수의학석사학위논문

Vaccination with a porcine reproductive and respiratory syndrome virus vaccine at 1-day-old improved growth performance of piglets

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Abstract

Vaccination with a porcine reproductive and respiratory syndrome virus vaccine at 1-day-old improved growth performance of piglets

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Porcine reproductive and respiratory syndrome (PRRS) is the one of the most serious viral diseases of swine throughout the world. We showed in the study that PRRSV modified live-virus (MLV) vaccine was to evaluate the efficacy of this commercial PRRSV MLV vaccine when administered to one-day-old piglets under field conditions based on clinical, virological, immunological, and pathological criteria. A population of 40 one-day-old piglets in each individual three farms, which had undergone production losses due to respiratory diseases related to PRRSV, was designed to 20 vaccination pigs and the other 20 as unvaccinated pigs. The vaccinated groups in three farms were intramuscularly inoculated with a 2.0ml dose of Fostera PRRSV MLV vaccine respectively, whereas unvaccinated groups were performed by inoculation of 2.0 ml dose of PBS. Compared to unvaccinated group, the vaccinated groups turned out that the overall growth performance was significantly enhanced and the lung lesions were reduced effectively under field conditions at 182 days of age. Moreover, the study

demonstrated that the vaccinated one-day-old piglets can trigger significantly the elicitation of both humoral and cell-mediated immune responses (as measured by anti-PRRSV antibody titers and number of PRRSV-specific IFN-γ-SC) compared to the unvaccinated piglets. These results suggest that inoculation PRRSV MLV vaccine to one-day-old piglets is efficient in improving growth performance from one day all the way to 182 day in endemic farms suffering with PRRSV-2 infection or PRRSV-1 and PRRSV-2 infection.

Keywords:
1-day-old pig; porcine reproductive and respiratory syndrome virus; Vaccination; Maternally derived antibodies

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LIST OF ABBREVIATIONS

CMI	Cell mediated immunity
ELISA	Enzyme-linked immune-sorbent assay
IFN-γ-SCs	Interferon gamma secreting cells
IHC	Immunohistochemistry
IL	Interleukin
ISH	*in situ* hybridization
NAb	Neutralizing antibody
MLV	Modified live virus
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
PMWS	Postweaning multisystemic wasting syndrome
PRDC	Porcine respiratory disease complex
PRRS	Porcine reproductive and respiratory syndrome
Th	Helper T lymphocyte
$TCID_{50}$	Median tissue culture infective dose

Abstract

A porcine reproductive and respiratory syndrome virus (PRRSV) modified live-virus (MLV) vaccine was evaluated under field conditions for registration as recommended by the Republic of Korea's Animal, Plant & Fisheries Quarantine & Inspection Agency. A single dose of the vaccine was administered to 1-day-old piglets and their growth performance was monitored under field conditions. Three separate farms were selected based on their history of PRRSV-associated respiratory diseases. On each farm, 40 pigs were randomly allocated to one of two treatment groups: (i) vaccinated (n = 20) and (ii) unvaccinated (n = 20) pigs at 1 day of age. Vaccinated pigs showed an increase in their market weight by 6.23 kilograms per pig compared to the unvaccinated pigs (98.01 kilograms in vaccinated group vs. 91.78 kilograms in unvaccinated group; $P < 0.05$) and exhibited a decrease in mortality rate by 6.7%P (3.3% in vaccinated group vs. 10% in unvaccinated group; $P < 0.05$). The pigs had a sufficiently mature immune system for the vaccine to elicit humoral and cell-mediated immunity (as measured by anti-PRRSV antibodies and PRRSV-specific interferon-γ secreting cells, respectively) at 1 day of age even in the presence of maternally derived antibodies. The results presented in this study demonstrate that the PRRSV MLV vaccine is effective in improving growth performance from day 1 all the way to day 182 in endemic farms suffering with PRRSV-2 infection or both PRRSV-1 and PRRSV-2 infection.

Keywords:

1-day-old pig

Porcine reproductive and respiratory syndrome virus

Vaccination

Maternally derived antibodies

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most economically important disease to the global swine industry. PRRS is a combination of reproductive disorders in sows and gilts such as abortions, premature farrowing, mummified and stillborn fetuses, and respiratory disorders with poor growth performance in pigs from nursery to finishing period (Zimmerman et al., 2012). The causative agent for PRRS is the PRRS virus (PRRSV), which is a single-stranded, enveloped RNA virus belonging to the *Arteriviridae* family. There are two main types, PRRSV-1 (European origin) and PRRSV-2 (North American origin) (Snijder and Meulenberg, 1998).

In Korean farms, PRRSV vaccines are typically administered into pigs at 21 days of age. However, the age of the first infection with PRRSV in Korean farm has recently been getting younger. During a two-year period between 2015-2016, serum samples from 352 weaned pigs at 3 to 5 weeks of age were examined by real-time polymerase chain reaction (PCR) at the Department of Veterinary Pathology, Seoul National University. PRRSV was detected in 153 cases compared to 67 out of 347 samples from the previous two years (2013-2014). Theoretically, all these cases could have been prevented through vaccination 21-day-old which is currently recommended by manufactures. Early vaccination however has its own caveats, (i) the immune system may not be mature enough to efficiently respond to the vaccination and (ii) maternally derived antibodies may interfere with the efficacy of vaccination. Recently, a commercial PRRSV modified live-virus (MLV) vaccine (Fostera^{TM}PRRS, Zoetis, Parsippany, New Jersey, USA) was licensed for 1-day-old vaccination in 2015 (http://www.zoetis.com). However, no in-depth evaluation of the efficacy of this vaccine when administered to 1-day-old piglets has been performed under field

conditions. The objective of this study was to evaluate the efficacy of this commercial PRRSV MLV vaccine when administered at day 1 under field conditions based on clinical, virological, immunological, and pathological criteria.

2. Materials and methods

2.1. Farms history

The clinical field trial was conducted on 3 separate farms; farms A (230-sow), B (420-sow), and C (230-sow), respectively. The 3 farms are one-site and continuous production systems. The three selected farms had suffered recent losses due to respiratory diseases by co-infection with PRRSV-1 and PRRSV-2 in farm A and B, and by infection with PRRSV-2 in farm C in post-weaning and growing pigs.

Prior to the beginning of this study, post-weaning pigs from all farms were submitted to the Department of Veterinary Pathology in Seoul National University. Lung and tonsil were pooled, homogenized, and cultured in MARC-145 cells and porcine alveolar macrophages for field PRRSV isolation.

2.2. Experimental design

In each of the three farms, ten healthy pregnant sows, (parity = 1 or 2) at 7 days antepartum were randomly selected, and allocated to treatment and pen using the random number generation function (Excel, Microsoft Corporation, Redmond, Washington, USA). Sows were housed in individual crates with an empty crate between each sow to minimize shed of vaccine virus to controls. From the ten sows, five sows were designated as vaccinated group while the other five were designated as unvaccinated group. After farrowing, four healthy newborn piglets (two male and two female) from each one of the 5 sows designated as vaccinated pigs and 4 healthy newborn piglets (2 male and 2 female) from each of the 5 sows designated as unvaccinated pigs using the random number generation function (Excel, Microsoft Corporation. Pigs in VacA (n = 20, male = 10 and female = 10), VacB (n = 20, male = 10 and female = 10), and VacC (n = 20, male = 10 and female = 10) groups from farms A, B, and C, respectively, were intramuscularly injected with a 2.0 mL dose of PRRSV MLV vaccine (Fostera^{TM} PRRS, Zoetis, Lot No. 169588, Serial No. 163540/159469, Expiration date 28NOV17,

PRRS Potency 4.5 $\log_{10}$ $TCID_{50}$/dose) at 1 day old. Pigs in UnVacA (*n* = 20, male = 10 and female = 10), UnVacB (*n* = 20, male = 10 and female = 10), and UnVacC (*n* = 20, male = 10 and female = 10) groups from farms A, B, and C, respectively, were intramuscularly injected with a 2.0 mL dose of (PBS, 0.01M, pH 7.4) at 1 day old. Blood samples from all vaccinated pigs and unvaccinated pigs were collected by jugular venipuncture at 1 (prior to vaccination), 7, 14, 21, 35, 49, 70, 91, 140 and 182 days of age.

