Antibody response and maternal immunity upon boosting PRRSV-immune sows with experimental farm-specific and commercial PRRSV vaccines

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ABSTRACT

The porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in sows and respiratory disease in pigs of all ages. Despite the frequent use of vaccines to maintain PRRSV immunity in sows, little is known on how the currently used vaccines affect the immunity against currently circulating and genetically divergent PRRSV variants in PRRSV-immune sows, i.e. sows that have a pre-existing PRRSV-specific immunity due to previous infection with or vaccination against the virus. Therefore, this study aimed to assess the capacity of commercially available attenuated/inactivated PRRSV vaccines and autogenous inactivated PRRSV vaccines – prepared according to a previously optimized in-house protocol – to boost the antibody immunity against currently circulating PRRSV variants in PRRSV-immune sows.

PRRSV isolates were obtained from 3 different swine herds experiencing PRRSV-related problems, despite regular vaccination of gilts and sows against the virus. In a first part of the study, the PRRSV-specific antibody response upon booster vaccination with commercial PRRSV vaccines and inactivated farm-specific PRRSV vaccines was evaluated in PRRSV-immune, non-pregnant replacement sows from the 3 herds. A boost in virus-neutralizing antibodies against the farm-specific isolate was observed in all sow groups vaccinated with the corresponding farm-specific inactivated vaccines. Use of the commercial attenuated EU type vaccine boosted neutralizing antibodies against the farm-specific isolate in sows derived from 2 farms, while use of the commercial attenuated NA type vaccine did not boost farm-specific virus-neutralizing antibodies in any of the sow groups. Interestingly, the commercial inactivated EU type vaccine boosted farm-specific virus-neutralizing antibodies in sows from 1 farm. In the second part of the study, a field trial was performed at one of the farms to evaluate the booster effect of an inactivated farm-specific vaccine and a commercial attenuated EU-type vaccine in immune sows at 60 days of gestation. The impact of this vaccination on maternal immunity and on the PRRSV infection pattern in piglets during their first weeks of life was evaluated. Upon vaccination with the farm-specific inactivated

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vaccine, a significant increase in farm-specific virus-neutralizing antibodies was detected in all sows. Virus-neutralizing antibodies were also transferred to the piglets via colostrum and were detectable in the serum of these animals until 5 weeks after parturition. In contrast, not all sows vaccinated with the commercial attenuated vaccine showed an increase in farm-specific virus-neutralizing antibodies and the piglets of this group generally had lower virus-neutralizing antibody titers. Interestingly, the number of viremic animals (i.e. animals that have infectious virus in their bloodstream) was significantly lower among piglets of both vaccinated groups than among piglets of mock-vaccinated sows and this at least until 9 weeks after parturition.

The results of this study indicate that inactivated farm-specific PRRSV vaccines and commercial attenuated vaccines can be useful tools to boost PRRSV-specific (humoral) immunity in sows and reduce viremia in weaned piglets.

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1. Introduction

Control of porcine reproductive and respiratory syndrome (PRRS) is a major challenge to all people involved in the swine industry. The causative agent of PRRS, the PRRS virus (PRRSV), is a positive-sense single-stranded RNA virus that belongs to the family of Arteriviridae, order Nidovirales (Meng, 2000). PRRSV infections are associated with respiratory distress in swine of all ages and reproductive failure in the breeding stock. Clinical signs reported in PRRSV-infected gilts and sows include late-term abortion (days 107–110 of gestation), early farrowing (days 110–112 of gestation) and high numbers of stillborn, mummified or weakborn piglets (Wensvoort et al., 1991; Collins et al., 1992). Losses in the breeding and farrowing, the nursery and the grower–finisher phase due to PRRSV impose a substantial economic burden on pork producers (Neumann et al., 2005). Historically, PRRSV isolates are divided into two genotypes: a European (EU, type I) and a North American (NA, type II) genotype. The genetic, antigenic and pathogenic variability (Bautista et al., 1993; Nelson et al., 1993; Meng et al., 1995; Halbur et al., 1996) within each genotype is high and is increasing in time. This genetic drift presents a real challenge for PRRSV control at herd and regional level (Meng, 2000).

