Abstract

The objective of this study was to evaluate the diversity and persistence of porcine reproductive and respiratory syndrome virus (PRRSV) in a swine herd where American type of modified live vaccine (MLV) was used. The study was carried out on the farrow-to-finish farm with 2 500 sows, which had a history of endemic PRRSV manifested as periodic mini-outbreaks of PRRS. The farm has been endemically infected with PRRSV-EU since 1998. The vaccination programme with the MLV was first applied in 2001 and consisted of vaccinating all sows every six months and all gilts 60 d before farrowing. All incoming gilts were also vaccinated twice with a two-week interval. During the monitoring period, PRRSV-EU type was detected in 28 of 63 samples collected from two to three-month-old piglets. PRRSV-US type was detected in 20 samples. Nineteen samples were positive for both EU and US types of PRRSV at the same time. The results of RT-PCR testing serum samples from 58 sows were negative. ORF5 RT-PCR products from samples containing both PRRSV-EU and PRRSV-US types were sequenced. Phylogenetic analyses, showed a close relationship to the American genotype of PRRSV strains from the monitored farm to the known vaccine strain V2332 present in the American type PRRS MLV. The presence of the PRRSV-US in the two-three-month-old piglets indicates that the American type of vaccine virus has spread from vaccinated sows to the non-vaccinated piglets. Simultaneous presence of both PRRSV-EU and US strains in 19 samples suggests very low cross protection between the American type PRRS MLV and very diverse Lithuanian EU type field strains. In this situation an increased chance for inter-genotypic recombination can be a threat.

Key words: swine, PRRS virus, vaccination, phylogenetic analysis.

Porcine reproductive and respiratory syndrome virus (PRRSV) infection is considered one of the most important diseases in swine production today, being responsible for significant economic losses to the swine industry worldwide. One of the main characteristic features of PRRSV is its high variability. Several studies have described significant genetic differences between PRRSV isolates from Europe and North America (1, 9). Two genotypes of PRRSV are currently recognised: American (PRRSV-US) - and European (PRRSV-EU) - type strains. Both genotypes are only 55%-80% identical at the nucleotide level (17) and thought to derive from a common ancestor (8). Although PRRS is sometimes clinically similar in North America and in Europe, the strains of both genotypes differ in virulence (5) and antigenic (2, 11) and genetic properties (4, 8, 10). In spite of the fact that immune response to PRRSV is still not completely understood, some vaccines made from American- or European-type strains are being produced and marketed. Several studies have reported controversial results about the efficacy of vaccination with heterologous strains (7). It seems that American-type vaccines are more effective in protecting against infections caused by American-type field strains and less effective against European-type field infections (20). However, it is obvious that some degree of protection against the heterologous genotype can be achieved (6).

PRRSV-US field strains have been originally detected in North America and then introduced to European pig herds by the use of American type modified live vaccines (MLV) containing that type of the virus. On some occasions the virus of American type MLV had reverted to virulent strain after vaccinating naïve herds (12).

In Lithuania, PRRS outbreaks were first observed in 1997, when new pig breeds were imported from Western Europe and introduced into local pig farms. However, the latest studies suggest that PRRSV must have been present in Lithuania before that time (15). Lithuanian PRRSV ORF5 and ORF7 sequences have been shown to belong to the genetic subtype 2 of PRRSV-EU genotype, grouping viruses, which are exceptionally different from the known strains circulating in Central and Western Europe (14, 15). It has to be noted that PRRSV-US genotype virus had also
been introduced into one Lithuanian pig herd through the use of a modified – live vaccine made from US type isolate. The objective of this study was to evaluate the diversity and persistence of PRRSV strains in a swine herd where American type MLV was used and where exceptionally diverse Lithuanian PRRSV strain was detected before introducing the heterologous vaccine.

**Material and Methods**

**Samples collection and RNA extraction.** The study was carried out on the farrow-to-finish farm with 2 500 sows, which had a history of endemic PRRSV infection manifested as periodic mini-outbreaks of PRRS. The farm has been infected with PRRSV-EU since 1998. The vaccination with American type PRRS MLV was first applied in 2001 to all population of sows every six months and to all gilts 60 d before farrowing. Prior to introduction into the herd, all the new incoming gilts were also vaccinated two times with an interval of two weeks. In 2003, the vaccination programme was stopped due to low efficacy and regulatory issues.

