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Pathological and Immunological Evaluation of Porcine Reproductive and Respiratory Syndrome Modified-Live Virus and Subunit Vaccines

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A dissertation submitted in partial fulfillment of
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Abstract

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Porcine reproductive and respiratory syndrome (PRRS) is linked to economically important disease in modern swine industry causing reproductive loss and respiratory disease. PRRS virus (PRRSV) is further divided into two major types, PRRSV-1 and PRRSV-2. PRRSV-1 and

PRRSV-2 were first isolated in Europe and North America respectively around 1991, both strains are still dominant in that discovered continent. But both PRRSV-1 and PRRSV-2 has appeared together in Asian countries including Korea and caused more complicated disease. PRRS vaccination, while it does not completely prevent PRRSV infection, is one of the most powerful methods for managing PRRS. Vaccination with a homologous strain is more effective than vaccination with a heterologous strain and modified live vaccines has reduced production and economic losses and wild type virus shedding. Currently, four Modified Live Virus Vaccines (MLV), two based on PRRSV-1 and two based on PRRSV-2 are commercially available in the Korean market. However, no studies have been performed yet to assess the efficacy of these 4 PRRSV MLV vaccines against heterologous PRRSV-1 and PRRSV-2 field viruses. For this reason, this study evaluated the efficacies of two different MLV 1 commercial PRRS vaccines and two different MLV 2 commercial PRRS vaccines′ efficacies in growing pigs through PRRSV-1 and PRRSV-2 challenges. Either MLV 1 vaccines reduced lung lesions and PRRSV positive cells only in homologous PRRSV-1 challenges but either MLV 2 vaccines showed cross protection not only homologous PRRSV-2 but also heterologous PRRSV-1. With these results, studies were expanded to test PRRSV-1 and PRRSV-2 co-infected commercial farm comparing MLV 1 with MLV 2 vaccine efficacies. Regardless of the type of MLV, all vaccinated groups generally exhibited improved growth rate compared to the unvaccinated pigs but as the previous

study indicated, either of the MLV 2 vaccines had a better overall growth rate compared to the pigs vaccinated with either of the MLV 1 vaccines. MLV 1 vaccination failed to reduce any type of PRRSV-1 or PRRSV-2 viremia while MLV 2 vaccines decreased PRRSV-2 viremia. Taken together, MLV 2 vaccines can be more efficacious than MLV 1 vaccines in PRRSV-1 and PRRSV-2 co-infected farms.

Although PRRS MLV vaccines has been more efficacious than killed vaccines, there has been growing concerns about the safety fo live vaccines, especially breeding farms. To meet this demand, relatively recently PRRS subunit vaccine was developed claiming little adverse effects appearing vaccines using whole virus for antigen but no comparative field studies between PRRS subunit vaccines and PRRS MLV were done before. In this study, the efficacy of a porcine reproductive and respiratory syndrome subunit and a modified-live virus vaccine against respiratory diseases in endemic farms were compared. Three farms were selected based on their history of respiratory diseases caused by co-infection with both PRRSV-1 and PRRSV-2. Pigs vaccinated with the PRRS subunit vaccine had a similar or better efficacy against respiratory disease and had a better growth performance compared to those vaccinated with the PRRS MLV vaccine. But at the peak of PRRSV-1 and PRRSV-2 viremia, both PRRS subunit vaccine and MLV's neutralizing antibodies and T-cell responses against PRRSV-1 and PRRSV-2 were at low levels suggesting that either vaccine is only able to provide a partial protection against co-circulating PRRSV-1 and

PRRSV-2.

Lastly, effectiveness of a commercial PRRSV subunit vaccine against heterologous PRRSV-1 and PRRSV-2 challenge in late-term pregnant gilts were investigated. Regardless of the challenge strain's genotype, the vaccinated gilts carried their pregnancies to term and farrowed between days 114 and 115 of gestation while unvaccinated gilts aborted between days 105 and 110 of gestation. The vaccinated gilts had a significantly lower level of PRRSV viremia and significantly higher levels of virus-neutralizing antibodies and interferon-γ-secreting cells compared with the unvaccinated gilts. These results revealed that vaccination in late-term pregnancy with PRRSV subunit vaccine was efficacious against reproductive failure due to heterologous PRRSV-1 and PRRSV-2 infection.

Keywords: Porcine reproductive and respiratory syndrome; Co-infection; MLV vaccine; Killed vaccine; Subunit vaccine

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER IV..

LIST OF FIGURES

CHAPTER I.

CHAPTER III.

LIST OF ABBREVIATIONS

ADWG	Average daily weight gain
dpc	days post-challenge
dpv	days post-vaccination
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Ennzyme-linked immunospot assay
IFN-r-SC	Interferone-r secreting cells
ISH	In-situ hybridization
MLV	Modified-live virus vaccine
NA	Neutralizing antibody
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCV2	Porcine cirocovirus type 2
PCR	Polymerase chain reaction
PRRSV	Porcine reproductive and respiratory syndrome virus

General Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first reported in 1987 in USA and highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) reported in 2006 in China. PRRS is still unresolved disease in modern swine industry throughout the world.

PRRS virus is a small, enveloped, positive-sense single-stranded RNA virus. The virion has an infectious RNA genome of about 15 kb in size, consisting of a proteinaceous nucleo capsid coated with lipid-containing envelope with 5 or 6 structural proteins. N protein is a good candidate for virus-specific antibody detection but its immunological protection has not been demonstrated. The two main envelope proteins are the non-glycosylated matrix (M) envelope protein and GP5. The heterodimer GP5/M is necessary for the formation of virions, but GP5/M alone is not sufficient for viral infectivity.

PRRSV is classified into two main types, PRRSV-1 and PRRSV-2, genetically. PRRSV-1 and PRRSV-2 were first isolated in Europe and North America respectively around 1991, both strains are still dominant in that discovered continent. But both PRRSV-1 and PRRSV-2 has appeared together in Asian countries including Korea and caused more complicated disease. So, co-vaccination with MLV1 and MLV2 together are worth studying to protect both strains.

Clinical sings include late-term abortion in sow and respiratory disease in growing pigs but HP-PRRS strains kill the fetus by passing the placenta with moderate efficiency in the mid term pregnancy and abortion rates of 40-100 percent and sow mortality of about 10 percent are recorded in Southeast Asia.

Studies have shown that once the PRRS virus mutates, Modified live vaccines are no longer able to neutralize virus and vaccines' virus strain can be shed to other pigs and compromise detecting natural infection serologically from live PRRS vaccines. Killed vaccines are free from viral shedding but generally do not have neutralizing capabilities and often do not confer immune protections in pigs.

Vaccination with whole virus regardless of whether Modified live vaccines or Killed vaccines may induce antibody dependent enhancement (ADE) . To avoid these whole virus vaccine side effects, reverse vaccinology and genetic engineering techniques were employed in developing PRRS Subunit vaccine.

This thesis was therefore designed to investigate, 1) the efficacies of each type of MLV vaccines against PRRSV-1 and PRRSV-2 virus challenge in growing pigs, 2) the efficacies of modified live virus (MLV) vaccines against respiratory disease in pig farms co-infected with PRRSV-1 and PRRSV-2, 3) the comparison of efficacies of Subunit vaccine with MLV vaccines in respiratory disease, 4) the effectiveness of Subunit vaccine against heterologous PRRSV-1 and PRRSV-2 in late-term pregnant gilts.

Literature Review

1. Porcine reproductive and respiratory syndrome virus

1.1. Historical Background

Porcine reproductive and respiratory syndrome (PRRS) was initially reported in the USA in 1987 and continues to be important agent of late-term reproductive losses, neonatal respiratory disease, reduced growth rate, and increased mortality in modern swine industry (1, 2).

There were reports from Canada of antibodies in serum in 1979, but no virus was identified in these samples and there were no reports of the disease (3). A clinically similar outbreak was reported in Germany in November 1990, but no common association was found between the outbreaks in Germany and the United States (4). In 1991, an etiology of previously unrecognized RNA viruses was established that satisfies Koch's postulates (5, 6).

Since 1987, when the PRRS virus was first reported, the clinical signs of PRRSV infection have expanded, and respiratory symptoms and pneumonia in breeding sow and nursery / growing pigs are often complicated by single or multiple bacteria. In addition, cases of reproductive diseases and neonatal

deaths from PRRSV have been reported even in the PRRSV vaccinated group (7). The clinical signs became more aggravated in Highly Pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) reported in 2006 in China (8). So, the goal of the PRRS Virus Control efforts is to have a collaborative effort that is applied to the study of the PRRS virus in order to continue to develop tools and management strategies that can reduce the economic impact for producers.

The economic loss due to PRRSV is estimated to be between $6.25 and $15.25 USD per pig marketed in Europe and North America (9, 10).

