VIRAEMIA AND SEROCONVERSION IN PIGLETS FOLLOWING VACCINATION WITH PRRSV-EU TYPE VACCINE – A FIELD OBSERVATION

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Abstract

One of the methods for controlling porcine reproductive and respiratory syndrome (PRRS) is to use modified live vaccines (MLV’s). This report presents field observations on the profiles of seroconversion obtained by ELISA, and viraemia measured by RT-PCR, in piglets vaccinated with Porcilis PRRS (Intervet) in a farrow-to-finish herd with 400 sows where a Polish wild type PRRSV strain has circulated since 2001. In February 2003, a vaccination program started involving immunization of sows every 6 months and of piglets at around 40 d of age. Twelve piglets were ear-tagged at 14 d and bled every 2-3 weeks until 132 d of life. The majority of piglets had detectable maternal antibodies against PRRSV at day 14. As maternal antibodies waned, most (10 of 11) pigs were seronegative at the time of vaccination. However, one pig, which had the highest levels of maternal antibodies, remained positive (S/P ≥ 0.5) until day 43. After vaccination at 41 d of age, most of the pigs seroconverted between 21 and 42 d post-vaccination (dpv). One pig did not seroconvert until the end of the study. Viraemia was not detected by PCR until 2 dpv, when vaccine virus was identified by nucleotide sequencing. Porcilis viraemia was detected intermittently up to 42 dpv. At 68 and 92 dpv viraemia caused by the wild type strain was detected in some animals. In summary, it can be concluded that vaccination program protected pigs up to 108 d of life against the infection with the local virulent strain. Yet, our findings of viraemia with Polish field virus in vaccinated pigs also indicate that the high levels of sequence divergence between the Porcilis PRRS modified live vaccine and Polish field strains may reduce vaccine efficacy.

Key words: PRRSV, vaccine, PCR, ELISA, nucleotide sequencing.

Porcine reproductive and respiratory syndrome (PRRS) is an important disease of swine throughout the world, and is characterized by reproductive failure in gilts and sows, and by respiratory tract illness that can be especially severe in neonatal and nursery-age pigs. PRRSV is a recently emerged disease, and appears to have occurred almost simultaneously across the world: in the US and Canada in 1987 (5), in Japan in 1989 (18), in Germany in 1990 (7). PRRS is caused by a virus (PRRSV) that has been classified with lactate dehydrogenase elevating virus (LDV) of mice, equine arteritis virus (EAV) and simian haemorrhagic fever virus (SHFV) in the Arteriviridae family (3). Sequence analysis of the GP5 structural glycoprotein revealed only 55% amino acid identity between the prototype strains isolated in North America (VR2332) and Europe (Lelystad strain). These prototype strains define the two genotypes of PRRSV which are currently recognized: American (US) and European (EU). Initially the genotypes were restricted to their place of emergence but in the late 1990’ies PRRSV-US was introduced in Europe through the use of a modified live vaccine (MLV) (8) and PRRSV-EU was introduced in North America (15).

One of the methods of PRRS control is the use of vaccination with the modified live (MLV) or inactivated vaccines. Generally, inactivated PRRSV vaccines appear to have relatively low efficacy. MLVs appear to be more efficacious, but are associated with safety concerns (2, 11). Furthermore, even for MLVs, there are contradictory reports on vaccine efficacy, with low efficacy assumed to be due to restricted cross protection between the genotypes and even between strains that belong to a given genotype (1, 6, 10, 16, 23). This report presents preliminary field observations on the profiles of seroconversion and viraemia in piglets vaccinated with Porcilis PRRS in a herd where a Polish wild type PRRSV strain circulated.

Material and Methods

Farm “A” was a Polish farrow-to-finish herd with 400 sows. Piglets were weaned at the 28th d of age. At approximately day 70 of age, the weaners were moved to the finishing sector. The farm has been endemically infected with PRRSV-EU since 2001. PRRSV infection was associated with a low farrowing rate (76.9%), high pre-weaning mortality (11.6%) and
respiratory problems in 7.7% of the nursery pigs. In February 2003, a vaccination program was started that included immunization of sows every 6 months and of nursery piglets at about the 40th d of age. In September 2003, twelve 14-day-old piglets, each from a different litter, were randomly selected and ear tagged. They were bled at the day of selection and every 2-3 weeks afterwards, until day 132 of age. The piglets were vaccinated intramuscularly with a single dose of Porcilis PRRS vaccine (Intervet, the Netherlands) at day 41 of age. One dose of the vaccine contained at least 10^{4.0} TCID_{50} of the DV strain representing European genotype of PRRS.