The most commonly used strategy to combat PRRSV is to force back the virus through vaccination. Commercial attenuated and inactivated vaccines, either based on EU or NA type PRRSV, are routinely used in gilts, sows and growing pigs in order to reduce the negative effects of PRRSV infection. Commercial attenuated PRRSV vaccines offer clear protection against (re-) infection with homologous virus variants (Nielsen et al., 1997; Plana-Duran et al., 1997; Labarque et al., 2004; Martelli et al., 2007; Zuckermann et al., 2007) and the use of these vaccines in PRRSV-affected populations was reported to reduce the number of persistently infected and shedding pigs (Cano et al., 2007). Unfortunately, they seem to protect less completely and inconsistently against heterologous viruses (Murtaugh et al., 2002; Labarque et al., 2004; Kimman et al., 2009; Geldhof et al., 2012). In addition, there have been some safety issues with the current generation of commercial attenuated vaccines. It has been described that the vaccine virus may spread transplacentally and/or cause reproductive failure in sows and gilts (Nielsen et al., 2001). Moreover, the vaccine virus may revert to virulence and cause pathology (Mengeling et al., 1999; Nielsen et al., 2001, 2002) and there is even evidence of recombination between attenuated vaccine virus and circulating PRRSV variants in the field (Li et al., 2009). In contrast with the attenuated vaccines, the commercial inactivated vaccines are generally safe. A significant drawback however is that they appear to have limited efficacy. While some studies suggest a correlation between the use of these vaccines and an improved farrowing rate, reduction in return to oestrus and an increase in the number of piglets weaned per sow in endemically infected populations (Papatsiros et al., 2006), several other studies report that commercial inactivated vaccines do not protect gilts against an experimental PRRSV challenge (Scortti et al., 2007; Zuckermann et al., 2007). Interestingly however, it was recently demonstrated that an inactivated PRRSV vaccine can be developed that offers partial protection against experimental challenge after 2 vaccinations (Vanhee et al., 2009). Moreover, it was shown that this vaccine can be easily adapted to farm-specific PRRSV variants (Geldhof et al., 2012), which is interesting since virus heterogeneity can compromise vaccine efficacy and since the degree of protection induced by vaccination is linked to the homology between the vaccine virus and the field virus to which the pigs are exposed (Labarque et al., 2004).

Different immune mechanisms seem to be involved in protection against PRRSV and there is ample evidence that the PRRSV-specific antibody response may play a significant role in this process. It has for instance been shown that virus-neutralizing antibodies can protect pregnant sows against PRRSV-associated reproductive failure and transplacental spread, as well as against virus replication in tissues and viremia upon challenge with infectious virus (Osorio et al., 2002; Lopez and Osorio, 2004). In addition, passive transfer of sufficient amounts of virus-specific neutralizing antibodies can prevent viremia in young weaned pigs (Lopez et al., 2007). Conceivably, virus-specific (neutralizing) antibodies are also of major importance for maternal immunity: it is well known that the colostrum is the primary source of protective antibodies and other (pathogen-specific) immune factors in newborn piglets (Nechvatalova et al., 2011). The current generation of commercial attenuated vaccines can induce antibodies that neutralize homologous virus variants (Charermtantanakul et al., 2006; Scortti et al., 2006; Okuda et al., 2008). Similarly, the recently developed
experimental inactivated PRRSV vaccine has the capacity to induce neutralizing antibodies in naïve animals (Vanhee et al., 2009; Geldhof et al., 2012). This is in contrast with the commercially available inactivated vaccines, which are not able to induce a robust (neutralizing) antibody response (Zuckermann et al., 2007; Vanhee et al., 2009; Geldhof et al., 2012). Despite the knowledge on their antibody-inducing capacity and their frequent use to control the disease in breeding herds, little is known on how the commercially attenuated and inactivated vaccines may boost the (neutralizing) antibody response against currently circulating PRRSV variants in PRRSV-immune sows, i.e. sows that have a pre-existing PRRSV-specific immunity due to previous infection with or vaccination against the virus. It is also not known to what extent the recently developed farm-specific inactivated PRRSV vaccines may boost antibody immunity against newly emerging virus variants in PRRSV-immune animals.

The present study aimed to evaluate the antibody immunity against currently circulating PRRSV variants in PRRSV-immune sows upon booster vaccination with farm-specific inactivated PRRSV vaccines and commercial PRRSV vaccines. A first experiment examined the effect of booster vaccination with these vaccines on the antibody response in non-pregnant, PRRSV-immune replacement sows under experimental conditions. Upon vaccination, virus-specific as well as virus-neutralizing antibody concentrations in the blood were monitored and animals were checked for viremia. A second experiment focussed on the antibody response in pregnant, PRRSV-immune sows after booster vaccination under field conditions. This experiment was performed on a farm where PRRSV is endemic, despite regular vaccination against the virus. Virus-specific as well as virus-neutralizing antibody concentrations in the blood of the sows were monitored and animals were checked for viremia. After parturition and transfer of colostrum to the piglets, the same parameters were also monitored in their offspring.