2. Etiology

PRRSV is a small, enveloped, positive-sense single-stranded RNA virus. The virion has an infectious RNA genome of about 15 kb in size, consisting of a proteinaceous nucleo capsid coated with lipid-containing envelope with 5 or 6 structural proteins. Virions are small and varied shapes ovale form with a diameter of about 50 to 70 nm, and the surface of the virions has unclear surface protrusions (11, 12). Similar to other arteriviruses, the genomic organization of PRRSV consists of approximately 15,000 nucleotides grouped into approximately 11 ORFs (1a, 1b, 2a, 2b, 3, 4, 5a, 5, 6, 7 and short transframe ORFs) expressed from genomic and subgenomic (sg) mRNAs

(sgmRNAs). ORFs 1a and 1b form 80 percent of the genome and encode viral transcription, replication, and immunomodulation machinery for protein cleavage, homologous recombination, and RNA replicase. ORF1a and ORF1b are translated as large polypeptides, which are then condensed into about 12 nonstructural proteins proteolytically (nsp) (13, 14).

2.1. Nonstructural proteins

One of two large polyproteins translated from the full-length genomic RNA molecule encodes PRRSV nsps. ORF1a is translated into a large roughly 260 to 277 kDa polyprotein (pp) 1a that is proteolytically cleaved into smaller active proteins including four proteases (nsp1-alpha, nsp1-beta, nsp2, and nsp4) that cleave ORF1a- and ORF1b-encoded protein. Besides proteolytic activity, nsp1-alpha and nsp1-beta can make virulence by directly blocking type 1 interferon synthesis or inhibiting the signaling pathway (15). Due to variable in-frame deletions that are commonly found in extremely virulent strains, Nsp2 exhibits substantial scale polymorphisms. But, the nsp2 region does not seem to contribute to the virulence (16).

2.2. Structural proteins

N protein is a good candidate for virus-specific antibody detection and disease diagnosis because it is the most abundant and antigenic among the

protein in the virion. Four to five domains of antigenic importance for the N protein have been identified, which are structurally common antigenic sites to European and North American strains. It is expressed at high levels in infected cells and accounts for 20-40% of the total protein content of virions. It is involved in nuclear shuttling and nucleolus localization and may impact nuclear processes during replication, likely through the processing of ribosomal RNA precursors and ribosome biogenesis (17). Although it can help in immunodiagnosis due to its large amount of expression and high antigenicity, immunological protection has not been demonstrated. The two main envelope proteins are the non-glycosylated matrix (M) envelope protein, which has little signal sequence and accumulates in the endoplasmic reticulum, and GP5, which forms a disulfide-linked heterodimer with M and integrates into the virion envelope. After the signal peptide is cleaved, GP5 contains an ectodomain approximately 30 amino acids length and is expected to contain 2-5 N-linked glycans. The heterodimer GP5/M is necessary for the formation of virions, but GP5/M alone is not sufficient for viral infectivity (18, 19). The glycoproteins 29-30 kDa GP2, 45-50 kDa GP3, and 31-35 kDa GP4 are present in low amounts and shape a complex of trimeric envelope protein. For their assembly and incorporation in the virion and for viral infectivity, the presence of all three proteins is required (Wissink et al. 2005). The trimeric structure intervene infection alone or together with GP5 (18, 19). CD163 mediates the infection of permissive porcine cells by interacting with minor envelop glycoprotein complexes (18, 20, 21).

3. Epidemiology

3.1.Persistent infection

Persistent infection with PRRSV has been reported in animals through transmission experiments and virus detection. Studies have detected infectious virus in 100 - 165 DPI, especially from tonsil or lymphoid tissues (22, 23, 24). Persistent infection occurs regardless of the age of the pig at the time of infection or whether the fetus has been exposed in the Uterus (25, 26). The mechanism by which the virus survive even in the presence of an active immune response has not been identified, but it has been found to be independent of the mutation (27, 28).

3.2. Vertical transmission

The viremic Sows transmit PRRSV to the fetus through the placenta, causing the fetus to die or deliver weak or normal-looking newborn piglets (5, 29, 30, 31). While PRRSV can replicate to fetuses 14 days or more of gestation period, fetal infections during the first two thirds of pregnancy are uncommon, as most PRRSVs pass through the placenta efficiently only in the last trimester of pregnancy (32, 33, 34, 35, 36). Transit is independent of the reproductive virulence of the virus isolate. When vaccinated at the age of 90 days of gestation, it seems that the virulence and the placenta passage

are not correlated as the highly pathogenic virus or the low pathogenic virus pass through the placenta equally (37).

3.3. Horizontal transmission

Not only vertical transmission but also introducing new susceptible animals can help virus remain constantly in the herd (Jeffrey et al. 2019). A significant proportion of the population will easily become infected when susceptible and infectious pigs are combined, such as when weaning. It was reported that 80–100% of pigs in three swine herds were infected by 8–9 weeks of age (38) and 96% of market hogs sampled from 50 herds turned out to be positive (39). However, Houben reported different seroconversion times within litters showing as early as 6–8 weeks of age seroconversion, but others 12 weeks of age and the end of the monitoring period, still free of PRRSV infection (40).

4. Pathogenesis

PRRSV replicates cells exhibiting CD163 receptor for PRRSV binding, internalization, and replication in a subset of monocyte-derived cells (41). Co-expression of cell surface sialoadhesin (Sn, CD169) can increase viral

internalization, although it is not necessary (42).

4.1. Postnatal PRRS

PRRSV virus infection can be classified into three distinct stages: acute infection, persistence, and extinction phage (43).

Acute infection occurs after exposure and spreads rapidly in the lung and lymphatic tissues, which are the major sites of replication. Viremia appears 6 to 48 hours after exposure to the virus (44, 45). Certain PRRSV strains can display atypical tissue tropisms, such as neurotropic PRRSV strains that replicate in the brain (46, 47). Pig age at the time of PRRSV exposure affects disease, as young pigs replicate the virus at higher titers and have a longer period of viremia and shedding when compared to older pigs (48).

The persistent phase is characterized by the disappearance of clinical symptoms, a gradual decrease in the replication of PRRSV in the lymph nodes and tonsils, reduced viral shedding, which is observed at the end of viremia (22, 49).

The onset of the extinction phase varies from pig to pig, but can take up to 250 days after exposure, which begins when viral shedding stops and viral clearance completed (50). In PRRSV-infected macrophages, cell death is caused by necrosis and apoptosis, including caspase activation and a mitochondria-mediated pathway. (51, 52). The activation of higher levels of

bystander cell death in various tissues is a hallmark of the HP-PRRS strain. (53).

4.2. Reproductive PRRS

All gestation period of fetuses are susceptible to PRRSV infection but, PRRSV effectively infects the fetus by crossing the placenta of a pregnant sow only in the third trimester of pregnancy (30). However, a small number of strains, including some HP-PRRS strains, have been reported to kill the fetus by passing the placenta with moderate efficiency in the midterm pregnancy (54, 55). Resistance to PRRSV transit at the placenta seems to be two way. Intra fetal or intra amniotic inoculation of fetuses with PRRSV 45 - 50 days of gestation did not result in passage of PRRSV from fetus to dam (32). The reason for resistance to PRRSV transit at the placenta during early and mid gestation and for efficient transit during late gestation may be due to numbers of PRRSV permissive cells in the fetal placenta and other fetal tissues. It was demonstrated that during early and mid gestation, the predominant macrophage phenotype is the less permissive CD163+Sn−, whereas in late gestation it is the highly permissive CD163+Sn+. Fetuses are infected by transit of PRRSV from the dam to fetal through placenta or by transmission from adjacent intrauterine PRRSV infected fetuses (56). PRRSV replicates to its highest titers in fetal thymus, tonsils, and lymph nodes (57, 58), contributing to fetal death or, if pregnancy

continues to term, birth of PRRSV infected piglets (34, 58). Lesions are observed in a minority of PRRSV infected fetuses (30), raise a question whether viral replication in fetuses is the sole or significant cause of fetal death (57). In PRRS virus crossing study, inoculating PRRSV into 90th day of pregnancy gilt showed apoptosis directly from CD163+Sn+ cells and found that there was a correlation with bystander cell death in both maternal endometrium and fetal placenta (57). The amount of PRRSV-infected macrophages in the endometrium, the PRRSV load in each fetal thymus, and the risk of fetal death have a strong correlation with the number of direct and bystander cell death (59).

5. Clinical signs

5.1. Sows and boars

In sows which are at 21-109 days of gestation, 1-3 percent of litters may be lost during the stage of acute illness. This occurs as abortions or irregular returns to estrus or impregnant sows (2). Acute exacerbations of endemic diseases such as agalactia, incoordination and/or sarcoptic mange, atrophic rhinitis or cystitis/pyelonephritis are irregularly observed in sows of acute disease (60). During acute disease, mortality in sows is usually 1-4% and is often associated with pulmonary edema and/or cystitis/nephritis (61).