Serum was tested for PRRSV antibodies by indirect ELISA and for PRRSV RNA by RT-nested PCR. The antibody test was an indirect in-house ELISA with recombinant PRRSV ORF7 antigen produced in E. coli (Stadejek et al., manuscript in preparation). ELISA results were expressed as sample to positive (S/P) ratios with values of 0.5 and above being considered as positive reaction. This ORF7 ELISA is currently used at the National Veterinary Research Institute, Puławy, for routine PRRSV serodiagnostic testing.

RT-PCR was performed essentially as described previously (20). RNA was extracted directly from serum samples using the "Total RNA Prep Plus" kit according to the manufacturers protocol (A&A Biotechnology, Gdynia, Poland). Following extraction, the RNA was eluted in 100 µl of RNAse free water. Total RNA was used as template in a single-tube reverse transcription nested PCR specific for ORF5 of EU-type PRRSV. In the first step, 5 µl of 22% trehalose was used to store and maintain the following mixture in the lid of 0.2 ml Eppendorf tubes: 20 pmol of each inner primers ORF5F (5’ATGAGATGTTCTCACAAATTGGGGCG3’) and ORF5R (5’CTAGGGCTCCCCATGTGCTAGCGGAAGT3’) (22), 1 µl of dNTPs (10 mM), and 0.25 µl of Taq Polymerase (1.25 U, Fermentas, Vilnius, Lithuania). The tubes were left to dry for 2 h at room temperature prior to storage. In the next step, RT-PCR was performed in the bottom of the tubes containing the dried, trehalose-treated reagents within the lid. Amplification was carried out in 50 µl volumes containing 5 µl of RNA and the following reagents: 5 µl of 10x PCR buffer (100 mM Tris-HCl (pH 8.8), 500 mM KCl, 0.8% Nonidet P40) (Fermentas), 5 µl of MgCl2 (25 mM, Fermentas), 2 µl of dNTPs (10 mM, Sigma), 5 pmol of each outer primers EUORF5B (5’CAATGAGGTGGCCACAACC3’) and EUORFSC (5’TATGTIATGCTAAGGCGTAGCAG3’) (11), 1 µl of 10% Triton X-100 (Sigma), 0.5 µl (2.5 U) of Taq DNA polymerase (Fermentas), 0.25 µl (10 U) of RNasin (Promega, Madison, WI, USA), and 0.5 µl (100 U) of MMLV reverse transcriptase (Life Technologies). Mineral oil was used to prevent evaporation of the PCR reactions. Thermal cycling was as follows: 42°C for 30 min, 95°C for 5 min, and then 20 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min. The tubes were subsequently inverted several times and then centrifuged briefly before being returned to the thermocycler for nested PCR, using 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min. A single extension step of 72°C for 10 min completed the amplification process. The RT-PCR resulted in an amplicon of 606 bp. All the obtained amplicons were gel purified and sequenced (20). The resulted nucleotide sequences were compared to each other using Lasergene program package (DNASTar).

Results

Detailed results are presented in Table 1. At day 14 of age, 41.6% (5/12) piglets had maternal antibodies (S/P>0.5). As expected, the maternal antibodies declined gradually over time, so that at days 30 and 43 of age, 25% (3/12) and 9% (1/11) pigs, respectively, were seropositive. None of the serum samples collected before vaccination contained PRRSV. Pig 202 died before vaccination. Most of the vaccinated pigs seroconverted between days 21 and 42 post-vaccination (pv). Pig 266 did not seroconvert until the end of the observation period. In serum samples obtained at day 2 through 92 pv, PRRSV was detected in the serum of 6 pigs, with viraemia appearing to be intermittent. Together, 11 serum samples were positive in nested RT-PCR. Nucleotide sequence analysis revealed that pigs sampled between days 2 and 42 pv were viraemic with the vaccine strain (A-VAC). The sequences of all 7 amplicons were identical to Porcilis PRRS.