At weaning (approximately 21 days of age), vaccinated pigs and unvaccinated pigs stayed on site in their farms based on the Korean field study protocol. They were housed by treatment, a minimum of four pens per treatment, 5 pigs per pen (in the same barn). The five selected pigs from each sow were randomly assigned to one of four pens such that each pen contained pigs from only one litter using the random number generation function (Excel, Microsoft Corporation). Pens were randomly assigned to litters/treatments with an empty pen between each occupied pen to minimize the shedding of the vaccine virus to the controls. Five pigs from vaccinated groups (one pig from each sow) and unvaccinated groups (one pig from each sow) were randomly selected from each farm for euthanasia and necropsy at 91 days of age. Lung samples were collected from all the remaining pigs at the time of. All methods were approved by the Seoul National University Institutional Animal Care and Use Committee.

2.3. Clinical observation

Following vaccination, the pigs were monitored weekly for their physical condition and scored weekly for clinical respiratory disease severity using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (Halbur et al., 1995). Observers were blinded to the vaccination status.

2.4. Growth performance

The live weight of each pig was measured at 1 (prior to vaccination), 21(at weaning), 70, 91, 140, and 182 days of age. The average daily weight gain (ADWG; gram/pig/day) was analyzed over five time periods: (i) between 1 and 21 days of age, (ii) between 21 and 70 days of age, (iii) between 70 and 91 days of age, (iv) between 91 and 140 days of age, and (v) between 140 and 182 days of age. ADWG during the different production stages was calculated as the difference between the starting and final weight divided by the duration of the stage. Data from dead or removed pigs were excluded in the calculation.

2.5. Quantification of PRRSV RNA

Total RNA was extracted from 250 μL of serum sample by using 750 μL of TRIzol LS (Invitrogen, San Diego, California, USA), following the manufacturer's instructions. All serum samples were tested for PRRSV-1 and PRRSV-2 genomic cDNA and copy number were quantified by real-time PCR as previously described (Wasilk et al., 2004). For PRRSV-1 field isolate, the forward and reverse primers were 5'- TGGCCAGTCAGTCAATCAAC-3' and 5'-AATCGATTGCAAGCAGAGGGAA-3', respectively. For PRRSV-2 field isolate, the forward and reverse primers were 5'-TGGCCAGTCAGTCAATCAAC-3' and 5'-AATCGATTGCAAGCAGAGGGAA-3', respectively (Wasilk et al., 2004, Park et al., 2014). For the vaccine virus, the forward and reverse primers were 5'-CTTGACACAGTTGGTCTGGTTACT-3' and 5'-GTTCTTCGCAAGCCTAATAACG-3', respectively (Park et al., 2014). Real-time PCR for the field and vaccine viruses was performed as previously described (Park et al., 2014). The real-time PCR was considered positive as having a cycle threshold (Ct) level <37 cycles.

2.6. Serology

The serum samples were tested using the commercially available PRRSV enzyme-linked

immunosorbent assay (ELISA; HerdCheck PRRS 3XR™, IDEXX Laboratories Inc., Westbrook, Maine, USA). Serum samples were considered positive for PRRSV antibody when the S/P ratio ≥0.4, according to the manufacturer's instructions.

Serum virus neutralization tests were performed with the field virus, as previously described (Yoon et al., 1994, Wu et al., 2001, Zuckermann et al., 2007). The presence of virus-specific cytopathic effect (CPE) in each well was recorded after incubating for 5 days. The presence of virus in wells without CPE was further determined by immunofluorescence microscopy using an SDOW17-FITC conjugate. The neutralizing antibody titers of each serum were determined as the reciprocal of the highest dilution in which no evidence of virus growth was detected. Serum samples were considered to be positive for neutralizing antibody if the titer was greater than 2.0 (log_2) (Zuckermann et al., 2007).

2.7. Enzyme-linked immunospot (ELISPOT) assay

The numbers of PRRSV-specific interferon-γ secreting cells (IFN-γ-SC) were determined in peripheral blood mononuclear cells (PBMC) as previously described (Meier et al., 2003; Diaz and Mateu, 2005; Park et al., 2014) with some modifications. Briefly, 5×10^5 PBMC was plated in 96-well microplate precoated with swine specific IFN-γ antibody (10 μg/mL, MABTECH). Cells were stimulated with field virus at multiplicity of infection (MOI) of 0.01 as the recall antigen for 20 h incubation at 37°C in a 5% CO_2 atmosphere. Unstimulated cells and phytohemagglutinin (10 μg/mL)-stimulated cells were used as negative and positive controls, respectively (Meier et al., 2003; Diaz and Mateu, 2005; Park et al., 2014). The spots on the membranes were read by an automated ELISPOT Reader (AID ELISPOT Reader, AID GmbH, Strassberg, Germany). The results were expressed as the numbers of IFN-γ-SC per 10^6 PBMC.

2.8. Pathology

The estimation of macroscopic lung lesions (ranging from 0 to 100% of the affected lung) was based on the percentage of the volume of the entire lung and the percentage volume from each lobe added to the entire lung score (Halbur et al., 1995). Microscopic lung lesion and in situ hybridization (ISH) were performed on three blocks of lung tissues, which included eight pieces of lung: two piece from the right cranial lobe, two from the right middle lobe, one from the ventromedial part of the right caudal lobe, one from the dorsomedial part of the right caudal lobe, one from the midlateral part of the right caudal lobe, and one from the accessory lobe of each pig. The choice of lung tissues was based on the presence of macroscopic lesions. Microscopic lung lesions were scored blindly on a scale from 0 (normal) to 4 (severe diffuse) by two pathologists (Halbur et al., 1995).

ISH was performed to detect PRRSV-1 and PRRSV-2 in lung tissues as previously described (Han et al., 2013). Three sections were cut from each of three blocks of tissue from one entire pulmonary lobe of each pig. In each slide, 10 fields were randomly selected, and the number of positive cells per unit area (0.95 mm^2) was analyzed with the NIH Image J 1.51r Program (http://imagej.nih.gov/ij/download.html) (Halbur et al., 1996a). The mean values were also calculated.

2.9. Statistical analysis

The data from each farm was analyzed independently because of different time and types of PRRSV infection in growing pigs in 3 farms. The data (the respiratory scores, ADWG, logarithm transformed serology, logarithm PRRSV RNA quantification, the macroscopic and microscopic lung lesion scores, and IHC scores) was analyzed with a general linear mixed model. A general linear mixed model was adjusted with different time intervals. The fixed effects of the generalized linear mixed model included treatment and time point. The random effects of the model included room and animal. A value of $P < 0.05$ (two-tailed) indicates a statistical significance.

3. Results

3.1. Virus isolation

PRRSV-1 was isolated from tissue homogenates; SNUVR160525 (GenBank no. KY883681) from farm A and SNUVR160605 (GenBank no. KY883683) from farm B. PRRSV-2 was isolated from tissue homogenates; SNUVR160590 (GenBank no. KY883689, lineage 5) from farm A, SNUVR160593 (GenBank no. 883690, lineage 5) from farm B, and SNUVR160313 (GenBank no. KY883686, lineage 1) from farm C based on analysis of the open reading frame 5 (ORF5) (Shi et al., 2010). The vaccine strain (P129, GenBank no. AF494042) and field PRRSV-1 viruses share 54.3% (farm A) and 53.3% (farm B) identity for ORF5 amino acid sequence, respectively. The vaccine strain (P129) and field PRRSV-2 viruses share 91.0% (farm A), 86.5% (farm B), and 86.5% (farm C) identity for ORF5 amino acid sequence, respectively.