For acute highly pathogenic PRRS (HP-PRRS), abortion rates of 40-100 percent and sow mortality of about 10 percent are recorded in Southeast Asia (54). Clinical signs of infected sows or gilts range from asymptomatic to loss of appetite, fever, lethargy, pneumonia, agalactia, discoloration of the ears and vulva, subcutaneous and hindlimb swelling, delayed estrus after weaning, and rarely death (62).

In addition to anorexia, lethargy, and respiratory clinical symptoms, boars acutely infected with PRRSV may lack libido and have a variable decrease in semen quality (63). Sperm deformity occurs 2-10 weeks after virus infection, and it is not known whether the fertilization rate is damaged, but motility decreases and acrosome abnormalities are seen (36).

5.2. Suckling pigs

Severe dyspnea and tachypnea occur in neonatal pigs infected with PRRSV in utero or around birth and can also show periocular edema, conjunctivitis, eyelid edema, blue ear discoloration, inappetence, fever, diarrhea of the cutaneous erythema, sweating, rough hair coats, bleeding post injection, anorexia, and symptoms of the central nervous system. Clinical symptoms in neonatal pigs infected with PRRSV differ markedly in incidence and severity, with the most characteristic clinical signs being dyspnea (thumping) and tachypnea. Mortality in PRRSV-infected neonatal pigs can reach 100 percent

(64).

5.3. Nursery pigs

Fever, pneumonia, lethargy, inability to survive, and a marked rise in mortality from single to multiple concurrent bacterial infections was characterized by porcine reproductive and respiratory syndrome virus infection in weaned pigs. The pronounced rise in mortality associated with PRRSV infections in immature swine is driven by complicated bacterial infections. Coinfection with single or several bacteria and, less commonly, with other viruses are significant complicating factor of PRRSV infection and is clinically relevant to the increase in postweaning mortality from 1–2% to 10–15% while all other variables tend to remain unaffected (65).

5.4. Grow finishing pigs

Infection of the porcine reproductive and respiratory syndrome virus in grow finishing pigs is usually only characterized by a temporary inappetence and fever. PRRSV seroconversion in finished pigs can be the only evidence of infection (35).

6. Lesions

PRRSV infection-related gross lesions vary greatly and can rely on the isolation of the virus, swine genetic variations, and stress factors (environmental and endemic bacterial and viral flora) (Halbur et al. 1995). Lung lesions are various from none to severe complicated interstitial pattern and are commonly complicated by lesions resulting from concurrent bacterial infection (62).

Consistent gross and microscopic lesions are observed in the lungs and lymph nodes 4 to ≥28 DPI. There is interstitial pneumonia that varies in severity. Distribution may be cranioventral to diffuse, consistency varies from slightly firm and resilient to moderately firm and rubbery, color varies from tan to dark red purple, and pulmonary edema varies from mild to severe with separation of lobules. In HP PRRS, the lungs may be hemorrhagic.

Mainly, the lymph nodes of young pigs are affected a lot, and they are significantly enlarged and the gross lesion are various (32). When exposed to PRRS, in the beginning nodes are enlarged, edematous, tan, and moderately firm and in HP PRRS may be hemorrhagic. Later, nodes are becoming firm and white or light tan in a nodular or diffuse pattern.

In many cases in PRRS reproductive diseases, small or normal weight

neonatal pigs are delivered at the end of pregnancy, but at various rates, autolyzed dead pigs and mummified pigs are expelled before term. A thick brown mixture of meconium and amniotic fluid may be coated with dead pigs, which shows fetal discomfort and/or hypoxia (67). Lesions are unusual in fetuses and stillborn pigs and seldom lead to a definitive PRRS diagnosis. In fetuses with little autolysis in the uterus, PRRSV-specific gross lesions are best seen but it is more commonly observed in litters that are infected with PRRSV during pregnancy and die right after farrowing or euthanized for autopsy (29).

7. Immunity

7.1. Innate immunity

A classical innate antiviral immunity like interferon releasing and inflammatory cytokine production are not occurred in PRRSV infection were first reported by Van Reeth and colleagues at Ghent University in Belgium (68). Recently it was explained that PRRSV nonstructural protein 1 (nsp1) inhibits the activation of transcription of interferon (15). The innate immune response to PRRSV is affected by virus strains while the adaptive immune response is not delayed in model cell culture systems may supports these explanations. Also, PRRSV cause pig immune system to release

immunosuppressive cytokines including IL-10, IL-4, and transforming growth factor β (TGF β), which help PRRSV escape innate immunity and invite secondary bacterial or other virus infection.

7.2. Humoral immunity

Depending on the antibody detection method, IgM antibodies against PRRSV can be detected at 5 dpi and IgG antibodies at 7 to 10 dpi (32). Humoral immunity to PRRSV appears to be very durable once acquired. However, Nucloecapsid antibodies can break down faster even if the virus is present in lymphatic tissue. Since many serological diagnoses are based on the nucleocapsid antigen, antibodies are not detected several months after exposure, which can be misdiagnosed as non-immune status (69). Antibody-secreting plasma cells and PRRSV-specific B memory cells are located in various lymphoid tissues, especially the lymph nodes connected to the lungs, genital areas, spleen and tonsils (70).

PRRSV induced Antibody Dependent Enhancement was reported and it appears to be related that PRRSV infect macrophages and neutralizing antibody levels against PRRSV are usually very low (71). But other studies showed PRRSV neutralizing antibody titer in swine serum has nothing to do with viral growth and there was a positive correlation between antibody titer and inhibition of infection (72). Also, comparing simian cells and

porcine alveolar macrophages neutralization specificity revealed no evidence of enhanced infection of macrophages (73). Hence, further studies are requried to the ADE of PRRSV infection for better vaccine designs especially for subunit vaccines.

Neutralizing antibodies appears 9 to 105 days after infection (32) and are believed to be core of virus elimination but it's funcion is still arguable because neutralizing antibodies mostly show after viremia disappered (74). Maternal driven antibody exist without PRRS infection for 6-10 weeks and this passive immunity proved to protect piglets from homologues PRRSV (75) but naturally infected pigs can also neutralize heterologous PRRSV (76). Mainly PRRS antibodies are generated against ORF6 and 7 (77) but trimeric complex arising from GP2, GP3, GP4 envelope glycoproteins seems to neutralize virus (18) and GP5 is not required for macrophage infection and anti-GP5 antibodies do not neutralize infection (78). Antibodies against PRRSV don't block or may help PRRSV infection because Antibody Dependent Enhancement (ADE) with Fc receptor uptake and PRRSV replication in the presence of Neutralizing antibody were observed (79).

7.3. Cellular immunity

After PRRSV infection, antigen-specific lymphocyte proliferation begins at 28 days after infection, peaks at 49 days and decreases 77 days after infection (80). This proliferative response can be inhibited by CD4 and MHC II antigen antibodies, suggesting that the reaction is dependent on CD4 + T

lymphocytes (81).

Interferon-r ELISPOT has shown a consistent T-cell response to PRRSV infection using live PRRSV in leukocyte cultures, but its importance is unclear (82). PRRSV infecion can produce Th1 mediated immune response was proved using PRRS infected pigs exposed cutaneously to dinitroflurobenzene but the source of interferon-r is difficult to determine since it is produced by Th1 helper T cells, activated cytotoxic T cells, and natural killer cells. Differences of MHC I haplotypes are linked to resistance or susceptibility to PRRSV infection, cytotoxic T-lymphocyte (CTL) seems to affect resistance to PRRSV infection (83). T cell responses are important for resistance to PRRSV infection as regulatory T cells and IL-10 are associated with delayed immune activation and sustained infection (84).

7.4. Persistent infection

Unlike other viral disease, PRRSV infection is lasting longer for 4 to 6 weeks and after that even convalescent nonviremic pigs can infect newly introduced naive sentinel pigs. This means pigs are remain infected though viremia resolved (85). Studies showed that PRRSV can be detected up to 186 days in test groups and irregularly up to 251 days in individual pigs (50). These persistent infection observations are consistent with the escaping innate immunity and with the poor B- and T-cell responses Persistent

infections are common feature of PRRSV belonged arteriviruses, especially lactate dehydrogenase elevating virus (LDV) of mice and equine arteritis virus (EAV) (86). LDV virus evoke life long viremia but PRRSV has limited time of viremia and there are neither delayed infection nor cyclical viremic wax and wane. The exact mechanisms of PRRSV persistent infection were not revealed (87).

8. Modified Live vaccine

PRRS vaccines had been broadly classified Modified live vaccines and inactivated killed vaccines. Studies have shown that once the PRRS virus mutates, live vaccines are no longer able to neutralize virus, which would further complicate the epidemic situation in pigs and hinder any preventive effects of the vaccine against PRRS (88).

Vaccination, while it does not completely prevent PRRSV infection, is one of the most powerful methods for managing PRRS. Vaccination with a homologous strain is more effective than vaccination with a heterologous strain and modified live vaccines has reduced production and economic losses and wild type virus shedding (89).

