



Efficacy of a type 2 PRRSV modified live vaccine (PrimePac™ PRRS) against a Thai HP-PRRSV challenge

Chaitawat Sirisereewan¹ · Yonlayong Woonwong^{1,2} · Jirapat Arunorat¹ · Roongtham Kedkovid¹ · Teerawut Nedumpun³ · Sawang Kesdaangakonwut¹ · Sanipa Suradhat^{4,5} · Roongroje Thanawongnuwech^{1,5} · Komkrich Teankum¹

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Abstract

The Chinese highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) has caused a severe threat to the pig population in Southeast Asian countries. The purpose of this study was to investigate the efficacy of a type 2 PRRSV modified live vaccine (PrimePac™ PRRS, lineage 7) against a Thai HP-PRRSV (10PL01, lineage 8). Three-week-old PRRSV-free pigs were randomly assigned into three groups. Vaccinated challenged group (group 1, $n = 16$) was immunized with PrimePac™ PRRS vaccine at 3 weeks old. The unvaccinated challenged group (group 2, $n = 16$) was injected with PBS at 3 weeks old, and unvaccinated unchallenged group (group 3, $n = 10$) was served as a negative control. At 9 weeks old, all groups, except the negative control group, were challenged with the Thai HP-PRRSV. All pigs were monitored daily during 10 days post-infection (dpi) and were necropsied at 10 and 17 dpi. The results revealed that vaccinated challenged pigs showed significantly lower ($p < 0.05$) mean rectal temperatures, clinical respiratory scores, lung lesion scores, and levels of virus load in serum and lung tissue compared with the unvaccinated challenged pigs. Moreover, vaccinated challenged pigs exhibited PRRSV-specific serum neutralizing antibodies at the end of the experiment. Our findings indicated that the studied type 2 PRRSV vaccine provided partial protection against the Thai HP-PRRSV infection based on the body temperature, levels of viremia, and the severity of lung lesions. These results demonstrated that partial protection of PrimePac™ PRRS vaccine might be useful for controlling HP-PRRSV infection in the endemic area.

Keywords HP-PRRSV · Pigs · Modified live vaccine · PrimePac™

Introduction

Porcine reproductive and respiratory syndrome (PRRS) has been recognized as a highly contagious swine disease, leading to massive economic losses about \$664 million annually in the US swine industry (Holtkamp et al. 2013). PRRS is caused by PRRS virus (PRRSV) classified into type 1 (EU) and type 2 (US) genotypes. PRRSV belongs to the family Arteriviridae and genus *Arterivirus* (Cavanagh 1997; Meulenberg et al. 1997). The clinical outcomes of PRRSV infection are reproductive failure in breeding herd and respiratory disorder in nursery to finishing pigs. In 2006, massive outbreaks of highly pathogenic PRRSV (HP-PRRSV) with discontinuous 30 amino acid deletion in NSP 2 emerged in China resulting in high mortality and morbidity rates in infected pigs (Tian et al. 2007). After the first outbreak in China, HP-PRRSV rapidly spread to Southeast Asian countries including Vietnam, Laos, Cambodia, Philippines, Myanmar, and Thailand (Jantafong

✉ Komkrich Teankum
Komkrich.t@chula.ac.th

¹ Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Henri-Dunant Rd., Bangkok 10330, Thailand

² Department of Farm Resources and Production Medicine, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen, Nakorn Pathom, Thailand

³ Interdisciplinary Program in Medical Microbiology, Graduate School, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand

⁴ Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Henri-Dunant Rd., Bangkok 10330, Thailand

⁵ Center of Emerging and Re-emerging Infectious Diseases in Animals, Faculty of Veterinary Science, Chulalongkorn University, Henri-Dunant Rd., Bangkok 10330, Thailand

et al. 2015). Interestingly, recent surveillances of genetic diversity of PRRSV circulating in Thai swine industry showed that HP-PRRSV has been reported as the dominant virus in Thailand (Jantafong et al. 2015). Thus, it is speculated that HP-PRRSV could be a major threat to the Thai swine industry.

To protect pigs from HP-PRRSV, attenuated live vaccines based on highly pathogenic PRRSV strains provided completely protection against HP-PRRSV challenge in China (Leng et al. 2012; Yu et al. 2015). However, these vaccines were not allowed for use in other countries including Thailand. Therefore, commercially available type 2 PRRS vaccines, belonging to lineage 5 and lineage 8, were proven under the experimental condition and reported to be effective against HP-PRRSV (Do et al. 2015; Lager et al. 2014; Wei et al. 2013). Unfortunately, cross-protection between vaccine strains and field strains was limited (Do et al. 2015; Lager et al. 2014). In addition, the safety is of concern. It has been documented that lineage 5 and lineage 8 PRRSV vaccines are able to revert to virulence in many regions (Opriessnig et al. 2002; Shi et al. 2010). Interestingly, type 2 PRRS vaccine (PrimePac™ PRRS) belongs to lineage 7 proved to be more closely related to cluster of HP-PRRSV than type 2 PRRS vaccine derived from lineage 5 (Shi et al. 2010). It is possible that PrimePac™ PRRS vaccine could be used effectively to protect pigs against heterologous HP-PRRSV infection. Moreover, the efficacy of PrimePac™ PRRS has not been reported against HP-PRRSV previously.

