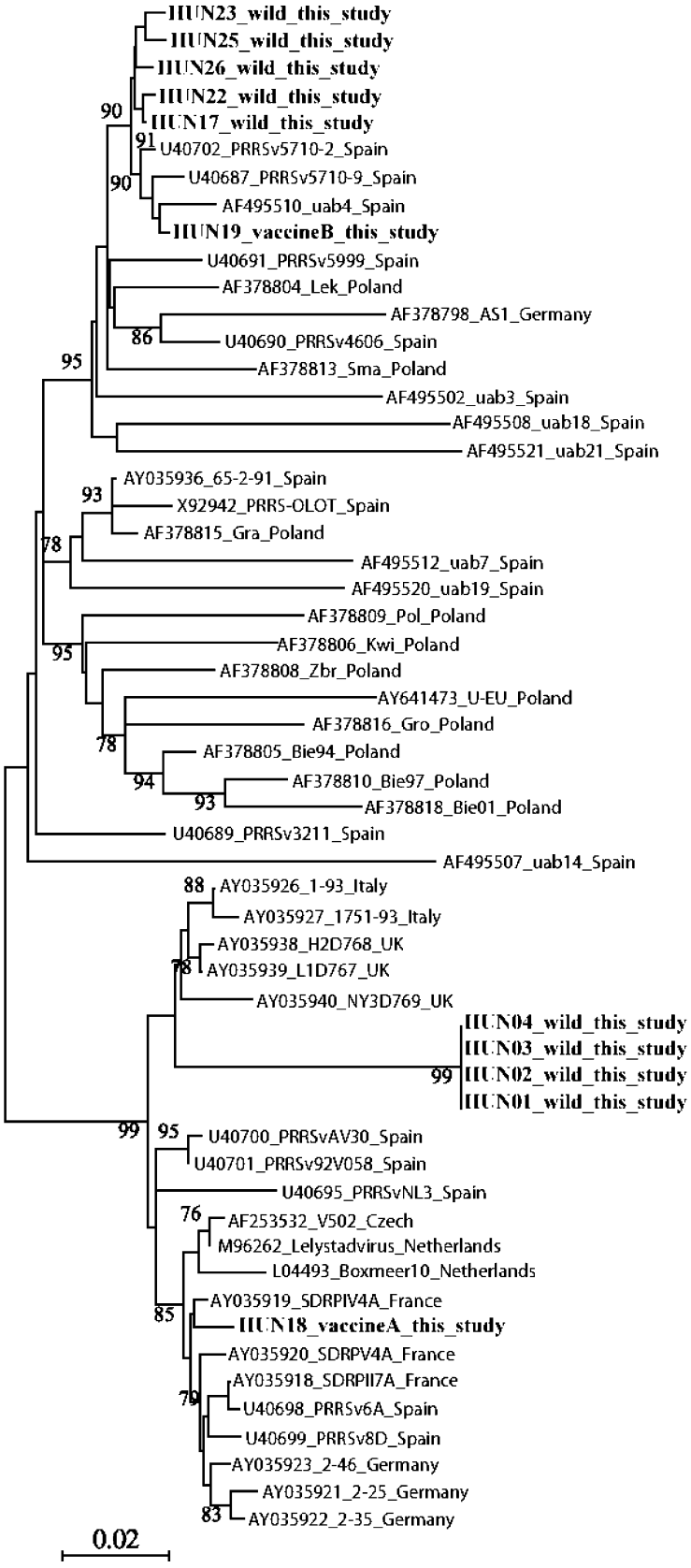
5 (ORF5), the least conserved ORF of PRRSV, which encodes an envelope-associated glycoprotein (GP5) and is a target for neutralizing antibodies and cellular immune responses [9]. The nucleotide sequence of a 423-bp portion of the ORF5 was determined. In order to detect/exclude the presence of additional PRRSV strains in the tested samples, a PCR targeting the ORF7 gene was used by applying the following primers PRRS1: 5′-CCA GCC AGT CAA TCA RCT GTG-3′ and PRRS2: 5′-GCG AAT CAG GCG CAC WGT ATG-3′, and carried out as described for the ORF5 PCR; this PCR amplified a 301-bp fragment of the nucleocapsid gene. Additional PRRSV sequences were obtained from the GenBank and used for the phylogenetic analysis of the detected viruses. The phylogenetic trees were prepared by applying the neighbour-joining method using 2-parameter distance (MEGA2.1; [6]).