2. Materials and methods

2.1. Virus isolates

PRRSV-isolates were obtained from 3 farms, showing clinical signs compatible with PRRS in sows and growing pigs despite vaccination against PRRSV. Isolate 07V063 was isolated from fetal tissue, while PRRSV-isolates 08V194 and 08V204 were isolated from the serum of a 14- and a 4-week-old piglet, respectively. All 3 isolates were isolated and cultured on porcine alveolar macrophages (PAM) and subsequently adapted to growth on MARC-145 cells by repeated passages. Sequencing of ORF2-7 of these isolates was performed as described before (Delrue et al., 2010; Geldhof et al., 2012). Nucleotide sequences were submitted to Genbank under accession numbers: [GU737264], [07V063; [GU737265], [08V194]; and [GU737266], [08V204]. Sequencing data allowed their classification as EU type virus and pointed out they did not originate from attenuated vaccine viruses used in the farms (Farms 07V063 and 08V204: Porcilis® PRRS, Intervet at 60 days of pregnancy and Ingelvac® PRRS MLV, Boehringer Ingelheim at 6 days of lactation; Farm 08V194: Ingelvac® PRRS MLV, Boehringer Ingelheim every 4 months). MARC-145 grown stocks of PRRSV 07V063 (2 passages on PAM + 2 passages on MARC-145), 08V194 (2 passages on PAM + 4 passages on MARC-145) and 08V204 (2 passages on PAM + 3 passages on MARC-145) were prepared for experimental vaccine preparation.

2.2. Vaccines

Binary ethylenimine (BEI) -inactivated vaccines, based on the farm-specific PRRSV isolates 07V063, 08V194 and 08V204, were prepared (07V063i, 08V194i and 08V204i). Vaccine preparation and quality control were performed as described before (Vanhee et al., 2009; Geldhof et al., 2012). Each vaccine dose consisted of 1 mL BEI-inactivated, MARC-145-grown virus ($10^8$ TCID$_{50}$ on MARC-145 cells) mixed with 1 mL o/w Suvaxyn (an oil-in-water diluent, normally used in the commercial pseudorabies virus vaccine Suvaxyn Aujeszký, Fort Dodge Animal Health). BEI-inactivated vaccines were administered intramuscularly in the neck muscles behind the ear.

The commercial PRRSV vaccines used in this study include an attenuated EU type vaccine (Porcilis® PRRS, Intervet, $\geq 10^9$ TCID$_{50}$/2 mL), an attenuated NA type vaccine (Ingelvac® PRRS MLV, Boehringer Ingelheim, $\geq 10^9$ TCID$_{50}$/2 mL) and an inactivated EU type vaccine (Progressis®, Merial, strain P120: $\geq 2.5$ log IF Units). The commercial vaccines were administered according to the manufacturer’s instructions.

2.3. Vaccination of non-pregnant PRRSV-immune replacement sows under experimental conditions

2.3.1. Animals

Twenty-three to twenty-five non-pregnant culled sows were selected from each of the 3 PRRSV-positive farms and housed in A2 biosafety level animal facilities. At that time, all 3 farms applied a vaccination scheme using commercial vaccines (farm 07V063 and farm 08V204: EU type attenuated vaccine at 60 days of pregnancy, NA type attenuated vaccine at 6 days of lactation; farm 08V194: NA type attenuated vaccine every 4 months) in sows. Weaners, growers and finishing pigs were not vaccinated. Upon arrival of the animals in the animal facilities, serum samples of all selected sows were tested and found negative in virus isolation assays on alveolar macrophages and MARC-145 cells.