MLV vaccines are used in piglets from three weeks of age or sows and gilts 3-6 weeks prior to breeding and mass vaccination whole breeding herd

including boars every 3-4 month periodically (OIE, 2004). The vaccination of seronegative replacement breeding stock 60-90 days before incoming to the farm is recommended. Weak points of MLV vaccination are MLV vaccines' virus strain can be shed to other pigs and compromise detecting natural infection serologically and live PRRS vaccines may mutate in pigs, which will even complicate the symptoms. However, the use of entire herd mass vaccination with MLV vaccine in the face of outbreaks resulted in a quick stabilizaion of farm than Live virus inoculation protocol (90).

8.1. MLV type 1

PRRSV-1 is subdivided into 4 subtypes with a nucleotide variation up to 30%. Four commercial MLV1 vaccines (Porcilis PRRS, UNITSTRAIN PRRS, ReproCyc PRRS EU, Ingelvac PRRSFLEX EU) are categorized into subtype 1 and currently used in European and Asian countries. Studies showed that all four MLV1 vaccines have partial protection ability against highly pathogenic PRRSV-1 subtype 3 Lena strain by lowering feverish time but MLV1 vaccination has little effect on viremia and lung lesion when challenged with PRRSV-2 (91). Vaccination of pigs with MLV1 did not reduce the level of viremia and lung lesions after challenge with PRRSV-2 (92) while the same MV1 vaccine did reduce the levels of viremia and lung lesions post-challenge with a different PRRSV-2 strain. MLV1 vaccines which provided partial to improved protection and breeding performance in

gilt and sows against even heterologous PRRSV-1, provided only limited cross protection in gilt against PRRSV-2 (93).

These results mean that MLV1 vaccinations give predictable protection only against PRRSV-1 rather than PRRSV-2 if ever.

8.2. MLV type 2

PRRSV-2 is subdivided into 9 lineages with up to 21% nucleotide variation. Two MLV2 vaccines (Fostera PRRS, Ingelvac PRRS MLV) were effective against HP-PRRSV-2 challenge in growing pigs and same lineage Fostera PRRS provided slightly better performance than different lineage Ingelvac PRRS MLV did in body temperature, levels of viremia, and number of IFN-r -SC (94).

In Korea, the MLV2 vaccination against heterologous PRRSV-2 has been efficacious since it was first launched 20 years ago (93) and surprisingly the same MLV2 vaccine still works against currently isolated heterologous PRRSV-2 strains (95). But MLV2 vaccine was not able to reduce the levels of viremia against European heterologous PRRSV-1 subtype 1 (Lelystad-like) strains (92, 96).

MLV2 vaccination on pregnant sows increased breeding performance and IFN-r-SC response while reducing maternal viremia and the level of PRRSV-2 in fetal thymus against a heterologous PRRSV-2 challenge (97,

98). But cross protection on reproductive disease against same PRRSV-1 strain were various between two MLV2 vaccines and even same MLV2 vaccine's cross protection levels on reproductive and respiratory signs were also different (93, 98).

8.3. Co-Vaccination of MLV 1 and MLV 2

As PRRSV-1 and PRRSV-2 were first isolated in Europe and North America respectively around 1991, both strains are still dominant in that discovered continent. But both PRRSV-1 and PRRSV-2 has appeared together in Asian countries including Korea and caused more complicated disease (99). So, co-vaccination with MLV1 and MLV2 together are worth studying to protect both strains. Unfortunately, studies related to co-vaccination with MLV1 and MLV2 brought inconsistant results. In PRRSV-1 and PRRSV-2 dual challenge test, one report found that co-vaccination of pigs at four weeks of age provided partial protection against both strains' respiratory disease (100) but other report revealed that co-vaccination provided protection against only PRRSV-1 respiratory disease (101). In this study, MLV1 and MLV2 vaccines' co-vaccination on pigs significantly decreased the efficacy of the MLV2 vaccine but the MLV1 vaccine's efficacy was not affected. In contrast to these co-vaccination efficacy against respiratroy disease, another study of co-vaccination with MVL1 and MLV2 vaccines against reproductive failure from PRRSV-1 and

PRRSV-2 showed dual protection possibility in sows and gilts (97). These discrepancies among studies of co-vaccination with MLV1 and MLV2 against respiratory and reproductive disease are thought to be contributed by the extreme antigenic diversity of PRRSV (102).

9. Killed vaccine

9.1. Commercial PRRSV KV

Killed vaccines generally do not have neutralizing capabilities and often do not confer immune protections in pigs. They are more costly and are mostly used to induce immune responses in sows (103) or used to boost previously vaccinated with Live vaccines. Currently it's uncommon vaccinating only one time with Killed vaccine in conventional swine farm. High serum prevalence of PRRSV farm with a number of 1100 sows including gilts showed improved breeding performance and weaned piglet ratio when vaccinated 18 month continuously with killed PRRSV vaccine (Progressis ®, Merial, type 1 PRRSV)(104). This may imply repeated vaccination with killed virus vaccine can increase immunity against PRRSV but other study using same vaccine came out fail in virus clearance when challenged with Lelystad virus (type 1 PRRSV) even with increased IFN-gamma secretion and 8 fold virus neutralization antibody titer (92). Similarly, a killed PRRSV

vaccine (PRRomiSe ®, Intervet, type 2 PRRSV) injected two times on PRRSV infected pigs didn't lower viral shedding although it increased VN titer (16 fold) and IFN - gamma (105).

Taken together, aside from the safety of killed PRRSV vaccines, improving its efficacy is necessary. So, there has been many attempts to improve its efficacies incorporating various inactivation methods, changing adjuvants, nano particle vaccine delivery systems and gene engineering technologies like vector vaccines or subunit vaccines.

9.2. Subunit PRRS vaccines

According to the study by Yoon K-J et al. (71) and recent studies on PRRSV, vaccination with whole virus regardless of whether attenuated or inactivated may induce antibody dependent enhancement (ADE), similar to the human dengue virus (DV), feline infectious peritonitis virus (FIPV) and equine infectious anaemia virus (EIAV). If administered with conventional PRRS vaccines, allergic reactions often develop in pigs after PRRSV has invaded the lungs (106). This means that once PRRSV has entered the body fluids (humoral) and combine with antibodies, there is a greater chance that it will attack immune cells of the host, like monocytes and macrophages, and further worsen the condition (107). PRRSV with antibody dependent enhancement may induce further mutations and adaptations, creating new

strains and new outbreaks of infectious disease that are very difficult to tackle with the use of conventional live and killed PRRS vaccines (107). To avoid these whole virus vaccine side effects, reverse vaccinology (108) and genetic engineering techniques were employed in developing PRRS Subunit vaccine. Subunit vaccine use a small portion of proteins believed to induce neutralizing anbodies such as non-structural nucleoprotein of ORF7 and non-structural protein ORF1b of the PRRSV to induce cell-mediated immune response and may employ other bacteria as vaccine delivery platform (109).

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Chapter I

Comparison of four commercial modified-live porcine reproductive and respiratory syndrome virus (PRRSV) vaccines against heterologous Korean PRRSV-1 and PRRSV-2 challenge

Abstract

The efficacy of four commercial porcine reproductive and respiratory syndrome virus (PRRSV) modified-live vaccines (MLV), against PRRSV-1 and PRRSV-2 challenge was evaluated and compared in growing pigs. Two of the vaccines were based on PRRSV-1 and two on PRRSV-2. There were no significant differences between each of the two PRRSV-1 MLV vaccines and the two PRRSV-2 MLV vaccines respectively based on virological, immunological, and pathological evaluations. Vaccination with either of the PRRSV-1 MLV vaccines resulted in reduced PRRSV-1 but not PRRSV-2 viremia. Additionally, vaccination with either of the PRRSV-1 MLV vaccines resulted in reduction of lung lesions and PRRSV-1 positive cells in PRRSV-1 challenged pigs but had no significant effect in PRRSV-2 challenged pigs. In contrast, vaccination with either of the two PRRSV-2 MLV vaccines resulted in the reduction of both PRRSV-1 and PRRSV-2 viremia. PRRSV-2 MLV vaccines were also able to effectively reduce lung lesions and PRRSV positive cells after challenge with either PRRSV-1 or PRRSV-2. Our data suggest that while vaccination with PRRSV-1 MLV vaccines can be effective against PRRSV-1, only PRRSV-2 MLV vaccines can protect against both Korean PRRSV-1 and PRRSV-2 challenge under conditions of this study

Keywords comparison, modified-live virus vaccine, porcine reproductive and respiratory syndrome virus, vaccination

Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first described as a 'mystery swine disease' in 1987 in USA (1) and as 'blue ear disease' in 1990 in Europe (2). The first cases of PRRS in Korea were detected in 1994. Since then, PRRS has rapidly become one of the most impactful global swine diseases causing devastating economical losses to the swine industry worldwide. PRRS is characterized by reproductive failure in breeding females and respiratory disease in pigs of all ages (3). The causative agent for PRRS is the PRRS virus (PRRSV). PRRSV is a single stranded positive sense RNA virus that belongs to the family of Arteriviridae in the order Nidovirales with two distinct species based on antigenic and pathogenic differences, PRRSV-1 of European origin and PRRSV-2 of North American origin (4,5).

