

Efficacy of commercial genotype 1 porcine reproductive and respiratory syndrome virus (PRRSV) vaccine against field isolate of genotype 2 PRRSV



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ABSTRACT

Although several recent studies have found that type 1 porcine reproductive and respiratory syndrome virus (PRRSV) modified live virus (MLV) vaccine showed appreciable levels of cross-protection against type 2 PRRSV infection, the possibility of cross-protection between two genotype of PRRSV is still controversial. To determine potential protective efficacy against hetero-genotype field strain of PRRSV and to improve understandings of the mechanisms underlying performance improvement after infection in vaccinated animals, piglets were vaccinated with type 1 PRRSV MLV vaccine and challenged with type 2 field strain of PRRSV. As a result, vaccinated animals gained on average 8.45 kg in comparison to 4.77 kg measured in non-vaccinated animals during a 3-week period after viral challenge, which shows using a certain PRRSV vaccine could be clinically effective against heterologous genotypic virus challenge. In vaccinated animals, viremia was reduced and cleared rapidly, whilst viral load was much higher and reduced more slowly, indicating rebound viremia in non-vaccinated animals. The titers of neutralizing antibody against the type 2 PRRSV did not exceed the protective level in any animal from both vaccinated and control groups. Instead, antibody avidity of vaccinated animals was much higher than in the control group clearly. Furthermore, a strong negative correlation between antibody avidity and viremia was noted in 80% of vaccinated animals. Through those results from tests evaluating degree of antibody maturation and its relevance with clearing viremia, it could be suggested that non-neutralizing antibodies induced by vaccination prior to challenge might play a key role in protection against PRRSV infection, especially in early time course.

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1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) infection is the leading cause of economic loss to the swine industry worldwide (Meier et al., 2003; Neumann et al., 2005). Infection by PRRSV causes respiratory disease and growth retardation in nursery and grower–finisher pigs and reproductive failure in sows and boars (Lunney and Chen, 2010). Two distinct PRRSV genotypes

have been identified: type 1 (European genotype, EU) and type 2 (North American genotype, NA). Genetic similarity between type 1 and type 2 isolates ranges from 55% to 63% for non-structural proteins and from 61% to 81% for structural proteins (Nelsen et al., 1999). Furthermore, the genetic, antigenic, and pathogenic variability within each genotype is constantly increasing over time (Meng, 2000).

Historically, type 1 and 2 PRRSVs were mainly distributed in Europe and North America, respectively (Shi et al., 2010). However, type 1 PRRSV has been introduced to the countries outside Europe including North America and Asia causing economic losses (Fang et al., 2007; Dewey et al., 2000) and Asia (Lee et al., 2010; Thanawongnuwech et al., 2004). Similar to the type 1 PRRSV, type 2 has been spread worldwide (Shi et al., 2010). Intermingled

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existence of the two genotypes at the same farms has also been identified in many parts of the world (Ropp et al., 2004; Lee et al., 2010).

Although commercial modified live virus (MLV) and inactivated PRRSV vaccines have been licensed for use for some time, the efficacy of vaccines against genetically heterologous PRRSV field isolates is controversial and clinical protection is generally considered unsatisfactory under field conditions (Mengeling et al., 2003; Scotti et al., 2007).

Unfortunately, because of its high costs, it is economically challenging to use two or more PRRSV vaccines simultaneously. Also, there is no multi-strain live or combined killed PRRSV vaccines due to safety and efficacy problems (Mengeling et al., 2013; Geldhof et al., 2012). As needs for novel PRRSV vaccine having efficacy of cross-protection are growing, some recent studies have revealed that vaccinated gilts exposed to heterologous strains of PRRSV during gestations can display appreciable levels of cross-protection against different strains, even between type 1 and type 2 PRRSV strains (Opriessnig et al., 2005; Roca et al., 2012). Likewise, in a recent field trial performed by our laboratory to evaluate the efficacy of commercial type 1 PRRS MLV vaccine in gestating sows, significant improvement of reproductive performance and reduced levels of viral load during early infection were identified. This was observed in sows suffering from both type 1 PRRS and heterologous type 2 PRRS (data not shown). Although the protective effect achieved through vaccination in our recent study was considerable, the underlying mechanisms of action responsible for the shortened viremic period and reduced viral load in vaccinated animals remain unclear.