At the time of selection, each farm also submitted 50 serum samples: 10 of sows, 10 of gilts, and 10 of 3-, 7-, and 10-week-old piglets. Serologic testing via immunoperoxidase monolayer assay (IPMA) revealed high titers of PRRSV-specific antibodies in animals of all age groups. Selected sera with high PRRSV-specific antibody titers were used for virus isolation on alveolar macrophages. The virus isolation assays revealed that PRRSV was circulating at the 3 farms. Sequencing of ORF7 of the virus isolates revealed that all viruses had the same background as the viruses that had been isolated earlier on the respective farm and that were used for vaccine preparation.
2.3.2. Experimental setup

The sows from each herd were randomly assigned to five different groups (Table 1). Groups 07V063CON, 08V194CON and 08V204CON (n = 3 or 5) served as mock-vaccinated control groups, receiving 1 mL RPMI-1640 in 1 mL o/w Suvaxyn. The sows of group 07V063i, 08V194i and 08V204i (n = 5) were vaccinated with a BEI-inactivated autogenous PRRSV vaccine based on the herd-specific PRRSV isolate. Groups 07V063PROi, 08V194PROi and 08V204PROi (n = 5) received 2 mL of a commercial EU type inactivated PRRSV vaccine (Progressis®). Groups 07V063INatt, 08V194INatt, 08V204INatt (n = 5) and 07V063INatt2, 08V194INatt2, 08V204INatt2 (n = 5) were vaccinated with an EU-(Porcilis® PRRS) and an NA-(Ingelvac® PRRS MLV) type attenuated vaccine, respectively. In all groups, vaccination was performed only once (single shot), one week upon arrival of the animals in the animal facilities. Following vaccination, the sows were monitored clinically by visual inspection on a daily basis. The injection site was examined for local inflammation. Animals were bled at 0, 1, 2 and 3 weeks post vaccination and serum was collected for detection of virus-specific and virus-neutralizing antibodies via IPMA and seroneutralization (SN) tests, respectively. Serum samples were also examined for the presence of infectious virus using virus isolation assays on PAM and MARC-145 cells.

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2.4. Vaccination of pregnant PRRSV-immune sows under field conditions

2.4.1. Animals/Farm

The herd used in this experiment was a 340-sow farrow-to-finish farm, located in West-Flanders (Belgium). The farm suffered from endemic PRRSV infection since a severe PRRSV-outbreak in 2007. At the start of the experiment, circulation of the PRRSV-isolate 07V063 in the herd was confirmed by virus isolation and sequencing of ORF7. The owners agreed to participate in a field trial to test the antibody response in 30 pregnant, PRRSV-immune sows after vaccination with a farm-specific BEI-inactivated vaccine or an attenuated EU type PRRSV vaccine regularly used on the farm (Porcilis® PRRS, Intervet) and to evaluate the protection by colostral immunity in their offspring.

2.4.2. Experimental setup

Thirty pregnant sows were randomly assigned to 3 different groups (Table 1). Ten sows were mock-vaccinated (1 mL RPMI-1640 in 1 mL o/w Suvaxyn) and served as a control group (group 07V063CON2). Ten sows were vaccinated with a BEI-inactivated autogenous PRRSV vaccine, based on the PRRSV variant isolated on the farm (group 07V063i2). Ten sows were vaccinated with an attenuated EU type PRRSV vaccine (Porcilis® PRRS, Intervet) (group 07V063PROatt2). All vaccines were administered once at 60 days of gestation. Following vaccination, the sows were monitored clinically by visual inspection on a daily basis. Blood was taken from all sows at the time of vaccination, 2 weeks later, and at the beginning and end of the lactation period. After parturition, 4 piglets were randomly selected from each sow and blood samples were taken from these animals at fixed time-points (3, 5, 7 and 9 weeks of age). Virus-specific and virus-neutralizing antibody titers were determined via IPMA and SN tests, respectively. Serum samples were examined for the presence of infectious virus using virus isolation assays on PAM and MARC-145 cells.

2.5. Serological examination and virus isolation assays

PRRSV-specific antibody titers in serum samples were determined using IPMA assays as described by Wensvoort et al. (1991). IPMA tests were performed on MARC-145 cells infected with the MARC-145-adapted PRRSV isolates 07V063, 08V194 or 08V204. For each animal group, IPMAs were performed on cells infected with the virus variant isolated on the corresponding farm. Virus-neutralizing antibodies were detected by SN tests on MARC-145 cells as described before (Geldhof et al., 2012), using the MARC-145-adapted isolates 07V063, 08V194 or 08V204. For each animal group, SN tests were performed with the virus variant isolated on the corresponding farm.

To detect viremia (i.e. the presence of infectious virus in the bloodstream), presence of infectious PRRSV in serum samples was tested using virus isolation assays as described before (Labarque et al., 2000). To check the sensitivity of the PAM and different passages of MARC-145...
cells, all used cell batches/pasages were assayed in virus
titrations using PRRSV stocks with known virus titers.