Induction of specific immune responses requires approximately 4 weeks after infection (Lopez and Osorio 2004; Loving et al. 2015; Yoon et al. 1994). Infection after adequate immune responses in vaccinated pigs (4–6 weeks post-vaccination) may be useful for assessment of vaccine efficacy. The goal of this study was to determine the efficacy of a type 2 PRRS modified live vaccine (PrimePac™ PRRS) against a Thai HP-PRRSV (10PL01) based on clinical, immunological, virological, and pathological parameters under an experimental condition.

Materials and methods

Viruses and cells

The Thai type 2 HP-PRRSV (10PL01) was isolated from an outbreak farm in Phitsanulok province (Ayudhya et al. 2012) and was kindly provided by the Chulalongkorn University, Veterinary Diagnostic Laboratory (CU-VDL). The virus was cultured and titrated in MARC-145 cells (Thanawongnuwech et al. 1998), and stored at $-80\text{ }^{\circ}\text{C}$ until needed.

Experimental design

Forty-two 3-week-old castrated pigs from a PRRS-negative farm were allocated into three groups and identified by ear tag. Vaccinated challenged group (group 1, $n = 16$) was immunized with the assigned modified live PRRS vaccine (PrimePac™, Batch No. A600AD03) at 3 weeks old according to the manufacturer's instructions. The unvaccinated challenged group (group 2, $n = 16$) was injected with a phosphate-buffer solution (PBS) at 3 weeks old, and unvaccinated unchallenged group (group 3, $n = 10$) was served as a negative control. Prior to vaccination, all serum samples were tested and found PRRSV seronegative using a commercial ELISA (IDEXX PRRS X3 antibody test, USA), and PRRSV genetic-free in sera using a modified qRT-PCR. At 6 weeks after vaccination, all groups except the negative control group (group 3) were intranasally challenged with 2 ml of viral suspension containing 10^4 TCID₅₀/ml of the Thai HP-PRRSV lineage 8 (10PL01). Necropsy was performed on 10 days post-infection (dpi) and on 17 dpi or whenever found moribund or dead pigs. All methods and animal studies were conducted under the approval of Chulalongkorn University Animal Care and Use Committee, Chulalongkorn University (Animal Use Protocol No. 1431086).

Clinical examinations

Animals were monitored daily during the subsequent 10 dpi for physical condition, rectal temperatures, and clinical respiratory disease. The clinical respiratory scores were estimated using the previously described protocol (Sirisereewan et al. 2017). All pigs were given a score of clinical respiratory signs ranging from 0 to 3: 0 = normal; 1 = mild respiratory distress; 2 = moderate respiratory distress; 3 = severe respiratory distress with cyanosis.

Serological examinations

Blood and serum samples were collected at -45 , -39 , -35 , -28 , -21 , -14 , -7 , 0 , 2 , 4 , 7 , 10 , 14 , and 17 dpi. PRRSV-specific antibody response was tested using a commercial PRRSV ELISA test kit (IDEXX PRRS X3 Ab test, USA). Serum samples were considered positive for PRRSV antibody when the S/P ratio was greater than 0.4, according to manufacturer's instruction.

Serum virus neutralization (SVN) test were performed with the challenged virus at 0 and 14 dpi, as previously described (Sirisereewan et al. 2017). Positive samples were considered to be positive for neutralizing antibodies (NAs) if the titer $\geq 1:2$ ($1\log_2$). The presence of PRRSV was confirmed by IPMA assay as previously described (Thanawongnuwech et al. 1998).

Quantification of PRRSV RNA in the sera and lung tissues

RNA was extracted from serum samples and lung tissue using an RNA extraction test kit (NucleoSpin, Germany). Quantification of PRRSV RNA in sera was performed by a real-time PCR as previously described (Egli et al. 2001).

Isolation of peripheral blood mononuclear cell and flow cytometry

Peripheral blood mononuclear cells were isolated based on the previous protocols (Suradhat et al. 2015). In vitro activation was performed in a flat-bottomed 96-well plate by seeding 1×10^6 cells of peripheral blood mononuclear cells (PBMCs) into each well, and then cultured with 0.01 multiplicity of infection (m.o.i.) of the Thai prototype PRRSV of genotype 2 (01NP1) or only media or mock infected MARC-145 lysate for 48 h prior to harvesting for fluorescent staining and flow cytometric analyses. Details of the staining step and staining protocol for Treg, IL-10 and IFN- γ were described previously (Suradhat et al. 2016). All flow cytometric analyses were performed using the FC 500 MPL System (Beckman Coulter, CA, USA).