In most European countries PRRSV-1 is the prevalent species (6), while the majority of PRRS cases in North American countries are caused by PRRSV-2 only (7). PRRSV-1 is rarely considered an important economic pathogen in the US (personal communication to Dr. Aaron J. Lower, Carthage Veterinary Service Ltd.). In contrast, the Korean farmland appears to be a region in which both PRRSV-1 and PRRSV-2 are equally prevalent with both species causing serious clinical problems (8). Currently in Korean farms, the most common method of controlling PRRSV infection is through vaccination. Although modified-live virus (MLV) vaccines so far have

provided limited protection against heterologous field strains (9,10), they are widely used and considered to be the most effective tool in controlling PRRSV infection.

Currently, four MLVs, two based on PRRSV-1 and two based on PRRSV-2 are commercially available in the Korean market. However no studies have been performed yet to assess the efficacy of these 4 PRRSV MLV vaccines against heterologous PRRSV-1 and PRRSV-2 field viruses. The objective of this study was to evaluate and compare the efficacy of these four PRRSV MLV vaccines against single heterologous PRRSV-1 and PRRSV-2 challenge based on clinical, virological, immunological, and pathological criteria under the same experimental conditions.

Materials and methods

Virus

PRRSV-1 (SNUVR090485, pan-European subtype 1) and PRRSV-2 (SNUVR090851, lineage 1) were used as challenge inocula (11,12). Nucleotide homology of open reading frame 5 genome from PRRSV challenge viruses was compared with the vaccine viruses from 4 PRRSV MLV vaccines (Table 1).

Table I. Percentage nucleotide homology of open reading frame 5 genome from PRRSV challenge viruses used in this study compared with the vaccine viruses from 4 PRRSV modified-live virus vaccines

	PRRSV-1 (JN315686)	PRRSV-2 (JN315685)	Porcilis PRRS (AY743931)	UNISTRAIN PRRS (GU067771)	Ingelvac PRRS MLV (AF066183)	Fostera PRRS (AF494042)
PRRSV-1	100	59	87.9	88.1	61.1	61.1
PRRSV-2	59	100	59.5	59.3	85.9	87.2
Porcilis PRRS	87.9	59.5	100	93.3	61.4	61.6
UNISTRAIN PRRS	88.1	59.3	93.3	100	61.1	60.6
Ingelvac PRRS MLV	61.1	85.9	61.4	61.1	100	91.3

Experimental design

A total of 132 colostrum-fed, cross-bred, conventional piglets were purchased at 14 days of age from a commercial PRRSV-free farm. All piglets were negative for PRRSV according to routine serological testing and real-time reverse transcriptase polymerase chain reaction (PCR) as previously described (13).

Pigs were divided into 11 groups (12 pigs per group) and assigned into 11 rooms using the random number generation function (Excel, Microsoft Corporation, Redmond, Washington, USA). The pigs within each group were housed in same room (Table 2). Five groups (in 10 separate rooms) were challenged with PRRSV-1, five groups (in 10 separate rooms) were challenged with PRRSV-2 and one group (in two separate rooms) was used as control using the random number generation function (Excel, Microsoft Corporation). At -35 days post challenge (dpc, 28 days of age), pigs were injected intramuscularly on the right side of the neck with 2 mL of Porcilis PRRS (Vac1A/Ch1 and Vac1A/Ch2 groups ; Lot No. D353A07, MSD Animal Health, Boxmeer, the Netherlands), UNISTRAIN PRRS (Vac1B/Ch1 and Vac1B/Ch2 groups; Lot No. 61WK-B, Hipra, Amer, Spain), Ingelvac PRRS MLV (Vac2A/Ch1 and Vac2A/Ch2 groups; Lot No. 245-659A, Boehringer Ingelheim Vetmedica, St. Joseph, Missouri, USA), and Fostera PRRS (Vac2B/Ch1 and Vac2B/Ch2 groups; Lot No. A405013B, Zoetis, Parsippany, New Jersey, USA) according to the manufacturer's instructions. Pigs in the

UnVac/Ch1, UnVac/Ch2, and UnVac/UnCh groups were administered an equal volume of phosphate buffered saline (PBS, 0.01M, pH 7.4).

At 0 dpc (63 days of age), pigs in the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, Vac2B/Ch1, and UnVac/Ch1 groups were inoculated intranasally with 3 mL of PRRSV-1 inoculum (105 TCID50/mL of SNUVR090485, second passage in alveolar macrophages). Pigs in the Vac1A/Ch2, Vac1B/Ch2, Vac2A/Ch2, Vac2B/Ch2, and UnVac/Ch2 groups were inoculated intranasally with 3 mL of PRRSV-2 inoculum (105 TCID50/mL of SNUVR090851, second passage in alveolar macrophages). Pigs in the UnVac/UnCh group served as negative controls and were not exposed to either the vaccine or virus. Upon challenge, pigs in the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, Vac2B/Ch1, and UnVac/Ch1 groups were randomly assigned into 10 out of 22 rooms. Pigs in the Vac1A/Ch2, Vac1B/Ch2, Vac2A/Ch2, Vac2B/Ch2, and UnVac/Ch2 groups were randomly assigned into 10 out of 22 rooms. Each room contained 6 pens and each pig was housed individually in a pen. In each of the 10 rooms, allocation of pens to treatment was in accordance with a randomized complete block design with one-way treatment structure. Blocking was based on pen location. A block comprised of four pens located near each other. The experimental unit for treatment was the individual animal. Within each block, one pen was randomly assigned to each treatment group. Pigs in the UnVac/UnCh group were randomly placed into 12 pens in the two remaining rooms.

Following PRRSV challenge, the physical condition and the rectal temperature of each pig was monitored daily. Blood samples were collected at -35, -21, 0, 7, 10, and 14 dpc. Pigs were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 7 and 14 dpc as previously described (14). All of the methods were previously approved by the Seoul National University Institutional Animal Care and Use, and Ethics Committee.

Table II. Experimental design and results of lesion score and porcine reproductive and respiratory syndrome virus (PRRSV) RNA within lung lesion at 7 and 14 days post challenge (dpc) Different letters (a, b, and c) indicate significant ($P < 0.05$) difference among groups.

Groups	PRRSV		dpc	Lung lesion score		PRRSV-positive cells within lung lesion	
	Vaccination (28 days)	Challenge (63 days)		Macroscopic	Microscopic	PRRSV-1	PRRSV-2
Vac1A/Ch1	Porcilis PRRS	PRRSV-1	7	14.2 ± 4.9^{b}	0.7 ± 0.8^{ab}	3.7 ± 1.0^{b}	0 ± 0
			14	7.5 ± 2.7^{b}	0.5 ± 0.8^{ab}	0.7 ± 0.8^{b}	0 ± 0
Vac1B/Ch1	UNISTRAIN PRRS	PRRSV-1	7	16.7 ± 7.5^{b}	0.8 ± 0.8^{ab}	3.7 ± 0.8^{b}	0 ± 0
			14	8.3 ± 4.1^{b}	0.7 ± 0.5^{ab}	0.5 ± 0.5^{b}	0 ± 0
Vac2A/Ch1	Ingelvac PRRS MLV	PRRSV-1	7	18.3 ± 4.1^{b}	0.8 ± 0.6^{b}	0 ± 0^{c}	0 ± 0
			14	4.2 ± 4.9^{b}	0.3 ± 0.6^{b}	0.5 ± 0.5^{b}	0 ± 0
Vac2B/Ch1	Fostera PRRS	PRRSV-1	7	16.7 ± 5.2^{b}	0.7 ± 0.5^{b}	0 ± 0^{c}	0 ± 0
			14	3.3 ± 5.2^{b}	0.2 ± 0.4^{b}	0.3 ± 0.8^{b}	0 ± 0
UnVac/Ch1	None	PRRSV-1	7	30.8 ± 8.0^{a}	1.6 ± 0.5^{a}	20.3 ± 3.7^{a}	0 ± 0
			14	24.2 ± 6.6^{a}	1.4 ± 0.5^{a}	9.7 ± 3.5^{a}	0 ± 0
UnVac/UnCh	None	None	7	0.8 ± 2.0^{c}	0.3 ± 0.5^{b}	0 ± 0^{c}	0 ± 0
			14	1.7 ± 4.1^{b}	0.2 ± 0.4^{b}	0 ± 0^{b}	0 ± 0
Vac1A/Ch2	Porcilis PRRS	PRRSV-2	7	58.3 ± 14.7^{a}	3.7 ± 0.4^{a}	0 ± 0	45.5 ± 12.2^{a}
			14	45.8 ± 6.6^{a}	3.3 ± 0.8^{a}	0 ± 0	34.8 ± 6.2^{ab}
Vac1B/Ch2	UNISTRAIN PRRS	PRRSV-2	7	55.8 ± 10.2^{a}	3.8 ± 0.4^{a}	0 ± 0	43.8 ± 7.0^{a}
			14	47.5 ± 7.6^{a}	3.3 ± 0.4^{a}	0 ± 0	35.3 ± 6.3^{ab}
Vac2A/Ch2	Ingelvac PRRS MLV	PRRSV-2	7	32.5 ± 5.2^{b}	2.0 ± 0.6^{b}	0 ± 0	30.8 ± 9.2^{a}
			14	21.7 ± 6.8^{b}	1.8 ± 0.8^{b}	0 ± 0	23.8 ± 5.0^{ab}
Vac2B/Ch2	Fostera PRRS	PRRSV-2	7	31.7 ± 7.5^{b}	2.1 ± 0.2^{b}	0 ± 0	29.2 ± 7.0^{a}
			14	18.3 ± 7.5^{b}	1.6 ± 0.5^{b}	0 ± 0	22.2 ± 6.7^{b}
UnVac/Ch2	None	PRRSV-2	7	63.3 ± 8.2^{a}	3.8 ± 0.4^{a}	0 ± 0	45.2 ± 13.7^{a}
			14	46.7 ± 8.2^{a}	3.3 ± 0.5^{a} C	0 ± 0	36.0 ± 14.0^{a}
UnVac/UnCh	None	None	7	0.8 ± 2.0^{c}	0.3 ± 0.5^{c}	0 ± 0	0 ± 0^{b}
			14	1.7 ± 4.1^{c}	0.2 ± 0.4^{c}	0 ± 0	0 ± 0^{c}