3.2. Clinical observation

The mean respiratory scores were significantly lower ($P < 0.05$) in pigs from the VacA group compared to pigs from the UnVacA group at 49, 63, 70, and 77 days of age (Fig. 1A). The mean respiratory scores were significantly lower ($P < 0.05$) in pigs from the VacB group compared to pigs from the UnVacB group at 49, 56, and 63 days of age (Fig. 1B). The mean respiratory scores were significantly lower ($P < 0.05$) in pigs from the VacC group compared to pigs from the UnVacC group at 35, 42, and 49 days of age (Fig. 1C).

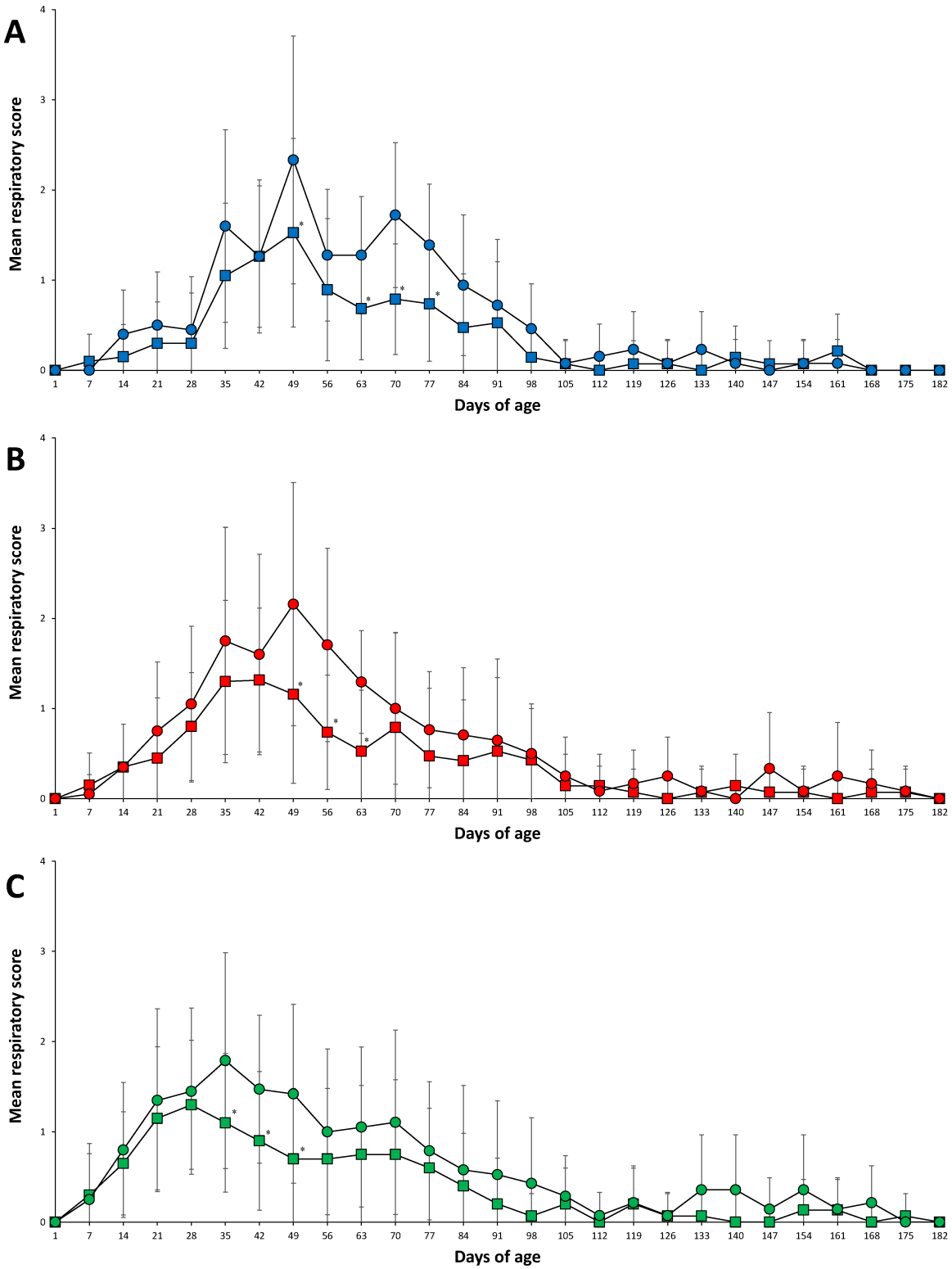

Fig. 1. Mean respiratory score from farm A (A) in VacA (■) and UnVacA (●), farm B (B) in VacB (■) and UnVacB (●), and farm C (C) in VacC (■) and UnVacC (●) groups. Variation is expressed as the standard deviation. *Significant difference ($P < 0.05$) between vaccinated and unvaccinated group within the same farm.

3.3. Growth performance

In farm A, there was no significant difference in body weight between the VacA (average weight 1.74 kg ± 0.30) and UnVacA (average weight 1.71 kg ± 0.31) groups at 1 day of age. After vaccination, the ADWG was significantly higher ($P < 0.05$) in pigs from the VacA group compared to pigs from the UnVacA group during the 70-91, 91-140 and 140-182 days period. In farm B, there was no significant difference in body weight between the VacB (average weight 1.70 kg ± 0.30) and UnVacB (average weight 1.73 kg ± 0.28) groups at 1 day of age. In farm C, there was no significant difference in body weight between the VacC (average weight 1.85 kg ± 0.31) and UnVacC (average weight 1.87 kg ± 0.33) groups at 1 day of age. After vaccination, the ADWG was significantly higher ($P < 0.05$) in pigs from the VacC group compared to pigs from the UnVacC group during the 21-70, 70-91 and 91-140 days periods. The overall growth performance (1 to 182 days of age) of pigs in the vaccinated group was significantly higher ($P < 0.05$) compared to pigs in the unvaccinated group at all 3 farms (Table 1).

Table 1. Data (mean ± standard deviation) for average daily weight gain (ADWG), mortality rate, pathology, and in situ hybridization (ISH) between vaccinated and unvaccinated animals on 3 farms.

	Age (day)	Farm A		Farm B		Farm C	
		VacA	UnVacA	VacB	UnVacB	VacC	UnVacC
ADWG (gram/pig/day)	1-21	127 ± 29	137 ± 23	139 ± 38	134 ± 34	157 ± 33	153 ± 32
	21-70	462 ± 94	417 ± 108	368 ± 64	341 ± 34	480 ± 91*	407 ± 76
	70-91	746 ± 167*	534 ± 164	631 ± 222	535 ± 165	660 ± 55*	585 ± 108
	91-140	589 ± 65*	653 ± 79	645 ± 79	616 ± 45	732 ± 51*	692 ± 49
	140-182	703 ± 86*	613 ± 88	583 ± 78	564 ± 90	547 ± 72	581 ± 87
	1-182	549 ± 18*	508 ± 16	496 ± 25*	463 ± 25	550 ± 39*	520 ± 34
Market weight (kg)		101.0 ± 3.2*	93.7 ± 3.0	91.5 ± 4.6*	85.5 ± 4.6	101.5 ± 7.0*	96.1 ± 6.1
Mortality rate		1/20	2/20	1/20	3/20	0/20	1/20
Lung lesion scores							
Macroscopic	182	16 ± 7.60*	32 ± 8.18	17 ± 9.93*	35 ± 13.76	10 ± 8.56*	25 ± 9.72
Microscopic	182	0.9 ± 0.26	1.2 ± 0.36	1.0 ± 0.53	1.5 ± 0.65	0.6 ± 0.49	1.0 ± 0.53
ISH scores							
PRRSV-1	182	0.6 ± 0.97	0.6 ± 0.92	0.6 ± 0.89	0.7 ± 0.85	0 ± 0	0 ± 0
PRRSV-2	182	1.1 ± 1.19	0.9 ± 0.92	0.9 ± 0.83	0.9 ± 1.04	0.9 ± 0.93	0.6 ± 0.89

*Significant difference ($P < 0.05$) between vaccinated and unvaccinated groups within the same farm.