Data representing mean percentage (\pm SEM) of the cytokine-producing cells from pigs in the same group, were calculated (% cytokine-producing cells obtained from the PRRSV-cultured PBMC – % cytokine-producing cells obtained from the mock-cultured PBMC).

Pathologic examination

The gross lung lesions and histopathological changes were evaluated using the previously described protocol (Halbur et al. 1995). All lungs were scored to estimate the percentages of lungs affected by pneumonia, and the sections of lung tissue were scored ranging from 0 (normal) to 4 (severe) to estimate the severity of lung lesions.

Statistical analyses

Data were analyzed using analysis of variance (ANOVA) followed by Tukey's multiple comparison tests and Kruskal-Wallis followed by Dunn's multiple comparison tests and pair *t* test. All statistical analyses were performed using GraphPad Prism for Windows (GraphPad Software Incorporated, San Diego, CA, USA).

Results

Clinical examination

Following HP-PRRSV challenge, all challenged pigs exhibited clinical signs related to HP-PRRSV infection including depression, anorexia, cough, and high fever (104–107 °F) starting from the first dpi (Fig. 1a). Vaccinated challenged pigs had lower mean rectal temperature compared to the unvaccinated challenged pigs at 9 and 10 dpi ($p < 0.05$). The clinical respiratory scores in vaccinated challenged pigs were significantly lower ($p < 0.05$) than those of the unvaccinated challenged pigs at 2, 5, 7, 8, 9, and 10 dpi (Fig. 1b). In addition, all challenged pigs exhibited transient decrease in the number of total white blood cells compared to the pre-infection levels. However, the leucocyte numbers rebounded to the pre-infection levels at 4 dpi in the vaccinated challenged pigs, but remained at lower levels in the unvaccinated challenged pigs (Fig. 1c). Pigs in the negative control group remained normal both rectal temperature and respiratory signs throughout the observation period.

Quantification of PRRSV RNA in sera and lung tissue

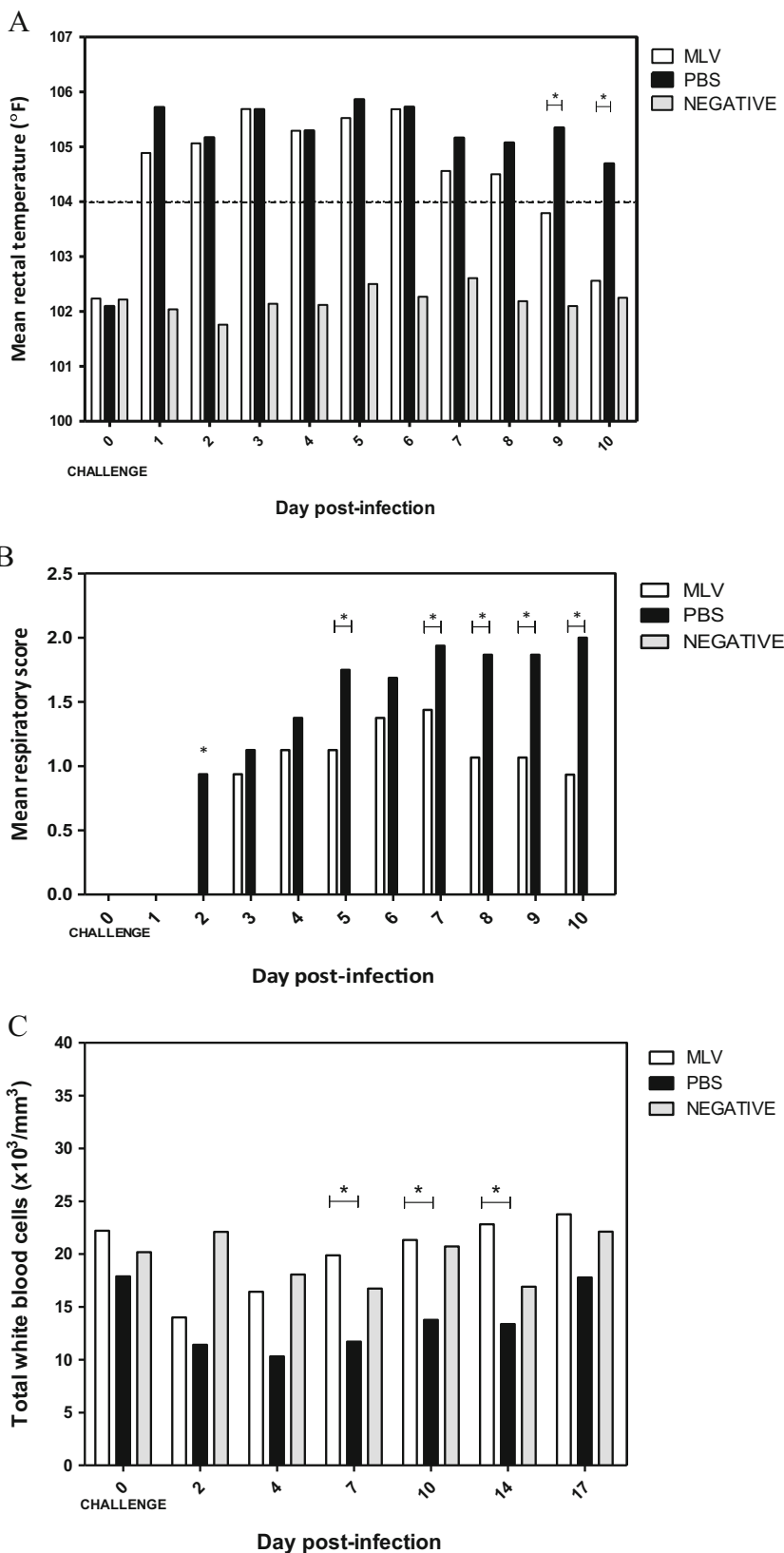
Following vaccination, viremia in pigs receiving MLV was sporadically detected in the serum at 3 days post-vaccination (dpv) to 21 dpv (Fig. 2a). In group-2 pigs receiving PBS, PRRSV were not detected in the serum until after challenged. Viremia was initially detected in all challenged pigs as early as 2 dpi and peaked at 4–7 dpi. Vaccinated challenged pigs exhibited lower levels ($p < 0.05$) of viremia in the serum compared to unvaccinated challenged pigs at 7 and 14 dpi and faster reduction of viremia was observed (Fig. 2a). HP-PRRSV was not detected in the negative control pigs throughout the observation period.