The serological investigations carried out in July 2003 indicated a high ratio of PRRSV-seropositive sows in the first affected barn (62 positives out of the 84 investigated), and the antibody titres against the virus were also high according to the evaluation protocol of the ELISA test. The PRRSV-detecting PCR tests yielded positive results using lung and tonsil samples collected from necropsied

pigs. These findings confirmed that the farm had experienced a PRRS outbreak. The biosecurity measures were immediately intensified, and no new pigs were allowed to enter the farm. The number of abortions and the pre- and post-weaning mortality rates decreased for approximately three months. However, the extended serological investigations demonstrated the spread of the virus in the herd, i.e., 60% positivity was found among the investigated 90 growing pigs. In order to reduce the potential losses, vaccination of the breeding stock with a commercially available live vaccine (vaccine A) was initiated in early 2004, and this was continued until May 2004. In July 2004, an onset of respiratory disease among the already PRRSV-seropositive (thus, infected) fattening pigs prompted the vaccination of this age group, too, which continued until October 2004, but, due to cost considerations, a different live vaccine (vaccine B) was used. However, the farm returned to using vaccine A between November 2004 and April 2005. In late 2004, a sudden rise in abortions, stillbirths, and early farrowing occurred in spite of the vaccination and strict herd management. Additionally, respiratory symptoms including severe coughing associated with lethargy among unvaccinated growing and fattening pigs occurred more frequently between January and April 2005, when several samples were positive for PRRSV by PCR (Fig. 1). Again, due to economical constraints, vaccine B has been used to immunize the breeding stock since June 2005. Ultimately, the clinical PRRS disappeared from the farm.

The results of the two PCR assays were consistent, and the detected viruses proved to be type 1 PRRSV by nucleotide sequence analysis. The four ORF5 sequences (HUN01-HUN04, originating from different pigs) obtained in 2003 showed a 100% sequence identity with one another and formed a separate branch on the phylogenetic tree of their respective sequences (Fig. 2). Importantly, a PRRSV (HUN17) was detected in early 2005 that originated from the offspring of a sow that had been vaccinated several times with vaccine A. The nucleotide sequence of HUN17 had 89.5% identity compared with HUN01-HUN04 sequences and showed 98.8% nucleotide identity with the GenBank-deposited sequence of vaccine B. Therefore, PCR amplification and sequencing of the partial ORF5 of both vaccine A and B were carried out by directly sampling the vaccine vials. In the prepared nucleotide alignment, vaccine A showed a 100% and vaccine B a 99.1% identity to its GenBank counterpart data, and further, vaccine B showed strong nucleotide identity with HUN17 (99.3%). The ORF5 nucleotide sequences of the PRRSVs detected in February–March 2005 from lung and tonsil samples of dead pigs that had shown the clinical signs of respiratory distress grouped around the sequence of vaccine B, but apparently showing a tendency of divergence, i.e. decreasing level of nucleotide identity ranging from 99.76 to 99.05% between

Fig. 2. Phylogenetic tree of partial ORF5 nucleotide sequences of PRRSV strains originating from clinical samples, vaccine strains, and from the GenBank database, constructed by the neighbour-joining method using 2-parameter distance. Bootstrap values were calculated for 100 replicates. The names of the sequences obtained in this study are shown in bold, and their details are provided in Table 1


HUN23_wild_this_study
HUN25_wild_this_study
HUN26_wild_this_study
HUN22_wild_this_study
HUN17_wild_this_study
U40702_PRRSv5710-2_Spain
U40687_PRRSv5710-9_Spain
AF495510_uab4_Spain
HUN19_vaccineB_this_study
U40691_PRRSv5999_Spain
AF378804_Lek_Poland
AF378798_AS1_Germany
U40690_PRRSv4606_Spain
AF378813_Sma_Poland
AF495502_uab3_Spain
AF495508_uab18_Spain
AF495521_uab21_Spain
AY035936_65-2-91_Spain
X92942_PRRS-OLOT_Spain
AF378815_Gra_Poland
AF495512_uab7_Spain
AF495520_uab19_Spain
AF378809_Pol_Poland
AF378806_Kwi_Poland
AF378808_Zbr_Poland
AY641473_U-EU_Poland
AF378816_Gro_Poland
AF378805_Bie94_Poland
AF378810_Bie97_Poland
AF378818_Bie01_Poland
U40689_PRRSv3211_Spain
AF495507_uab14_Spain
AY035926_1-93_Italy
AY035927_1751-93_Italy
AY035938_H2D768_UK
AY035939_L1D767_UK
AY035940_NY3D769_UK
HUN04_wild_this_study
HUN03_wild_this_study
HUN02_wild_this_study
HUN01_wild_this_study
U40700_PRRSvAV30_Spain
U40701_PRRSv92V058_Spain
U40695_PRRSvNL3_Spain
AF253532_V502_Czech
M96262_Lelystadvirus_Netherlands
L04493_Boxmeer10_Netherlands
AY035919_SDRPIV4A_France
HUN18_vaccineA_this_study
AY035920_SDRPV4A_France
AY035918_SDRPII7A_France
U40698_PRRSv6A_Spain
U40699_PRRSv8D_Spain
AY035923_2-46_Germany
AY035921_2-25_Germany
AY035922_2-35_Germany
0.02

HUN17/HUN22 and HUN17/HUN26, respectively. Notably, these viruses were detected almost five months after the last dose of vaccine B on the farm.

