Antibody reaction in immunologically naïve replacement gilts vaccinated with an attenuated PRRSV live vaccine

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Key words
Pig, PRRSV, vaccination, ELISA, RT-PCR

Summary
Objective: Serological testing of blood samples is commonly known as an approved method to diagnose infectious diseases. Likewise it is used for monitoring infectious diseases as it is fast and cost-effective. Nevertheless interpretation of results can be difficult, especially when the samples were taken from animals, which received a vaccination prior to the serological examination. This is mainly due to the fact, that not every vaccination induces a measurable antibody reaction. In this recent case gilts were vaccinated with an attenuated live vaccine and the serum samples were negative in the ELISA. The question aroused which serological reaction to a vaccine is expected under field conditions.

Material and methods: In order to clarify this question a group of 28 gilts negative for porcine reproductive and respiratory syndrome (PRRS) virus (from a verifiable PRRSV-negative stock) were vaccinated with an attenuated PRRSV vaccine and blood samples were taken on days 0, 2, 4, 8 and 15 post vaccinationem (p. vacc). To provide a reliable means of diagnosis the samples were tested with an antibody ELISA and RT-PCR.

Results: A replication of the vaccine virus was demonstrated via RT-PCR in 100% of the animals 4 days p. vacc. The first samples classified positive in the ELISA were detected 8 days p. vacc. On day 15 p. vacc. a positive serological result was obtained for all animals.

Conclusion: The vaccination with an attenuated PRRSV vaccine provides a pronounced antibody reaction under field conditions. In case of a negative serological reaction in recently vaccinated pigs all steps from the manufacturing process through to the implementation of the vaccine have to be critically evaluated. Likewise correct laboratory studies and the assessment of diagnostic results need to be scrutinized.

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Schlüsselwörter
Schwein, PRRSV, Impfung, ELISA, RT-PCR

Zusammenfassung


Ergebnisse: Eine Vermehrung des Impfvirus konnte 4 Tage p. vacc. bei 100% der Tiere nachgewiesen werden. Der früheste Nachweis von Antikörpern gegen PRRSV war im ELISA an Tag 8 p. vacc. möglich und an Tag 15 p. vacc. erwiesen sich alle Tiere als serologisch positiv.


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Introduction

Serology is a fast and cost-effective method for diagnostics as well as for monitoring purposes in large samples from live pigs. However, the interpretation of serological findings from samples collected in the field is difficult and often unsatisfying. For the majority of the agents the antibody reaction following an infection cannot be differentiated from maternally derived antibodies or antibodies induced by vaccination. Moreover, the interpretation of serological findings is complicated when vaccination is only inducing a weak antibody response (3, 6).

In a recent case of a classical PRRS outbreak in a sow herd which had been regularly vaccinated with an attenuated live-virus vaccine on a 4-month interval, the question aroused whether these pigs had been successfully vaccinated or not. A sampling of the affected sows would have been senseless since it was highly likely that a wildtype virus infection already induced an antibody response that could not be differentiated from a reaction prior to the vaccination. Therefore, the PRRS outbreak was diagnosed by the detection of PRRSV applying RT-PCR technique to sera from weak-born piglets sampled within a few hours after birth. For further investigation of the question indicated above, blood samples from eight replacement gilts, originating from a PRRSV-free multiplier herd and still housed in the acclimatisation unit of the particular farm, were collected 5 weeks after their vaccination. The gilts received the same attenuated live-virus vaccine as used for the sow herd. The ELISA test (IDEXX PRRS X3) revealed a positive result for one of the gilts but negative results for all the others (OD values 0.007 to 0.057). These serological findings resulted in a discussion about the number of pigs that can be expected to be seropositive after vaccinating immunologically naïve gilts with an attenuated live-virus vaccine against PRRS.