In the present study, non-immunized or vaccinated animals were challenged with type 2 field strain PRRSV. Rectal temperatures, body weight, and viral serum loads were monitored during the early stage of infection. In addition, serological evaluations were performed including virus neutralization tests (VNT) and conventional and avidity ELISAs. Serum levels of interferon- γ (IFN- γ), an important antiviral cytokine in PRRSV infection (Zinkernagel et al., 1996; Meier et al., 2003), and interleukin-10 (IL-10), a known cytokine synthesis inhibitory factor (Moore et al., 2001), were determined. Based on these findings, we evaluated the implications of vaccination in protective efficacy against heterologous genotype field strain of PRRSV and suggested antibody avidity as a novel indicator of protective efficacy in PRRSV vaccinology.

2. Materials and methods

2.1. Animals, vaccination and viral challenge

Twenty-two weaned specific pathogen-free piglets (4 weeks of age) were housed in an isolated animal research facility and randomly assigned to two experimental groups. All piglets were confirmed as free of both PRRSV antigen and antibody in pre-experimental screens. Piglets were either vaccinated with a single dose (2 ml) of Amervac-PRRS[®] vaccination and challenged (VC group) or non-immunized and challenged (NC group) by intramuscular mock-vaccination with 2 ml of phosphate buffered saline. Both groups were challenged intranasally with 1 ml ($10^{5.0}$ TCID₅₀/ml) KKU-PP2013 per pig 28 days after vaccination. At 3, 5 and 7 days post challenge (dpc) two piglets per group were sacrificed.

PRRSV MLV vaccine (Amervac-PRRS[®] Hipra Spain) was administered via intramuscular route, following the manufacturer's recommendations. NA type PRRSV isolate KKU-PP2013 (GenBank accession number KJ010490) used to challenge animals was isolated from commercial pigs and propagated in MARC-145 cells as described previously (Kim et al., 1993). Tenth passage viruses were

used for viral challenge. The ORF5 nucleotide sequence of KKU-PP2013 shared only 60.5% identical residues to the ORF5 of the vaccine strain.

All procedures were approved by the Institutional Animal Care and Use Committee (Konkuk University) (Approval No. KU14101).

2.2. Clinical evaluations

Vaccine efficacy against the hetero-genotype PRRSV challenge was evaluated by taking rectal temperatures and measuring body weights of all piglets from dpc 0 to 21.

2.3. Serum sample preparation

Blood samples were collected with sterile disposable syringes at 0, 3, 5, 7, 10, 14, 21, and 28 dpc. Samples were centrifuged at $3200 \times g$ for 10 min and supernatants were collected and stored at -70°C for further analysis. Serum separation was performed within 8 h of sample collection and all transportation and centrifugation procedures were performed at 4°C .

2.4. Quantification of serum viral load

Extraction of viral nucleic acids from serum samples were carried out using the commercially available viral nucleic acid extraction kit (Viral Gene-spin, Intron Biotechnology, South Korea) following the manufacturer's instructions.

Quantitative analysis of PRRSV viremia was conducted in duplicate using TaqMan real-time reverse transcription PCR as described previously (Kleiboeker et al., 2005) using serum samples collected at 0–28 dpc. The standard curve was constructed with 10-fold serially diluted PRRSV isolate KKU-PP2013 (5.78×10^5 TCID₅₀/ml) propagated in MARC-145 cells.

2.5. Serological examinations

2.5.1. Avidity ELISA

In order to determine the anti-PRRSV antibody titer in serum, conventional ELISA was performed in duplicate on collected serum samples using a commercially available ELISA kit (Idexx PRRS 3X, Idexx, United States). Samples showing S/P (sample-to-positive) ratio, calculated from OD value, over 0.4 (0.25 in OD value) was considered as seropositive. All procedures were conducted according to the manufacturer's instructions.

PRRSV-specific antibody avidity was determined accordingly but with modifications. Briefly, the binding of low-avidity antibody was disrupted with 6.5 M urea washes after the antibody binding step. Optical density (OD) values measured in conventional and avidity ELISA were compared and the relative avidity index (RAI) was calculated.

$$\text{RAI} = \frac{\text{OD value in avidity ELISA}}{\text{OD value in conventional ELISA}}$$