Clinical observation

Clinical observation of respiratory symptoms was recorded daily using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (15). Observers were blinded to vaccination and challenge status. Rectal temperatures were also recorded daily at the same time by the same personnel.

Serology

Serum samples that were collected were tested using a commercially available PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS X3 Ab test, IDEXX Laboratories Inc, Westbrook, Maine, USA). Serum samples were considered positive for PRRSV antibody if the S/P ratio was ^{3} 0.4, according to the manufacturer's instructions.

Quantification of PRRSV RNA

RNA was extracted from serum samples that were collected to quantify PRRSV genomic cDNA copy numbers, as previously described (13). PRRSV-1 forward and reverse primers were 5′-TGGCCAGTCAGTCAATCAAC-3′ and 5′-AATCGATTGCAA GCAGAGGGAA-3′, respectively. PRRSV-2 forward and reverse primers

were 5′-TGGCCAGTCAGTCAATCAAC-3′ and 5′-AATCGATTGCAAGCAGAGGGAA-3′, respectively (13).

For the Porcilis PRRS vaccine virus, the forward and reverse primers were 5′-TGTAGACAACCGGGGGAGAG-3′ and 5′-CTAGGCCTCCCATTGCTCAG-3′, respectively. For the UNISTRAIN vaccine virus, the forward and reverse primers were 5′-GTTGCCCAGCCATTTTGAC-3′ and 5′-CACGCTGCTGAGTACATACC-3′, respectively [16]. For the Ingelvac PRRS MLV vaccine virus, the forward and reverse primers were 5′-CTAACAAATTTGATTGGGCAG-3′ and 5′-AGGACATGCAATTCTTTGCAA-3′, respectively (16). For the Fostera PRRS vaccine virus, the forward and reverse primers were 5′-CTTGACACAGTTGGTCTGGTTACT-3′ and 5′-GTTCTTCGCAAGCCTAATAACG-3′, respectively (17). Real-time PCR was performed to quantify PRRSV genomic cDNA copies (13,16-18).

Enzyme-linked immunospot (ELISPOT) assay

The numbers of PRRSV-specific interferon-r secreting cells (IFN-r-SC) were determined in peripheral blood mononuclear cells (PBMC) using challenging PRRSV-1 and PRRSV-2 as previously described (17,19,20).

Pathology and in situ hybridization

The total amount of microscopic lesions in the lung sections was scored blindly ranging from 0 (normal) to 4 (severe) by two pathologists (15). In situ hybridization (ISH) for the detection and differentiation of PRRSV-1 and PRRSV-2 nucleic acids in lung tissues was performed and analyzed morphometrically with the NIH Image J 1.51r Program (http://imagej.nih.gov/ij/download.html) as previously described (21).

Statistical analysis

Continuous data included rectal temperature, PRRSV RNA (log10 of the number of PRRSV genomic copies per mL quantified by real-time PCR), PRRSV antibody titer, and number of IFN-r-SC (measured by ELISPOT assay). Continuous data were analyzed using Tukey's multiple comparisons test for comparison between groups in order to estimate the difference at each time point. Discrete data (clinical signs, lung lesion scores, and ISH scores) were analyzed with the Kruskal-Wallis test. When the Kruskal-Wallis test was significant, the Mann-Whitney test was performed to determine the significant differences between the groups. A value of $P < .05$ was considered significant.

Results

Clinical observation

There were no observable clinical signs after vaccination and before challenge in any of the pigs from all 6 groups. In PRRSV-1 challenged groups, the mean rectal temperature was significantly ($P < 0.05$) lower in pigs from the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, Vac2B/Ch1, and UnVac/UnCh groups at 2 to 5 dpc compared to pigs from the UnVac/Ch1 group. The mean rectal temperature was significantly lower ($P < 0.05$) in pigs from the Vac2A/Ch1 and Vac2B/Ch1 groups at 6 dpc compared to pigs from the UnVac/Ch1 group (Figure 1A). The mean respiratory scores were significantly ($P < 0.05$) lower in pigs from the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, Vac2B/Ch1, and UnVac/UnCh groups at 2 to 8 dpc compared to pigs from the UnVac/Ch1 group. The mean respiratory scores were significantly ($P < 0.05$) lower in pigs from the UnVac/UnCh group compared to pigs from the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, and Vac2B/Ch1 groups at 5 and 6 dpc (Figure 2A).

In PRRSV-2 challenge groups, the mean rectal temperature was significantly ($P < 0.05$) lower in pigs from the Vac2A/Ch2, Vac2B/Ch2 and UnVac/UnCh groups at 2 to 10 dpc compared to pigs from the Vac1A/Ch2 and Vac1B/Ch2 groups. The mean rectal temperature was significantly ($P < 0.05$) lower in pigs from the Vac2A/Ch2, Vac2B/Ch2, and UnVac/UnCh groups at 2 to 8 dpc compared to pigs from the UnVac/Ch2 group. The mean rectal temperature was significantly ($P < 0.05$) lower in pigs from the Vac1A/Ch2 and Vac1B/Ch2 groups at 3 dpc compared to pigs from the UnVac/Ch2 group. The mean rectal temperature was significantly ($P < 0.05$)

lower in pigs from the UnVac/UnCh group at 2 to 7 dpc compared to pigs from the Vac2A/Ch2 and Vac2B/Ch2 groups (Figure 1B). The mean respiratory scores were significantly ($P < 0.05$) lower in pigs from the Vac2A/Ch2, Vac2B/Ch2, and UnVac/UnCh groups at 2 to 8 dpc compared to pigs from the Vac1A/Ch2, Vac1B/Ch2, and UnVac/Ch2 groups. The mean respiratory scores were significantly ($P < 0.05$) lower in pigs from the Vac1A/Ch2 and Vac1B/Ch2 groups at 2, 5, 6, and 7 dpc compared to pigs from the UnVac/Ch2 group. The mean respiratory scores were significantly ($P < 0.05$) lower in pigs from the UnVac/UnCh group at 2, 4, 5, 6, and 7 dpc compared to pigs from the Vac2A/Ch2 and Vac2B/Ch2 groups. Pigs in the UnVac/UnCh group maintained normal rectal temperatures and respiratory signs throughout the study (Figure 2B).

Quantification of PRRSV RNA

Genomic copies of the vaccine virus were detected in the sera of vaccinated pigs -21 dpc (14 days post vaccination) but, thereafter, no genomes of the vaccine strain were detected throughout the rest of the experiment. In the PRRSV-1 challenged groups, pigs from the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, and Vac2B/Ch1 groups had significantly ($P < 0.05$) lower genomic copies of PRRSV-1 in their sera compared to pigs from the UnVac/Ch1 group at 7 to 14 dpc. There was no significant difference in genomic copies of PRRSV-1 in the sera of pigs from the Vac1A/Ch1,

Vac1B/Ch1, Vac2A/Ch1, and Vac2B/Ch1 groups (Figure 3A).

In the PRRSV-2 challenged groups, pigs from the Vac2A/Ch2 and Vac2B/Ch2 groups had significantly ($P < 0.05$) lower genomic copies of PRRSV-2 in their sera compared to pigs from the Vac1A/Ch2, Vac1B/Ch2, and UnVac/Ch2 groups at 7 to 14 dpc. There was no significant difference in genomic copies of PRRSV-2 in the sera of pigs from the Vac1A/Ch2, Vac1B/Ch2, and UnVac/Ch2 groups. PRRSV-1 was not detected in PRRSV-2 challenged pigs and vice versa. No PRRSV of any genotype was detected in the sera of pigs from the UnVac/UnCh group throughout the experiment (Figure 3B).

