THE EFFECT OF MATERNALLY-DERIVED ANTIBODIES AGAINST ANTIBODY PRODUCTION IN VACCINATED PIGLETS WITH MODIFIED -LIVE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS VACCINES

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จุฬาลงกรณ์มหาวิทยาลัย
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A Thesis Submitted in Partial Fulfillment of the Requirements
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ผลกระทบของแอนติบอดี้ที่ถ่ายทอดจากแม่ต่อการตอบสนองทางภูมิคุ้มกันของลูกสุกรที่ฉีดวัคซีนป้องกันโรคพีอาร์อาร์เอสชนิดเชื้อเป็น

น.ส.สุนิตย์ มีบำรุง

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Field of Study	Veterinary Pathobiology
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สุนิตย์ มีบำรุง : ผลกระทบของแอนติบอดี้ที่ถ่ายทอดจากแม่ต่อการตอบสนองทางภูมิคุ้มกันของลูกสุกรที่ฉีดวัคซีนป้องกันโรคพีอาร์อาร์เอสชนิดเชื้อเป็น. (THE EFFECT OF MATERNALLY-DERIVED ANTIBODIES AGAINST ANTIBODY PRODUCTION IN VACCINATED PIGLETS WITH MODIFIED -LIVE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS VACCINES) อ.ที่ปรึกษาหลัก : ผศ. น.สพ. ดร. เดชฤทธิ์ นิลอุบล

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาการตอบสนองภูมิคุ้มกันแบบสารน้ำภายหลังการทำวัคซีนป้องกันโรคพีอาร์อาร์เอสชนิดเชื้อเป็นในลูกสุกรที่มีระดับของภูมิคุ้มกันที่ถ่ายทอดจากแม่สู่ลูกในระดับต่ำ (SN titer < 8) และสูง (SN titer □ 8) รวมทั้งศึกษาผลการใช้วัคซีนป้องกันโรคพีอาร์อาร์เอสต่างไอโซเลตระหว่างแม่สุกร และลูกสุกรต่อการสร้างภูมิคุ้มกันแบบสารน้ำภายหลังการทำวัคซีนในลูกสุกร ร่วมกับประเมินอัตราการเลี้ยงรอด และอัตราการเจริญเติบโตในลูกสุกร ลูกสุกรจำนวน 120 ตัว จากฟาร์มสุกร 3 ฟาร์ม ได้แก่ ฟาร์ม 1 (สุกรแม่พันธุ์ใช้วัคซีน Ingelvac® PRRS MLV: MLV1) ฟาร์ม 2 (สุกรแม่พันธุ์ใช้วัคซีน Prime Pac® PRRS MLV: MLV2) และฟาร์ม 3 (ปลอดโรคพีอาร์อาร์เอส) ลูกสุกรที่มีภูมิคุ้มกันที่ถ่ายทอดจากแม่ในระดับสูงจากฟาร์ม 1 และ 2 ฟาร์มละ 24 ตัว แบ่งเป็น 6 กลุ่ม ได้แก่ H1, H1-1, H1-2, H2, H2-1 และ H2-2 ลูกสุกรที่มีภูมิคุ้มกันในระดับต่ำจากฟาร์ม 1 และ 2 ฟาร์มละ 24 ตัว แบ่งเป็น 6 กลุ่ม ได้แก่ L1, L1-1, L1-2, L2, L2-1 และ L2-2 ลูกสุกรจากฟาร์ม 3 จำนวน 24 ตัว ถูกแบ่งเป็น 3 กลุ่ม ได้แก่ N, N-1 และ N-2 โดยกลุ่ม H1, L1, H2, L2 และ N เป็นลูกสุกรที่ไม่ได้รับการฉีดวัคซีน กลุ่ม H1-1, L1-1, H2-1, L2-1 และ N-1 ลูกสุกรได้รับวัคซีน MLV1 ที่อายุ 2 สัปดาห์ และกลุ่ม H1-2, L1-2, H2-2, L2-2 และ N-2 ลูกสุกรได้รับวัคซีน MLV2 ที่อายุ 2 สัปดาห์ พบว่าไม่พบการตายจากการติดเชื้อไวรัสพีอาร์อาร์เอสในลูกสุกรทุกกลุ่มที่ได้รับวัคซีน MLV1 และ MLV2 แต่อย่างไรก็ตามลูกสุกรในกลุ่ม H1 และ H2 มีอัตราการเลี้ยงรอดเท่ากับ 100% ถึงแม้ว่าจะไม่ได้รับวัคซีนก็ตาม ลูกสุกรที่ได้รับวัคซีน MLV1 และ MLV2 มีอัตราการเจริญเติบโตสูงกว่าลูกสุกรที่ไม่ทำวัคซีนอย่างมีนัยสำคัญ จากผลตรวจหาระดับภูมิคุ้มกันพบการเพิ่มขึ้นของระดับภูมิคุ้มกันต่อเชื้อไวรัสพีอาร์อาร์เอสในลูกสุกรทุกกลุ่มที่ทำวัคซีน แต่อย่างไรก็ตามกลุ่มที่มีภูมิคุ้มกันที่ถ่ายทอดจากแม่ในระดับต่ำมีการตอบสนองที่เร็ว และสูงกว่ากลุ่มที่มีภูมิคุ้มกันที่ถ่ายทอดจากแม่ในระดับสูง นอกจากนี้ยังตรวจพบนิวทรัลไลซิ่งแอนติบอดีต่อเชื้อไวรัสจากวัคซีน MLV1 และ MLV2 ในระดับสูงที่สุดจากกลุ่ม L1-1 และ L2-2 ตามลำดับ นิวทรัลไลซิ่งแอนติบอดีต่อเชื้อไวรัสที่แยกได้จากฟาร์ม 1 (THA_SP/RB_S1/P1/0120-18) และฟาร์ม 2 (THA_WC/RB_F165/20-22) ตรวจไม่พบการตอบสนองทางภูมิคุ้มกันต่อเชื้อดังกล่าวในลูกสุกรที่ได้รับวัคซีนทุกกลุ่มการทดลอง จากผลการศึกษาบ่งชี้ว่า ระดับของภูมิคุ้มกันที่ถ่ายทอดจากแม่สู่ลูกไม่ได้มีผลต่อการสร้างภูมิคุ้มกันในลูกสุกรภายหลังการทำวัคซีนอย่างเด่นชัด แต่การทำวัคซีนสามารถลดอัตราการตายที่อาจสืบเนื่องมาจากการติดเชื้อไวรัสพีอาร์อาร์เอส และช่วยเพิ่มอัตราการเจริญเติบโตในลูกสุกรในฝูงที่มีการติดเชื้อไวรัสพีอาร์อาร์เอสได้ถึงแม้จะมีการใช้วัคซีนต่างไอโซเลตของเชื้อไวรัสพีอาร์อาร์เอสในลูกสุกรก็ตาม ความแตกต่างทางพันธุกรรมระหว่างเชื้อไวรัส และไวรัสจากวัคซีนไม่ได้มีผลต่อการป้องกันโรคพีอาร์อาร์เอสในการศึกษานี้ การพิจารณาเพื่อเปลี่ยนวัคซีนควรพิจารณาจากการตอบสนองทางภูมิคุ้มกันทั้งแบบสารน้ำ และพึ่งเซลล์ร่วมกับประสิทธิภาพการผลิต ในกรณีนี้ถึงแม้การเปลี่ยนวัคซีนในลูกไม่ได้มีผลต่อประสิทธิภาพการผลิต และการสร้างภูมิคุ้มกัน แต่การใช้วัคซีนหลายเสตรนอาจมีผลต่อความหลากหลายทางพันธุกรรมของเชื้อ และการควบคุมโรคในอนาคต

สาขาวิชา	พยาธิชีววิทยาทางสัตวแพทย์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2563	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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5975315331 : MAJOR VETERINARY PATHOBIOLOGY

KEYWORD: Porcine reproductive and respiratory syndrome virus, Maternally-derived antibodies, humoral antibody, Modified lived virus vaccine, Pig

Sunit Mebumroong : THE EFFECT OF MATERNALLY-DERIVED ANTIBODIES AGAINST ANTIBODY PRODUCTION IN VACCINATED PIGLETS WITH MODIFIED -LIVE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS VACCINES. Advisor: Asst. Prof. Dachrit Nilubol, D.V.M.Ph.D.

The humoral antibody responses following vaccination with different lineage of modified live genotype 2 porcine reproductive and respiratory syndrome virus (PRRSV) vaccines (MLVs) were investigated. Moreover, the mortality and growth performance in pigs were observed. Twenty-four pigs with high maternally derived antibody (MDA) levels (SN titer 8) from each PRRSV positive Herd 1 and Herd 2 were allocated to the H1, H1-1, and H1-2 groups (Herd 1) and H2, H2-1, and H2-2 groups (Herd 2). Twenty-four pigs with low MDA levels (SN titer <8) were allocated to the L1, L1-1, and L1-2 (Herd 1) and L2, L2-1, and L2-2 groups (Herd 2). Twenty-four pigs from the negative herd (Herd 3) were allocated to 3 groups: N, N-1, and N-2 groups. The H1, H2, L1, L2, and N groups were unvaccinated. The H1-1, H2-1, L1-1, L2-1, and N-1 groups were vaccinated with MLV (Ingelvac® PRRS MLV) at 2 weeks of age. The 5 other groups: H1-2, H2-2, L1-2, L2-2, and N-2 groups were vaccinated with MLV2 (Prime Pac® PRRS MLV) at 2 week of age. The antibody responses were measured by ELISA and serum neutralization (SN). The mortality was not observed in all vaccinated groups. Additionally, pigs with high MDA levels from the H1 and H2 groups did not exhibit the mortality throughout the study. ADG in pigs vaccinated with each MLV1 and MLV2 was significant higher than that in unvaccinated pigs. However, pigs vaccinated with MLV2 showed the highest values of ADG. Seroconversion was observed in all vaccinated groups with no significance difference in antibody titers between pigs with low and high MDA levels. However, pigs with low MDA levels showed the earliest and highest antibody level post vaccination. The highest SN-titer against MLV1 isolate and MLV2 isolate was observed in pig from the L1-1 and L2-2 groups, respectively. The field PRRSV-2 isolates: THA_SP/RB_S1/P1/0120-18 and THA_WC/RB_F165/20-22, the SN titers against these isolates were not observed in vaccinated groups. These results indicated that MDAs might not affect the immune response post vaccination. PRRSV-2 MLVs reduced the mortality and improved growth performance of pigs in the endemically PRRSV infected herds. Although, the vaccine was changed in weaning pigs, the antibody responses were detected post vaccination in both pigs with low and high level of MDAs. Moreover, the genetic similarity between vaccine and field virus was not related to the protection. Vaccine selection should depend on the induction of immune response and protection against heterologous PRRSV infection.

Field of Study: Veterinary Pathobiology Student's Signature .....

Academic Year: 2020 Advisor's Signature .....

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Sunit Mebumroong

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CHAPTER I

INTRODUCTION

Importance and Rationale

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most important pathogens currently affecting the swine production worldwide since its emergence in late 1980s (Done et al.,1996). This virus causes the disease characterized by reproductive impairment or failure in breeding animals including increase in premature farrowing, late term abortion, stillborn pigs, mummified fetuses and weak piglets, and respiratory disease in pigs from nursery to finishing. It is also the most important virus associated with porcine respiratory disease complex (PRDC) in pigs especially in nursery and growing pigs which triggering the negative results to herd productions (Rose et al.,2003; Fablet et al.,2016).

PRRSV is an enveloped, positive-sense single stranded RNA virus belonging to the genus Arterivirus and family Arteriviridae in the order Nidovirales (Lunney et al.,2016). The PRRSV genome is about 15 kb in length and contains ten open reading frames (ORFs), including ORFs 1 to 7 (Johnson et al.,2011; Meulenberg et al., 1997; Wu et al.,2001). ORF1 (ORF1a and ORF1b) comprises approximately 80% of the whole genome of PRRSV which encodes non-structural proteins that are necessary for viral replication. The other 6 ORFs (ORFs 2–7) encode six structural proteins, including glycoproteins (GP) 2-5, and the M and N proteins. Two additional structural proteins, E and ORF5a, have also been discovered (Johnson et al., 2011; Lunney et al.,2016; Wu et al., 2001). ORF5 of PRRSV is the most variable region, playing important roles in genetic variation and protection, and it contains a neutralizing epitope (Murtaugh et al., 1998; Gonin et al., 1999; Plagemann et al., 2002).

Recently, PRRSV has been divided into two genotypes consisting of PRRSV-1 (European genotype, Lelystad virus) and PRRSV-2 (North American genotype, VR-2332) both of which are of high genetic diversity (Nilubol et al.,2013; Brar et al.,2015; Stadejek et al.,2013). Based on phylogenetic analysis, the high diversity of PRRSV has been

observed, four subtypes for PRRSV-1 and nine lineages for PRRSV-2 (Shi et al,2010; Stadejek et al.,2017). There are many factors contribute to the diversity of PRRSV such as quasispecies characteric of PRRSV, the replication with no proof reading activity of RNA polymerase, the recombination event and host immune selection (Murtaugh et al. ,2010) . Furthermore, the co-existence of PRRSV-1 and PRRSV-2 has been increasingly reported in several countries, especially in Asia, including China, Korea, Vietnam, and Thailand (Chen et al.,2011; Nilubol et al., 2013). The concurrent diversity and co-infection of both PRRSV genotypes have raised questions concerning control methods involving vaccination program, as to which types of PRRSV MLV should be used in the herd (Chaikhumwang et al.,2015; Nilubol et al.,2014).

Currently, various types of PRRSV vaccines have been developed and implemented to swine herd due to the economic losses caused by PRRSV outbreaks. The commercial PRRSV vaccine including killed vaccine (KV), subunit vaccines and modified live virus vaccine (MLV) are available in many countries. However, several researches indicated that the CMI response which is essential for complete production against PRRSV infection, generated by MLV (Madapong et al.,2020). Therefore, MLV is more efficient and preferable (Murtaugh et al.,2011; Nilubol et al.,2014) to control the disease in both experimental and field trials worldwide since its first introduction in 1994. However, the limitations of PRRS MLV have been discussed. Several vaccination trials indicated that MLV influences the genetic diversity of PRRSV in vaccinated herd and it provides only partial protection or no protection against heterologous isolates (Charerntantanakul,2012; Nilubol et al.,2014; Sun et al.,2018), the disease still occurs and remains difficult to control (Shi et al.,2010).

In addition to the diversity of PRRSV isolates causing by the use of MLV, the factors impairing vaccine efficacy under field conditions are not yet well known. Maternally derived antibodies (MDAs) present in the piglets that born to infected or vaccinated sow herds is considered as one factor affecting immune response in PRRS MLV vaccinated piglets (Gelhof et al.,2013; Fablet et al.,2016). Moreover, vaccination time in the piglets is one of critical factors that affects the immune response due to the

high MDA level, the maturation of piglet immune system and also health condition of the piglets.

In PRRSV vaccinated breeding herds, the immunization of the piglets when they still have the high level of MDAs (PRRSV-specific antibodies) must be considered (Fablet et al. ,2016; Rose and Andraud, 2017) . According to previous research, the piglets with no PRRSV-neutralizing antibodies at the time of vaccination, the vaccine viremia, rapid gamma interferon and seroconversion showed rapid responses within 7 to 14 day-post vaccination but the piglets with high levels of PRRSV-neutralizing antibodies did not show immune response (Fablet et al. ,2016) . Similar to results available for Aujeszky's disease and Equine Viral Arteritis, caused by virus belonging to the same family as PRRSV.

Taken altogether, in addition to the strain of PRRS, it seems likely that MDAs present in piglets from infected or vaccinated herds could interfere with PRRS MLVs. However, there is little information on PRRS Vaccination facing MDAs. Therefore, the main objective of this study is to investigate the effect of MDAs inducing by PRRSV MLV vaccination in sow herd on humoral immune response in the piglets given the same and different lineage of PRRSV MLVs.