2.5.2. Viral neutralization test

Viral neutralization test (VNT) was performed for 90 min at 37°C with two-fold serially diluted, heat-inactivated (30 min at 56°C) test serum in the presence of 200 TCID₅₀ of KKU-PP2013. Following incubation, the serum-virus mixture was added to MARC-145 cells and the cells were seeded into microtiter plates and then cultured for 5 days. Neutralizing antibody (nAb) titers were determined by observing the cytopathic effects on MARC-145 in combination with results from the immunofluorescent assay (IFA). IFA was performed as previously described (Kim et al., 2007) using mouse anti-PRRSV monoclonal antibody (type 1 PRRSV specific; Median diagnostics,

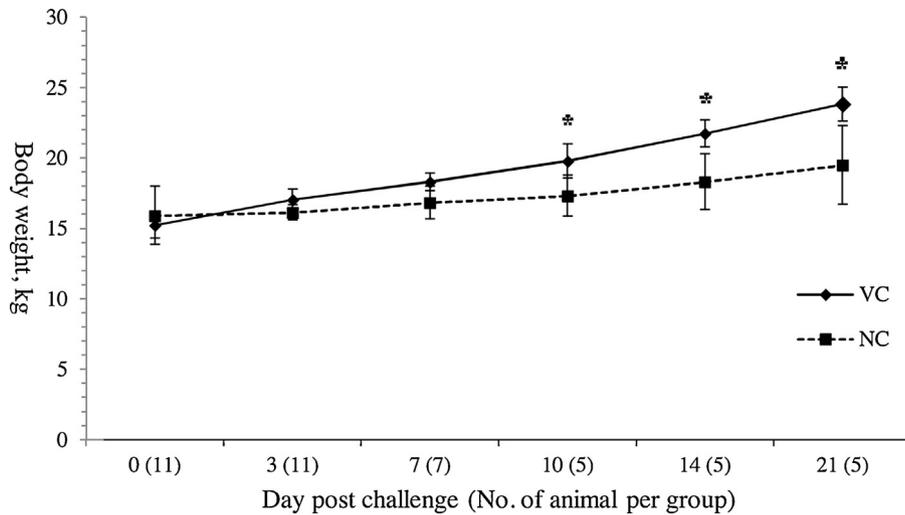


Fig. 1. Mean body weight recorded in both groups after challenge. VC group showed higher weight gain than NC group, with the increasing differences between two groups in the course of time.

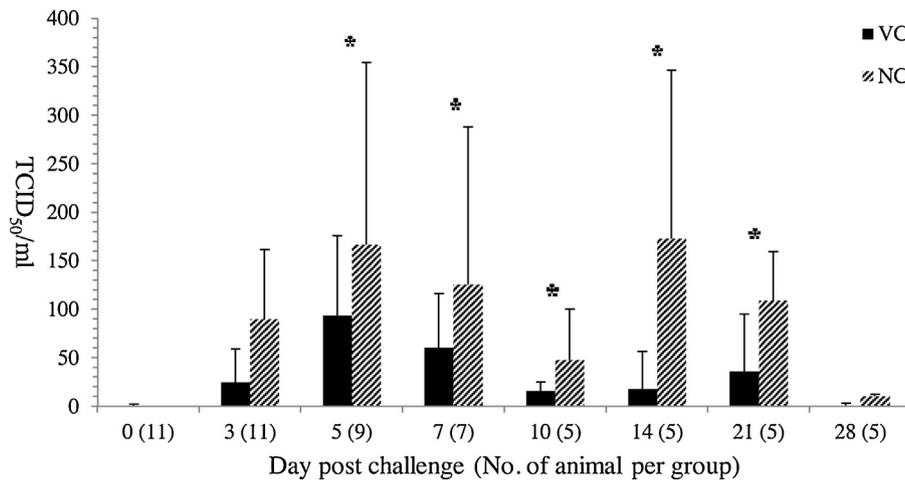


Fig. 2. Mean serum viral loads. Viremia peaked at 5 dpc and was cleared at 10 dpc in VC group. In the NC group, viral load was much higher, showing rebound at 14 dpc.

South Korea) and fluorescein isothiocyanate conjugated goat anti-mouse IgG (Jenobiotech, South Korea).

2.5.3. Measurement of cytokine levels in serum

Levels of IFN- γ and IL-10 were quantified from serum samples in duplicate using Porcine IFN- γ Quantikine ELISA Kit and Porcine IL-10 Quantikine ELISA Kit (R&D Systems, United States) respectively, following the manufacturer's instructions.