2.6. Statistical analysis

The serology of the sow sera was analyzed using a
Friedman test, followed by Dunn’s multiple comparisons
test to determine significant differences compared to
the day of vaccination at different time-points post vaccination
in all groups.

In the second experiment, a two-tailed Fisher’s exact
test was used to detect significant differences in the
number of piglets with maternal virus-neutralizing anti-
bodies in the 3 piglet groups. The same test was used to
determine significant differences in the number of viremic animals in the 3 piglet groups. A Kruskall-Wallis test,
followed by Dunn’s multiple comparisons test, was
performed to detect significant differences in maternal
virus-neutralizing antibody titers between the 3 piglet
groups.

An overall p value of 0.05 was taken as the level of
statistical significance. All statistical analyses were
performed using GraphPad Prism version 5.0a (GraphPad
Software, San Diego, California, USA).

3. Results

3.1. Vaccination of non-pregnant PRRSV-immune
replacement sows under experimental conditions

3.1.1. Clinical signs and detection of infectious PRRSV in sow
serum samples

All sows remained in good health after they were
vaccinated. No local or systemic vaccine side effects were
noted throughout the trial period. At all time-points, the
serum samples of all animals were PRRSV-negative as
determined by virus isolation assays on PAM and MARC-
145 cells.

3.1.2. Antibody response

On the day of vaccination, all animals were positive for
PRRSV-specific antibodies and many also for virus-
neutralizing antibodies. Upon vaccination, no significant
increase in virus-specific or virus-neutralizing antibodies was seen in the mock-vaccinated groups 07V063CON,
08V194CON and 08V204CON. When compared to the day
of vaccination, significantly higher virus-specific antibody
titers were observed in group 07V063i at 2 and 3 weeks
post vaccination (Fig. 1). Upon vaccination, group 07V063i showed a significant increase in virus-neutralizing anti-
bodies at 2 and 3 weeks post vaccination reaching mean
values of 7.0 log_{2} and 7.2 log_{2}, respectively (Fig. 2). In group
08V194i, the virus-neutralizing antibody titers were
significantly higher at 2 and 3 weeks post vaccination,
reaching mean values of 5.2 log_{2} and 5.3 log_{2}, respectively (Fig. 2). Group 08V204i showed a significant increase in
virus-neutralizing antibodies at 2 and 3 weeks post vaccination, reaching mean values of 5.7 log_{2} and
4.9 log_{2}, respectively (Fig. 2). For group 08V204PROi,
virus-specific antibody titers were significantly increased at 2 and 3 weeks post vaccination (Fig. 1). No differences in

virus-neutralizing antibodies were observed at any time-
point in groups 07V063PROi and 08V194PROi. Group
08V204PROi showed a significant increase in virus-
neutralizing antibodies at 3 weeks post vaccination,
reaching a mean value of 3.3 log_{2} (Fig. 2). Upon vaccination, virus-specific antibodies were significantly increased at 2
and 3 weeks post vaccination for group 08V204PORatt
(Fig. 1). A significant increase in virus-neutralizing antibodies was detected in group 08V194PORatt at 2
and 3 weeks post vaccination, reaching mean values of
2.7 log_{2} and 2.8 log_{2} respectively, and in group
08V204PORatt at 2 weeks post vaccination, reaching a
mean value of 4.8 log_{2}. No increase in virus-neutralizing antibodies was detected in 07V063PORatt (Fig. 2).
Upon vaccination with Ingelvac® PRRS MLV, no differences in
virus-specific or virus-neutralizing antibody titers were
observed in the groups 07V063INGatt, 08V194INGatt and
08V204INGatt, at any time-point tested (Figs. 1 and 2).

3.2. Vaccination of pregnant PRRSV-immune sows under field
conditions

3.2.1. Clinical signs and detection of infectious PRRSV in sow
serum samples

Upon vaccination, the appetite of all sows remained
normal and no local or general reactions were observed.
One of the mock-vaccinated sows died during parturition.
Eight piglets died during nursery and the finding of traumatic lesions in the head and/or body together with
milk-filled stomachs indicated that 5 of these piglets were
crushed to death by the dams. Routine diagnosis, including
a virus isolation assay for PRRSV detection, was performed
on tissue samples of the 3 remaining piglets, but samples
were negative for all tested pathogens. No infectious
PRRSV was isolated from any of the sow serum samples
(either on PAM or MARC-145 cells).