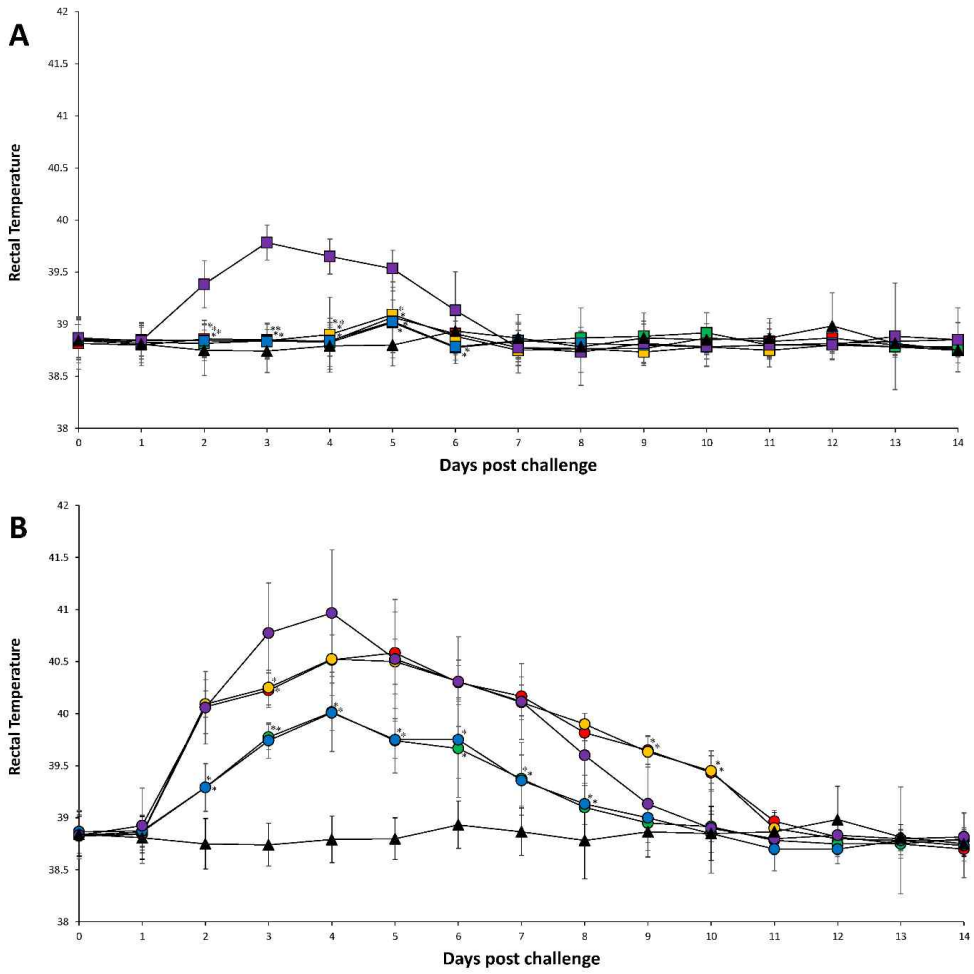

Figure 1. Mean rectal temperature. (A) PRRSV-1 challenged groups from the Vac1A/Ch1 (■), Vac1B/Ch1 (■), Vac2A/Ch1 (■), Vac2B/Ch1 (■), UnVac/Ch1 (■), and UnVac/UnCh (▲). (B) PRRSV-2 challenged groups from the Vac1A/Ch2 (●), Vac1B/Ch2 (●), Vac2A/Ch2 (●), Vac2B/Ch2 (●), UnVac/Ch2 (●), and UnVac/UnCh (▲). Variation is expressed as the standard deviation. Significant difference between vaccinated challenged and unvaccinated challenged groups within the same PRRSV type challenge is indicated as P value <0.05*.

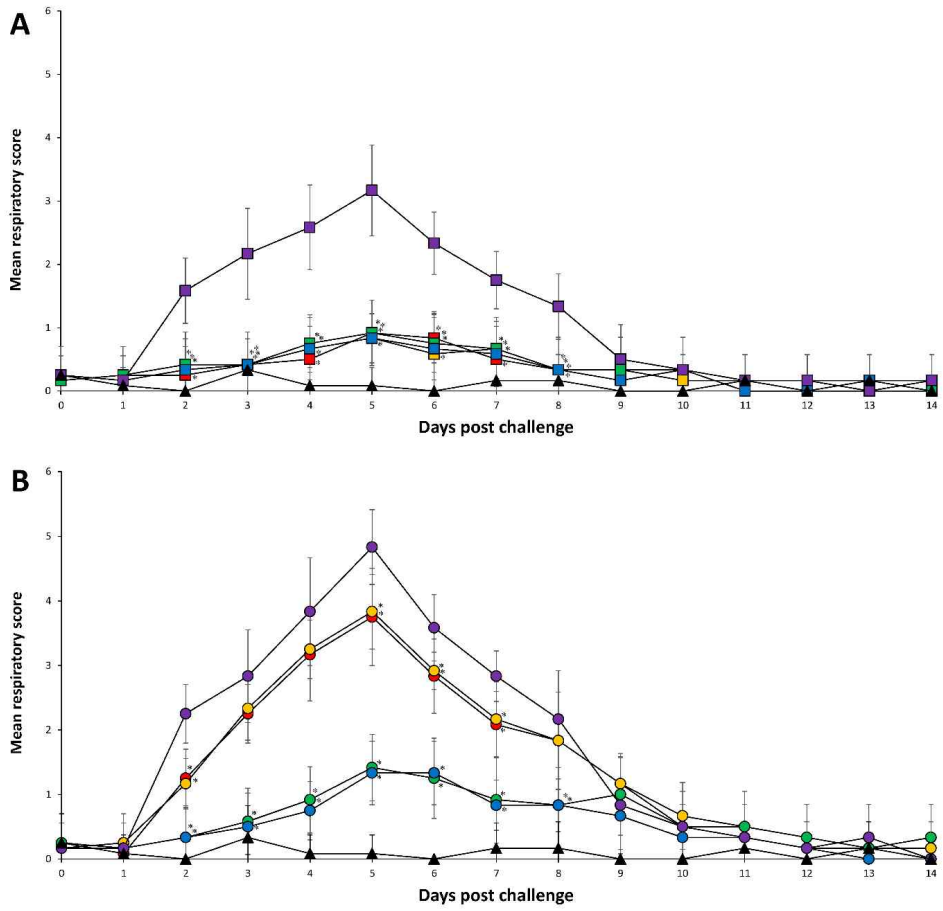

Figure 2. Mean respiratory score. (A) PRRSV-1 challenged groups from the Vac1A/Ch1 (■), Vac1B/Ch1 (■), Vac2A/Ch1 (■), Vac2B/Ch1 (■), UnVac/Ch1 (■), and UnVac/UnCh (▲). (B) PRRSV-2 challenged groups from the Vac1A/Ch2 (●), Vac1B/Ch2 (●), Vac2A/Ch2 (●), Vac2B/Ch2 (●), UnVac/Ch2 (●), and UnVac/UnCh (▲). Variation is expressed as the standard deviation. Significant difference between vaccinated challenged and unvaccinated challenged groups within the same PRRSV type challenge is indicated as P value <0.05*.