Based on the collected data, the following possibilities should be considered: i. The newer, outbreak-causing viruses originated from the virus stock of vaccine B; ii. A field PRRSV strain that had high genetic similarity to the vaccine B PRRSV strain had been introduced onto the farm. The analysis of the alignment of the amplified ORF7 nucleotide sequences of the selected representative viruses (HUN01, HUN04, HUN17, HUN18, HUN19, and HUN23) supported the findings based on the ORF5 phylogeny (data not shown). Besides revealing complete nucleotide identity between HUN01/HUN04, and between HUN17/HUN23, the same single nucleotide mutation was detected between the sequences of HUN19/HUN17, and HUN19/HUN23, but the AAC $\rightarrow$ AAT change of the codon at residue N_{85} (referring to the translation of GenBank accession number DQ009649 nucleotide sequence) did not result in an amino acid substitution.

Considering the epidemiological data and the results of the phylogenetic analyses, it is likely that the newer viruses evolved from the PRRSV strain (existing as a quasispecies in the host [4]) of vaccine B. This could happen either by reversion or by random mutations, although recombination among the different coexisting PRRSV strains on the farm cannot be excluded, as such events have been documented [10, 12, 16]. The PRRSV ORF5 is a recognized site for recombination, with the preferential occurrence of such phenomena between strains with complete sequence identity of shorter stretches flanked by longer regions with a high percentage of identity [16]. The viruses that occurred simultaneously on the farm (i.e. HUN01-04 type, vaccines A and B) before detecting vaccine "B-like" viruses did not meet this criterion (data not shown). However, in order to explore the possibility of recombination, more comprehensive sequence analyses (sequencing parts of distant genes) would be necessary.

There are PRRSV ORF5 sequences in the GenBank that show similarity to the newer viruses, but it is noteworthy that vaccine B is among those having the highest genetic relatedness to them and, importantly, no "B-like" viruses unrelated to vaccination with vaccine B have as yet been detected in Hungary (unpublished). The mere re-detection of a harmless vaccine virus should be excluded for several reasons: all of the new PRRSVs originated from unvaccinated diseased or dead pigs without the apparent contribution of other viral pathogens; these viruses showed high spreading capacity among unvaccinated pigs, and furthermore, one of the detected strains was able to propagate in a sow that had been vaccinated several times with vaccine A. The detected viruses showed a remarkable tendency for genetic divergence, and notably, the new strains displaced the original ones since "HUN01-04-like" viruses were not detected after the appearance of the newer variants. This is a described characteristic for field PRRSV strains [7].

Our study apparently demonstrates that using a single or short-term vaccination protocol against PRRS in part of a herd does not provide a protective level of herd immunity. Moreover, the modified live vaccine viruses transmit to unvaccinated pigs. In order to reduce potential losses due to the disease, biosecurity has to be improved, and measures to achieve a protective herd immunity

against PRRS that is as homogeneous as possible have to be pursued. However, vaccination has limitations: in practice, there is no homologous PRRS vaccine available for PRRS field viruses, since extremely rapid genetic changes of these pathogens are observed in infected herds. The importance of the genetic and antigenic homology of vaccine/field viruses is further supported by our observations. Although the efficacy of vaccine A versus post-infection immunity cannot clearly be documented because the farrowing rate and piglet mortalities came back to base-line numbers before the use of vaccine A, it was shown that vaccine A did not prevent infection, replication, and clinical disease apparently caused by vaccine "B-like" PRRS viruses. However, since the production parameters in sows came back to background before the second use of vaccine B – and it has been the case for a year now – it is likely that both vaccination with vaccine B and post-infection immune responses against vaccine "B-like" viruses induced protective immunity.

In order to fully elucidate the background of the above findings, more comprehensive studies, such as experimental infection of pigs with the PRRSV variants involved and more extensive analyses of the viral sequences would be useful. Since we have recently detected a virtually identical incident on another pig farm (unpublished), we believe that the observations presented should be of interest to vaccine producers, diagnosticians, and practitioners.

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