At first necropsy, levels of viral load in lung tissue had no significant differences in both challenged groups at 10 dpi. However, the vaccinated challenged pigs had lower levels of viral load in lung tissue ($p < 0.05$) at 17 dpi (Fig. 2b). HP-PRRSV was not detected in the lung tissue of negative control pigs throughout the experiment.

Serological examinations

All pigs had no seroconversion to PRRSV based on the ELISA results at the time of vaccination. Seroconversion was first shown at 14 dpv in the group receiving MLV immunization. Following the HP-PRRSV challenge, vaccinated challenged pigs had significantly higher ($p < 0.05$) anti-PRRSV antibody titers than those of unvaccinated challenged pigs at 4 and 7 dpi (Fig. 3a). Pigs in the negative control group remained PRRSV seronegative throughout this experiment.

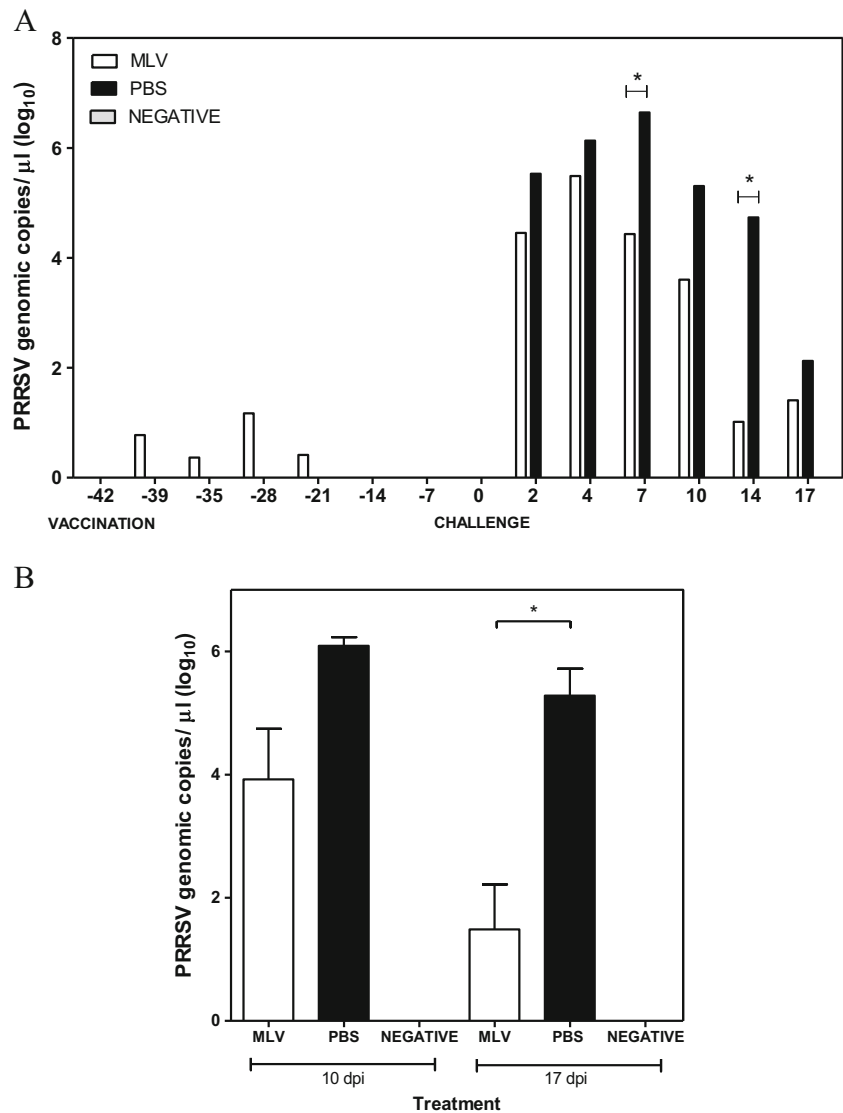
Fig. 1 Mean rectal temperature (a), mean respiratory score (b), total white blood cells (c) from the experimental pigs following HP-PRRSV challenge. Statistical analyses were performed using one-way ANOVA followed by Tukey’s multiple comparison test. An asterisk indicates the difference ($p < 0.05$) between the group receiving MLV and PBS