2.6. Statistical analysis

Statistical significance in all experiments was determined using Mann-Whitney *U* test, except for data from RAI calculations and avidity-virus load correlations. Data from RAI calculations were analyzed using Fisher's exact test. Avidity-virus load correlation was verified with Spearman's rho test. With the exception of Figs. 4 and 5 data are presented as mean \pm standard deviation (SD). *P*-values under 0.05 were considered significant. All statistical analysis was performed using IBM SPSS Statistics for Windows (version 21.0).

3. Results

3.1. Clinical parameters

Rectal temperatures of piglets were measured and recorded at 0, 2 and 3 h post challenge (hpc) and 2, 3, 5, 7, 14 and 21 dpc. Differences in rectal temperature between VC and NC groups were not statistically significant, except at 0 hpc. Sporadic high rectal temperatures were observed in the VC group from 2 dpc onwards, resulting in higher standard deviations (data not shown).

With regards to body weight, the VC group showed improved weight gain, with the differences in weight gain steadily increasing between VC and NC groups with increasing dpc (Fig. 1).

3.2. Serum viral load

Screening tests by conventional PCR at 3 dpc confirmed infection with type 1 PRRSV in VC groups. No transmission of the vaccine strain to the NC group were detected and all antigen detected in VC group animals was of type 2 vaccine strain PRRSV. The qualitative positive PRRSV antigen rates between groups were of no significant difference. Viremia caused by KKU-PP2013 challenge was quantified using real-time PCR. In both groups, viremia was detected from dpc 3 to 21, however, different trends in serum viral load between

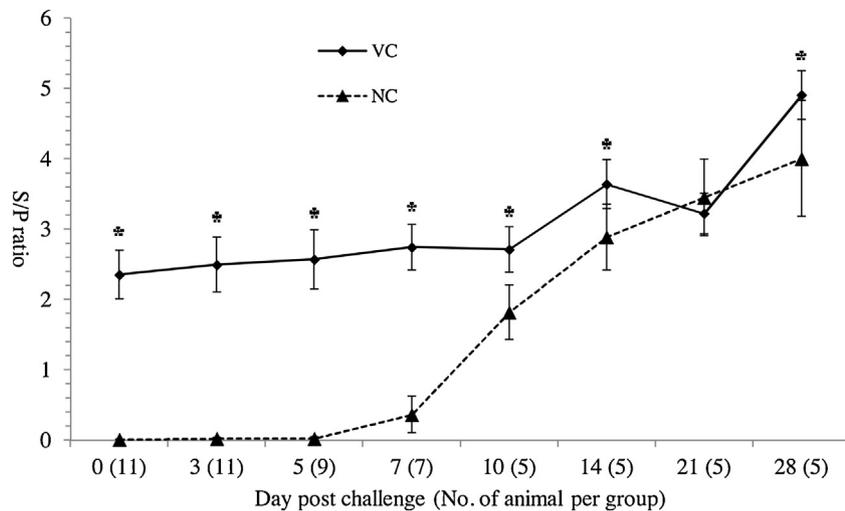


Fig. 3. Mean S/P ratios of both groups measured by conventional ELISA. Antibody titers of VC animals were significantly elevated in comparison to that of NC animals.

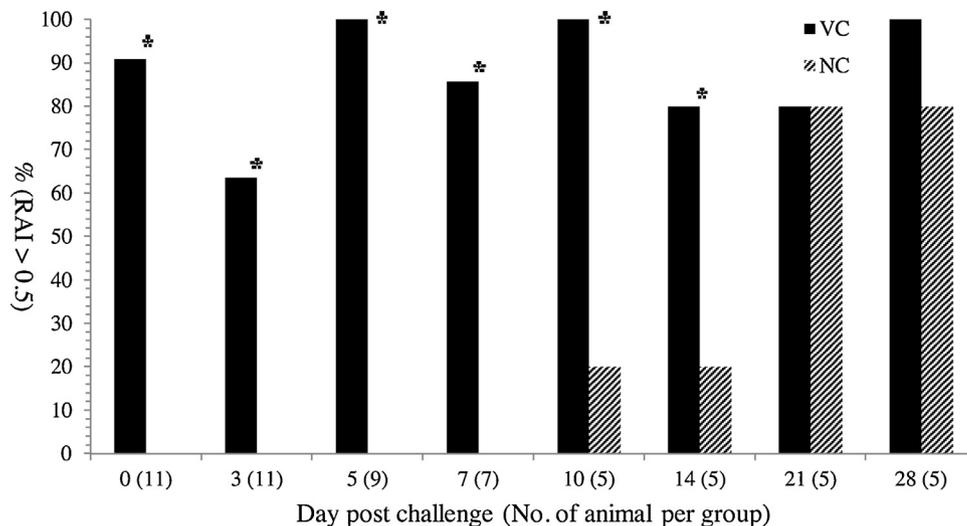


Fig. 4. Percentage of animals with $RAI \geq 0.5$. The extent of antibody maturation is presented as RAI value. VC groups showed much higher rates of RAI-positive ($RAI > 0.5$) piglets.