3.2.2. Antibody response

3.2.2.1. Sows. On the day of vaccination, all animals were
positive for PRRSV-specific antibodies and the majority
also for virus-neutralizing antibodies. The sows of group
07V063CON2 showed no significant increase in virus-
specific or virus-neutralizing antibodies at any time-point
post vaccination (Figs. 3 and 4). Upon vaccination, a
significant increase in virus-specific antibody titers was
observed at 2 weeks post vaccination and at the end of
lactation in group 07V063i2 (Fig. 3) and a significant
increase in virus-neutralizing antibody titers was observed
at 2 weeks post vaccination, reaching a mean titer of
5.6 log_{2} (Fig. 4). Upon vaccination with Porcilis® PRRS, no
significant increase in virus-specific or virus-neutralizing
antibody titers was observed at any time-point.

3.2.2.2. Piglets. Virus-specific antibodies were found in
serum samples taken from piglets born to all sows. At 3
weeks of age, the virus-specific antibody titers of piglets
from sow group 07V063i2 were significantly higher than
those of piglets of sow group 07V063CON2, but this was
not the case for the 07V063PORatt2-derived piglets. While
the virus-specific antibody titers in the majority of piglets
Days post vaccination
from sow groups 07V063i2 and 07V063PORatt2 decreased or remained stable over time, the virus-specific antibody titers in most piglets derived from the 07V063CON2 sow group increased toward the end of the trial (data not shown). Four piglets derived from 3 sow groups 07V063CON2 had a low virus-neutralizing antibody titer at 3 weeks of age and only 2 piglets from 1 sow of group 07V063CON2 had virus-neutralizing antibody titers at 5 weeks of age. No virus-neutralizing antibodies against 07V063 were detected in any of these piglets at other time-points. At 3 weeks of age, 100% (37/37) and 66% (25/38) of piglets from sow groups 07V063i2 and 07V063PORatt2 had virus-neutralizing antibody titers, respectively. Virus-neutralizing antibody titers had disappeared in a majority of the piglets of sow group 07V063PORatt2 (29% positive, 11/38) by 5 weeks of age, while 81% (30/37) of piglets of sow group 07V063i2 were still positive at that time. At 7 and 9 weeks of age, 2 piglets of 1 sow of group 07V063i2 still had virus-neutralizing antibodies against 07V063. At these time-points, no virus-neutralizing antibodies were detected in the serum of piglets derived from any other sow. When compared with group 07V063CON2 and 07V063PORatt2 piglets, a significantly higher number of animals with virus-neutralizing antibodies was observed in group 07V063i2 piglets at 3 and 5 weeks of age. Also, the virus-neutralizing antibody titers in group 07V063i2 piglets were significantly higher at 3 and 5 weeks compared to group 07V063CON2 and 07V063PORatt2 piglets (Fig. 5).

3.2.3. Detection of infectious PRRSV in piglet serum samples

The results of virus isolation on PAM cells from piglet serum samples are shown in Table 2. Infectious PRRSV was isolated from 2 serum samples of 3-week-old piglets of 1 sow of group 07V063CON2, resulting in a PRRSV-positive percentage of 6% in this group. At that time, no infectious virus could be isolated from any of the piglets of group 07V063i2 and 07V063PORatt2 sows. In the 07V063i2 and 07V063PORatt2 piglet groups, the first viremic animals were only observed at 7 weeks of age. At 9 weeks, infectious PRRSV was isolated from 64%, 21% and 19% of piglets from group 07V063CON2, 07V063PORatt2 and 07V063i2 sows, respectively. When compared to group 07V063CON2 piglets, the number of viremic animals in group 07V063i2 and group 07V063PORatt2 piglets was significantly lower at 7 and 9 weeks of age.