HP-PRRSV-specific serum neutralizing antibodies were not detected in all pigs at the challenged day (0 dpi).

Interestingly, SN titers were observed in pigs receiving MLV immunization (6/8) at 14 dpi and significantly increased

Fig. 2 PRRSV genomic copies in the sera (a) and lungs (b) of experimental pigs. Pigs were immunized with MLV or PBS, and challenged with HP-PRRSV at 0 dpi. Lung samples were collected at 10 and 17 dpi. Statistical analyses were performed using one-way ANOVA followed by Tukey's multiple comparison test. An asterisk indicates the difference ($p < 0.05$) between the group receiving MLV and PBS



compared to 0 dpi (Fig. 3b). Pigs in the negative control group had no neutralizing antibodies throughout this study.

Frequency of PRRSV-specific interferon- γ -secreting cells, IL-10, and Treg in PBMC

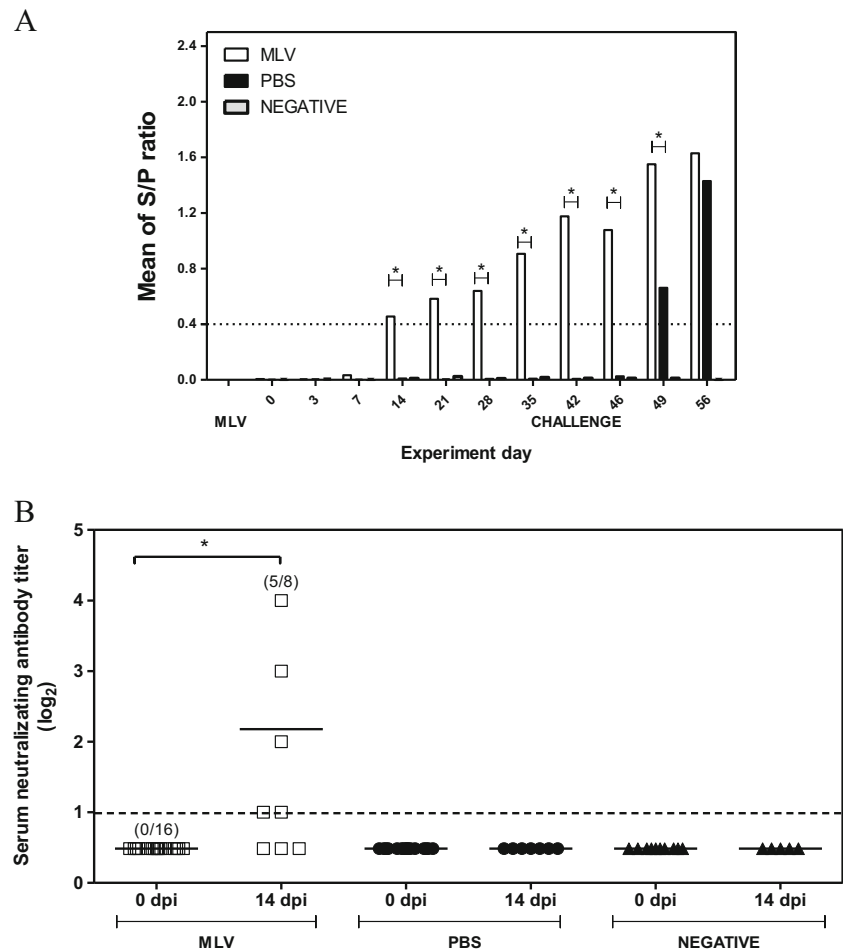
Following MLV vaccination and HP-PRRSV infection, MLV-immunized pigs induce the numbers of PRRSV-specific IL-10-producing cells starting from 7 dpv and, then, gradually declined at 28 dpv. MLV-immunized pigs significantly enhanced IL-10 production at 56 dpv or 14 dpi compared to the PBS-immunized pigs (Fig. 4a). The numbers of PRRSV-specific Treg in the MLV-receiving group were higher than those of the PBS-receiving group during the pre-challenged and post-challenged periods (Fig. 4b). However, the numbers

of PRRSV-specific CD3⁺IFN- γ ⁺ cells had no difference in MLV-immunized pigs and PBS-immunized pigs throughout the observation period (Fig. 4c).