From several experimental studies it is known that the infection with PRRS wildtype virus as well as the application of PRRS live-virus vaccines induces an antibody response detectable by ELISA methods within 10 to 14 days (5, 10–12). The serological reaction peaks at approximately 4 weeks post infection (p.i.) in pigs infected with wildtype virus and in those which received an attenuated live-virus vaccine (7, 11, 12). Using RT-PCR technique the earliest detection of PRRSV published is 24 hours p.i. in case of an infection with wildtype virus (9), whereas this information is not available for attenuated vaccine viruses. Vaccinated pigs remain viraemic until 22 days p. i. (11).

Hence these results from experimental studies are not in accordance with the results of serological testing in the aforementioned case, it has been discussed in how many pigs a vaccination with an attenuated PRRSV under field conditions is usually inducing a response detectable with ELISA and RT-PCR technique. Weak serological reactions are well known from the immunisation of pigs with inactivated vaccines (e.g. against Mycoplasma hyopneumoniae) as well as attenuated live vaccines (e.g. against Lawsonia intracellularis) (1, 3, 6).

The study presented here was performed with the objective to find out how many immunologically naïve gilts will show an antibody reaction detectable by ELISA after vaccination with Porcilis® PRRS. Furthermore, the determination of the time of vaccination to potential seroconversion was examined. The replication of the vaccine virus was monitored by RT-PCR technique and confirmed by sequencing.

Material and methods

In a conventional farrow-to-finish herd with 300 sows, a batch of 28 replacement gilts delivered the day before from a multiplier herd, which is negative for PRRSV (Cat. IV [2]), were included in the study. The gilts were from the same origin the “non-responder batch” came from. Both batches of gilts were housed in an acclimatisation barn with indirect (air) contact to sows selected for slaughtering. The gilts had an age of approximately 180 days.

On study day 0 the gilts received an intramuscular injection of 2 ml Porcilis® PRRS (Intervet Deutschland GmbH, MSD Animal Health, Unterschleissheim, Germany), in 15 of the gilts the vaccine with batch number A143E01 and in 13 gilts the vaccine with batch number A144ED01 was used. On study days 0, 2, 4, 8 and 15 blood samples were collected from the jugular vein of each pig.

Immediately after sampling, all blood samples were transported to the Field Station for Epidemiology for further processing. Each sample was centrifuged and the serum was transferred into a sterile plastic cup (Eppendorf, Hamburg, Germany) for storage at −20 °C. All serum samples were examined for PRRSV-specific antibodies using the HerdChek® PRRSV X3 (IDEXX Laboratories AG, Berne, Switzerland). The test was conducted according to the manufacturer’s instructions.

The ELISA used in this study is designed to detect the presence of IgG antibodies specific to PRRSV in both porcine serum and porcine plasma samples. The test does not allow the differentiation between PRRSV strains, because the microtiter plates are coated with antigen of genotype 1 (NA) and genotype 2 (EU) of the PRRSV. A microtitration format has been configured by coating recombinant PRRSV antigens on the plate. After incubation of the test the antibodies present in the serum form a complex with the coated antigen. Subsequently unbound material is removed from the wells, an anti-porcine/horseradish peroxidase conjugate is added, which binds to any porcine antibody still present in a well. By adding substrate into the wells a change in the liquids’ color can be detected when antibodies against PRRSV were present in the samples. The extinction is correlated with the amount of specific antibodies in the test item, i. e. serum or plasma sample. The detailed procedure of the ELISA is described in the HerdChek® PRRSV X3-Handbook (IDEXX).

The nominal cut-off for a positive result in this assay is a sample/positive control (S/P) ratio of 0.40 as determined by the manufacturer. If the S/P ratio is less than 0.40, the sample has to be
classified as “negative” for PRRSV antibodies. If the S/P ratio is greater than or equal to 0.40, the sample is classified as “positive” for PRRSV antibodies.

An analysis to detect PRRSV specific genome fragments was performed by using RT-PCR technique. RNA of all serum samples collected on study days 0, 2, 4, 8 and 15 was extracted manually using the QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany). The manufacturer provides a protocol for purification viral RNA from 140 µl plasma, serum, cell-free body fluids and cell-culture supernatants by means of spin columns and a micro-centrifuge.