groups were noted. In the VC group, viremia peaked at 5 dpc and reduced rapidly and was almost cleared at 10 dpc. In the NC group, viral load was much higher and reduced much slower indicating rebound viremia at 14 dpc (Fig. 2).

3.3. Serological examinations

3.3.1. Avidity ELISA

In conventional ELISA, antibody titers of VC animals were significantly elevated in comparison to NC animals at all time points, except at 21 dpc (Fig. 3). High antibody titer at 0 dpc could be explained by the presence of pre-developed antibodies through vaccination.

To evaluate the degree of antibody avidity maturation, OD values from conventional and avidity ELISA were compared. Appearance rates of highly avidity-matured antibody is presented as RAI value and VC groups showed much higher rates of RAI-positive ($RAI > 0.5$) piglets (Fig. 4). Interestingly, 80% of VC animals (4 out of 5 animals) which survived until the end of the experiment showed shortened viremic periods with rapid serum viral load reductions. A strong negative correlation was observed between degrees of antibody avidity and viremia (Fig. 5). In these animals

a trend for reduced serum viral load was noted when RAI values peaked and vice versa. In NC piglets, no such correlation was observed due to the scarce amount of avidity-matured antibodies.

There were no significant differences between groups with regards to nAb titer. Mean nAb titers were below protective level (2^3) for the duration of the entire experiment (data not shown).

3.3.2. Serum cytokine levels

Serum cytokine levels were measured by quantitative ELISA. VC piglets showed a more active $IFN-\gamma$ response early after viral challenge than NC piglets. This trend continued in VC animals, peaking at 7 dpc, thereafter levels converged with NC group (Fig. 6).

Elevated levels of IL-10 were detected in NC animals compared to VC animals but no significant differences between the two groups were observed except at 7 and 14 dpc (Fig. 7).

4. Discussion

Although this experiment was performed against only one type 2 PRRSV isolate, VP-046, MLV vaccine showed a certain degree of efficacy against hetero-genotype PRRSV in the early course of infection. It was impossible, however, to observe significant differences