Macroscopic lung lesions

The severity of PRRSV-induced lung lesions was characterized by multifocal, tan-mottled areas, with irregular and indistinct borders. The gross lung lesion scores had no significant differences among groups at 10 dpi. However, gross lung lesion scores were significantly lower in vaccinated challenged pigs compared to unvaccinated challenged pigs at 17 dpi (Fig. 5a). No macroscopic lung lesions were observed in the negative control pigs throughout the experiment.

Fig. 3 Mean S/P ratios (a) and mean SN antibody titers (b) on 0 and 14 dpi of the experimental pigs. Pigs were immunized with MLV or PBS, and challenged with HP-PRRSV at 0 dpi. Statistical analyses were performed using one-way ANOVA followed by Tukey's multiple comparison test and Kruskal-Wallis followed by Dunn's multiple comparison tests. An asterisk indicates the difference ($p < 0.05$) between the group receiving MLV and PBS



Microscopic lung lesions

Microscopic lung lesions were characterized by thickening of alveolar septa with infiltration of mononuclear cells, type II pneumocyte hyperplasia and hypertrophy, and accumulation of macrophages in alveolar spaces. At 10 dpi, all challenged pigs exhibited PRRSV-specific lung lesions by developing interstitial pneumonia in all challenged groups. However, there were no significant differences between the two challenged groups at 10 dpi. Interestingly, vaccinated challenged pigs exhibited lower microscopic lung lesions at 17 dpi compared to 10 dpi. In contrast, the microscopic lung lesions had no significant difference in unvaccinated challenged pigs between 10 and 17 dpi (Fig. 5b).

Discussion

In this study, we hypothesized that the studied commercial type 2 vaccine was able to provide protection against the Thai HP-PRRSV challenge. Our results demonstrated that the newly introduced commercial type 2 PRRS MLV was

partly efficacious in protecting growing pigs against the Thai HP-PRRSV challenge. This finding is consistent with the previous studies that current commercial PRRS MLV vaccines had variations on their efficacy against the HP-PRRSV challenges depending on various factors (Do et al. 2015; Lager et al. 2014; Wei et al. 2013). It indicates that a PRRSV controlled strategy still requires the better efficacious vaccine and vaccination strategies against the HP-PRRSV infection.

PRRSV-induced immunosuppression causes porcine respiratory disease complex (PRDC) recognized as a major complication of PRRSV infection. Unfortunately, current commercial MLV vaccines could not provide complete protection against field strains of PRRSV. However, there are many advantages of PRRSV vaccination in PRRSV endemic areas. Evidently, PRRSV vaccination was able to reduce disease severity, virus shedding, and some clinical outcomes (Charoenchanikran et al. 2016; Do et al. 2015; Lager et al. 2014; Martelli et al. 2009; Park et al. 2014; Savard et al. 2016; Wei et al. 2013; Zuckermann et al. 2007). Thus, preventive vaccination programs seem meaningful and easy to use in PRRSV-positive herds in order to stabilize the herd immunity. They may also minimize the chances of having PRDC induced by PRRSV. Moreover, receiving PRRSV

Fig. 4 % PRRSV-specific CD3⁺ IL-10⁺ subpopulation (a), % PRRSV-specific CD3⁺IFN- γ ⁺ subpopulation (b), and % PRRSV-specific CD4⁺ CD25⁺ FoxP3⁺ subpopulation (c) of the experimental pigs. Pigs were immunized with MLV or PBS, and challenged with HP-PRRSV at 0 dpi. Statistical analyses were performed using one-way ANOVA followed by Tukey’s multiple comparison test. An asterisk indicates the difference ($p < 0.05$) between the group receiving MLV and PBS