Interestingly, at day 68 pv pigs 231 and 293 became viraemic and the sequence analysis showed that this virus was very similar to the wild type PRRSV strain present within the herd before the vaccine was applied (A-WT) (Fig. 1). In the analysed ORF5 fragment, this wild type strain shared only 82.6% identity with the vaccine, and represented a cluster of diverse field strains, as previously published (20) (Fig. 1). At day 92 pv pig 231 remained viraemic with wild type virus but also in pig 211 the same PRRSV strain was detected by PCR and DNA sequencing. The identity of all the wild type nucleotide sequences was above 98%.

Discussion

In Poland several MLV’s are being used, for example a vaccine based on an attenuated virus of American genotype, Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica) and three vaccines based on attenuated European genotype viruses, Porcilis PRRS (Intervet), Pyrsvac-183 (Syva) and Amervac (Hipra). Limited efficacy of American genotype MLVs against European wild type strains is a well known and described phenomenon (6, 21, 23).

However, in pigs vaccinated with Ingelvac PRRS MLV, subsequent infection with European type PRRSV is easily detected with serological tests or discriminatory RT-PCR, because of the very high level of genetic and antigenic differences between EU and US type viruses.
Table 1
Seroconversion (S/P) and viraemia in vaccinated piglets. Light shaded box indicates vaccine virus viraemia; dark shaded box indicates wild type PRRSV viraemia

<table>
<thead>
<tr>
<th>age (d)</th>
<th>14</th>
<th>30</th>
<th>43</th>
<th>62</th>
<th>82</th>
<th>108</th>
<th>132</th>
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<tr>
<td>dpv</td>
<td>2</td>
<td>21</td>
<td>42</td>
<td>68</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pig 202</td>
<td>2.11</td>
<td>0.88</td>
<td>died</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pig 205</td>
<td>0.49</td>
<td>0.06</td>
<td>0.12</td>
<td>0.44</td>
<td>1.89</td>
<td>2.49</td>
<td>1.90</td>
</tr>
<tr>
<td>pig 211</td>
<td>0.03</td>
<td>0.07</td>
<td>0.00</td>
<td>2.00</td>
<td>2.30</td>
<td>2.41</td>
<td>2.96</td>
</tr>
<tr>
<td>pig 222</td>
<td>0.35</td>
<td>0.12</td>
<td>0.09</td>
<td>0.49</td>
<td>1.54</td>
<td>1.55</td>
<td>1.08</td>
</tr>
<tr>
<td>pig 231</td>
<td>0.56</td>
<td>0.05</td>
<td>0.13</td>
<td>0.84</td>
<td>1.80</td>
<td>1.75</td>
<td>1.82</td>
</tr>
<tr>
<td>pig 258</td>
<td>1.93</td>
<td>0.74</td>
<td>0.40</td>
<td>1.80</td>
<td>2.41</td>
<td>0.96</td>
<td>died</td>
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<tr>
<td>pig 260</td>
<td>0.07</td>
<td>0.01</td>
<td>0.08</td>
<td>2.17</td>
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<td></td>
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<tr>
<td>pig 262</td>
<td>0.29</td>
<td>0.10</td>
<td>0.06</td>
<td>0.85</td>
<td>1.44</td>
<td>1.86</td>
<td>2.80</td>
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<tr>
<td>pig 266</td>
<td>0.43</td>
<td>0.12</td>
<td>0.06</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
<td>0.05</td>
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<tr>
<td>pig 279</td>
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<td>1.23</td>
<td>0.61</td>
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<tr>
<td>pig 284</td>
<td>0.39</td>
<td>0.13</td>
<td>0.04</td>
<td>0.30</td>
<td>2.82</td>
<td></td>
<td>1.71</td>
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<tr>
<td>pig 293</td>
<td>1.87</td>
<td>0.81</td>
<td>0.41</td>
<td>1.59</td>
<td>2.56</td>
<td>3.57</td>
<td>3.94</td>
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</table>

Fig. 1. Phylogenetic tree constructed with MegAlign program from Lasergene program package (DNASTAR) from sequences of 432 nt fragment of ORF5 of selected PRRSV strains downloaded from GenBank and obtained in presented studies (A-VAC and A-WT).
Thus, in this setting, the period of complete protection against heterologous challenge can be accurately examined (17, 19, Stadejek in preparation). In contrast, because the current serological assays cannot discriminate between antibodies induced by EU-type field and vaccine viruses, vaccination-challenge experiments with the European type vaccines and European type virulent PRRSV strains are much more difficult to evaluate, requiring RT-PCR and nucleotide sequencing. Yet, reports have emerged that EU type vaccines may have limited efficacy against divergent EU type field viruses (7). Because the field viruses that circulate in Poland are genetically amongst the most diverse in Europe, and generally are genetically very different from the currently available MLVs (22), examination of EU type MLV performance in Polish herds is of prime importance.