### Table 1

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</table>

1 Threshold cycle is the cycle in which the amplification line crosses the threshold (cut-off), PRRSV real-time PCR includes 45 cycles
2 The nominal cut-off for a positive result in this assay is a sample/positive control (S/P) ratio of 0.40 as determined by the manufacturer. If the S/P ratio is less than 0.40, the sample has to be classified as “negative” for PRRSV antibodies. If the S/P ratio is greater than or equal to 0.40, the sample is classified as “positive” for PRRSV antibodies.

nd = not detected, nt = not tested
At first, each sample is lysed by using a special buffer (included in the kit). Its function is to inactivate RNases and to isolate intact viral RNA. The concept of the spin column-system is to bind RNA to a silica-gel-based membrane in columns, after adding ethanol to the lysate of cells. Contaminants can be eliminated in two steps using two different washing buffers. The last step of the purification usually is the elution of RNA, which then can directly be used as a template for a real-time RT-PCR. The technique favoured in this study is based on Taqman® probes. It is a fast method with a very high sensitivity. It is a semi-quantitative method, which gives an advice about the virus load in each sample by showing the ct-values ("threshold cycle"). If the amplification line representing the amount of 'signal' within a particular reaction tube is passing the threshold (cut-off) very early, then it can be assumed that virus concentration in the corresponding sample was very high.

The AcuPig® PRRSV real time RT-PCR Kit (AnDiaTec, Kornwestheim, Germany) used for examination in this study is licensed by the national authority, FLI (Friedrich-Loeffler Institute, Insel Riems, www.fli.bund.de). To ensure the validity of results extraction- and amplification controls ran in parallel with each analysis.

Four of the serum samples with ct-values < 30 were sent to MWG Biotech AG (Ebersberg, Germany) for sequencing the PCR products. Two of the sequenced serum samples were taken on day 8 p. vacc. and the others were taken on day 15 p. vacc.

The target gene for sequencing was the ORF5-encoded major envelope glycoprotein (GP5) of PRRSV coding for one of the three major structural proteins. The sequenced PCR product offers a length of 600 bp.

Results

The results of RT-PCR and ELISA for all pigs are summarised in ►Table 1. The samples collected on study day 0 were RT-PCR negative. On study day 2 the RT-PCR revealed positive results in 12 gilts (42.8%) showing ct-values from 30.2 to 38.4. Two days later, on study day 4, all 28 gilts were RT-PCR positive with ct-values from 29.1 to 37.4. On study day 8 still 26 of the gilts (92.9%) were RT-PCR positive (ct-values 25.2–33.9) and the ELISA revealed a first positive result in one gilt that had been seronegative on study day 4. On the last study day (15) 21 of the gilts were RT-PCR positive but with ct-values showing a lowered virus load (30.6–40.7). ELISA testing revealed 100% positive pigs within a wide range of OD-values (►Table 1).

The results obtained by the sequencing indicate 100% identity to the EU vaccine strain in one case. The other three strains reveal 99.8% identity to the EU vaccine strain.

Discussion

The results clearly show that a replication of the PRRS vaccine virus, detected by RT-PCR on day 4 p. vacc. in 100% of the pigs, was inducing a marked serological reaction, detectable by ELISA technique in all study pigs on day 15 after vaccination. As described for experimental studies (4, 11) the results of this study clearly demonstrate that attenuated PRRS live-virus vaccines induce a serological response, detectable by standard ELISA technique also in pigs reared under conventional conditions. When interpreting the results it should be considered that the vaccine was applied exclusively to immunologically naïve pigs. Therefore, it cannot be stated that the results are valid for piglets vaccinated in the first weeks of life, when maternally derived antibodies are still present. Maternal antibodies are detectable by ELISA methods until 4–8 weeks of age (8). Whether interferences between maternally derived antibodies can impair the serological reaction provoked by vaccination with an attenuated live PRRS vaccine, needs to be verified in a further study.