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Literature Review

1) Porcine reproductive and respiratory syndrome (PRRS)

PRRSV is a highly economically significant viral disease of pig productions which causes the disease characterized by respiratory disorder in piglets and growing pigs, and reproductive failure in sows (Zimmerman et al.,2012). This disease has continued to have the negative economic impact in global swine industry since its emergence in the late 1980s (Done et al.,1996). Currently, PRRSV has established persistent infection and become endemic in many countries worldwide, including Thailand (Albina, 1997; Thanapongtharm et al.2014). The virus is the member of family Arteriviridae in the order Nidovilares. PRRSV is an enveloped single-stranded positive sense RNA virus. The PRRSV genome is approximately 15 kb in length and consists of ten open reading frames (ORFs), including ORFs 1 to 7 (Johnson et al.,2011; Meulenberg et al., 1997; Wu et al.,2001). ORF1 (ORF1a and ORF1b) comprises approximately 80% of the whole genome of PRRSV which encodes replicative enzymes. The other 6 ORFs (ORFs 2–7) encode six structural proteins, including glycoproteins (GP) 2-5, and the M and N proteins. Two additional structural proteins, E and ORF5a, have also been discovered (Johnson et al., 2011; Wu et al., 2001). ORF5 of PRRSV is the most variable region, playing important roles in genetic variation and protection, and it contains a neutralizing epitope (Murtaugh et al., 1998; Gonin et al., 1999; Plagemann et al., 2002). PRRSV was divided into two distinct genotypes; PRRSV1 (EU-genotype) and PRRSV2 (NA-genotype) (Brar et al.,2015; Nilubol et al.,2013). Recent phylogenetic studies using the ORF5 sequence is characterized by many groups including 4 subtypes for PRRSV1 and 9 lineages for PRRSV2 (Kang et al.,2018; Shi et al.,2010; Stadejek et al.,2017). Furthermore, the co-existence of PRRSV-1 and PRRSV-2 has been increasingly reported in several countries, especially in Asia, including China, Korea, Vietnam, and Thailand (Chen et al.,2011; Nilubol et al., 2013). The concurrent diversity and co-infection of both PRRSV genotypes have raised questions concerning control methods involving vaccination program, as to which types of PRRSV MLV should be used in the herd (Chaikhumwang et al.,2015; Nilubol et al.,2014). The multiple factors have been

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hypothesized to drive the genetic diversity of PRRSV such as the quasispecies characterics, the lack of proof reading of RNA polymerase in replication process, the genetic recombination and the selection of host immune system (Murtaugh et al.,2010). The diversity of PRRSV raises the concerning questions of the disease severity and the PRRSV control methods (Chaikhumwang et al.,2015).

The PRRSV control methods has been through the management of replacement stock which includes the acclimatization and vaccination with MLV prior to introduction to breeding herd that described in the clinical practice guideline (CPG) for PRRS in Thailand. Although, the different types of PRRSV vaccine: killed vaccine (KV), subunit vaccines, and MLVs, are commercially available in many country, vaccination with PRRSV-2 MLVs is preferable (nilubol et al.,2013). Although several control methods have been implemented to the herds, the disease occurs continuously in the herds and remains difficult to control (Shi et al.,2010).

The MLVs have been proved to be effective to control the infection with both genotypes of PRRSV and the MLVs are considered the most effective to reduce the clinical signs and the duration of viral shedding (Martelli et al.,2009; Murtaugh et al.,2011), but they cannot provide the completely protection against PRRSV. Moreover, PRRS MLV increased the PRRSV genetic diversity resulting in the emergence of variance isolates and the unsuccessful control program (Sun et al.,2018).

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2) Maternally-derived antibodies (MDAs)

MDAs or passive antibodies are transferred from dams to their offspring and protect the neonatal and young animals during the time of maturation of their immune system. The vast majority of MDAs are IgG isotype (Niewiesk, 2014). MDAs are induced during gestation by the infection or vaccination. In pigs, there are no antibodies transferred through the placenta from sows to their piglets. The piglets only receive passive immunity after birth, through the colostrum ingestion within the first 24 hours after birth. MDAs may persist up to eight weeks depending on the level of antibody in the colostrum. During first weeks of life, MDAs are essential for the survival of the

piglets. Their main function is to neutralize pathogen. The passive immunity (MDAs) in the term of PRRSV specific antibodies are important to protect the piglets from homologous PRRSV challenge. PRRSV-neutralizing antibodies (NAs) conferred sterilizing immunity in offspring which protect against viremia, viral replication in lungs, viral shedding through placenta (Labarque et al.,2000; Lopez et al.,2004). However, the NAs appears in low levels around 4 weeks post infection (Diaz et al. ,2005) . The presence of a 1:8 titer of PRRSV-NAs in serum is enough to block viremia but not protect against PRRSV replication and transmission (Lopez et al.,2007). The passive transfer of PRRSV-NAs at a higher concentration (SN titer of 1:32) induces full protection, but only in some of the young pigs (Lopez et al.,2007).

Unfortunately, MDAs have been observed to interfere with several vaccines, such as Influenza virus, Aujeszky's disease virus, Classical swine fever virus and PCV2. Also, the interference of MDAs has been proven for Equine viral arteritis, a disease caused by a virus belonging to Arterivirus. In the case of PRRS virus from previous reports, MDAs (PRRSV specific neutralizing antibodies; PRRSV specific NAs) have the negative effect on both humoral and cellular immune responses (Fablet et al.,2016). The PRRSV neutralizing antibodies inducing by MLV vaccination appeared early around 4 weeks post vaccination in the group of piglets that have low level of PRRSV neutralizing MDAs. In fact, leaflet of most vaccines include a special warning about MDA interference and how the timing of pig vaccination should be planned accordingly. The vaccination time for piglets should be adjusted to the level of MDAs specific to PRRSV.

3) **Humoral immune response against PRRSV infection**

PRRSV infection induces humoral antibody response within 5-9 day-post infection (DPI) without the presence of neutralizing antibodies (NAs). During primary response, IgM are predominant and can be detected until 42 DPI. IgG appear and peak around 3-6 weeks post infection and persist for months. This rapid humoral immune response is not the neutralizing antibodies and do not correlate with protection.

Neutralizing antibodies (NAs) are the crucial component of immune-mediated protection against most viral infection. The titers of NAs against PRRS virus are usually low (below 1:32) and do not confer cross-protection against heterologous isolates of PRRSV (Lee et al.,2014). PRRS specific NAs are consistently detected by day 28 DPI or later for both genotypes of PRRSV and directed against GP5, that contain the neutralizing epitopes (Gonin et al.,1999 and Nelson et al., 1993). Because of slow response, irregular appearance of PRRS specific NAs was unable to prevent the viremia.

Humoral antibodies against PRRSV can be detected in pigs at 5-9 day-post infection (DPI). During primary response, IgM are predominant and can be detected until 42 DPI. IgG appear and peak around 3-6 weeks post infection and persist for months. This rapid humoral immune response is not the neutralizing antibodies and do not correlate with protection.

Neutralizing antibodies (NAs) are the crucial component of immune-mediated protection against most viral infection. PRRS specific NAs can be detected around 28-42 DPI and the titers of NAs against PRRS virus are usually low (below 1:32). Moreover, PRRSV specific NAs is specific for homologous virus with partial neutralizing activity against heterologous viruses (Lee et al.,2014).

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4) PRRS modified-live virus (MLV) vaccine

PRRS modified-live virus (MLV) vaccine is well recognized for protective efficacy against PRRSV with several vaccination protocols. However, PRRS MLV vaccines have the limited efficacy against heterologous isolate of PRRSV and is one of the factors causing the genetic change of PRRSV (Lee et al.,2014; Neilsen et al,2001; Opriessnig et al,2002). At present, two MLV PRRSV vaccines representing types I and II are commercially available in Thailand. However, type II PRRSV MLV vaccine have been used preferentially because the manufacturer claims that it provides cross-protection against both types I and II. Although MLV vaccination has been used, many vaccinated herds have experienced sporadic disease outbreaks of PRRS (Nilubol et al.,2014).

Research problem

Do maternally-derived neutralizing antibodies (MDAs) mainly inducing by PRRSV MLV vaccination in sow herd have the effect on humoral immune response in the piglets given the same and different lineage of PRRSV MLVs?

Objectives of this study

To investigate the effect of MDAs mainly inducing by PRRSV MLV vaccination in sow herds on humoral immune response in the piglet given the same and different lineage of PRRSV MLVs.

Conceptual framework

To investigate the effect of MDAs on humoral immune response in piglets given PRRSV MLVs (IngelvacPRRS® and Prime Pac PRRS® MLV)

PHASE I Genetic characterization of field PRRSV-2 isolates from the studied herds

- ☐ Sample collection (Replacement gilts, nursery and finishing pigs)
- ☐ Detection of viral RNA using polymerase chain reaction (PCR)
- ☐ Genomic sequencing and phylogenetic analysis

PHASE II Evaluation of humoral immune responses in pigs with low and high MDA levels post vaccination

- ☐ Sample collection (Sows and nursery pigs)
- ☐ Polymerase chain reaction (PCR)
- ☐ ELISA
- ☐ Virus neutralization assay

Keywords

Porcine reproductive and respiratory syndrome, Maternally-derived antibodies, Humoral antibody, Modified lived virus vaccine, Pig

Advantages of Study

1) Opportunity to change timing of piglet vaccination, if virus neutralization antibodies are high it may be better to vaccinate after the antibody titers have decayed.
2) This knowledge will facilitate the design and implementation of a more successful PRRSV prevention and control program.

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CHAPTER II

MATERIALS AND METHODS

Material and methods

1) Ethics statement

All animal procedures were conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Research Council of Thailand according to protocols approved by Chulalongkorn University (IACUC number U1-07875-2561 and Protocol number 2031015).

2) Herd information

The study was conducted in three commercial swine herds located in Ratchaburi province. The herd was selected based on the permission of the herd owners and the serological information. In each herd, the serum samples were randomly collected from the replacement gilts and nursery to finishing pigs. The two PRRSV-positive swine herds: Herd 1 and Herd 2, and one PRRSV-negative (naïve) herd: Herd3 were recruited into this study. In each herd, it was operated in one-site farrow-to-finishing production facility. The breeding facilities were designated for breeding, gestation and farrowing activities. The farrowing units operated all-in/all-out by week and allow one week for downtime. The piglets were weaned at 3 weeks of age and move to nursery facilities. After nursery phase, the pigs were moved to the nursery house at approximately 9 weeks of age.

All sows were inseminated on-site using semen from PRRSV-free boars. The semen was confirmed PRRSV-negative result by PCR assay prior to insemination. The replacement gilts were internally produced and housed with nursery and finishing pigs. These replacement gilts were moved to the acclimatization facilities at 18 weeks of age for gilt developing and introduced to the herd at approximately 32 weeks of age.

Both PRRSV-1 and PRRSV-2 genotypes were detected in two PRRSV-positive herds. In each herd, the PRRSV control program was accomplished through

acclimatization of replacement gilts with the culling sows and PRRS MLV vaccination against PRRSV-2 prior to introduce to the breeding herd. Ingelvac PRRS® MLV (Boehringer Ingelheim, USA) and Prime Pac PRRS® MLV (MSD Animal Health, The Netherlands) were implemented to Herd 1 and Herd 2, respectively more than 3 years. The vaccination program included vaccination of all sows every three months and the piglets at 2 weeks of age. The replacement gilts were vaccinated with 2 doses of PRRSV-2 MLV at 18 and 22 weeks of age. The change of management was not observed in these studied herd.

In the PRRSV-negative herd, both types of PRRSV were not detected from replacement gilts and nursery to finishing pigs by RT-PCR assay and other serological tests (ELISA and virus neutralization (VN) test). There was no history of PRRS MLV vaccination. The status of this herd was not change before vaccination. The clinical signs of PRRSV infection was not observed.

The used piglets in each experimental herd were free from these major diseases including Enzootic pneumonia caused by *Mycoplasma hyopneumoniae*, Aujeszky's disease, Classical Swine Fever (CSF) Virus infection and Porcine Circovirus (PCV2) infection. All batch of the piglets was vaccinated against these diseases. Several control strategies were implemented to control the diseases with strict biosecurity.

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3) Experimental designs

<u>PHASE I</u> Genetic characterization of field PRRSV-2 isolates from the studied herds

The study was conducted in two PRRSV-2 positive herds from December 2017 to April 2020. Serum samples were randomly collected every 4 months: December 2017, April 2018, August 2018, December 2018, April 2019, August 201, December 2019 and April 2020. At each sampling time, 5 blood serum samples were collected from each of 3 population groups: replacement gilts, nursery pigs and finishing pigs. The sera were separated and assayed for the presence of viruses by polymerase chain reaction (PCR). Sequence reactions were performed at Biobasic Inc. (Ontario, Canada) using an ABI Prism 3730XL DNA sequencer. Pairwise sequence identity percentages

were further assessed. Phylogenetic tree was constructed from aligned nucleotide sequences based on ORF5 genes of PRRSV-2 isolates. Moreover, the field PRRSV-2 isolates which were characterized from each studied herd were used for neutralization assay in the next phase of experiment.

PHASE II Evaluation of humoral immune responses in pigs with low and high MDA levels post vaccination

The study was conducted in three studied herds: Herd 1, Herd 2 and Herd 3. In each of 2 PRRSV-positive herds, thirty multiparous (parity 2-5) sows were randomly selected from a batch of gestation (8 weeks after the last PRRSV-2 MLV vaccination). Blood samples were collected from all selected gestating sows at four weeks before farrowing time. The sera were separated and assayed for the presence of PRRSV-specific antibodies by ELISA and neutralizing antibodies (NAs) against field isolate of PRRSV-2 by virus neutralization (VN) test. Then, all selected gestating sows were moved to the farrowing house at 15 weeks of gestation. After parturition, all neonatal pigs from all selected gestating sows were weighed and received the colostrum to get the maternal immunity. The sixty piglets (2 piglets/sow) of 1.5±0.2 kg average body weight were selected and ear-tagged before cross fostering. They were bled at 7 days after birth to confirm the MDAs (PRRSV-specific antibodies) transfer level by ELISA and VN test. The twenty-four piglets with the highest level of MDAs (NA titer > 8) and twenty-four piglets with the lowest level of MDAs (NA titer < 8) were selected and allocated to 3 groups (8 pigs/group) including the group of non-vaccination, MLV1 vaccination and MLV2 vaccination that described in table 1.

In the PRRSV-negative (naïve) herd, fifteen multiparous (parity 2-5) sows were randomly selected from a batch of gestation. The serum samples were collected to confirm PRRSV-negative status by ELISA and VN test at four weeks before farrowing time. All piglets born to these selected sows were weighed at birth and also received the maternal antibody through the colostrum ingestion. The thirty piglets (2 piglets/sow) of 1.5±0.2 kg average body weight were randomly selected and ear-tagged. At 7 days of age, all these 30 piglets were bled and the sera were used to observe the MDAs transfer

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level before moving to the experimental facility. At weaning age, Twenty-four piglets were randomly allocated to 3 groups (8 pigs/group) that explained in table 1.

The experiment was observed until 10 weeks of age in nursery pigs. The blood serum samples from all pigs were repeatedly collected at 2, 3, 4, 6, 8, and 10 weeks of age. The sera were separated and assayed for the presence of PRRSV-specific antibodies by ELISA and neutralizing antibodies (NAs) against field isolate of PRRSV-2 and 2 different PRRSV-2 MLV isolates by virus neutralization (VN) test. Moreover, the viral RNA was detected using RT-PCR from serum samples.

The mortality rate was recorded. All pigs were weighed at 70 days of age. The average daily gain (ADG) was calculated at the end of experiment.

Table 1 Experimental design. Pigs were allocated into 15 groups. 10 treatment groups were vaccinated with MLV1 and MLV2 vaccines. Pigs from the H1-1, L1-1, H2-1, L2-1 and N-1 groups were vaccinated with MLV1 vaccine. Pigs from the H1-2, L1-2, H2-2, L2-2 and N-2 groups were vaccinated with MLV2 vaccine. The left 5 groups: H1, L1, H2, L2 and N was included as unvaccinated groups.