3.4. Mortality

In farm A, 1 vaccinated pig died of pneumonic pasteurellosis caused by *Pasteurella multocida* at 6 weeks of age, and 2 unvaccinated pigs died of Glasser's disease caused by *Haemophilus parasuis* at 6 and 7 weeks of age. In farm B, 1 vaccinated pig died at 6 weeks of age without any pneumonia or pathological lesions, 2 unvaccinated pigs died of pneumonic pasteurellosis and mycoplasmal pneumonia caused by *Pasteurella multocida* and *Mycoplasma hyopneumoniae* at 7 and 8 weeks of age, and 1 unvaccinated pig died of Glasser's disease caused by *Haemophilus parasuis* at 8 weeks of age. In farm C, 1 unvaccinated pig died of polyserositis caused by *Streptococcus suis* at 5 weeks of age (Table 1).

3.5. Quantification of PRRSV RNA in sera

No genomic copies of the vaccine virus and field virus were detected in the serum of pigs from either the vaccinated or the unvaccinated pigs at the time of vaccination (1 day of age) at all 3 farms. Vaccine virus genomes could be detected in all pigs from the VacA, VacB, and VacC groups up to 21 days of age. In the VacA group, vaccine virus genomes could be detected in the sera of 12/20 pigs at 35 days of age and 3/19 pigs at 49 days of age. In the VacB group, vaccine virus genomes were detectable in 10/20 pigs at 35 days of age and 2/19 pigs at 49 days of age. In the VacC group, vaccine virus genomes could be detected in 9/20 pigs at 35 days of age and 3/20 pigs at 49 days of age. No vaccine virus was detected in the serum of any of the unvaccinated pigs from all 3 farms throughout the experiment.

Pigs from the VacA group had a significantly lower ($P < 0.05$) number of genomic copies of PRRSV-1 in their blood compared to pigs from the UnVacA group at 35 days of age (Fig. 2A). Pigs from the VacB group had a significantly lower ($P < 0.05$) number of genomic copies of PRRSV-1 in their blood compared to pigs from the UnVacB group at 49 days of age (Fig. 2B). No genomic copies of PRRSV-1 were detected in the serum of any of the pigs from the VacC and UnVacC groups throughout the experiment (Fig. 2C).

There was no significant difference in genomic copies of PRRSV-2 in the blood between vaccinated and unvaccinated pigs on farm A (Fig 3A) and farm B (Fig. 3B) throughout the study. At 21 days of age, pigs from the VacC group had a significantly lower ($P < 0.05$) number of genomic copies of PRRSV-2 in their blood compared to pigs from the UnVacC group (Fig. 3C).

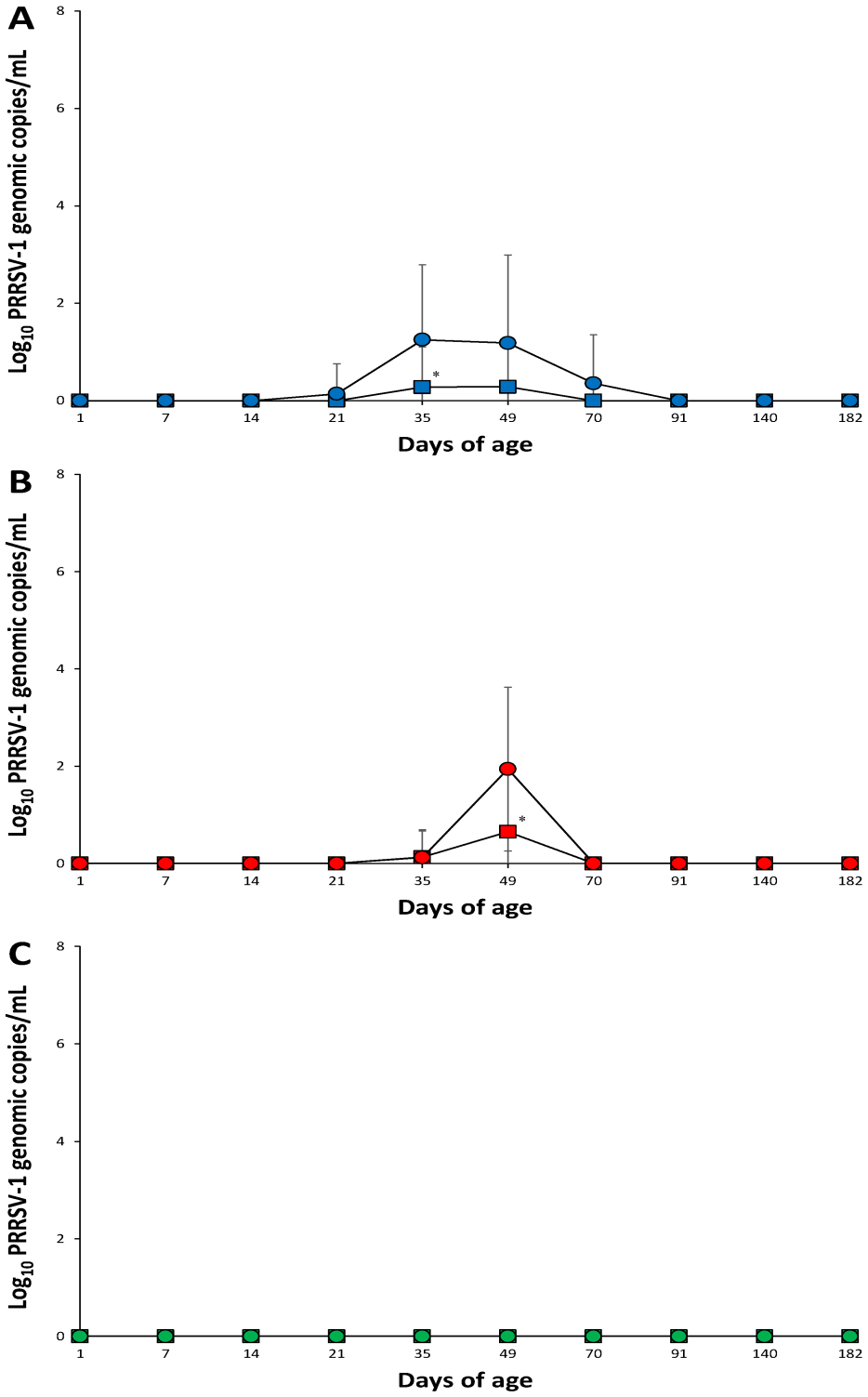

Fig 2. Mean values of the genomic copy number of PRRSV-1 in sera of pigs from farm A (A) in VacA (■) and UnVacA (●), farm B (B) in VacB (■) and UnVacB (●), and farm C (C) in VacC (■) and UnVacC (●) groups. Variation is expressed as the standard deviation. *Significant difference ($P < 0.05$) between vaccinated and unvaccinated group within the same farm.