4. Discussion

PRRSV is an economically important pathogen of pigs causing respiratory distress in piglets and reproductive failure in sows. PRRSV outbreaks on breeding farms manifest themselves in the form of late abortions, premature farrowing or birth of dead and/or weak piglets. Vaccination of sows is one of the strategies used to minimize the clinical and economic impact of PRRSV infections. However, the currently used vaccines appear to have variable degrees of success. In the last years, many pig farms routinely vaccinating their sows with the commercially available vaccines have suffered infections with new virus variants that escape the immunity induced by the current vaccine strains (Thanawongнуwech and Suradhat, 2010). The high genetic and antigenic variability of PRRSV clearly poses an important challenge for herd-level and regional control (Murtaugh et al., 1995; Kapur et al., 1996; Nelsen et al., 1999; Kimman et al., 2009). Although all the immune factors that can contribute to an effective protection and virus clearance have not yet been identified, several studies point out an important role of virus-neutralizing antibodies. Passive transfer of PRRSV-specific virus-neutralizing antibodies can protect pregnant sows against reproductive failure and confer sterilizing immunity in sows and offspring (Osorio et al., 2002). Similarly, passive transfer of sufficient amounts of virus-specific neutralizing antibodies can prevent viremia in young pigs.
SN antibody titer (log$_2$)

Days post vaccination
evaluate the antibody immunity against currently circulating PRRSV variants in PRRSV-immune sows upon booster vaccination with farm-specific inactivated PRRSV vaccines and commercial PRRSV vaccines.

A first experiment was performed to evaluate the serological response of non-pregnant replacement sows, originating from herds with active circulation of naturally occurring PRRSV-variants, upon vaccination with a farm-specific BEI-inactivated vaccine or either of 3 commonly used commercial vaccines (inactivated or attenuated). Upon vaccination, sows that received a farm-specific inactivated vaccine showed a significant increase in serum virus-neutralizing antibodies specific for the prevalent PRRSV-variant in their herd. Previous studies in piglets have shown that this type of vaccine can induce virus-specific neutralizing antibodies in naïve animals (Vanhee et al., 2009; Geldhof et al., 2012). In contrast, vaccination with the commercial inactivated vaccine gave variable results: the vaccine boosted the 08V204-specific, but not the 07V063- or 08V194-specific virus-neutralizing antibody response. Although it has been previously reported that these vaccines may boost virus-neutralizing antibody production in previously infected animals (Nilubol et al., 2004; Zimmerman et al., 2006), several studies suggest that the commercial inactivated vaccines do not significantly stimulate the virus-neutralizing antibody response in naïve animals (Zuckermann et al., 2007; Vanhee et al., 2009; Geldhof et al., 2012). Although substantial experimental proof is currently lacking, the apparent limited immunogenicity of the commercial inactivated vaccines may be related to the inactivation procedure, strain variability, antigenic dose, adjuvant, ... Also vaccination with the commercial attenuated vaccines gave variable results. Vaccination with the attenuated EU type vaccine

weaned pigs (Lopez et al., 2007). In addition, virus-specific neutralizing antibodies may significantly contribute to the colostral immunity that protects suckling piglets during their first weeks of life (Nechvatalova et al., 2011). Despite the frequent use of commercially available attenuated and inactivated vaccines for maintaining immunity in breeding herds, little is known on how these vaccines boost the antibody immunity against divergent (wild-type) virus variants in PRRSV-immune animals. This study aimed to

evaluate the antibody immunity against currently circulating PRRSV variants in PRRSV-immune sows upon booster vaccination with farm-specific inactivated PRRSV vaccines and commercial PRRSV vaccines.

A first experiment was performed to evaluate the serological response of non-pregnant replacement sows, originating from herds with active circulation of naturally occurring PRRSV-variants, upon vaccination with a farm-specific BEI-inactivated vaccine or either of 3 commonly used commercial vaccines (inactivated or attenuated). Upon vaccination, sows that received a farm-specific inactivated vaccine showed a significant increase in serum virus-neutralizing antibodies specific for the prevalent PRRSV-variant in their herd. Previous studies in piglets have shown that this type of vaccine can induce virus-specific neutralizing antibodies in naïve animals (Vanhee et al., 2009; Geldhof et al., 2012). In contrast, vaccination with the commercial inactivated vaccine gave variable results: the vaccine boosted the 08V204-specific, but not the 07V063- or 08V194-specific virus-neutralizing antibody response. Although it has been previously reported that these vaccines may boost virus-neutralizing antibody production in previously infected animals (Nilubol et al., 2004; Zimmerman et al., 2006), several studies suggest that the commercial inactivated vaccines do not significantly stimulate the virus-neutralizing antibody response in naïve animals (Zuckermann et al., 2007; Vanhee et al., 2009; Geldhof et al., 2012). Although substantial experimental proof is currently lacking, the apparent limited immunogenicity of the commercial inactivated vaccines may be related to the inactivation procedure, strain variability, antigenic dose, adjuvant, ... Also vaccination with the commercial attenuated vaccines gave variable results. Vaccination with the attenuated EU type vaccine
Porcilis® PRRS) increased 08V194- and 08V204-specific virus-neutralizing antibody titers in sows derived from the respective farms, but did not boost the 07V063-specific virus-neutralizing antibody titers in sows of farm 07V063. Vaccination with the attenuated NA type vaccine (Ingelvac® PRRS MLV) did not boost the virus-neutralizing antibody production against any of the farm-specific isolates. These results support the idea that current generation attenuated vaccines are not universally successful in stimulating the (heterologous) humoral immune response in PRRSV-immune animals. Although the absence of an anamnestic humoral immune response upon infection or re-vaccination may result from a lack in sufficient vaccine virus replication in PRRSV-immune animals, it is conceivable that antigenic differences between the vaccine and challenge virus contribute significantly to this phenomenon.