Breeding herds	PRRSV-2 MLV used in sow herd	MDAs titer level	PRRSV vaccines used in weaned pigs		
			No-vac	MLV1	MLV2
Herd 1	MLV1 (Ingelvac® PRRS MLV, PRRSV-2, Boehringer Ingelheim, USA)	High	H1 (n=8)	H1-1 (n=8)	H1-2 (n=8)
		Low	L1 (n=8)	L1-1 (n=8)	L1-2 (n=8)
Herd 2	MLV2 (Prime Pac® PRRS MLV (PRRSV-2, MSD Animal Health, The Netherlands)	High	H2 (n=8)	H2-1 (n=8)	H2-2 (n=8)
		Low	L2 (n=8)	L2-1 (n=8)	L2-2 (n=8)
Herd 3	No vaccine	-	N (n=8)	N-1 (n=8)	N-2 (n=8)

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4) Cell lines and viruses

Cell culture is necessary to isolate and propagate the field and vaccine viruses. For virus isolation and propagation, MARC-145 cell was used in this study. In this study, two PRRSV vaccine strains: VR2332 (Ingelvac® PRRS MLV, Boehringer Ingelheim, USA) and NEB-1 (Prime Pac® PRRS MLV, MSD Animal Health, The Netherlands) which are phylogenetically divergent from each other (Shi et al.,2010) was used in virus neutralization test, and the field isolates of PRRSV-2 from each herd was used in this study as well. Ingelvac PRRS® MLV (Boehringer Ingelheim, USA) belongs to sublineage 5.1 with the large number of international sequences. The vaccine-related sequences were also observed in this sublineage. The second vaccine, Prime Pac PRRS® MLV (MSD Animal Health, The Netherlands) belongs to lineage 7 with a few sequences. Field isolates of PRRSV-2 refer to THA_SP/RB_S1/P1/0120-18 and THA_WC/RB_F165/20-22 which were isolated from Herd 1 and Herd 2, respectively. The nucleotide and amino acid identities based on ORF5 gene between these field PRRSV-2 isolates and PRRSV-2 MLVs (Ingelvac® PRRS MLV, Boehringer Ingelheim, USA and Prime Pac® PRRS MLV, MSD Animal Health, The Netherlands) were summarized in Table 2.

The PRRSV-2 strains used in neutralization test was isolated and propagated in MARC-145 cells, and aliquots will be kept at -80° C to determine the virus titer (Madapong et al.,2017). To determine virus titer, 10-fold serial dilution was performed on virus stocks. The virus dilution in 96-well microtiter plate containing monolayer of MARC-145 cells was incubated for 48 hours and observed the CPE daily. The virus titer was calculated using Reed and Muench method as previously described (Christopher et al.,2001). The virus titer was expressed in 50% tissue culture infective dose ($TCID_{50}$) per ml. The starting virus amount of 100 $TCID_{50}$ per well was used.

5) Sample processing

5.1) Preparation of MARC-145 cells

MARC-145 was cultured in tissue culture flask (75 cm^2, Corning, USA) containing minimum essential media (MEM) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), antibiotics-antimycotics mixture (Gibco, USA) and glutamine (Gibco,

USA). MARC-145 cells were cultured at 37°C in a humidified atmosphere with 5% CO_2 until it reached monolayer morphology under inverted microscope. Then, the cells were washed with 1X PBS pH 7.4, followed by MEM before performing of virus isolation or propagation.

5.2) Preparation of blood sample inoculums

To prepare inoculum from blood sample, the sera were separated from blood samples by centrifugation at 2,500 rpm for 5 mins, then the aliquots were diluted with MEM (1:1 ratio) into 1 ml final volume and kept at -80°C for further used.

5.3) Preparation of PRRSV inoculums

To prepare inoculum form PRRSV vaccines, lyophilzed type II-PRRS MLV vaccines will be diluted with MEM into 1 ml final volume and kept at -80°C for further used.

5.4) Virus isolation and propagation

Monolayer MARC-145 cells were added with the inoculum. Then, cells were incubated for 1 hour in humidified CO_2 incubator and removed inoculums. Complete MEM was added, and cells were incubated for 3 – 5 days in humidified CO_2 incubator until the cytophatic effect (CPE) was observed under inverted microscope. PRRSV-infected cells appeared round-up morphology compared to the normal cells.

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5.5) Harvest virus

To collect virus, CPE-positive MARC-145 cells will be frozen at -80°C overnight and rapidly thawed at 37°C for 5 mins, two times. Cell culture containing virus was centrifuged at 2,500 rpm for 15 mins, 4°C and supernatant was collected, filtered (0.25μm), aliquoted and kept at -80°C for further used.

6) Laboratory analysis

6.1) PCR and sequence determination

PRRSV RNA was extracted from serum samples using the NucleoSpin® RNA virus kit (Macherey-Nagel Inc., PA, USA) in accordance with the manufacturer's instructions. cDNA was synthesized from the extracted RNA using M-MuLV reverse transcriptase (New England BioLabs Inc., MA, USA). PCR amplification was performed on the cDNA, and to amplify ORF5 of PRRSV-2 progeny, the following primers was utilized: ORF5 USF (5' - CCT GAG ACC ATG AGG TGG G - 3') and ORF5 USR (5' - TTT AGG GCA TAT ATC ATC ACT GG - 3'). PCR amplification was performed using a commercial kit (Go tag_ Green Master Mix, Promega, WI, USA). After the initial incubation at 95 °C for 2 min, the reactions were subjected to 35 cycles of PCR as follows: 95 °C for 30 s; 54 °C for 30 s; and 72 °C for 45 s, followed by a terminal, 5-min extension at 72 °C. Amplified PCR products were purified using a PCR purification kit (Macherey-Nagel, Germany). Sequence reactions will be performed at Biobasic Inc. (Ontario, Canada) using an ABI Prism 3730XL DNA sequencer.

6.2) Sequence analysis

The ORF 5 sequences of PRRSV-2 were used for sequence alignment and phylogenetic analysis. The nucleotide sequences of ORF 5 genes were aligned using CLUSTALW (Thompson et al.,1994). Amino acid sequences were aligned using BioEdit. Nucleotide sequence similarities (as percentages) were assessed (Forsberg et al.,2002; Nilubol et al,2013). A phylogenetic tree was constructed from the aligned nucleotide sequences based on ORF 5 genes by using neighbor-joining in MEGA 7 software. Neighbor-joining (NJ) trees were generated with a Kimura 2-parameter model using MEGA 7 (Tamura et al.,2007). The robustness of the phylogenetic analysis and the significance of the branch order were determined by bootstrap analysis with 1000 replicates.

6.3) Antibody detection

Serum samples were assayed for the presence of PRRSV-specific antibody by ELISA and SN assays. ELISA (HeardCheck PRRS X3, Idexx Laboratories Inc. , Westbrook, Maines, USA) was performed in accordance with the manufacturer' s instructions. The presence or absence of antibody was determined by calculating the sample-to-positive control (S/P) ratio of the test. The results were considered positive for PRRSV antibody when the S/P ratio was greater than 0.4. SN assay was performed to titrate PRRSV neutralizing antibodies (NAs) . PRRSV-NAs were quantified in serum samples from all vaccinated and unvaccinated pigs in MARC-145 cells against PRRSV-2 MLV isolates and field PRRSV-2 isolates as described previously. MARC-145 cells were cultured in 96-well, flat bottom plate for 2 days until monolayer-morphology was observed. Serially diluted supernatant containing virus was added in each well, incubated for 1 hour and completed MEM was added. CPE-positive cells were observed, and virus titers were calculated. Neutralization titers were expressed as the reciprocal of the highest serum dilution that completely inhibit virus infection (no CPE).

7) Data analysis

All statistical analyses were performed using the Statistical Analysis System(SAS) software, version 9.0 (2002, SAS Institute Inc. , Cary, NC, USA) . All differences in variables between treatment groups were considered significant when $P<0.01$. To determine the effect of vaccination on growth performance, average daily gain (ADG) between weaning and 10 weeks of age was calculated and expressed as the mean ± standard error of mean (mean± SEM) . An analysis of variance was used to compare growth performance between groups ($p < 0.01$) . Post-hoc pairwise comparisons were then performed using the Tukey test to adjust the p-values of these comparisons. The survival rate was calculated and compared between groups at the end of experiment. All ELISA values and SN titers reported as mean ±SEM.

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CHAPTER III

RESULTS

1) Phylogenetic analysis of PRRSV-2 isolates

To investigate the field isolates of PRRSV-2 in two studied herds: Herd1 and Herd 2 which were vaccinated with Ingelvac® PRRS MLV and Prime Pac PRRS® MLV in sows and piglets more than 5 years, the complete ORF5 genes of PRRSV-2 isolates collected in 2017, 2018, 2019, and 2020 were analyzed. Phylogenetic tree was constructed for PRRSV-2 isolates. A systematic classification of PRRSV-2 genotype has been conducted based on 195 sequences in database including field isolates of PRRSV-2 and PRRSV-2 MLV isolates.

PRRSV-2 was divided into nine lineages (1-9) in this system, which was used for PRRSV-2 classification in the present study. Phylogenetic analysis demonstrated the PRRSV-2 isolates collected from 2 studied herd between 2017 and 2020 were classified into two distinct lineages including 24 sequences in lineage 8.7 and 22 sequences in lineage 1 (Fig.1). The PRRSV-2 isolates collected from Herd 2 were classified only into lineage 8.7 since 2017. In contrast, the field isolates of PRRSV-2 collected from Herd 1 were classified into two lineages as previously described. However, the isolations of PRRSV-2 collected between 2018 and 2020 were not observed in lineage 8.7 in Herd 1 over time (Fig.1).

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The pairwise nucleotide and amino acid identity values between the field isolates from Herd 1 (THA_SP/RB_S1/P1/0120-18) and Herd 2 (THA_WC/RB_F165/20-22) were 83.58% and 84.07%, respectively (Table 2). THA_SP/RB_S1/P1/0120-18 isolated from Herd 1 was classified into lineage 1 which shared 83.25% and 83.74% nucleotide sequence identities with Ingelvac® PRRS MLV and Prime Pac® PRRS MLV, respectively. Furthermore, THA_WC/RB_F165/20-22 isolated from Herd 2 was classified into lineage 8.7, shared 87.89% and 88.23% nucleotide sequence identities with Ingelvac® PRRS MLV and Prime Pac® PRRS MLV, respectively, which its nucleotide sequence was more identical to Ingelvac® PRRS MLV and Prime Pac® PRRS MLV than that isolated from

Herd 1 (Table 2). The phylogenetic tree demonstrated that all isolates collected from Herd 2 were consistently observed in lineage 8.7 throughout the study and became dominant in PRRSV-2 genotype population in this studied herd over time (Fig.1).

Table 2 Nucleotide and amino acid identities based on ORF5 gene between vaccine isolates and field PRRSV-2 isolates.

		Nucleotide and amino acid identities				
Herd	**PRRSV-2 isolates**	Level of similarity	THA_SP/RB_S1/P1/0120-18	THA_WC/RB_F165/20-22	Ingelvac® PRRS MLV	Prime Pac® PRRS MLV
Herd 1	THA_SP/RB_S1/P1/0120-18	Nucleotide	100.00%	83.58%	83.25%	83.74%
		Amino acid	100.00%	84.07%	84.07%	84.07%
Herd 2	THA_WC/RB_F165/20-22	Nucleotide	83.58%	100.00%	87.89%	88.23%
		Amino acid	84.07%	100.00%	86.07%	92.53%

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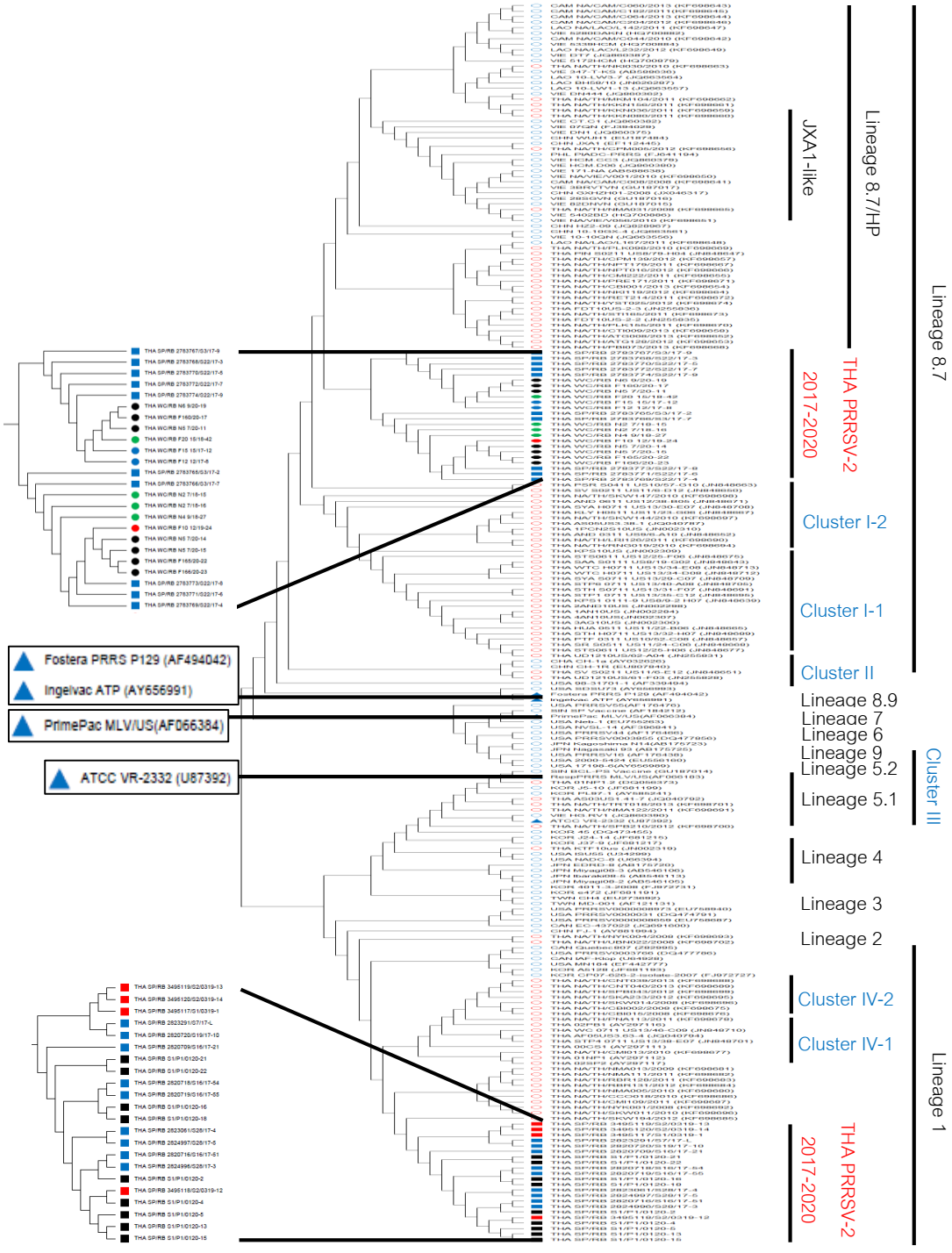


Figure1 Neighbor-joining tree of PRRSV-2 isolates based on the nucleotide sequences of complete ORF5 genes. Filled triangles represent PRRSV-2 prototype virus (VR-2332) and PRRSV-2 modified live vaccines (MLVs) . The rest filled squares and circles represent the field isolates of PRRSV-2 from Herd 1 and Herd 2, respectively. The color of filled squares and circles indicates the year of isolation. Blue, green, red and black indicate isolation of PRRSV-2 in 2017, 2018, 2019 and 2020, respectively.

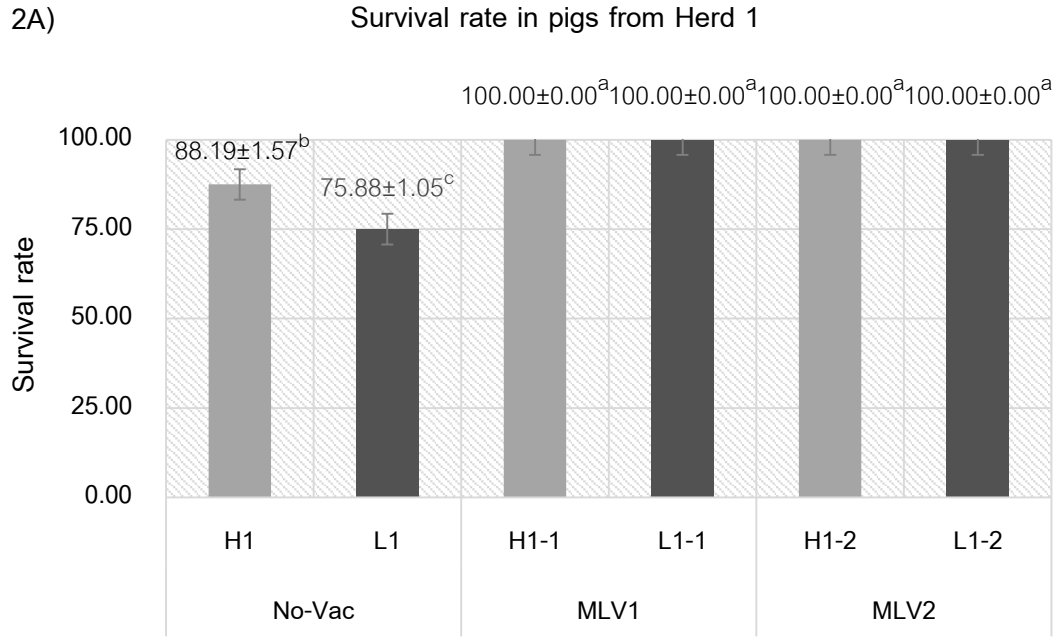
2) Mortality and growth performances

Pigs from Herd 1, the survival rates of pigs from all vaccinated group: H1-1, L1-1, H1-2, and L1-2 groups, were 100% , while the pigs in unvaccinated groups: H1 and L1 groups were 88.19±1.57% and 75.88±1.05%, respectively (Fig.2A). The survival rates of pigs in all vaccinated groups had significantly ($p < 0.001$) higher than that of pigs in unvaccinated groups. However, pigs with high level of MDAs from the H1 group had significantly ($p < 0.001$) higher, as compared to the pigs with low level of MDAs from the L1 group (Fig.2A). Pigs from Herd 2, the mortality was not observed in all PRRSV-2 MLV vaccinated groups: H2-1, L2-1, H2-2, and L2-2 groups. However, the survival rate in pigs which had high levels of MDAs in the H2 group was 100.00±00% as well. In contrast, the survival rate in pigs with low level of MDAs in the L2 group was 74.63±0.96% and it had significantly ($p < 0.001$) lower than that of pigs in the other groups from Herd 2 (Fig.2B). Pigs from Herd 3, there was only one group: N-2 group which the mortality was not observed entire the period of experiment. The survival rates in pigs from the N and N-1 groups were 63.94±2.72% and 85.31±1.11% , respectively (Fig.2C).