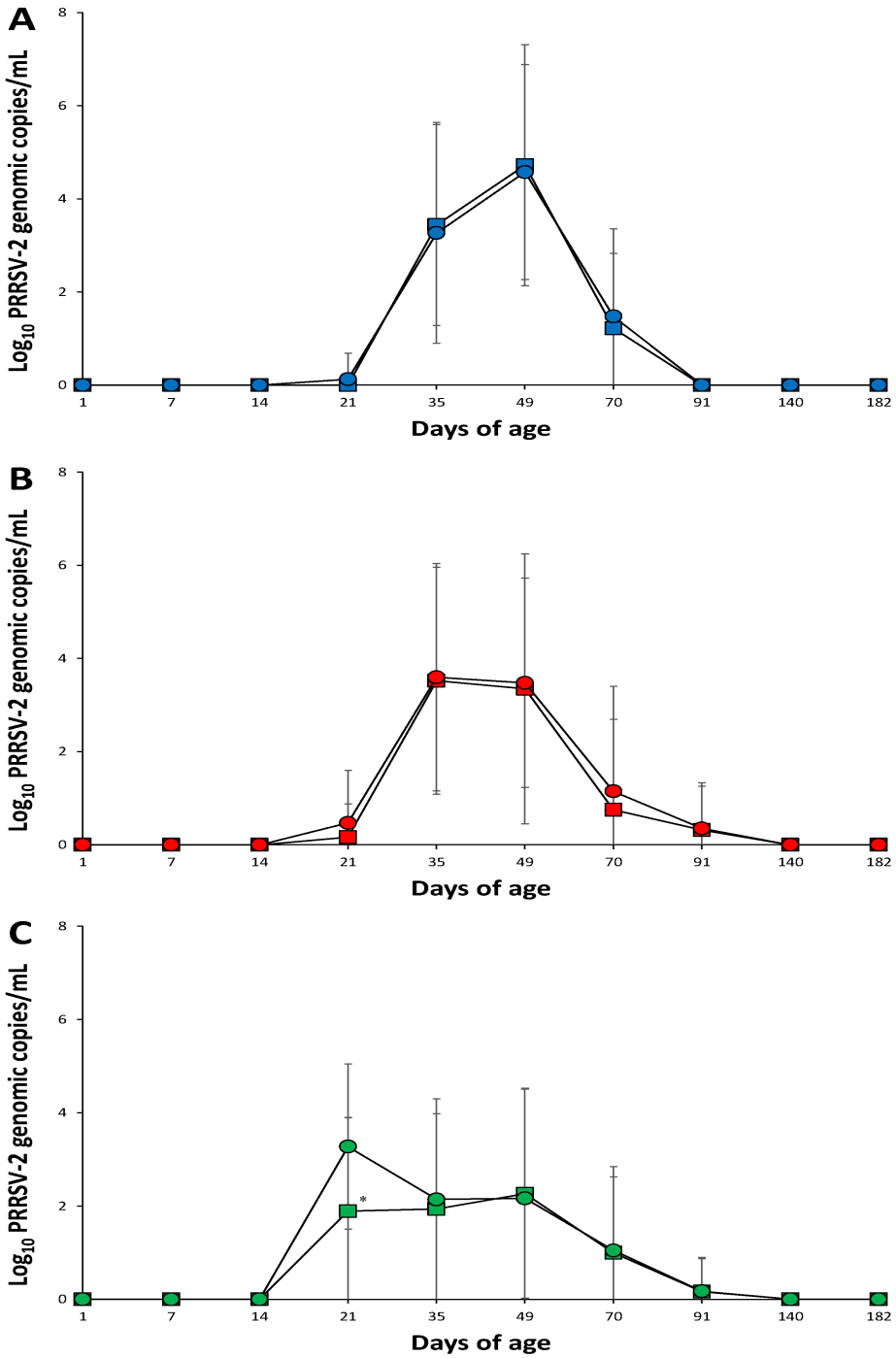

Fig 3. Mean values of the genomic copy number of PRRSV-2 in sera of pigs from farm A (A) in VacA (■) and UnVacA (●), farm B (B) in VacB (■) and UnVacB (●), and farm C (C) in VacC (■) and UnVacC (●) groups. Variation is expressed as the standard deviation. *Significant difference ($P < 0.05$) between vaccinated and unvaccinated group within the same farm.

3.6. Serology

Anti-PRRSV antibody titers were significantly higher ($P < 0.05$) in pigs from the VacA group compared to pigs from the UnVacA group at 21, 35, and 70 days of age (Fig. 4A). Anti-PRRSV antibody titers were significantly higher ($P < 0.05$) in pigs from the VacB group compared to pigs from the UnVacB group at 21 and 35 days of age (Fig. 4B). Anti-PRRSV antibody titers were significantly higher ($P < 0.05$) in pigs from the VacC group compared to pigs from the UnVacC group at 14 and 21 days of age (Fig. 4C).

Neutralizing antibody titers against PRRSV were not significantly different between vaccinated and unvaccinated pigs in farms A and B (Fig. 5A). Neutralizing antibody titers against PRRSV-2 were significantly higher ($P < 0.05$) in pigs from the VacC group compared to pigs from the UnVacC group at 35, 49, and 70 days of age (Fig. 5B).