**Results**

During the monitoring period, PRRSV-EU type was detected in 28 of 63 samples collected from two-three-month-old piglets. PRRSV-US type was detected in 20 samples. Nineteen samples were positive for both EU and US types of PRRSV. RT-PCR results from all serum samples of 58 sows were negative. Ten selected ORF5 PCR products from samples containing both PRRSV-EU and PRRSV-US types were sequenced and aligned with sequences available in the GenBank. Based on the sequences from the investigated farm and some selected EU-type ORF5 sequences from Lithuania, Belarus, Poland, and West European countries as well as US sequences, available in the GenBank, a phylogenetic tree was constructed (Fig. 1).
Fig. 1. A phylogenetic tree of the selected PRRSV strains downloaded from the GenBank and obtained in the present study, constructed with MegAlign programme from Lasergene package (DNASTAR). Clustal W algorithm was used for sequence alignment.

European genotype PRRSV sequences from the farm were clearly different from West European sequences, as it was expected, and were grouped with other sequences from Lithuania. American genotype PRRSV sequences where closely related to VR2332 and the American type MLV sequences. The obtained US type sequences from the monitored farm showed 97.4% similarity to the American type PRRS MLV.

Discussion

Experimental studies have indicated that homologous EU type MLV vaccine was capable to reduce clinical signs, shorten persistence of viraemia in challenged pigs, and increase number of weaned piglets from gilts challenged with a European strain of PRRS virus. The vaccine was shown to have an effect against the heterologous challenge, although the protection was not complete. Experience from the field has indicated that the vaccination with the EU type strain may reduce mortality losses and increase daily weight gain in finishing and nursery pigs in herds with chronic losses due to infection with European strain of the virus (6). However, others have had negative experiences especially with the use of US type MLV. This modified live vaccine was spreading from vaccinated to non-vaccinated animals and the vaccine strain could be associated with a pathogenic effect in herds (3). A vaccine-like strain isolated from the field was demonstrated to have a pathogenic effect after experimental inoculation of the late-term pregnant sows (12). Two single mutations in the genome have been linked to the attenuation of the vaccine strain and the subsequent reversion to virulence (16). Furthermore, the reverted US type vaccine strain was capable of the spreading to other herds (3).

Our study showed a close relationship of American genotype PRRSV strains from the investigated farm to strains V2332 or American type...
PRRS MLV strain, which clearly points at its origin from the vaccine used in the farm. The presence of the PRRSV-US in two-three-month-old piglets born 1-3 years after vaccination programme was stopped, indicates that the vaccine virus had spread from vaccinated sows to non-vaccinated piglets and was persisting in the herd. Furthermore, sequences were not identical to the American type PRRS MLV (97.4%) that could suggest the prolonged circulation of at least several years of the MLV related strain in weaners of this farm resulting in a significant antigenic drift. The fact that vaccine related PRRSV-US strain was circulating within the monitored farm for more than one year after the vaccination was stopped indicates that the vaccine strain partially reverted to virulence (12). Simultaneous presence of PRRSV-EU and US strains in 19 samples could suggest a very low cross protection between the US type MLV and the very diverse Lithuanian field strains (15). In this situation an increased chance for inter-genotypic recombination can be a threat.

Better understanding of the dynamics and persistence of the American type PRRS strains related to MLV in herds infected with wild type EU genotype PRRSV as well as the level of possible cross protection between different EU and US types of PRRSV strains is essential for the development of successful PRRSV control programmes. However, this study has showed that at least in two-three-month-old piglets infected with the very diverse PRRSV-EU field strain, the use of heterologous PRRSV-US genotype vaccine did not show good cross protection. The results of our study clearly indicate that both EU and US genotype PRRSV strains were present at the same time in one animal. This was the first report of PRRSV-US infection in Lithuanian swine herd, but the farm had been using vaccine containing that type of the virus. Considering that this American type MLV was the only vaccine related PRRSV-US strain circulating within the monitored farm for more than one year after the vaccination was stopped indicates that the vaccine virus had spread from vaccinated sows to non-vaccinated piglets and was persisting in the herd. Furthermore, sequences were not identical to the American type PRRS MLV (97.4%) that could suggest the prolonged circulation of at least several years of the MLV related strain in weaners of this farm resulting in a significant antigenic drift. The fact that vaccine related PRRSV-US strain was circulating within the monitored farm for more than one year after the vaccination was stopped indicates that the vaccine strain partially reverted to virulence (12). Simultaneous presence of PRRSV-EU and US strains in 19 samples could suggest a very low cross protection between the US type MLV and the very diverse Lithuanian field strains (15). In this situation an increased chance for inter-genotypic recombination can be a threat.

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References