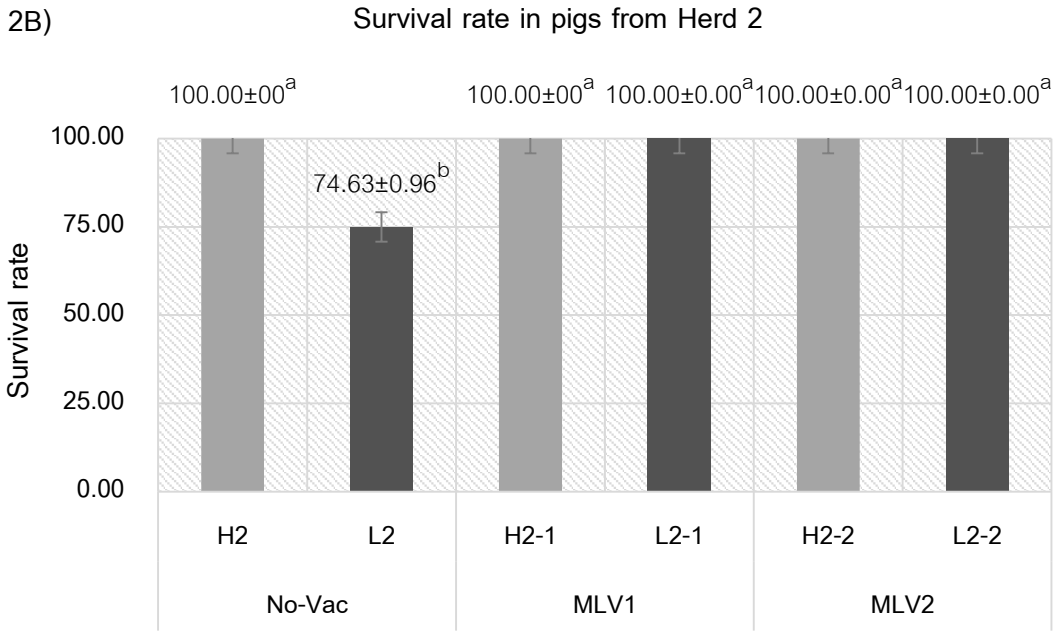
The body weight gain of pigs was monitored at the end of experiment. Pigs from Herd 1, pigs with low level of MDAs in unvaccinated group (L1 group) showed the lowest weight gain and ADG (Fig.3A). The pigs in all vaccinated groups: H1-1, L1-1, H1-2, and L1-2 had significantly ($p<0.001$) higher weight gain and ADG than those in pigs from the L1 group. Although, pigs in the H1 group were not vaccinated with PRRSV-2 MLV, there was no significant difference ($p<0.001$) in weight gain and ADG between the H1 group and the other vaccinated groups at the end of experiment (Fig.3A). Pigs with high level of MDAs vaccinated with MLV2 (Prime Pac® PRRS MLV) showed the highest growth performance (ADG) at the end of experiment (Fig.3A). Pigs from Herd 2, pigs in all vaccinated herds: H2-1, L2-1, H2-2, and L2-2 groups had significantly ($p<0.001$) higher weight gain and ADG than those in pigs from the unvaccinated group (L2 group). However, the pigs from the L2-1 groups exhibited the lower ADG, as compared to that in the unvaccinated pigs from the H2 group (Fig.3B). The ADG of pigs in the L2 group was

the significantly (p<0. 001) lowest in this studied herd (Fig. 3B) . Moreover, the unvaccinated pigs from herd 3 (N group) exhibited the lowest weight gain and ADG at the end of experiment (Fig.3C).

2A)



2B)



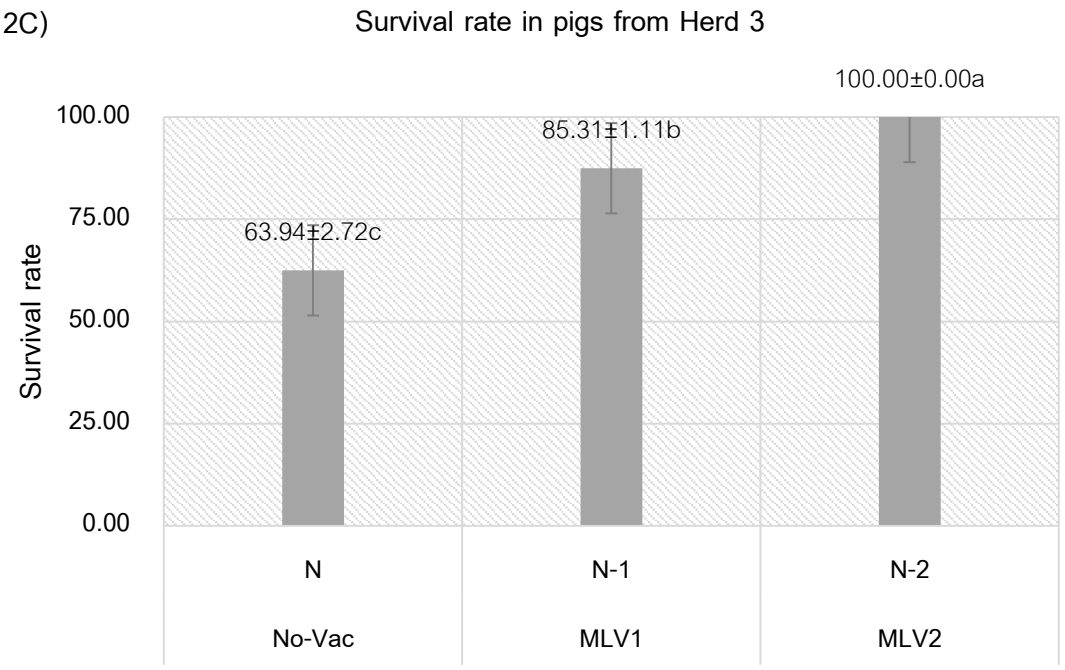
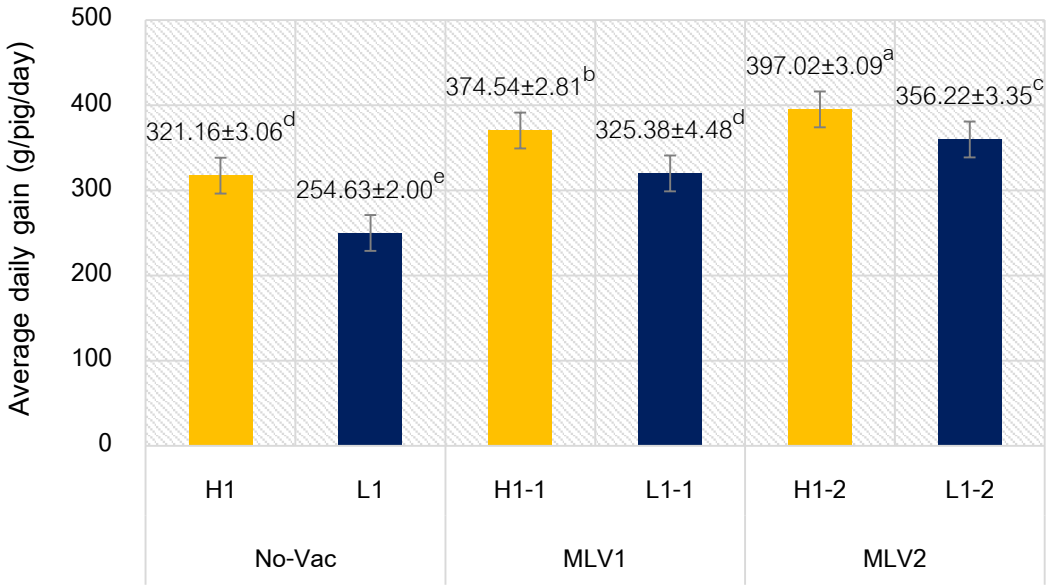
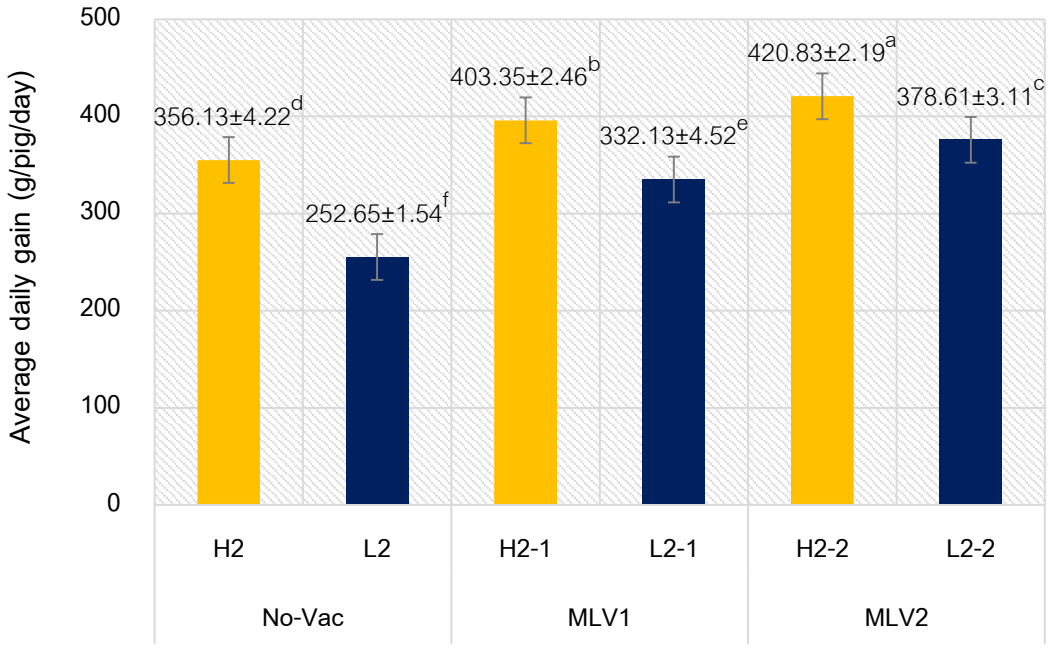


Figure 2. The survival rate of nursery pigs in each different herd: Herd 1, Herd 2, and Herd 3. (**2A**) The survival rates in 6 different PRRSV-2 MLV vaccinated groups: H1, H1-1, H1-2, L1, L1-1, and L1-2 groups from Herd 1 which sow herd were vaccinated with Ingelvac® PRRS MLV. (**2B**) The survival rates in 6 different PRRSV-2 MLV vaccinated groups: H2, H2-1, H2-2, L2, L2-1, and L1-2 groups from Herd 2 which sow herd were vaccinated with Prime Pac® PRRS MLV. (**2C**) The survival rates in 3 different PRRSV-2 MLV vaccinated groups: N, N-1, and N-2 groups from Herd 3 which sow herd were PRRSV negative. Variation is expressed as the standard deviation. Different letters in superscript indicate statistical significant difference (p-value <0.01) between groups.

3A) Mean values of average daily gain (ADG) in pigs from Herd 1



3B) Mean values of average daily gain (ADG) of pigs from Herd 2



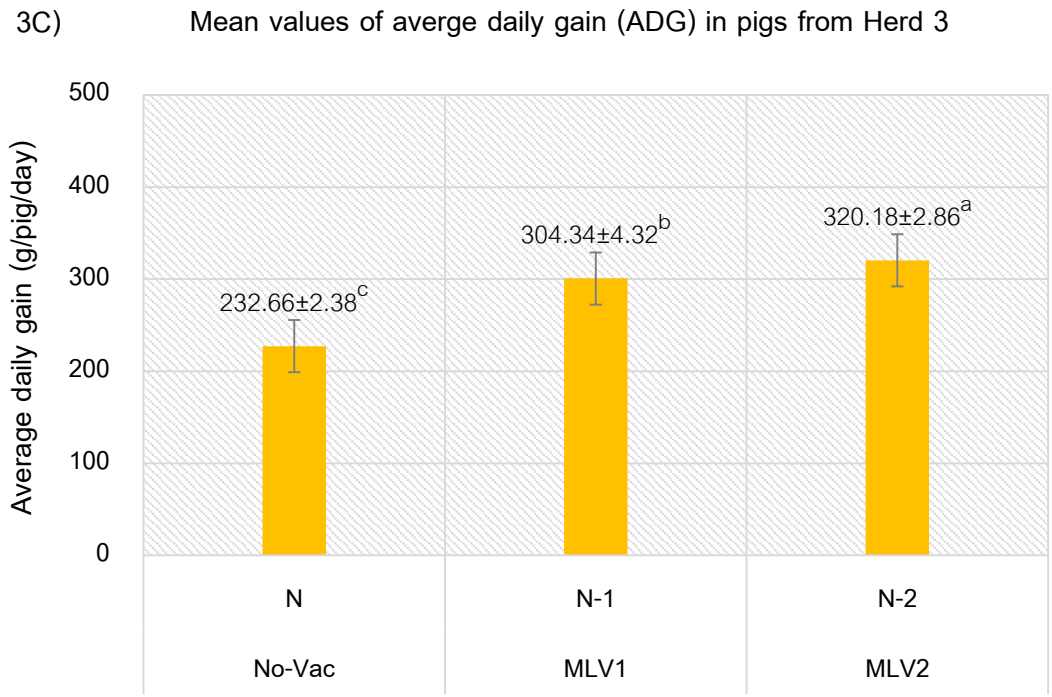


Figure 3 The mean average daily gain (ADG) of pigs in 3 different herds: Herd 1, Herd 2, and Herd 3. (**3A**) The mean ADG of pigs in the H1, H1-1, H1-2, L1, L1-1, and L1-2 groups from Herd 1 which sow herd were vaccinated with Ingelvac® PRRS MLV. (**3B**) The mean ADG of pigs in the H2, H2-1, H2-2, L2, L2-1, and L1-2 groups from Herd 2 which sow herd were vaccinated with Prime Pac® PRRS MLV. (**3C**) The mean ADG of pigs in the N, N-1, and N-2 groups from Herd 3 which sow herd were PRRSV negative. The ADG values are expressed as mean ± standard error of mean (SEM). Different letters in superscript indicate statistical significant difference (p-value <0.001) between groups.

3) Viremia (RT-PCR) in serum samples

Viral RNA of both genotypes of PRRSV: PRRSV-1 and PRRSV-2, was not detected in all pigs in each treatment groups before PRRSV-2 MLV vaccination. All pigs in the unvaccinated groups: N, H1, L1, H2, and L2 groups remained PRRSV-1 and PRRSV-2 PCR negative throughout the experiment.

Pigs from Herd 1, viral RNA was detected at 7 and 21 DPV in pigs from the L1-1 and L1-2 groups which all pigs in these 2 groups showed low levels of MDAs indicated by the SN titers against PRRSV-2 MLV1 (Ingelvac® PRRS MLV) and MLV2 (Prime Pac® PRRS MLV) isolates before PRRSV-2 MLV vaccination. The left 2 groups: H1-1 and H1-2 groups, exhibited the same pattern of viremia post vaccination, the PRRSV-2 RNA was only detected at 7 DPV.

Pigs from Herd 2, all vaccinated groups: H2-1, L2-1, H2-2, and L2-2 groups, showed the similar pattern of viremia which PRRSV-2 RNA was only detected at 7 DPV.