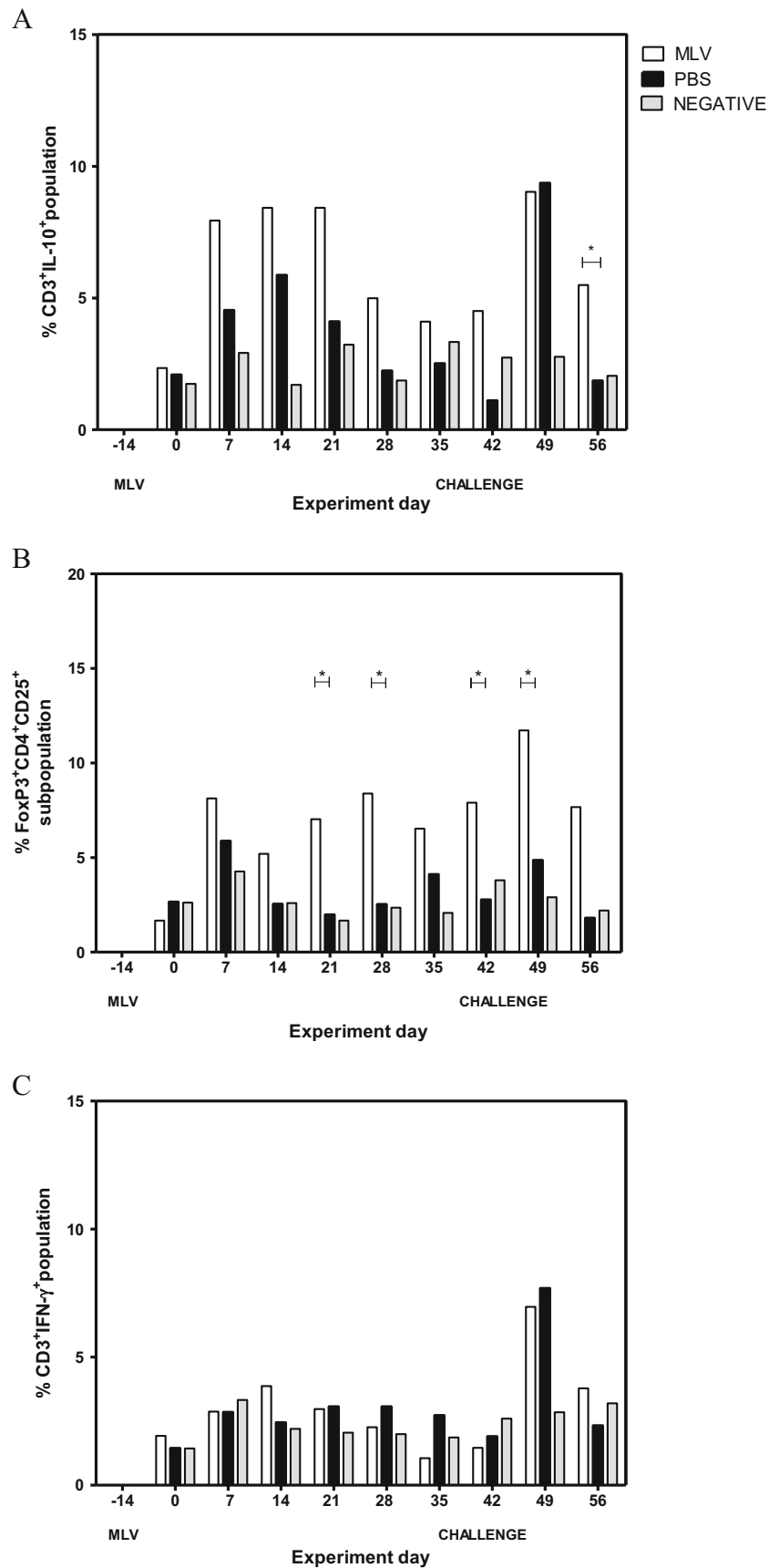
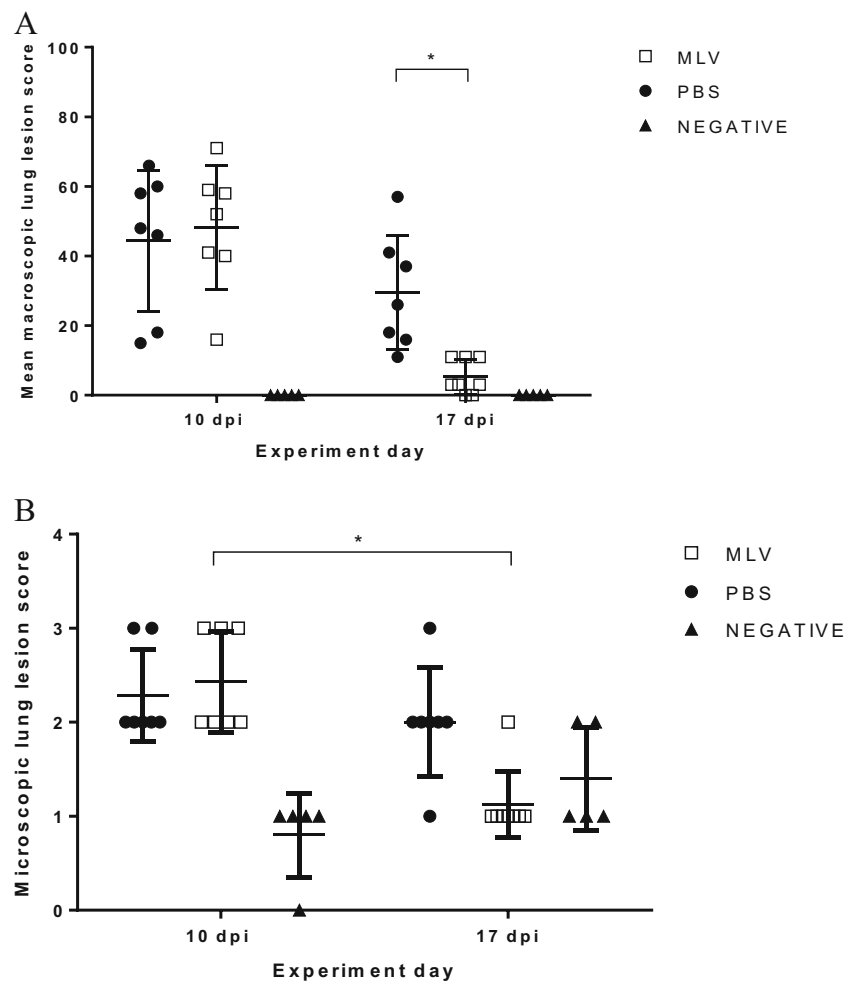