We combined ELISA, RT-nested PCR and DNA sequencing to monitor seroconversion and viraemia following vaccination with Porcilis PRRS vaccine in a group of 12 randomly selected piglets from 12 different litters in a herd infected with European type of PRRSV. We found that at the 14th d of age, maternal antibodies were present in only 5 piglets (S/P 0.56-2.25). This low level of maternal antibodies was surprising, given the extensive vaccination of the sows. However, it has previously been observed in our laboratory that sows do not respond with increased antibody levels to repetitive vaccination.

After vaccination at day 41 of age, 10 of 11 piglets had increased S/P ratios by day 21 pv. However, in only 5 pigs did the S/P ratios rise above the ELISA cut-off value. Johnson et al. (4) reported that in pigs vaccinated with attenuated PRRSV strains, S/P ratios did not exceed ELISA cut-off until day 42 pv, which was attributed to slow vaccine virus replication within the pig. To overcome this problem, we used a newly developed highly sensitive and specific peptide ELISA to specifically detect Porcilis PRRS antibodies, which confirmed that indeed, all pigs, except 266, seroconverted against the Porcilis vaccine (14).

The vaccine virus was detected in the serum of the vaccinated group up to day 42 pv. So, vaccinated animals could potentially shed the Porcilis vaccine virus for at least this period. Wills et al. (22) reported that pigs inoculated with a virulent PRRSV strain exhibited viraemia up to the 56th d post infection. Johnson et al. (4) reported that attenuated strains were present in serum up to the 35th (by virus isolation) or 49th (by RT-PCR) dpv.

In pigs 231 and 293, viraemia with wild type virus was detected by RT-nested PCR at day 68 pv, i.e. about day 38 after the pigs were transferred to the finisher area. Assuming a constant infection pressure with field virus in the finishing area, this observation shows that in these two animals, the immunity induced by the EU-type Porcilis vaccine lasted for less than 68 dpv, to day 108 of age. Furthermore, pig 231 remained viraemic with field virus at the 92nd pv, indicating that despite vaccination, some animals can be shedding field virus for more than three weeks. However, it should be stressed that virus shedding was not examined directly in the present study, and that shedding can in any case only be speculated upon RT-nested PCR results, because a positive RT-nested PCR result does not necessarily indicate presence of infectious virus.

We previously showed that pigs naturally infected with an American type vaccine-derived strain are completely protected against infection with wild type European strains for only about 40 d (21). In the present study, the European type vaccine protected pigs against infection with a field European PRRSV strain that are common in Poland for at most 68 d. This difference in the protection afforded by EU and US type MLVs appears surprisingly small. However, the field EU strains circulating in Poland are genetically very different from currently available EU type MLVs (22), and others have also reported that against such divergent EU-type strains, EU-type MLVs may have reduced efficacy (8).

Unpublished results from our laboratory showed that in farm A, non-vaccinated piglets from non-vaccinated sows seroconverted at the 35th d of age, and piglets from Porcilis PRRS vaccinated sows seroconverted at the 61th d of age, indicating field virus circulation in the nursery. Thus, Porcilis vaccination of piglets at day 41 of age extended the period in which they were protected against the locally circulating virulent strains up to the 108th d of age, and in effect eliminated virulent virus from the nursery. The observed improvement in the health status of nursery pigs following vaccination could be attributed to this vaccine effect.

In conclusion, while previous vaccination-challenge experiments with European type vaccines showed good protection (23), our findings indicate that low levels of antigenic relationship between the DV strain included in Porcilis PRRS MLV and field strains may reduce vaccine efficacy, a view supported by others (8). Further field studies employing a wider spectrum of field strains, and including measurements of infectious virus in serum as well as virus shedding, are needed to examine the role of genetic diversity of PRRSV-EU on EU type MLV efficacy.

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References