Pigs from Herd 3 which is the PRRSV negative, both 2 vaccinated groups: N-1 and N-2 groups, showed PRRSV-2 viremia at 7 and 21 DPV. At 35 and 49 DPV, the viremia was not detected.

4) Antibody response as measured by ELISA

The PRRSV-specific antibody responses were obviously different between unvaccinated and PRRSV-2 MLV vaccinated groups. Pigs from negative herd in unvaccinated group (N group) remained serologically negative throughout the study. The seroconversion was not observed in all pigs from the N group. At 0 DPV, pigs in all vaccinated groups: N-1 and N-2 groups, showed similar patterns of antibody responses post PRRSV-2 MLV vaccination, the seroconversion was detected as early as 7 DPV and reached a peak level at 49 DPV (Fig.6). However, there was no significant difference ($p<0.001$) in PRRSV-specific antibody titers between the N-1 and N-2 groups at the end of experiment (Fig.6).

Pigs from Herd 1 with high and low level of MDAs in the H1 and L1 groups, showed similar patterns of antibody responses post PRRSV-2 MLV vaccination which the seroconversion was not observed throughout the experiment (Fig.4). The PRRSV-

specific antibody responses were detected as early as 7 DPV in pigs with high and low level of MDAs from the H1-2 and L1-2 groups which was vaccinated with Prime Pac® PRRSV MLV at 3 weeks of age (0 DPV) , and their PRRSV-specific antibody titers reached their peaks at 49 DPV. However, there was no significant difference ($p<0.001$) in PRRSV-specific antibody titers between the H1-2 and L1-2 groups at the end of experiment (Fig.4). At, 21 DPV, pigs in the H1-1 group exhibited the seroconversion and their PRRSV-specific antibody titers reached the peaks at 49 DPV with no significant difference ($p<0.001$) compared to those in pigs from the H1-2 group at the end of experiment (Fig.4).

Pigs from Herd 2, at 21 DPV, the seroconversion was observed in pigs from different 4 vaccinated groups: H2-1, H2-2, L2-1 and L2-2 and reach their peaks at 49 DPV, and their antibody responses was significantly ($p<0.001$) stronger than those of the pigs from the unvaccinated groups (H2 and L2 groups) at the end of experiment. In contrast, the seroconversion was detected late at 35 DPV in pigs from the L2 group (Fig.5).

Pigs from Herd 3, PRRSV-specific antibody responses were obviously different between unvaccinated and PRRSV-2 MLV vaccinated groups. Pigs in unvaccinated group (N group) remained serologically negative throughout the study (Fig.6).

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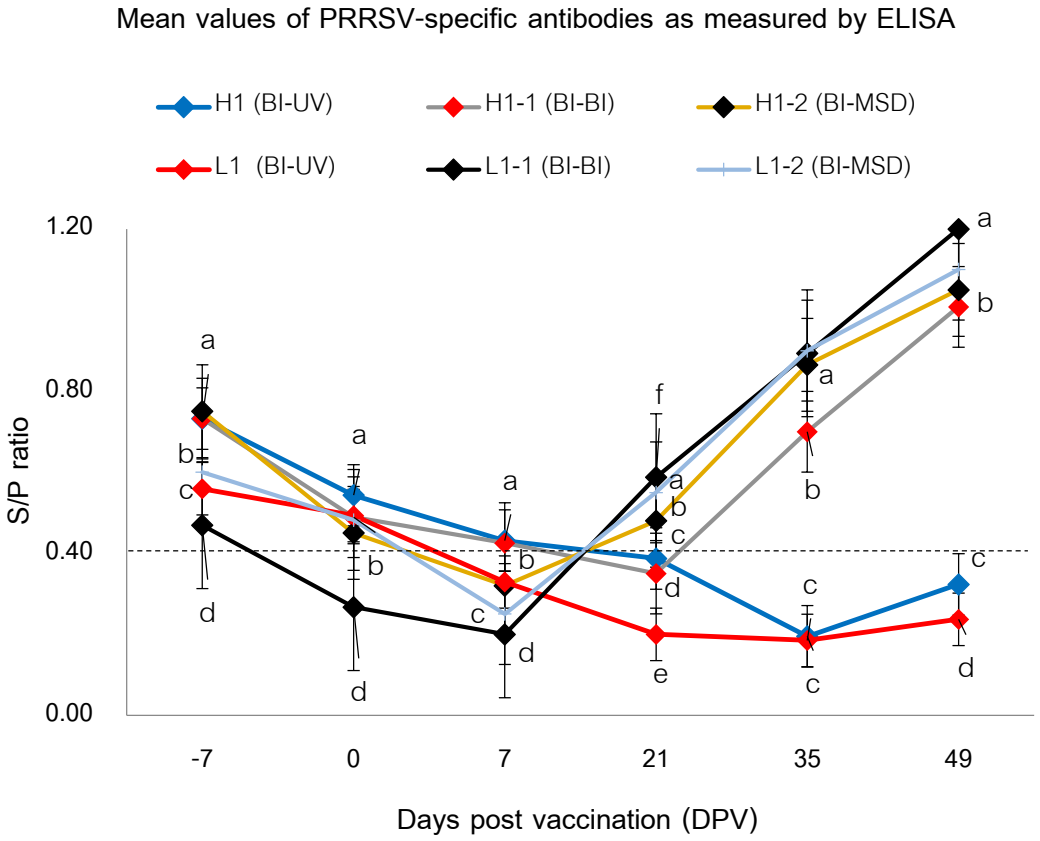

Figure 4 Mean values of PRRSV specific antibodies as measured by ELISA of 6 treatment groups: H1, H1-1, H1-2, L1, L1-1, and L1-2 groups from Herd 1. Antibody titers were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001). A dash line indicates the cutoff level (S/P ratio of 0.4). All serum samples collected at 0 DPV were PRRSV ELISA positive and PCR negative. Pigs in the H1 and L1 groups, their S/P ratio decreased continuously and PCR results remained negative throughout the study.

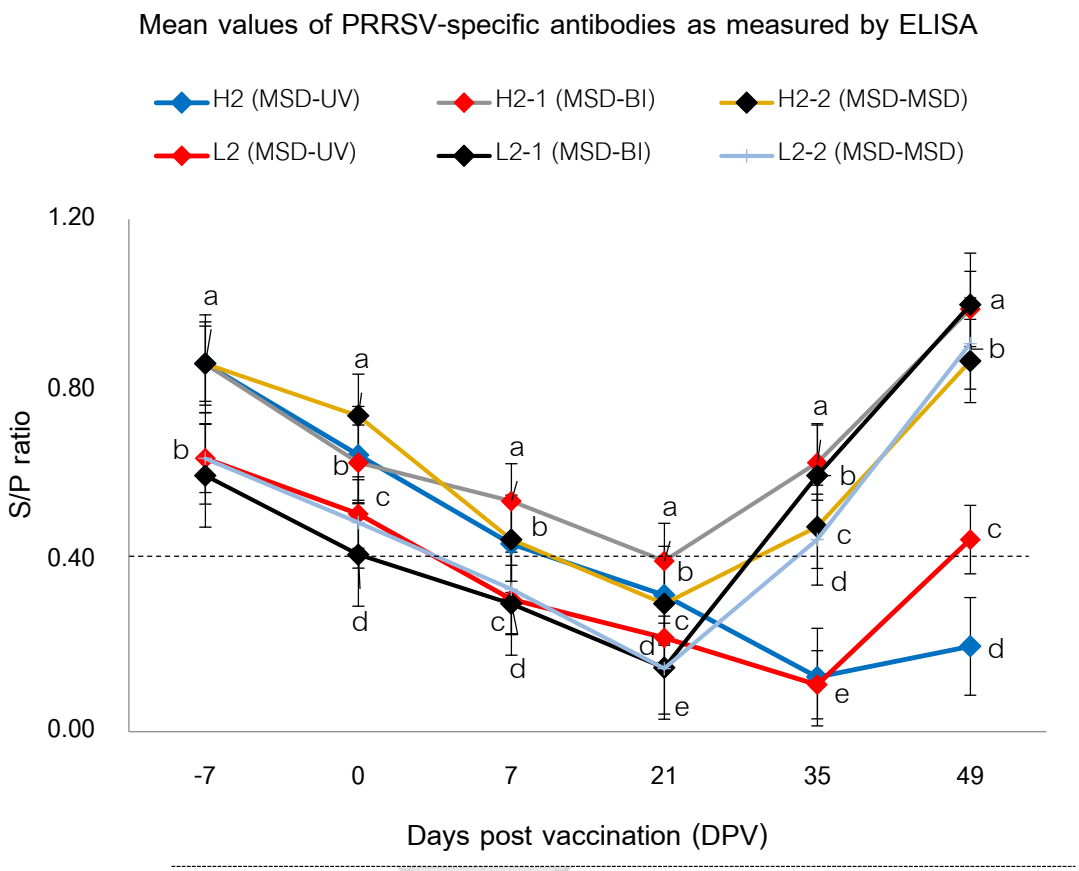

Figure 5 Mean values of PRRSV specific antibodies as measured by ELISA of 6 treatment groups: H2, H2-1, H2-2, L2, L2-1, and L2-2 groups from Herd 2. Antibody titers were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001). A dash line indicates the cutoff level (S/P ratio of 0.4). All serum samples collected at 0 DPV were PRRSV ELISA positive and PCR negative.

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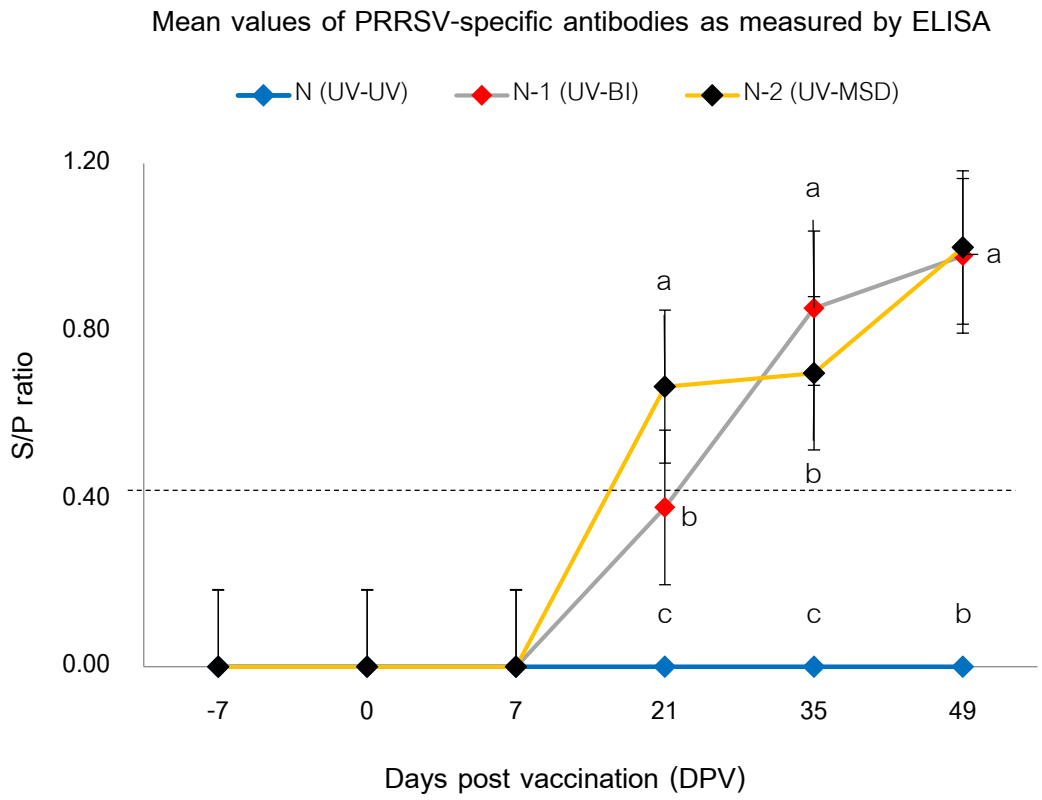

Figure 6 Mean values of PRRSV specific antibodies as measured by ELISA of 3 treatment groups: N, N-1, and N-2 groups from Herd 3 (negative herd). Antibody titers were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001). A dash line indicates the cutoff level (S/P ratio of 0.4). All serum samples collected at 0 DPV were PRRSV ELISA negative and PCR negative. Pigs in the N group remained ELISA and PCR negative throughout the study.

5) Antibody response as measured by SN assay

Pigs from negative herd (Herd 3) in unvaccinated group (N group) remained serologically negative throughout the study. Pigs in all vaccinated groups including the N-1 and N-2 groups showed the similar patterns of SN-titers against homologous viruses. The SN-titers against homologous viruses which referred to PRRSV-2 MLV isolates of each Herd 1 and Herd 2 were detected as early as 21 DPV and reached their highest levels at 49 DPV (Fig. 15 and Fig. 16). In the case of the heterologous field isolates of PRRSV-2: THA_SP/RB_S1/P1/0120-18 and THA_WC/RB_F165/20-22, the SN-titer against THA_SP/RB_S1/P1/0120-18 was observed at 21 DPV only in pigs from the unvaccinated group: N group, and slightly increased with significant ($p < 0.001$) higher than that in all vaccinated groups (Fig. 17). In contrast, the SN-titers against THA_WC/RB_F165/20-22 in the N and N-1 were not observed throughout the study (Fig.18). The SN-titer against THA_WC/RB_F165/20-22 was late detected at 35DPV and slowly increased in pigs vaccinated with Prime Pac® PRRSV MLV in the N-2 group (Fig.18).

Pigs with high and low level of MDAs from Herd 1 in unvaccinated groups: H1 and L1 groups showed the similar patterns of SN-titers against Ingelvac® PRRS MLV isolate, the SN levels gradually decreased and reached the lowest levels at 49 DPV (Fig.7). However, the SN-titers against Ingelvac® PRRS MLV isolate in the H1 group was significantly ($p < 0.001$) stronger than that in the L1 group entire experiment period (Fig.7). Pigs from the H1-1 and L1-1 groups were vaccinated with Ingelvac® PRRS MLV at 3 weeks of age (0 DPV) as previously described. At 0 DPV, the average SN levels in the H1-1 were significantly ($p < 0.001$) higher than those in the L1-1 group (Fig.7). The SN-titer in pigs from H1-1 gradually increased at 21 DPV and reached a peak level at 49 DPV. On the contrary, the SN-titer in pigs from L1-1 rapidly increased that could be detected as early as 7 DPV, and reached a peak at 49 DPV, and the highest level of SN-titer against Ingelvac® PRRS MLV isolate was observed in this group (Fig.7).

In the case of the Prime Pac® PRRSV MLV isolate, the SN responses against this strain rapidly increased at 7 DPV only in all pigs vaccinated with Prime Pac® PRRSV

MLV (H1-2 and L1-2 groups) (Fig.8). At 21 DPV, the SN titers in pigs from the H1-2 and L1-2 groups were significantly ($p<0.001$) higher than those from the H1, H1-1, L1 and L1-1 groups and reached a peak level at 49 DPV. The SN responses in pigs from the H1, H1-1, L1 and L1-1 groups were still low throughout the experiment (Fig.8).

The heterologous field isolate of PRRSV2: THA_SP/ RB_S1/ P1/ 0120-18 and THA_WC/RB_F165/20-22, all pigs from Herd 1 exhibited the similar pattern of SN-titers against field isolates of PRRSV2 that gradually decreased entire the period of experiment (Fig.9 and Fig.10). This situation indicated that there was no infection of field isolates of PRRSV-2 throughout the experiment.

Pigs with low and high level of MDAs from Herd 2 in unvaccinated groups: H2 and L2 groups, showed the similar pattern of SN responses against Prime Pac® PRRS MLV isolate. The SN titers in pigs from the H2 and L2 groups continuously decreased and reached the lowest levels at 49 DPV (Fig.12). The Pattern of SN-titers against Prime Pac® PRRS MLV isolate in the H2-1 was not different from those in the unvaccinated pigs (H2 group), it gradually decreased and reached the lowest level at 49 DPV with no significant difference in SN titers. In contrast, the SN-titers in pigs with low MDA level in L2-1 gradually increased at 7 DPV and then declined (Fig.12). Moreover, the SN-titers in pigs with low level of MDAs in L2-2, rapidly increased starting from 7 DPV and reached the peak levels at 49 DPV (Fig.12). The SN-titers in pigs from the H2-2 group decreased post PRRSV-2 MLV vaccination, then gradually increased at 21 DPV, and reached the highest level at 49 DPV and the highest level of SN-titers against Prime Pac® PRRS MLV isolate was observed in this group entire the experiment (Fig 12).