The first seropositive pig was detected by IDEXX PRRS X3 ELISA on study day 8, demonstrating that serological reactions have to be expected 7 days earlier than previously assumed. It can be suggested, that the IDEXX PRRS X3 allows an earlier detection of antibodies against EU genotype (vaccine) virus than an older ELISA, detecting antibodies against EU genotype virus from day 15 p. i. on (10). Certainly, there is a need for further studies to validate this statement. But nevertheless this result may be of special importance in forensic cases, when the time of PRRS virus introduction into a herd has to be estimated on the basis of serological findings.

The detection of PRRSV in 48% of samples obtained exactly 48 hours after first vaccination demonstrates the fast dissemination of the vaccine virus in pigs. The early detection of the vaccine virus results in the assumption that there is a comparability to an infection with the wildtype virus for which the earliest detection is described with 24 hours p. i. (9).

In the clinical case described above, it can be ruled out that a remarkable number of ELISA-negative results in samples collected until 5 weeks after the first vaccination of immunologically naïve replacement gilts are caused by the natural decline of antibody concentration. The antibody concentration is known to peak 4 weeks after vaccination (11) and, therefore, a lack of detectable antibody concentrations needs to be interpreted as either an unsuccessful vaccination or an incorrect implementation of the ELISA test in the laboratory.

In a case, where an unsuccessful vaccination is highly likely, a whole list of possible reasons for the vaccine failure has to be completed in an in-depth analysis, considering the entire process from manufacturing to application. The several steps of manufacturing the vaccine as well as the shipment from the manufacturer to the veterinary practice are in the responsibility of the manufacturer and usually are supervised by means of a quality management system. The release of vaccine batches to the German market is controlled by a national authority, Paul-Ehrlich-Institute (PEI, www.pei.de). In cases where the veterinary practitioner has doubts in the vaccine itself, e. g. the dosage and viability of the vaccine virus or the correct formulation of the adjuvans, he is obliged to report
the case to the PEI by using a pharmacovigilance reporting form (https://vetweb.pei.de/ta/index_form.php?PHPSESSID=7md0ji579l082iorc6l84snpb16jsdb#calendar). The PEI stores reference samples of all marketed vaccine batches for the duration of their shelf life. This is necessary to provide samples for testing in cases of pharmacovigilance.

Moreover, the storage conditions and storage period in the veterinary practice have to be checked. Vaccines have to be used earlier than the “best before” date and stored according to the manufacturer’s instructions. In order to ensure that the required temperature is accomplished refrigerators used for vaccine storage should be controlled on a regular basis by using a temperature-logger. For the storage of the lyophilisate of Porcilis® PRRS a temperature of 2–8 °C is required. In case of an unsuccessful vaccination the appropriate dissolving of lyophilisate and adjuvans as well as the time between dissolving and application needs to be carefully evaluated. According to the manufacturer’s guidelines the vaccine needs to be applied within 2 hours after dissolving the lyophilisate with the adjuvans. A prolonged storage of the dissolved vaccine will reduce the viability of the vaccine virus. The last step to be checked is the application of the vaccine. The length and diameter of the needles have to be appropriate for the size/body weight of the vaccinated pigs and the correct dosage (2 ml) of the vaccine has to be injected intramuscularly.

Another reason for not observing antibodies in properly vaccinated pigs can be an incorrect implementation of the ELISA test in the laboratory. Thus it is very important to control the quality of the entire testing procedure as it is described in the manufacturer’s instructions. At first it has to be checked whether the positive and negative controls have revealed valid results. Apart from a correct implementation/validation of the test it needs to be ensured that the test has been used earlier than its “best before” date and that it has been stored according to the manufacturer’s instructions. When a technical fault in the laboratory is unlikely, the next step should be ruling out a manufacturing fault during test production (e.g. antigen coating of the plates).

Conflict of interest

The authors confirm that they do not have any conflict of interest.

References


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