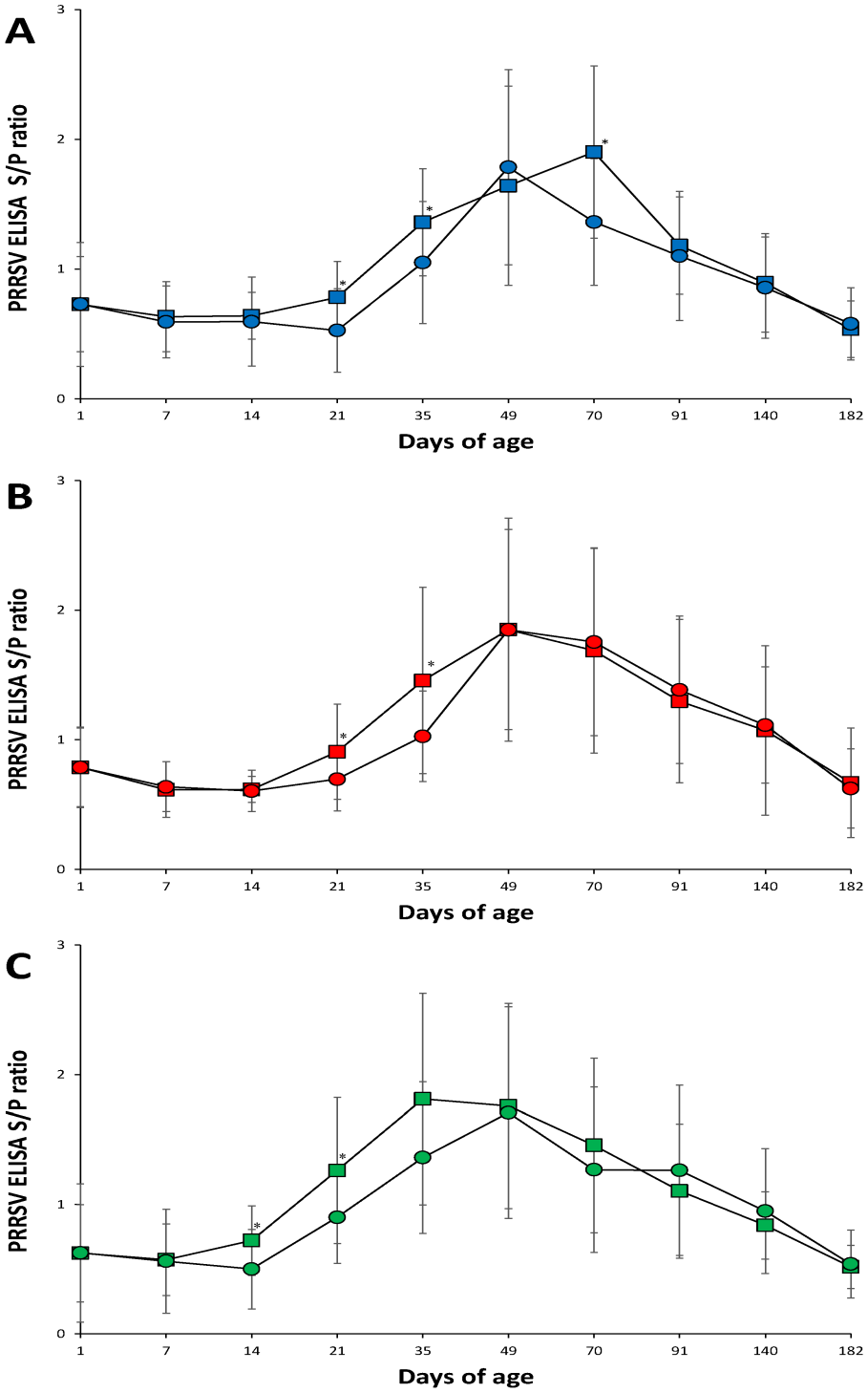

Fig 4. Mean values of the anti-PRRSV ELISA antibodies in sera of pigs from farm A (A) in VacA (■) and UnVacA (●), farm B (B) in VacB (■) and UnVacB (●), and farm C (C) in VacC (■) and UnVacC (●) groups. Variation is expressed as the standard deviation. *Significant difference ($P < 0.05$) between vaccinated and unvaccinated group within the same farm.

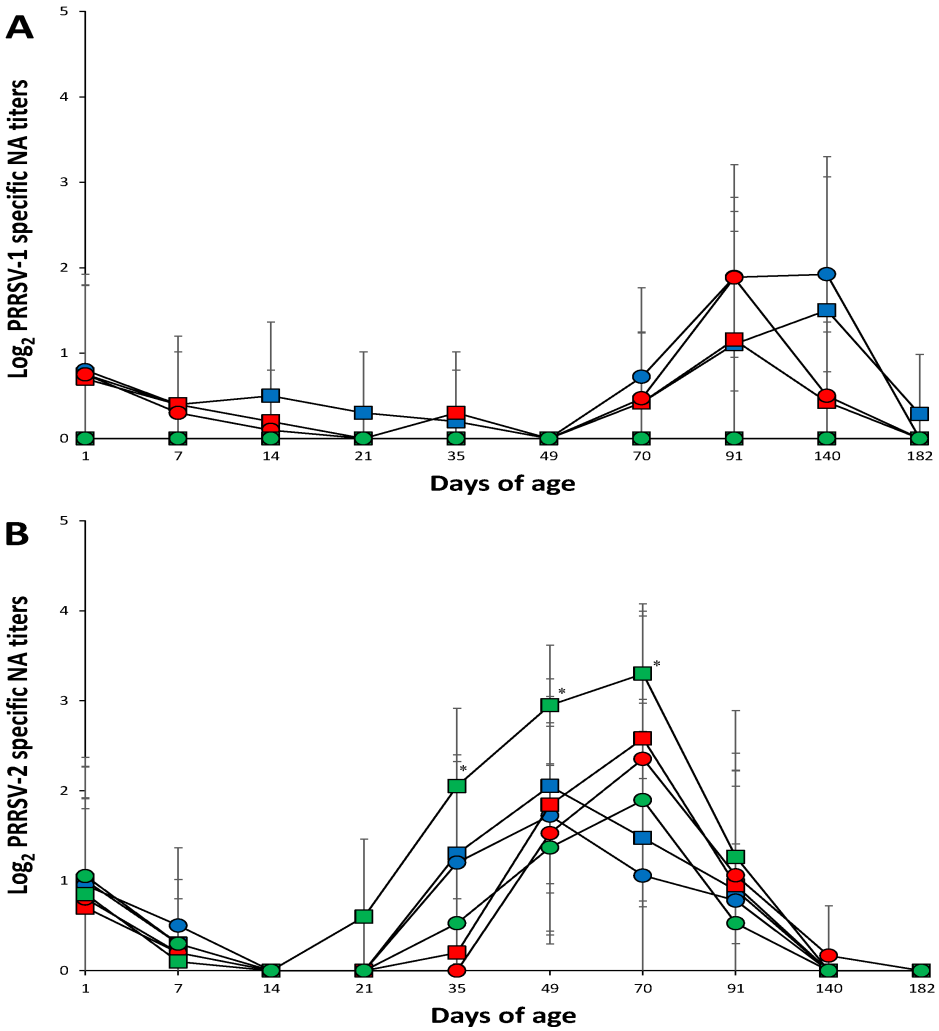

Fig 5. Mean values of PRRSV-1 (A) and PRRSV-2 (B) specific neutralizing antibody (NA) titers in sera of pigs from VacA (■), UnVacA (●), VacB (■), UnVacB (●), VacC (■), and UnVacC (●) groups. Variation is expressed as the standard deviation. *Significant difference ($P < 0.05$) between vaccinated and unvaccinated group within the same farm.

3.7. Interferon-γ secreting cells

Pigs from the VacA and VacB groups had a significantly higher ($P < 0.05$) number of PRRSV-1 specific IFN-γ-SC in PBMC compared to pigs from the UnVacA and UnVacB groups at 14, 21, and 35 days of age (Fig. 6A, B, and C).

Pigs from the VacA group had a significantly higher ($P < 0.05$) number of PRRSV-2 specific IFN-γ-SC in PBMC compared to pigs from the UnVacA group at 7, 14, 21, 35, 49, and 70 days of age. Pigs from the VacB and VacC groups had a significantly higher ($P < 0.05$) number of PRRSV-2 specific IFN-γ-SC in PBMC compared to pigs from the UnVacB and UnVacC groups, respectively, at 14, 21, 35, and 49 days of age (Fig. 7A, B, and C).