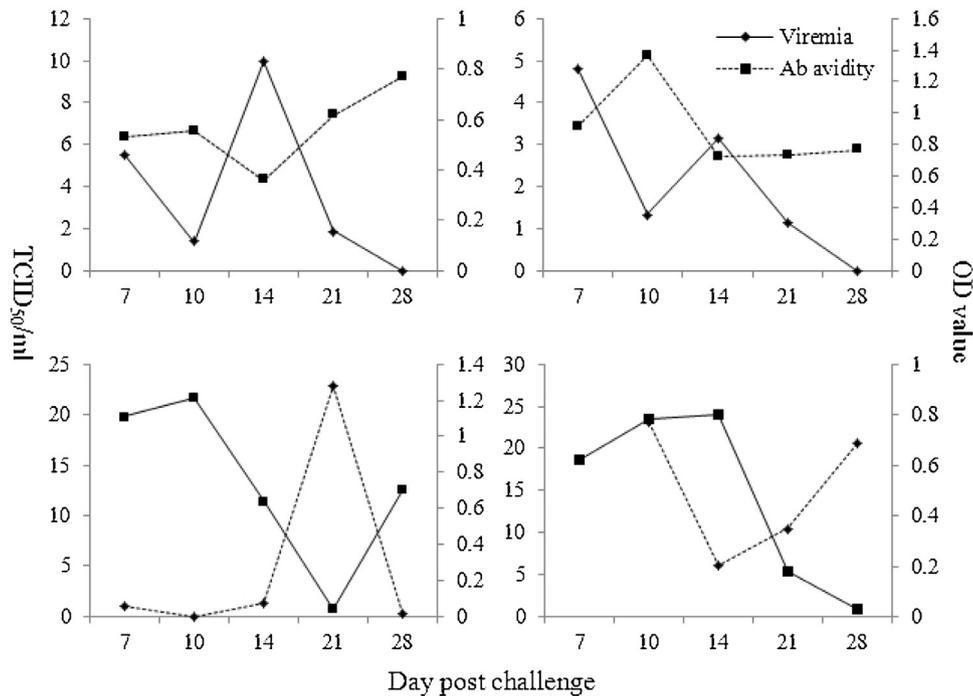


Fig. 5. Correlations between antibody avidity and virus load in serum. Each diagram depicts values for four individual pigs in NC group. In all animals presented, correlations were statistically significant ($P \leq 0.05$). A strong negative correlation was observed between the level of antibody avidity and viremia.

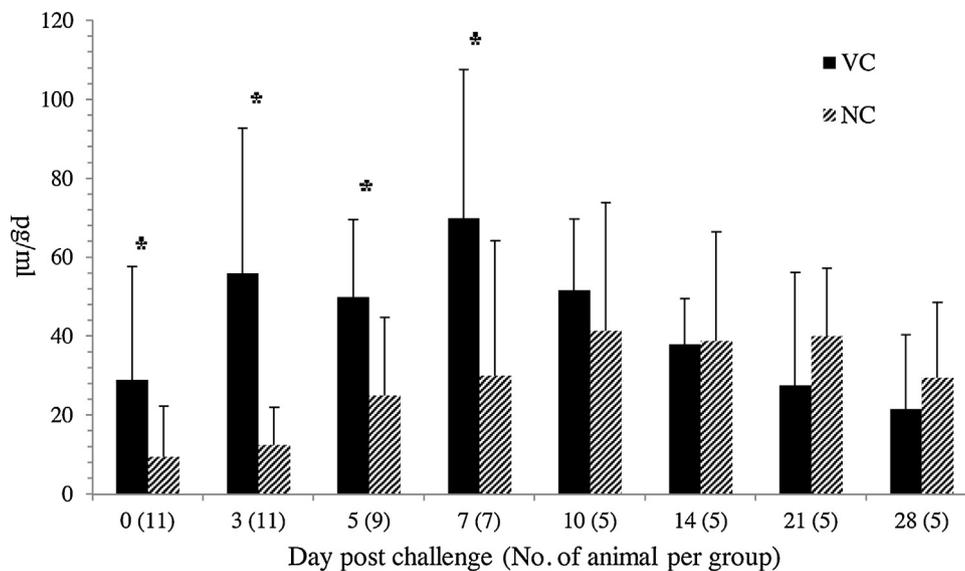


Fig. 6. Mean serum IFN- γ levels. VC piglets showed a more active IFN- γ response early after viral challenge than NC piglets, which peaked at 7 dpc and then converged with NC group levels.

in rectal body temperatures between groups, although weight gain was significantly improved in VC animals. Likewise, VC piglets displayed a decreased and shortened viremia compared to NC piglets. Collectively, these clinical parameters with respect to weight gain and serum viral load are in agreement with previous reports by Roca et al. (2012).

Several researchers have proposed that the nAb titer could be used as an indicative parameter to assess PRRSV efficacy in reducing clinical severity (Yoon et al., 1996; Lopez et al., 2007). Recently, however, a number of studies have indicated that elimination of PRRSV viremia often occurs before a nAb response was observed (Diaz et al., 2005; Molina et al., 2008). In the present study, nAbs were not detected early after infection. Although a huge amount of

antibody was detected by ELISA in VC animals, it was not able to clear PRRSV viremia by itself.

In the past, vaccine studies on human pathogenic viruses have focused on antibody avidity (Nair et al., 2009; Kemp et al., 2012; Kontio et al., 2012). Few studies, however, have dealt with avidity in PRRSV infection or vaccination. Interestingly, the present investigation revealed a negative correlation between antibody avidity and viral load. This relationship was unexpected because previous reports suggested that non-neutralizing antibody (non-nAb) produced against decoy epitopes do not grant PRRSV protection (Oleksiewicz et al., 2001; Fang et al., 2006; Mateu and Diaz, 2008). According to Oleksiewicz et al. (2002), however, epitopes recognized by B-cells are conserved between two genotypes of PRRSV.