In the case of the Ingelvac® PRRSV MLV isolate, the pigs from the unvaccinated groups (H2 and L2 groups) and the Prime Pac® PRRS MLV vaccinated groups (H2-2 and L2-2 groups) showed the similar pattern of SN-titers which were still low throughout the experiment (Fig.11). In contrast, the SN-titers against Ingelvac® PRRSV MLV isolate in pigs from the H2-1 and L2-1 groups were significantly ($p<0.001$) stronger than that in the H2, L2, H2-1 and L2-2 groups entire experiment period (Fig.11). However, the SN-

titer presented in pigs from the L2-1 group had significantly ($p<0.001$) higher than that in pigs from the H2-1 group throughout the experiment (Fig.11).

The heterologous field isolate: THA_WC/RB_F165/20-22, all pigs from Herd 2 exhibited the similar pattern of SN-titers against field isolates of PRRSV2 that gradually declined and reached their lowest levels between 35 and 49 DPV (Fig.14). Furthermore, in case of the other field isolate of PRRSV2: THA_SP/RB_S1/P1/0120-18, the SN-titers in pigs from the L2-1 rapidly increased during 0 to 7 DPV with the significantly ($p<0.001$) highest level entire the experimental period (Fig.13). The increasing of SN-titer was also observed in pigs vaccinated with Ingelvac® PRRSV MLV from the H2-1 group at 21 DPV with no significant difference in SN-titers between the H2-1 and L2-1 groups at the end of experiment (Fig.13). In contrast, the SN-titers in pigs from the left unvaccinated and Prime Pac® PRRSV MLV vaccinated groups slightly declined and reached their lowest levels between 35 and 49 DPV (Fig.13).

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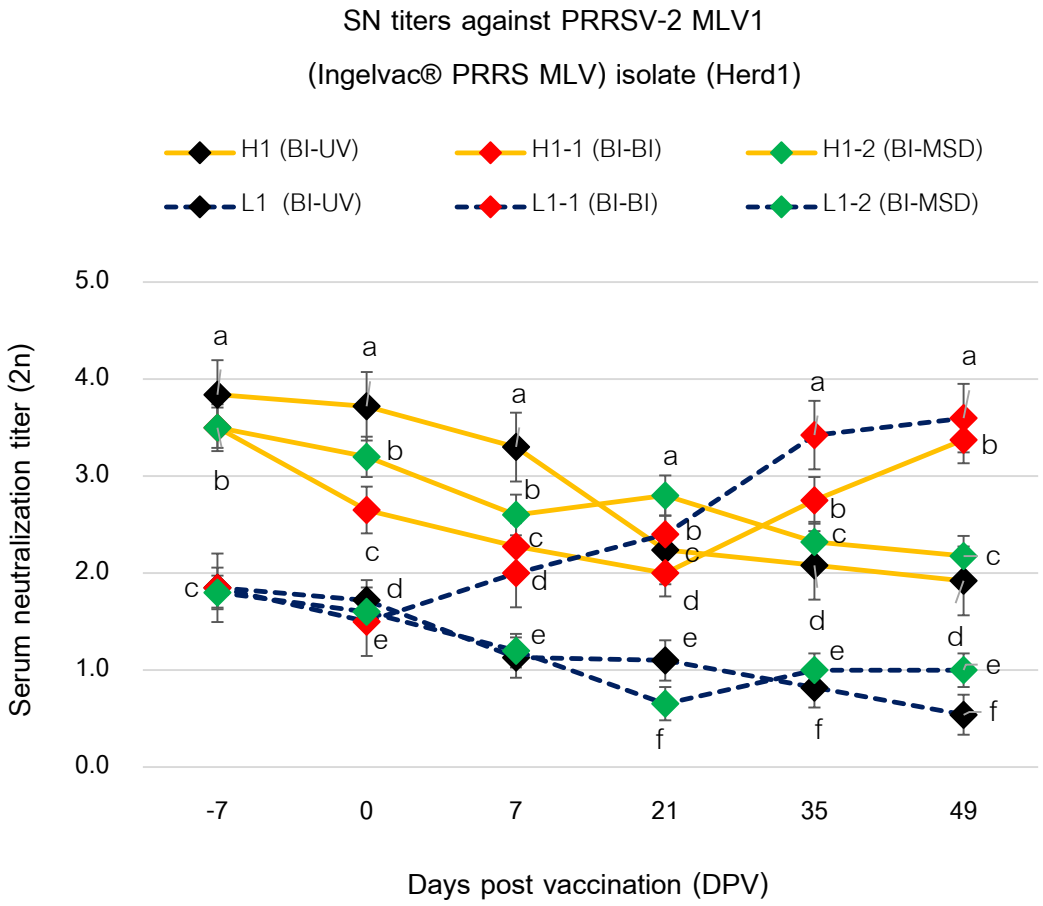

Figure 7 Antibody response as measured by serum neutralization (SN) assay using PRRSV-2 MLV1 isolate which referred to Ingelvac® PRRS MLV isolate including 6 groups: H1, H1-1, H1-2, L1, L1-1, and L1-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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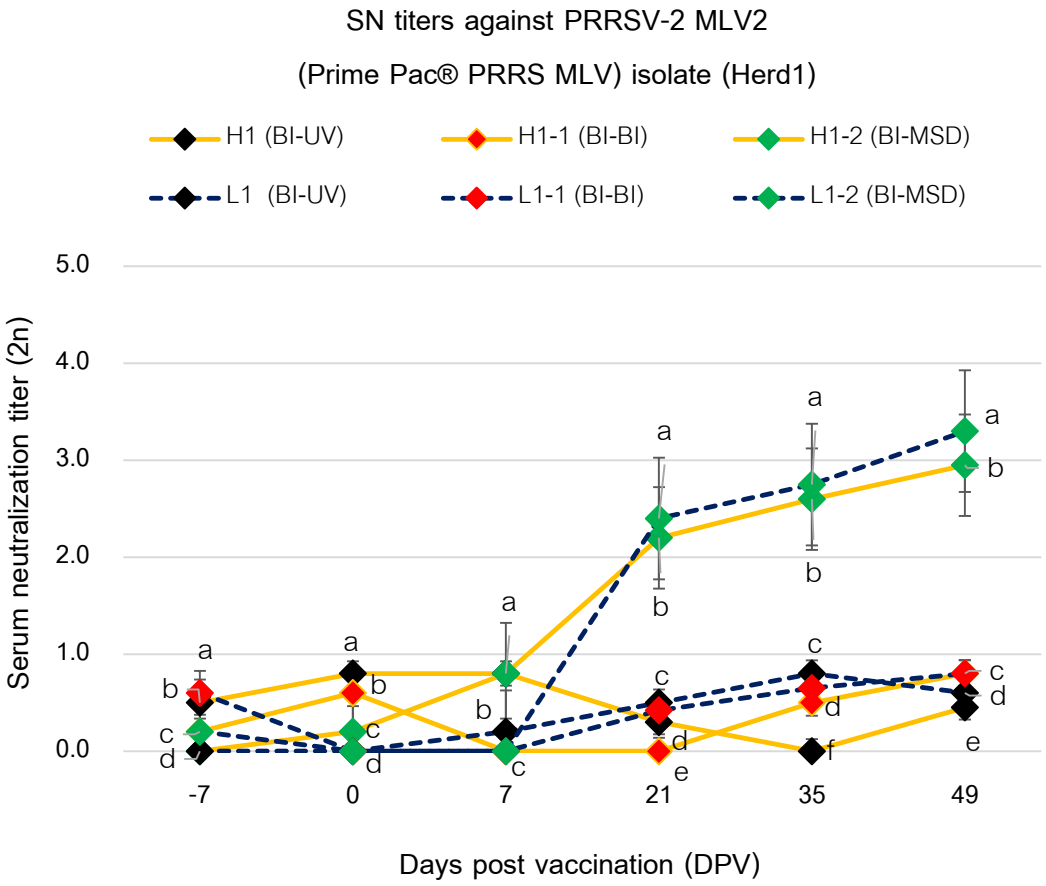

Figure 8 Antibody response as measured by serum neutralization (SN) assay using PRRSV-2 MLV2 isolate which referred to Prime Pac® PRRS MLV isolate including 6 groups: H1, H1-1, H1-2, L1, L1-1, and L1-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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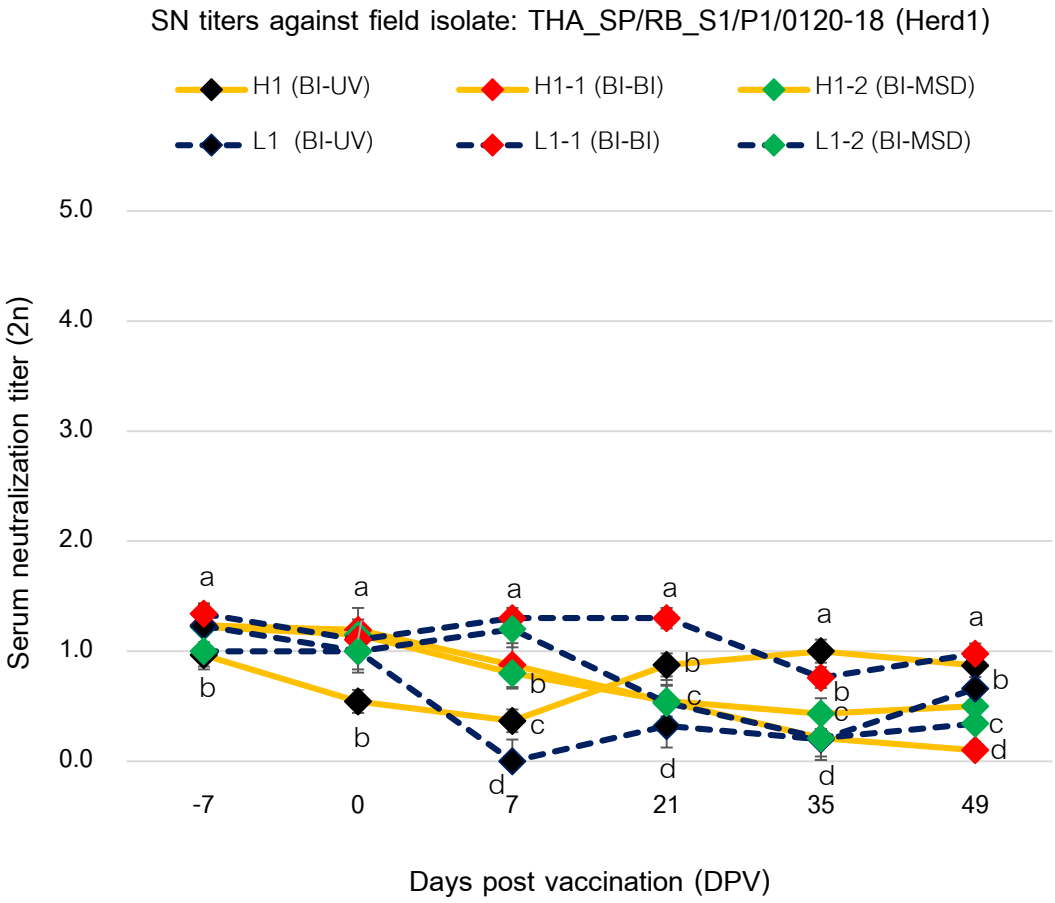

Figure 9 Antibody response as measured by serum neutralization (SN) assay using field PRRSV-2 isolated from Herd 1: THA_SP/RB_S1/P1/0120-18, including 6 groups: H1, H1-1, H1-2, L1, L1-1, and L1-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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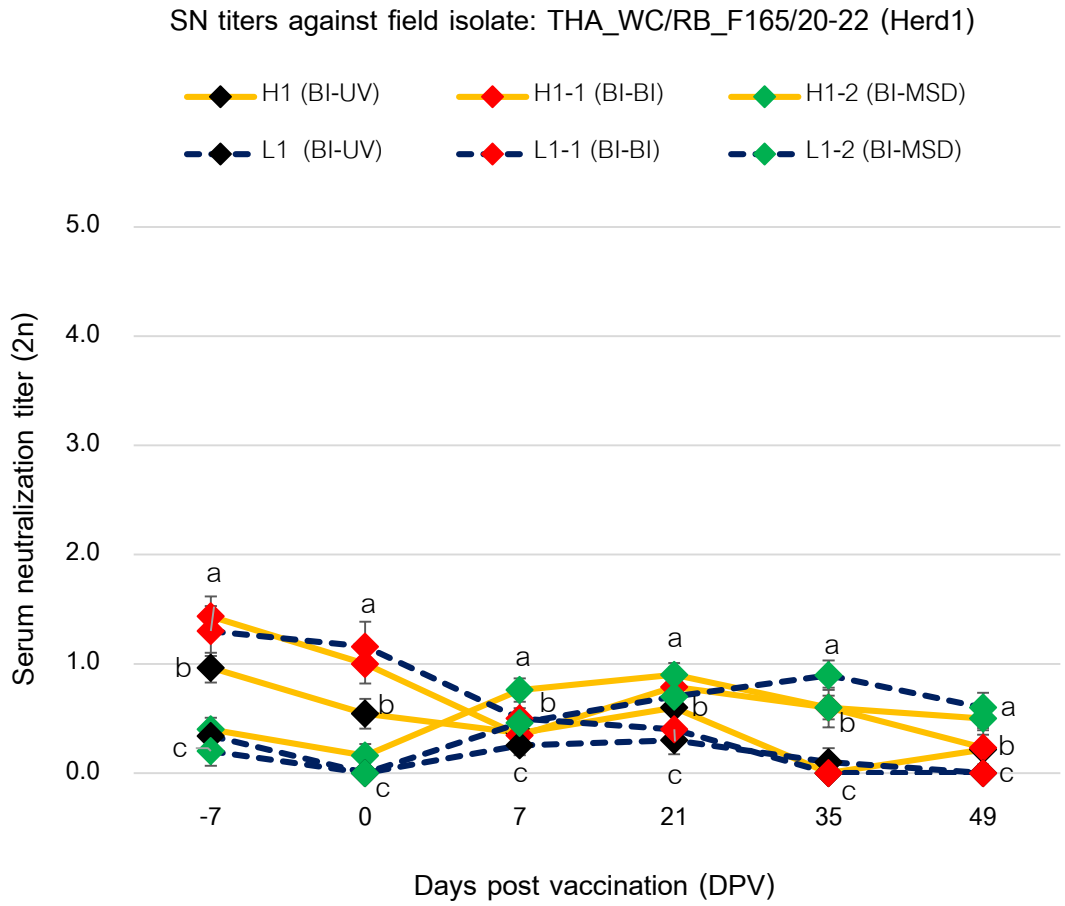

Figure 10 Antibody response as measured by serum neutralization (SN) assay using field PRRSV-2 isolated from Herd 2: THA_WC/RB_F165/20-22, including 6 groups: H1, H1-1, H1-2, L1, L1-1, and L1-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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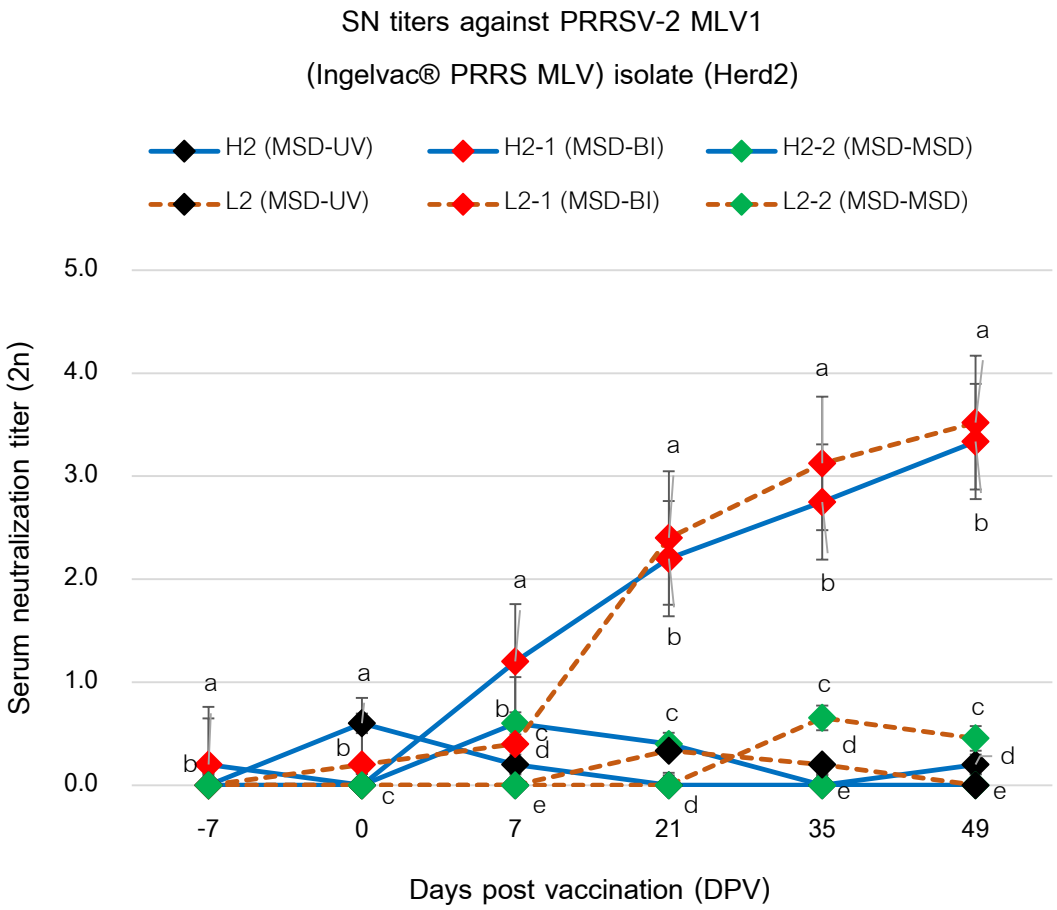