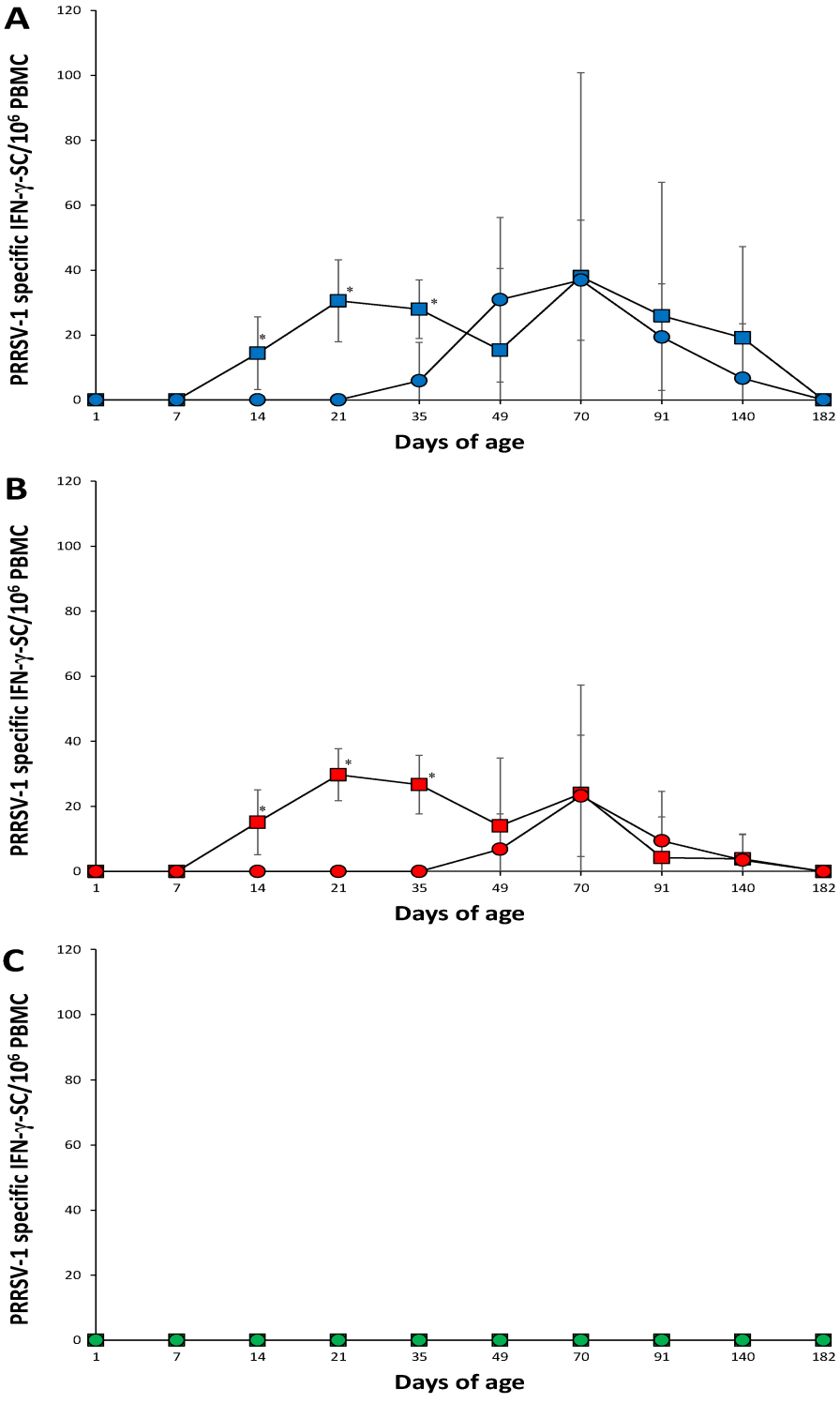

Fig 6. Mean values of the PRRSV-1 specific IFN-γ-SC/10^6 PBMC from farm A (A) in VacA (■) and UnVacA (●), farm B (B) in VacB (■) and UnVacB (●), and farm C (C) in VacC (■) and UnVacC (●) groups. Variation is expressed as the standard deviation. *Significant difference ($P < 0.05$) between vaccinated and unvaccinated group within the same farm.

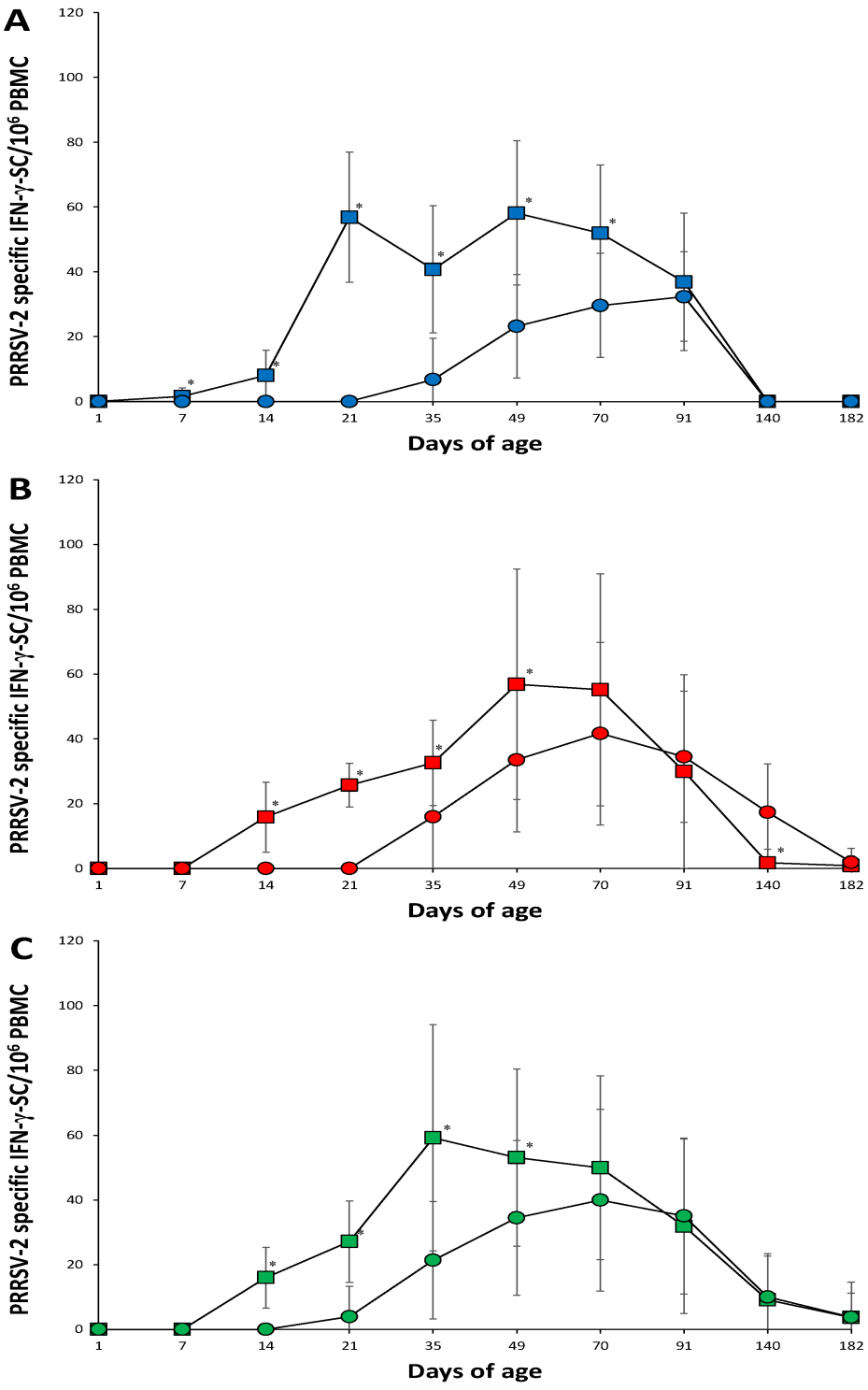

Fig 7. Mean values of the PRRSV-2 specific IFN-γ-SC/10^6 PBMC from farm A (A) in VacA (■) and UnVacA (●), farm B (B) in VacB (■) and UnVacB (●), and farm C (C) in VacC (■) and UnVacC (●) groups. Variation is expressed as the standard deviation. *Significant difference ($P < 0.05$) between vaccinated and unvaccinated group within the same farm.

3.8. Pathology

Pigs from the VacA, VacB, and VacC groups had significantly lower ($P < 0.05$) macroscopic lung lesion scores compared to pigs from the UnVacA, UnVacB, and UnVacC groups at 182 days of age. There was no significant difference in microscopic lung lesion scores and PRRSV positive cells scores between vaccinated and unvaccinated pigs in all 3 farms at 182 days of age (Table 1).

4. Discussion

A single-dose PRRSV vaccination of piglets at 1-day of age resulted in significantly improved growth performance and in reduced lung lesions under field conditions. The improved growth performance and the reduced lung lesions in vaccinated piglets indicated that piglets at 1 day of age are capable of mounting a protective immune response. In a previous study, pigs vaccinated at 21 days of age were protected against respiratory diseases (Park et al., 2014), further indicating that both day 1 and day 21 vaccination protocols were effective.