A second experiment investigated the impact of a BEI-inactivated autogenous vaccine and a commercial attenuated vaccine administered at 60 days of gestation on maternal immunity and on the PRRSV infection pattern in piglets during their first weeks of life.

In sows vaccinated with the farm-specific BEI-inactivated vaccine, 07V063-specific virus-neutralizing antibodies in the serum were significantly increased at 2 weeks post vaccination. In contrast, vaccination with Porcilis® PRRS did not significantly increase 07V063-specific virus-neutralizing antibody titers in the sow sera at any time point post vaccination. At 3 and 5 weeks postpartum, a significantly higher number of animals with 07V063-specific virus-neutralizing antibodies was observed in group 07V063i2 piglets compared to group 07V063CON2 and 07V063PORatt2 piglets. Also, the 07V063-specific virus-neutralizing antibody titers in group 07V063i2 piglets were significantly higher at 3 and 5 weeks compared to group 07V063CON2 and 07V063PORatt2 piglets.

The appearance of viremic animals in the 07V063i2- and 07V063PORatt2-derived piglet groups was delayed in time and the number of piglets that became viremic within the trial period was significantly reduced compared with the 07V063CON2-derived piglet group. Conceivably, the passive transfer of 07V063-specific virus-neutralizing antibodies in colostrum of 07V063i2-vaccinated sows to piglets contributed to the curtailment in the number of viremic piglets. In line with this, previous studies have shown that passive transfer of virus-neutralizing antibodies can protect young piglets against PRRSV viremia (Lopez et al., 2007). Despite a lower transfer of virus-neutralizing antibodies from sow to piglets in Porcilis® PRRS-vaccinated sows, results from our study indicate that the use of such a vaccine in sows still has positive effects in the nursery. These data suggest that also other colostrum-derived immune factors may play a role in the immunity of these piglets. As previously documented, not only antibodies, but also other immune factors/components (cytokines, immune cells, antibacterial proteins, lysozymes, ...) can be transferred via colostrum (Nguyen et al., 2007; Bandrick et al., 2008; Salmon et al., 2008; Nechvatalova et al., 2011). Data obtained in the current study illustrate the potential of maternal vaccination to protect piglets from PRRSV-infection during their first weeks of life. Further research will yield a better understanding of protective PRRSV-specific maternal immunity.

5. Conclusion

None of the currently available PRRSV vaccines is able to completely prevent respiratory infection, transplacental transmission and pig-to-pig transmission of wild-type virus variants or maintain immune protection in sows (Murtaugh et al., 2002). In spite of this, producers should not rule out using PRRSV vaccines as an aid to control clinical PRRSV. Vaccines have been used successfully to reduce the negative effects of PRRSV infection (Murtaugh et al., 2002; Kimman et al., 2009). In line with this, the results of the current study indicate that attenuated and inactivated vaccines can be useful tools to boost PRRSV-specific (humoral) immunity in PRRSV-immune sows and reduce viremia in weaned piglets. Farm-specific inactivated vaccines may prove useful in vaccination programs to boost the immunity in pregnant sows. Future research will allow optimization and simplification of the production process of the adaptable BEI-inactivated vaccines and give further insights into the mechanisms of protection induced by these vaccines.

Ethics statement

All animal experiments were approved by the local ethical committee of the Faculty of Veterinary Medicine, Ghent University. The FAGG and FAVV gave their permission (pharmaceutical preparation according to the cascade arrangement) for the field trial study.

Conflict of interest statement

The authors declare that they have no competing interests.

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