Figure 11 Antibody response as measured by serum neutralization (SN) assay using PRRSV-2 MLV1 isolate which referred to Ingelvac® PRRS MLV isolate including 6 groups: H2, H2-1, H2-2, L2, L2-1, and L2-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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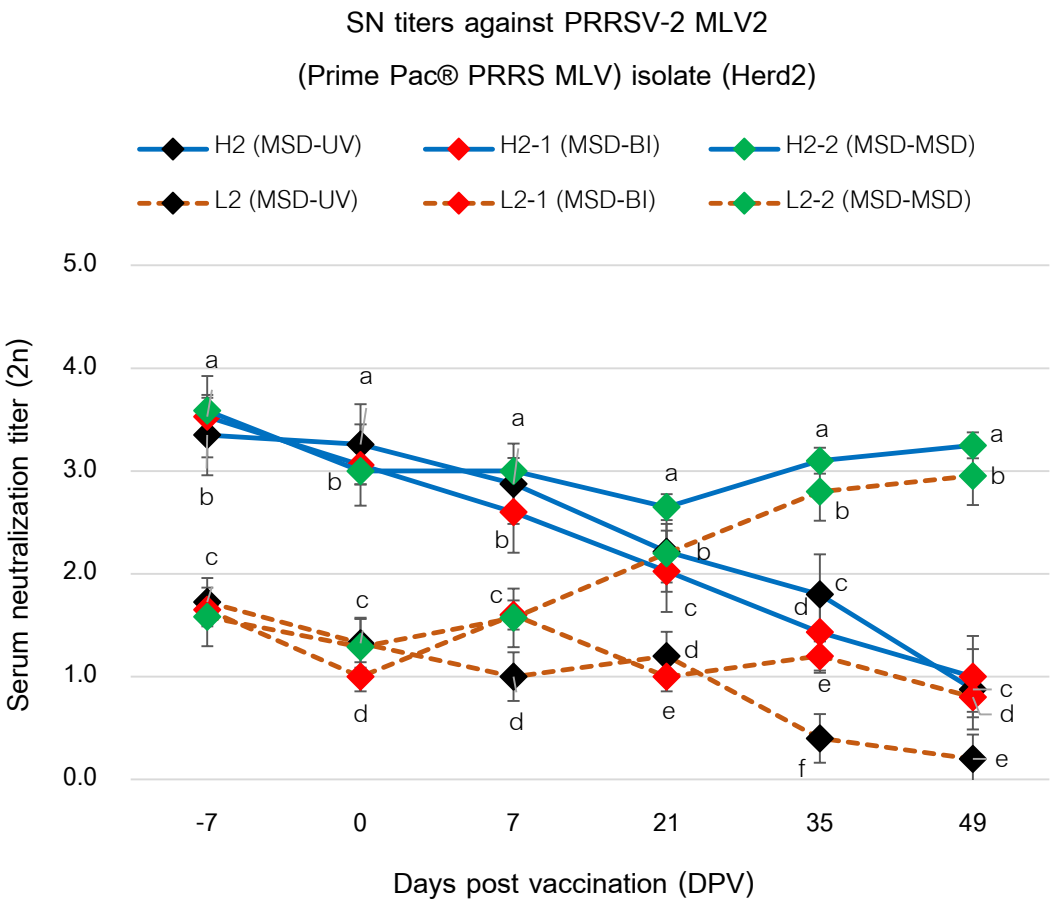

Figure 12 Antibody response as measured by serum neutralization (SN) assay using PRRSV-2 MLV2 isolate which referred to Prime Pac® PRRS MLV isolate including 6 groups: H2, H2-1, H2-2, L2, L2-1, and L2-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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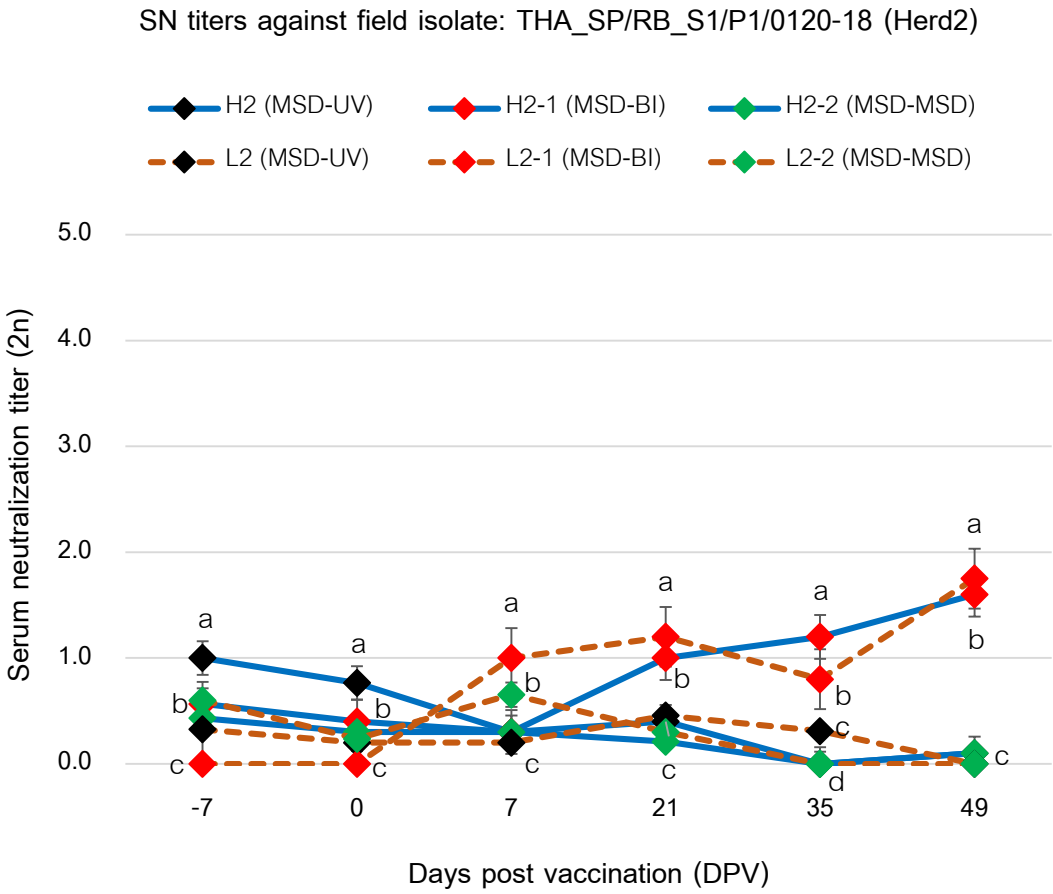

Figure 13 Antibody response as measured by serum neutralization (SN) assay using field PRRSV-2 isolated from Herd 1: THA_SP/RB_S1/P1/0120-18, including 6 groups: H2, H2-1, H2-2, L2, L2-1, and L2-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

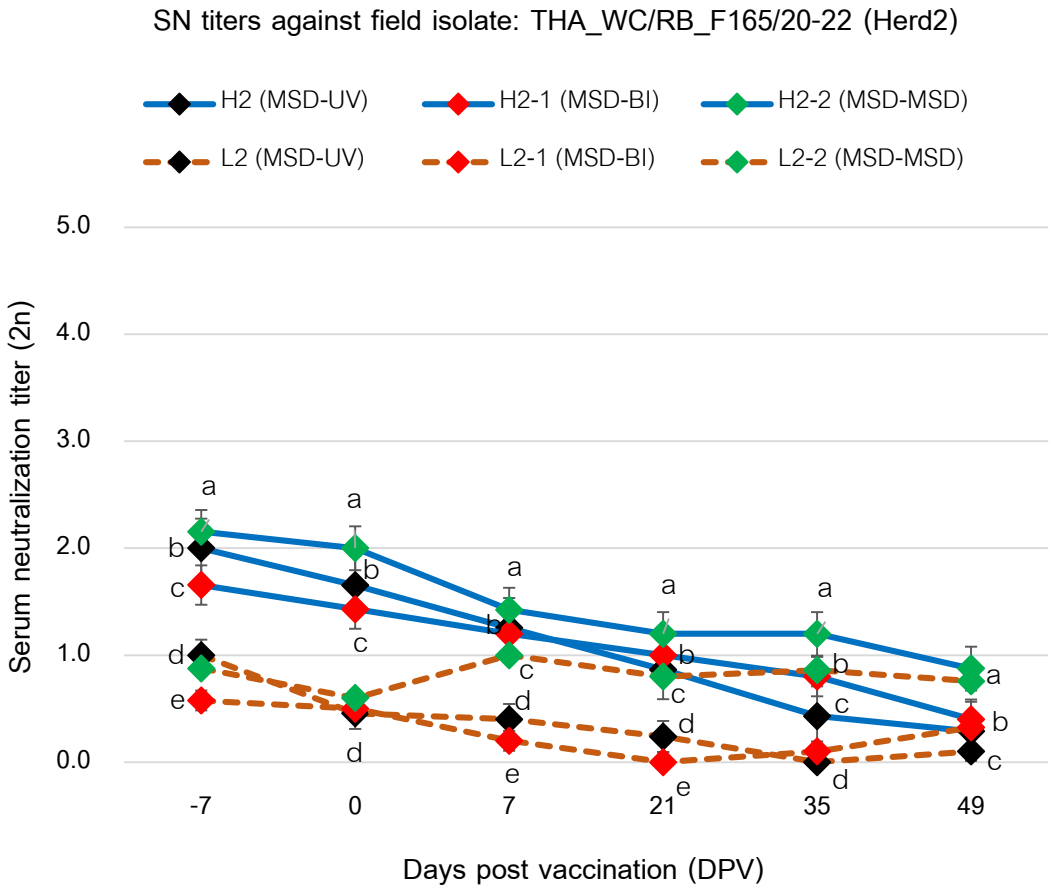

Figure 14 Antibody response as measured by serum neutralization (SN) assay using field PRRSV-2 isolated from Herd 2: THA_WC/RB_F165/20-22, including 6 groups: H2, H2-1, H2-2, L2, L2-1, and L2-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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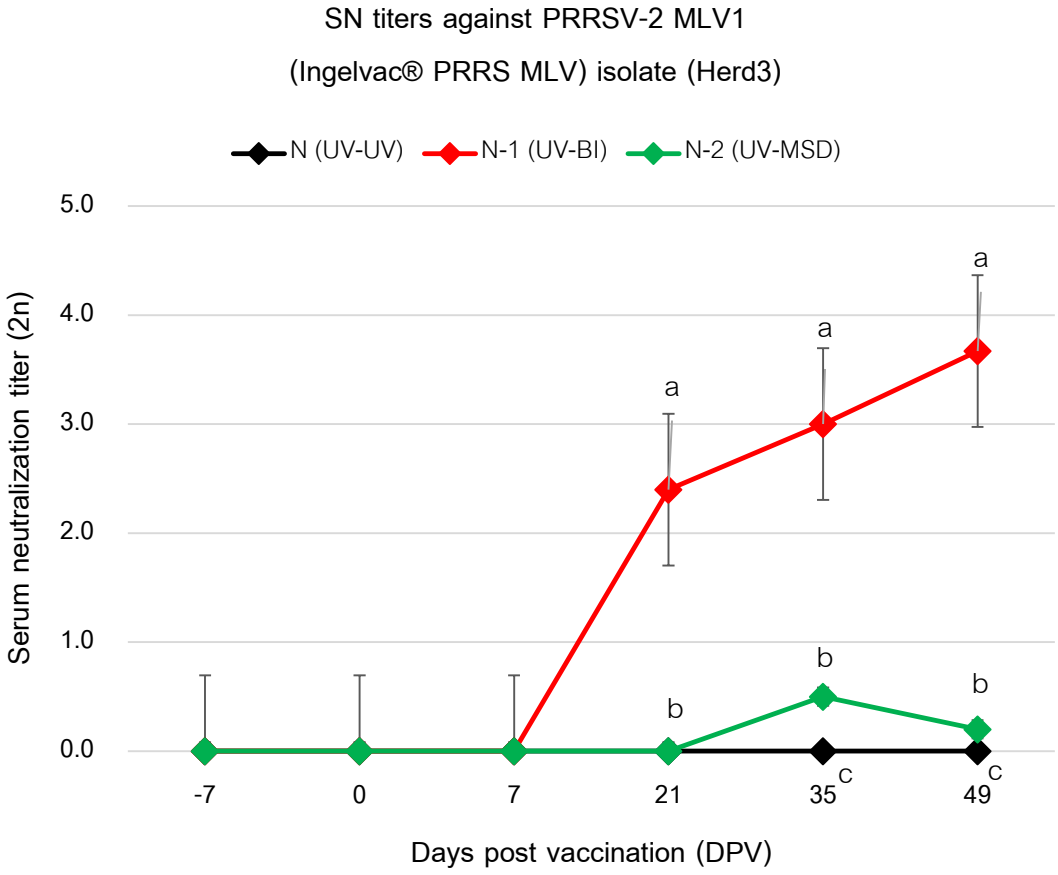

Figure 15 Antibody response as measured by serum neutralization (SN) assay using PRRSV-2 MLV1 isolate which referred to Ingelvac® PRRS MLV isolate including 3 groups: N, N-1, and N-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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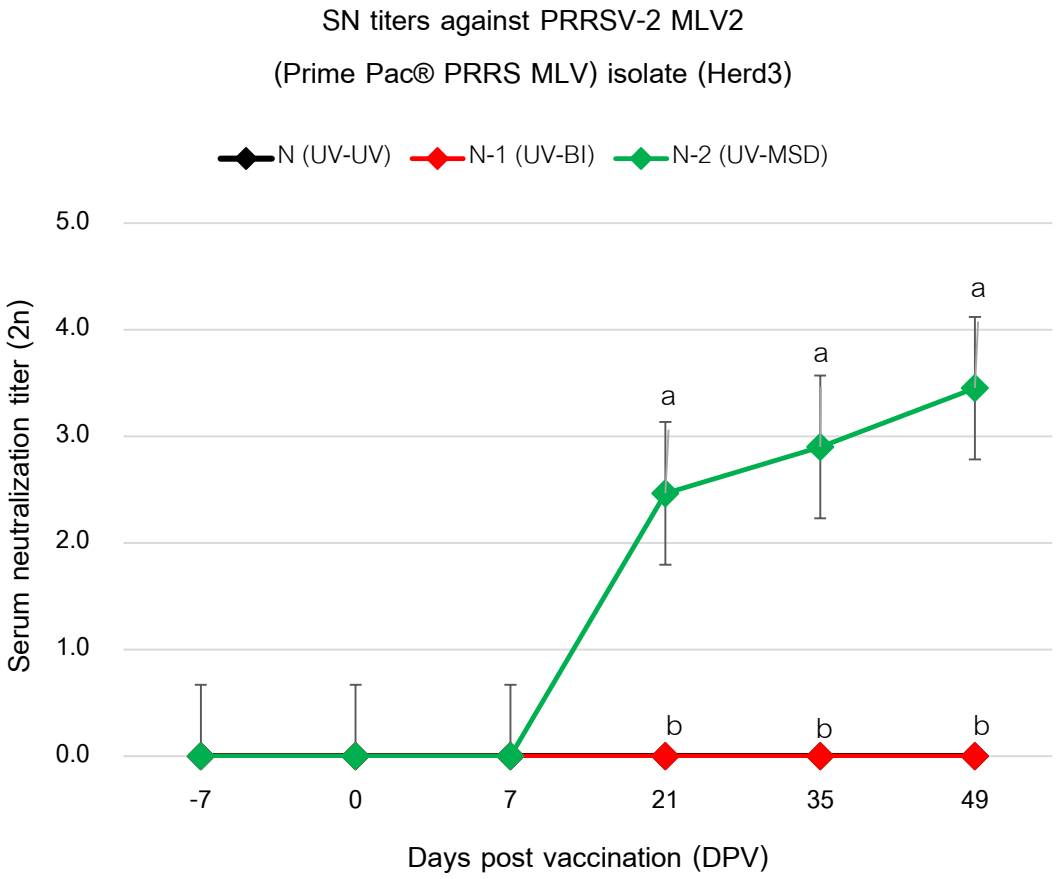