One of the main concerns for early vaccination has always been that the immune system may not be mature enough to respond to the vaccination efficiently. Anti-PRRSV ELISA antibodies and PRRSV-specific IFN-γ-SC were significantly higher in vaccinated pigs compared to unvaccinated pigs at 20 days post vaccination (21 days of age). These data indicate that the immune system of the 1 day old piglet is mature enough to elicit humoral (as measured by anti-PRRSV ELISA antibodies) and cell-mediated (as measured by IFN-γ-SC) immune responses after vaccination at 1 day of age. Our data are also supported by other studies, where vaccination of piglets at day 5 resulted in the development of a detectable humoral immune response and provided protection against PCV2 viremia and lymphoid lesions after challenge (O'Neill et al., 2011). In addition, vaccination of piglets at day 7 with a single-dose *Mycoplasma hyopneumoniae* vaccine induced protective immunity and protected against lung lesions after challenge (Reynolds et al., 2009; Wilson et al., 2013).

Another concern about early vaccination is whether the passively acquired maternal antibodies interfere with vaccination. Interference with PRRS vaccination depends on the initial levels of maternally derived NA. High maternally derived NA (≈ 7.0 $\log_2$) influence the course of the humoral and cell-mediated responses after vaccination with PRRS MLV at 21 days old (Fablet et al., 2016). In this field study we were unable to determine the effect of maternally derived NA and whether they interfere with PRRSV vaccination at 1 day old because of low levels of maternally derived NA (2 to 3

log_2) at the time of vaccination. A humoral immune response was clearly elicited when the PRRSV MLV vaccine was administered to 1-day-old pigs regardless of the levels of maternally derived antibodies in two of the farms. In farm C, the interference of maternal NA could not be accurately analyzed because 21-day-old pigs had already become infected with field PRRSV. Regardless, the PRRSV MLV vaccine used in this study was able to effectively overcome maternal immunity and induce anti-PRRSV antibodies even in the presence of maternally derived antibodies. The fact that these pigs had become infected with a field PRRSV at around 21 days of age might suggest that the anti-PRRSV antibody titers and the increase in the numbers of IFN-γ-SC which peaked at around 49 days of age, are mainly due to the PRRSV infection and not vaccination. However, anti-PRRSV antibody titers and the number of IFN-γ-SC were already significantly higher in vaccinated pigs prior to the PRRSV infection at 21 days of age and remained significantly higher even after PRRSV infection. These data clearly indicate that the humoral and cell-mediated immunity responses are mainly due to vaccination.

Improved growth performance and reduced mortality are also very clinically relevant because most of the economic losses due to PRRSV infection in herds are attributed to a decrease in growth performance and an increase in the mortality rate in post-weaning and growing-finishing pigs (Holtkamp et al., 2013). Therefore, growth performance and decrease in mortality are two of the most important parameters for the evaluation of a vaccine under field conditions. The economic benefit is calculated on the basis of market weight and mortality. Protection by the PRRS vaccine led to a healthy increase in the market weight by an average of 6.23 kilograms/pig (98.01 kilograms in the combined vaccinated groups vs. 91.78 kilograms in the combined unvaccinated groups; $P < 0.05$) and a decrease in the mortality rate by an average of 6.7%P (3.3% in the combined vaccinated groups vs. 10% in the combined unvaccinated groups; $P < 0.05$). The improved market weight of 6.2 kilograms/pig increased the revenue by approximately 14.57 US$ (exchange rate; US $1.00 = 1,141 Korean Won) per pig and the 6.7%P decrease in the mortality rate saved 15.75 US$ per pig. Total

economic benefit of using the PRRS vaccine is 30.32 US$/pig based on these two parameters alone.

The prevalence of the different PRRSV types varies from continent to continent. In Europe, PRRSV-1 is the most predominant virus type, while PRRSV-2 is more prevalent in North America. In Korea, however, both PRRSV types are prevalent and both cause respiratory disease in growing pigs (Choi et al., 2015). Theoretically, administration of multiple PRRSV MLV vaccines based on either of the two PRRSV types can be performed, but in reality, when inoculated concurrently, the PRRSV-1-based vaccine interferes with the effect of the PRRSV-2-based vaccine (Park et al., 2015a). Therefore, a single PRRSV vaccine that can be effective against both PRRSV types is clinically important. In this field study, we have shown that vaccination with a PRRSV MLV vaccine at 1 day old can protect growing pigs against both PRRSV-1 and PRRSV-2. These results are consistent with previous studies, where vaccination with the same PRRSV MLV vaccine at 3 weeks of age protected growing pigs against respiratory disease after either single challenge with PRRSV-1 or PRRSV-2 or dual challenge (Park et al., 2014, 2015a; Choi et al., 2016). In another study it was also shown that under field conditions, vaccination with the same PRRSV MLV vaccine at 3 weeks old leads to improved growth performance in growing pigs in farms where both PRRSV-1 and PRRSV-2 have circulated (Kang et al., 2017).

To our knowledge, this is the first field study to test the efficacy of a commercial PRRS vaccine administered to 1 day old piglets. Veterinarians have made great efforts towards preventing and controlling PRRSV infection. Vaccination is still the principal tool currently used to control PRRSV infection in pigs. The efficacy of the vaccine also depends on the timing of the vaccination in order to induce maximum protective immune response in piglets before they become naturally infected. An early vaccination regimen with immunization at 1 day of age can confer seroconversion and immune protection at an earlier time in life particularly around the time of weaning, when pigs from different litters are mixed together and are likely to face their first infection with PRRSV. These results

demonstrate that PRRSV vaccination at 1-day-old does not significantly affect the efficacy of the PRRSV MLV vaccine. The PRRSV MLV vaccine used in this study is effective in improving growth performance from day 1 all the way to day 182 in endemically infected farms. Therefore, PRRS vaccination at 1 day of age can be useful in protecting young piglets from early PRRSV infection.

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국 문 초 록

1일령 자돈에서 돼지 생식기 호흡기 증후군 생독 백신 접종시 성장률 개선

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돼지 생식기 호흡기 증후군 (PRRS)은 전세계적으로 돼지에게 걸리는 가장 심각한 바이러스 질병 중에 하나이다. 본 실험에서는 1일령 돼지에서 돼지 생식기 호흡기 증후군 바이러스 생독 (PRRSV MLV) 백신의 안전성과 접종시 성장률 향상을 확인하고자 하였다. 야외 임상 환경에서 생독 백신을 접종 한 후, 임상적, 바이러스적, 면역학적, 병리학적 기준으로 백신의 효능을 알아보고자 하였다. 실험은 돼지 생식기 호흡기 증후군 관련 호흡기 질병으로 인한 생산 감소로 경제적 피해를 보고 있는 3곳의 농장에서 진행되었다. 각 농장 당, 40마리의 1일령 자돈을 선발하여 20마리는 백신접종군으로, 나머지 20마리는 대조군으로 구분하였다. 백신접종군 돼지에는 한국에서 유통되고 있는 상용 백신 중 생독 PRRSV MLV 백신 (포스테라 PRRS, 한국조에티스) 을 2.0 ml씩 근육 내 접종 하였다. 반면에, 대조군 돼지에는 PBS를 2.0 ml씩 근육 내 접종 하였다. 그 결과, 백신접종군 성장률은 대조군과 비교하였을 때, 전반적으로 크게 증가되었으며 병리조직학적으로 폐병변이 효과적으로 감소되어 있는 것을 확인하였다. 또한 anti-PRRSV 항체가와 PRRS-specific IFN-γ-SCs 측정을 통해 백신접종이 1일령 돼지에게 체액성 면역뿐만 아니라 세포성 면역 반응을 효과적으로 유도할 수 있다는 사실을 확인하였다. 그러므로 Type-2 PRRSV에 감염되거나 또는 Type-1

PRRSV과 Type-2 PRRSV에 이중 감염 문제가 있는 농장에서 1일령 돼지에게 PRRSV 생독 백신의 접종은 성장률 향상과 감염 예방에 효과적인 것을 확인할 수 있었다.

주요어:

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