Fig. 5 Mean macroscopic lung lesion score (a) and microscopic lung lesion score (b) of the experimental pigs. Statistical analyses were performed using one-way ANOVA followed by Tukey's multiple comparison test and Kruskal-Wallis followed by Dunn's multiple comparison tests. An asterisk indicates the difference ($p < 0.05$) between the group receiving MLV and PBS



vaccine did improve growth performance in fattening pigs under the field condition (Lyo et al. 2016). On the contrary, strict farm managements are effective methods involving biosecurity, herd health monitoring, or depopulation and repopulation, etc. However, those are time-consuming and may have a negative economic impact. Nevertheless, effective PRRS control strategies should combine both PRRS vaccination programs and farm management in order to reduce the virus load and transmission within and among herds, particularly in PRRSV endemic areas.

According to the previous study, ORF5 sequence similarity between the PRRSV field strain and PRRSV vaccine strain was suggested for a better protective efficacy of the selected MLV vaccine (Labarque et al. 2004). However, vaccination with two genetically distant PRRS MLV vaccines, lineage 5 and 8, did not provide significant differences for protective efficacy against the HP-PRRSV belonging to lineage 8 (Do et al. 2015). It indicated that having the same genetic lineage was not a reliable parameter to guarantee the vaccine protection. In this study, we examined a newly introduced type 2 PRRSV vaccine of lineage 7 demonstrating that the studied type 2 PRRSV vaccine of lineage 7 was able to provide partial

protection against the Thai HP-PRRSV. This studied vaccine might become a vaccine of choices for controlling HP-PRRSV in endemic areas.

In this study, immunization with the MLV vaccine could not elicit PRRSV-specific cellular immunity and neutralizing antibodies prior to the HP-PRRSV challenge. No upregulation of PRRSV-specific IFN- γ -secreting cells possibly caused by the induction of IL-10 and Treg following the MLV immunization. Evidently, a reduction of IL-10 and Treg induced by the heterologous prime-boost immunization strategy could elicit PRRSV-specific IFN- γ -secreting cells prior to HP-PRRSV exposure (Sirisereewan et al. 2017). In this study, neutralizing antibodies were detected in the vaccinated pigs at 14 dpi, probably associated with cross-neutralizing antibody. Induction of cross-neutralizing antibody achieved by the MLV immunization might associate with cross-neutralizing epitopes located in the first 60 amino acids of the GP5 gene (Kim et al. 2013). Moreover, neutralizing antibody did play a major role on viremic reduction (Lopez and Osorio 2004; Yoon et al. 1996). In this study, reduction of HP-PRRSV viremia might be influenced by inducing PRRSV-

specific neutralizing antibodies. According to the previous study, the levels of viremia correlated with the severity of interstitial pneumonia (Han et al. 2013; Johnson et al. 2004), thus reducing the levels of viremia resulting in reduction of lung lesions and lung viral load in the late phase of infection.

It should be noted that this is the first experimental challenge study evaluating the commercial type 2 PRRSV vaccine (PrimePac™ PRRS), of lineage 7, against the Thai HP-PRRSV challenge (lineage 8). The vaccine efficacy was assessed based on clinical, virological, pathological, and immunological parameters and the vaccine, therefore, could be used in the field to control HP-PRRSV infection. Unfortunately, current commercial PRRSV vaccines still have the limitation against the HP-PRRSV as mentioned previously. Effective prevention and control of PRRSV must employ both vaccine and herd management implementation.

Conclusion

The modified live PRRSV vaccine (PrimePac™ PRRS) definitely could provide partial protection against the Thai HP-PRRSV challenge. Vaccinated challenged pigs showed significantly lower levels of rectal temperature, clinical respiratory scores, lung lesion scores, levels of HP-PRRSV viremia, and levels of HP-PRRSV load in the lung tissue compared to the unvaccinated challenged pigs. In addition, vaccinated challenged pigs exhibited PRRSV-specific serum neutralizing antibodies at the end of the experiment consistent with the presence of viremic reduction.

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Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest.

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