Figure 16 Antibody response as measured by serum neutralization (SN) assay using PRRSV-2 MLV2 isolate which referred to Prime Pac® PRRS MLV isolate including 3 groups: N, N-1, and N-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

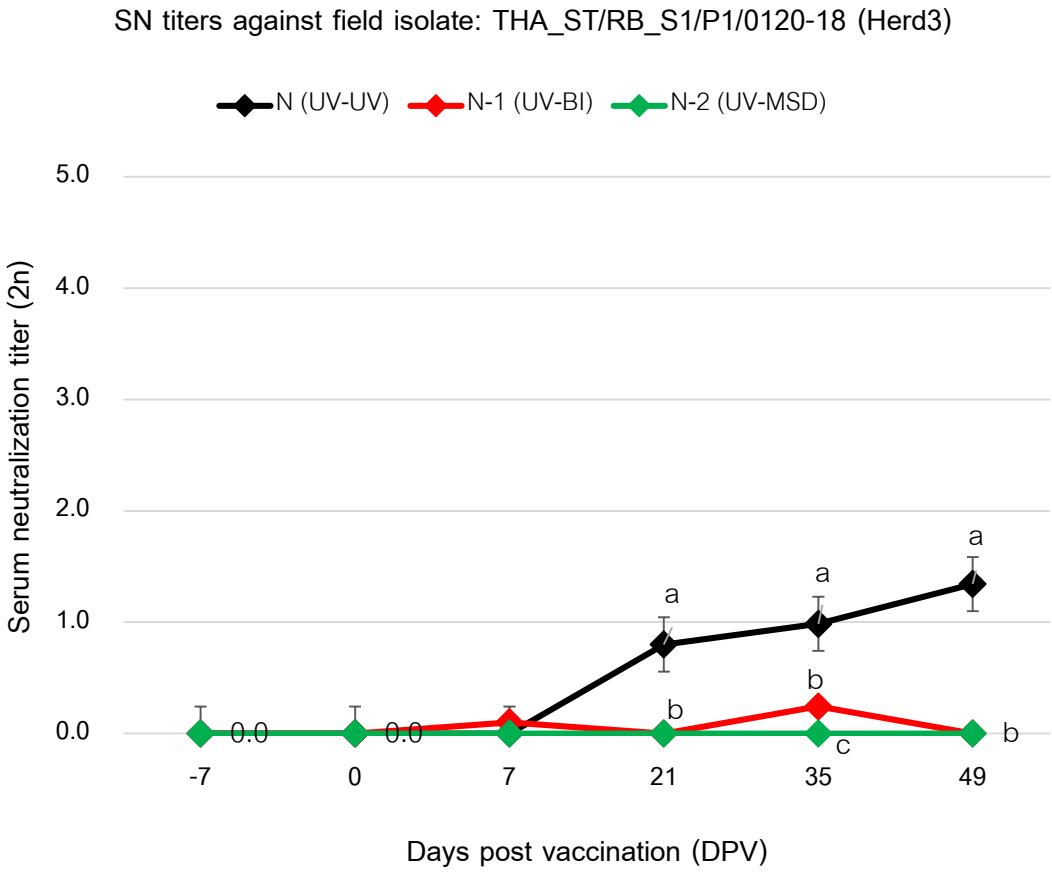

Figure 17 Antibody response as measured by serum neutralization (SN) assay using field PRRSV-2 isolated from Herd 1: THA_SP/RB_S1/P1/0120-18, including 3 groups: N, N-1, and N-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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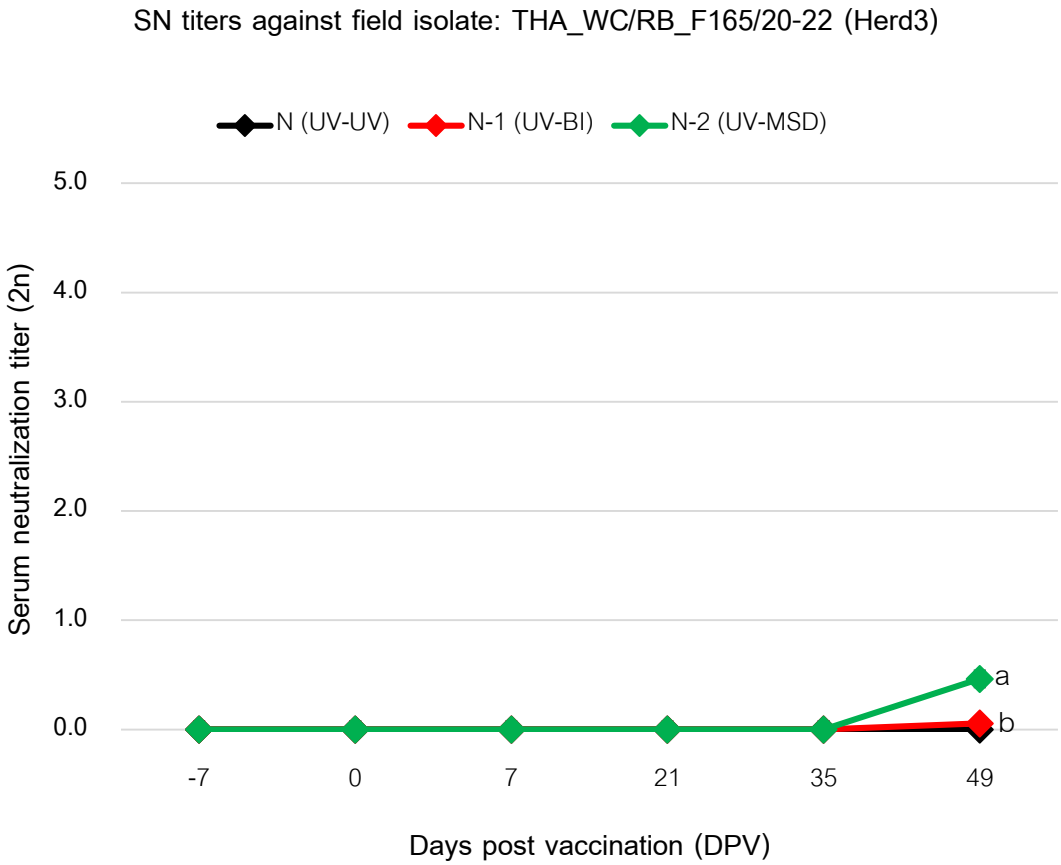

Figure 18 Antibody response as measured by serum neutralization (SN) assay using field PRRSV-2 isolated from Herd 2: THA_WC/RB_F165/20-22, including 3 groups: N, N-1, and N-2 groups. SN values were shown as mean ± standard error of mean (SEM). Different letters within the same day post vaccination (DPV) indicate the statistically differences between groups (p-value <0.001).

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CHAPTER IV

DISCUSSION

The present study was conducted to investigate the effect of MDAs on humoral immune response in piglets given 2 different lineage of PRRSV-2 MLVs: Ingelvac PRRS® MLV and Prime Pac PRRS® MLV produced by different manufactures. Moreover, the mortality and growth performances in nursery pigs were observed in all treatment groups.

Based on the results achieved, both PRRSV-2 MLV1 (Ingelvac PRRS® MLV) and PRRSV-2 MLV2 (Prime Pac PRRS® MLV) were able to reduce the mortality of pigs in all studied herds: Herd 1, Herd 2, and Herd 3, as compared to the unvaccinated groups. However, the pigs with high level of MDAs (SN titer □ 1: 8) which included in unvaccinated group: H2 groups, did not exhibit the mortality throughout the experiment. On the contrary, the pigs with low level of MDAs (SN titer < 1:8) in unvaccinated groups: L1, L2 and N groups showed the lowest survival rates at the end of experiment. Additionally, growth performance in pigs vaccinated with Ingelvac PRRS® MLV and Prime Pac PRRS® MLV from all studied herd had significantly higher values of body weight gain and average daily gain (ADG) comparing to those in unvaccinated pigs. These results are in line with the previous experiments that indicated that the vaccination with PRRSV-MLV significantly increased protection against the infection of field isolates of PRRSV in progeny pigs and it significantly improved the growth performance and reduced mortality caused by PRRSV-2 infection in a PRRSV-2 positive herd (Opriessnig et al.,2005 and Kritas et al.,2007). Interestingly, pigs with high level of MDAs (SN □ 1:8) in the H1 and H2 groups from all PRRSV positive herds: Herd 1 and Herd 2, was able significantly reduce mortality and increased ADG in nursery pigs, although it was not vaccinated with PRRSV-2 MLV at weaning age. This would imply that the MDAs in term of PRRSV-2 specific neutralizing antibodies is important for protection against the infection of field PRRSV-2 isolates in pigs from PRRSV positive herds. Moreover, the piglets should receive the maximum volume of colostrum within 24 hours after birth to

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achieve maximum level of MDAs in the newborn piglets (Labarque et al.,2000; Lopez et al.,2004). However, the efficacy of PRRSV-MLVs are considered because PRRSV-MLVs cannot provide the complete protection against the field heterologous isolates of PRRSV and they have no cross protection effect (Lee et al.,2014; Neilsen et al,2001; Opriessnig et al,2002). The inconsistent performance might be observed in PRRSV positive herds, although the PRRSV- MLVs were implemented to the sows and the piglets.

In this study, the use of different PRRSV MLVs was designated to use in weaning pigs in the H1-2, L1-2, H2-1, and L2-1 groups from 2 positive herds: Herd 1 and Herd 2. The achieved results indicated that the different PRRSV-2 MLVs implemented to weaning pigs were able to reduce the clinical losses associated with PRRSV infection. However, the use of different PRRSV-MLVs across a single production flow is generally discouraged due to the risk of recombination events (Murtaugh et al.,2002 and Li et al.,2009). Therefore, it would be best method to use a single PRRSV-MLV entire the production flow.

Furthermore, the field isolates of PRRSV-2 from 2 PRRSV positive herds was investigated using ORF5 gene of PRRSV-2 isolates collected between 2017 and 2020. The co-infection of PRRSV-1 and PRRSV-2 was observed in the pig population. However, PRRSV-2 was more dominant than PRRSV-1 in the studied herds. The phylogenetic analysis demonstrated that field PRRSV-2 isolates collected from 2 studied herds were classified into 2 distinct lineages: lineage 1 and lineage 8.7. All isolates collected from Herd 2 were consistently observed in lineage 8.7 throughout the study and became dominant in PRRSV-2 genotype population in this studied herd. On the contrary, the field isolates of PRRSV-2 collected from Herd 1 were classified into two lineages as previously described. However, all PRRSV-2 isolates collected between 2018 and 2020 were grouped in lineage 1 in this herd over time. PRRSV-2 isolates in lineage 8.7 collected in 2017 shared 83.25% and 83.74% nucleotide identities with PRRSV-2 isolates in lineage 1 collected during 2018 and 2020. These results indicated that the genetic variation of PRRSV-2 isolates was observed in the herd which used Ingelvac PRRS® MLV to control PRRSV infection for several years. This situation was in

line with the previous study in which the emergence of novel clusters of PRRSV-2 was detected after the introduction of Ingelvac PRRS® MLV into the herd, and one of the novel clusters became the endemic strain of that herd throughout the study (Nilubol et al.,2014). THA_SP/RB_S1/P1/0120-18 and THA_WC/RB_F165/20-22 were isolated from Herd 1 and Herd 2, respectively. THA_SP/RB_S1/P1/0120-18 was classified into lineage 1 and shared 83.25% and 83.74% nucleotide identities with Ingelvac PRRS® MLV and Prime Pac PRRS® MLV, respectively. Moreover, THA_WC/RB_F165/20-22 was more identical to Ingelvac PRRS® MLV and Prime Pac PRRS® MLV than field isolates of PRRSV-2 from Herd 1. The PRRSV-2 isolates from Herd 2 (THA_WC/RB_F165/20-22) shared 87.89% and 88.23% nucleotide identity values with Ingelvac PRRS® MLV and Prime Pac PRRS® MLV, respectively. Based on these results, it was interesting to noted that Ingelvac PRRS® MLV and Prime Pac PRRS® MLV was able to significantly affect mortality in nursery pigs post vaccination, although the field isolates of PRRSV-2 were not identical to both lineage of PRRSV-2 MLVs. These results suggested that the genetic similarity between vaccine and field isolates of PRRSV was not enough for vaccine selection. The induction of immune response and protection against heterologous PRRSV infection should be concerned (Madapong et al.,2017; Madapong et al.,2020).

Following the vaccination, the PRRSV-specific antibody response was detected without viremia in all pigs from unvaccinated groups throughout the experiment. In contrast, the viremia was observed in all vaccinated groups only at 7 to 21 DPV, then it disappeared. The PRRSV-specific antibody response in pigs with high level of MDAs from the H1-1 group was detected at 21 DPV which was slower than that in pigs with low level of MDAs. However, there was no significance difference ($p < 0.001$) in antibody titers in the vaccinated groups at the end of experiment. The pigs with low levels of MDAs expressed the earliest and highest antibody response post vaccination, as compared to the pigs with high MDA level from the other groups in each studied herds. However, the seroconversions were observed in all vaccinated groups and the PRRSV-specific antibody responses detected by ELISA in all vaccinated pigs were significantly higher those in unvaccinated pigs. These results of the present study indicated that the

MDAs did not obviously affect the immune response post vaccination. Both Ingelvac PRRS® MLV and Prime Pac PRRS® MLV could provide the protection against the infection of PRRSV in pigs with low and high PRRSV MDA levels, according to the production results, as previously described. However, the antibody response detected by ELISA was not the neutralizing antibody and did not correlate with the protection.

Neutralizing antibodies play an important role in protection against PRRSV infection. The high level of MDAs that presented in pigs from unvaccinated groups provided the protection against the PRRSV infection as shown in the production results. Pigs with low MDA levels which vaccinated with either Ingelvac PRRS® MLV or Prime Pac PRRS® MLV showed the highest SN titers against the homologous lineage of PRRSV-2 MLV. However, the pigs from H2-2 which had the high level of MDAs before vaccination and vaccinated with Prime Pac PRRS® MLV, showed the higher level of SN responses post vaccination as compare to those in pigs with low level of MDAs in Herd 2. Pigs from negative herd which included in vaccinated groups showed early response in the SN titers against the homologous lineage of PRRSV-2 MLV isolate. Pigs vaccinated with Prime Pac PRRS® MLV showed late detection of SN-titers against the homologous field PRRSV isolate, as compared to pigs vaccinated with Ingelvac PRRS® MLV. However, the mortality was not observed in pigs vaccinated with Prime Pac PRRS® MLV in this herd throughout the experiment. In case of heterologous field isolates of PRRSV-2: THA_SP/ RB_S1/ P1/ 0120-18 and THA_WC/ RB_F165/ 20-22, the SN titers against these 2 isolates were not observed in the vaccinated groups, excepted pigs in unvaccinated groups from negative herd. These results indicated that either low or high MDA levels did not affect the SN response post vaccination and the MDAs in term of PRRSV specific neutralizing antibody could provide the protection against the infection of PRRSV in unvaccinated pigs.

In this study, vaccination with PRRSV-2 MLVs reduced the mortality and improved growth performance of pigs in the endemically PRRSV infected herds. The MDA levels did not affect to immune response inducing by PRRSV-MLVs. Although, the vaccine was changed in weaning pigs to control the disease, the antibody responses

were detected post vaccination in both pigs with low and high level of MDAs. However, the increasing of genetic diversity should be concerned in the herd that used the different PRRSV MLVs across a production flow. Moreover, the genetic similarity between vaccine and field virus was not related to the protection. Vaccine selection should depend on the induction of immune response and protection against heterologous PRRSV infection.

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