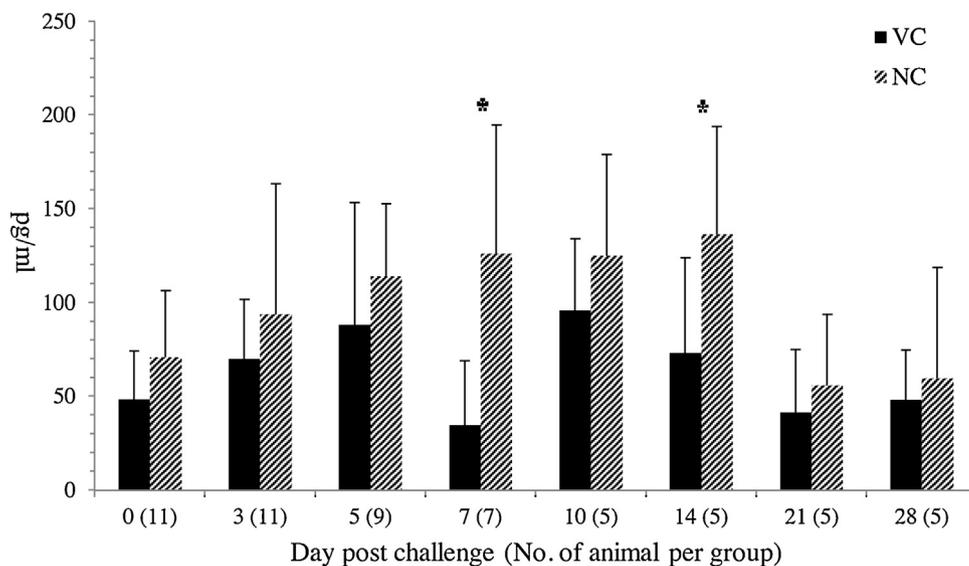


Fig. 7. Mean serum IL-10 levels. Elevated levels of IL-10 were detected in NC animals compared to VC animals but no significant differences between the two groups were observed except at 7 and 14 dpc.

Therefore, if non-nAbs play an important role in PRRSV protection, they may act in a cross-protective manner.

Unfortunately, there was a lack of correlation between nAb titer and antibody avidity. This phenomenon has already been observed in studies on measles and HIV vaccines (Bower et al., 2006; Kemp et al., 2012), indicating that the relationship between nAb titer and antibody avidity may be independent of their protective ability.

For numerous human pathogenic viruses, including measles virus, infected individuals or vaccine failures could be distinguished from vaccinated individuals and accurate estimations of vaccination time could be performed based on levels of antibody avidity (Paunio et al., 2000; Kontio et al., 2012). Furthermore, low levels of antibody avidity were measured in high-mortality pig groups and vice versa (unpublished findings). These findings imply that antibody avidity could be used as practical diagnostic index in field conditions. Clearly, follow-up studies on the kinetics of PRRSV infection and maturation of antibody avidity are required.

According to Klinge et al. (2009), the intensity of innate immunity might strongly influence the outcome of acute PRRSV infection. Increased serum levels of IFN- γ might be explained by the up-regulation of cells participating in cellular-mediated innate immune responses or by the responses of antigen specific T-cell activation as part of adaptive immunity. Presently, however, the cause of increased IFN- γ levels was not determined. Consequently, the role of highly avidity-matured antibody in IFN- γ secretion in response to PRRSV infection should be further investigated.

Together with previously reported theories, our findings suggest that non-nAbs, especially antibodies with high avidity, play an important role in reduction of serum viral load by stimulating an immune response such as activation of immune cells, not directly neutralizing PRRSV antigens. While few studies have investigated porcine antibody and its avidity maturation after infection, IgG2 was revealed to be the most important antibody subclass and it is in direct proportion to the age of individuals according to human antibody avidity studies (Lopatin and Blackburn, 1992; Nair et al., 2007). Thus, the likelihood of porcine mimicry to human antibody avidity traits should be further investigated, since presently whole antibody pool was detected without differentiating its isotypes and regardless of age-dependent avidity factors.

The study revealed a strong vaccination-mediated antibody response, even against heterogenotype PRRSV. Consequently, body weight gain was improved and reduced serum viral load was

observed. Furthermore, it was suggested that non-nAbs produced to target decoy epitopes might be important in cross-protection by vaccination. The ability of vaccine-mediated maturation of antibodies prior to PRRSV infection could be an indicator of protective efficacy. Furthermore, better understanding of the role of innate cellular immune responses in connection with vaccination-induced preformed antibodies in PRRSV vaccines may provide further insight into PRRSV vaccinology.

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