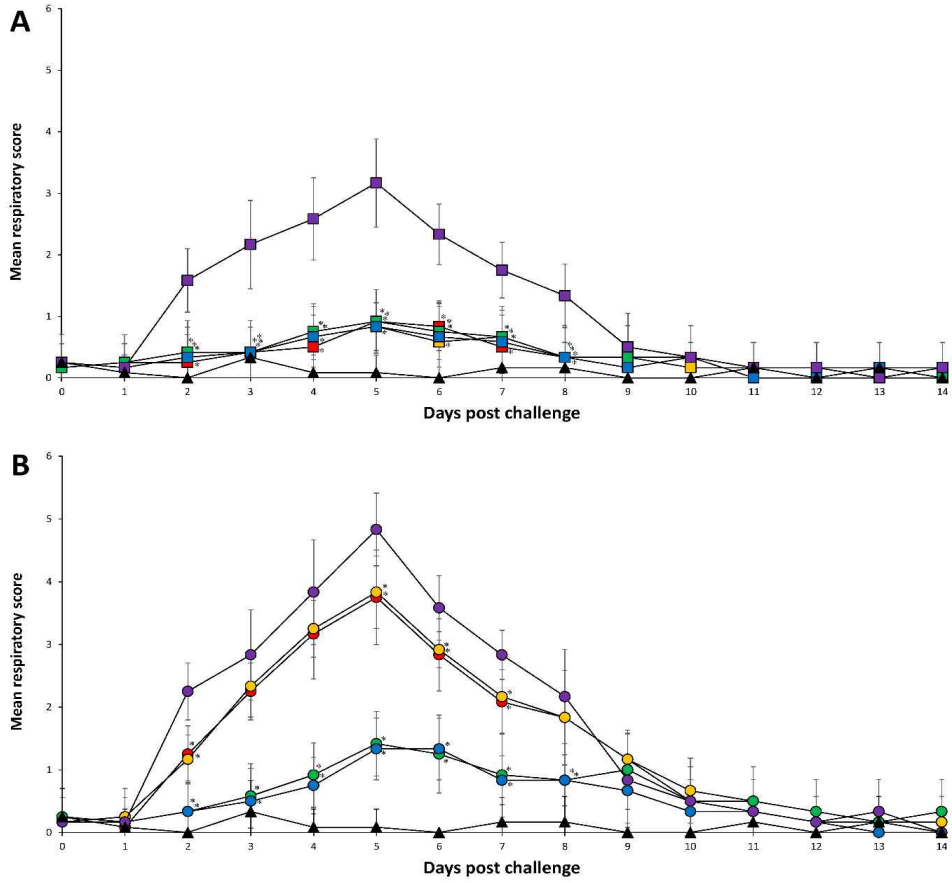

Figure 3. Mean values of the genomic copies number of PRRSV RNA. (A) PRRSV-1 challenged groups in serum from the Vac1A/Ch1 (■), Vac1B/Ch1 (■), Vac2A/Ch1 (■), Vac2B/Ch1 (■), UnVac/Ch1 (■), and UnVac/UnCh (▲). (B) PRRSV-2 RNA in serum from the Vac1A/Ch2 (●), Vac1B/Ch2 (●), Vac2A/Ch2 (●), Vac2B/Ch2 (●), UnVac/Ch2 (●), and UnVac/UnCh (p). Variation is expressed as the standard deviation. Significant difference between vaccinated challenged and unvaccinated challenged groups within the same PRRSV type challenge is indicated as P value <0.05*.

Serology

At the time of PRRSV vaccination (-35 dpc), pigs in all 11 groups were seronegative. Anti-PRRSV antibody titers were detected in vaccinated pigs only before challenge. In PRRSV-1 challenged groups, pigs from the Vac1A/Ch1, Vac1B/Ch1, and Vac2B/Ch1 groups had significantly ($P < 0.05$) higher anti-PRRSV antibody titers at 7 and 10 dpc compared to pigs from the Vac2A/Ch1 and UnVac/Ch1 groups. Pigs from the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, and Vac2B/Ch1 groups had significantly ($P < 0.05$) higher anti-PRRSV antibody titers at 14 dpc compared to pigs from the UnVac/Ch1 group (Figure 4A).

In PRRSV-2 challenged groups, pigs from the Vac1A/Ch2, Vac1B/Ch2, Vac2A/Ch2, and Vac2B/Ch2 groups had significantly ($P < 0.05$) higher anti-PRRSV antibody titers at 7 to 14 dpc compared to pigs from the UnVac/Ch2 group. Pigs from the Vac1A/Ch2, Vac1B/Ch2, and Vac2B/Ch2 groups had significantly ($P < 0.05$) higher anti-PRRSV antibody titers at 7 and 10 dpc compared to pigs from the Vac2A/Ch2 group. Anti-PRRSV antibody titers were not detected in any of the pigs from the UnVac/UnCh group throughout the study (Figure 4B).

Interferon-r secreting cells

In PRRSV-1 challenged groups, pigs from the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, and Vac2B/Ch1 groups had a significantly ($P < 0.05$) higher

number of PRRSV-1 specific IFN-r-SC compared to pigs from the UnVac/Ch1 group at -21, 0, 7, 10, and 14 dpc. Pigs from the Vac1A/Ch1 and Vac1B/Ch1 groups had a significantly ($P < 0.05$) higher number of PRRSV-1 specific IFN-r-SC compared to pigs from the Vac2A/Ch1 and Vac2B/Ch1 groups at 7 dpc. Pigs from the Vac1A/Ch1 and Vac1B/Ch1 groups had a significantly ($P < 0.05$) higher number of PRRSV-1 specific IFN-r-SC compared to pigs from the Vac2A/Ch1 group at 10 and 14 dpc (Figure 5A).

In PRRSV-2 challenged groups, pigs from the Vac2A/Ch2 group had a significantly ($P < 0.05$) higher number of PRRSV-2 specific IFN-r-SC compared to pigs from the Vac1A/Ch2, Vac1B/Ch2, Vac2B/Ch2, and UnVac/Ch2 groups at -21 dpc. Pigs from the Vac1A/Ch2, Vac1B/Ch2, Vac2A/Ch2, and Vac2B/Ch2 groups had a significantly ($P < 0.05$) higher number of PRRSV-2 specific IFN-r-SC compared to pigs from the UnVac/Ch2 group at 0, 7, 10, and 14 dpc. Pigs from the Vac2B/Ch2 group had a significantly ($P < 0.05$) higher number of PRRSV-2 specific IFN-r-SC compared to pigs from the Vac1A/Ch2 and Vac1B/Ch2 groups at 7 dpc. Pigs from the Vac2A/Ch2 and Vac2B/Ch2 groups had a significantly ($P < 0.05$) higher number of PRRSV-2 specific IFN-r-SC compared to pigs from the Vac1A/Ch2 and Vac1B/Ch2 groups at 10 and 14 dpc. In pigs from the UnVac/UnCh group, the mean numbers of PRRSV-1 and PRRSV-2 specific IFN-r-SC remained at basal levels (< 20 cells/106 PBMC) throughout the study (Figure 5B).

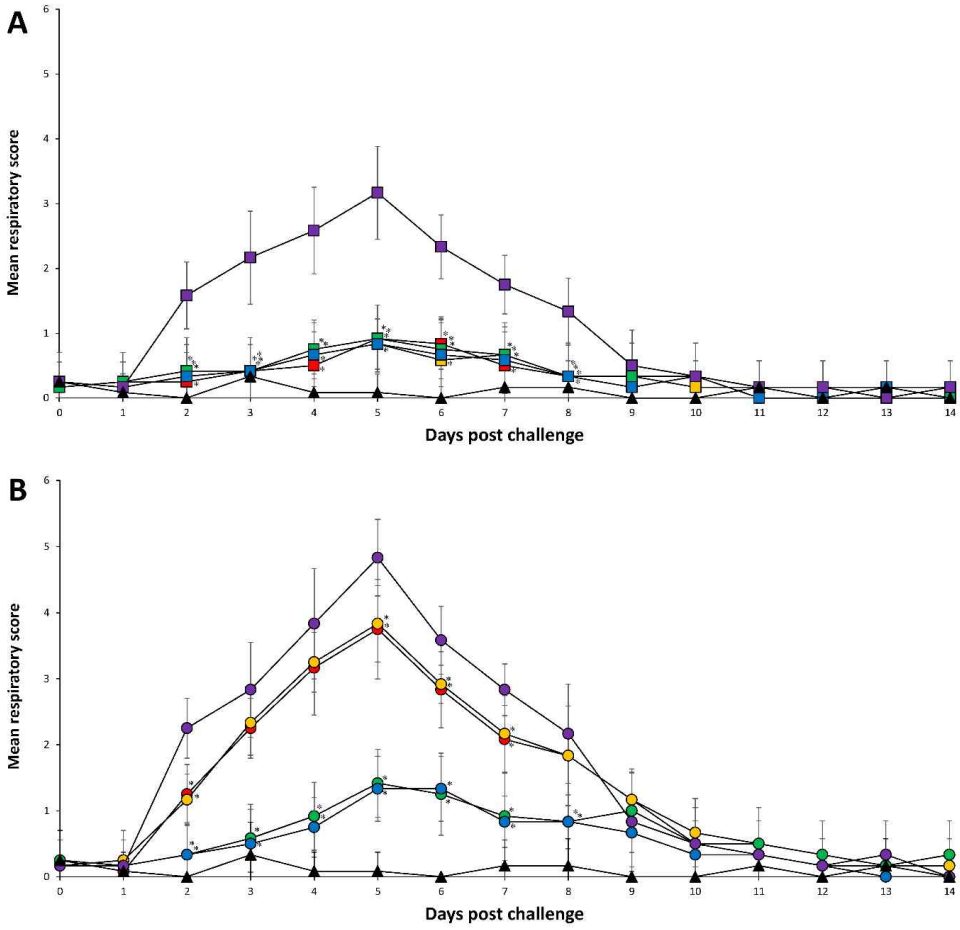

Figure 4. Mean values of the anti-PRRSV antibodies. (A) PRRSV-1 challenged groups from the Vac1A/Ch1 (■), Vac1B/Ch1 (■), Vac2A/Ch1 (■), Vac2B/Ch1 (■), UnVac/Ch1 (■), and UnVac/UnCh (▲). (B) PRRSV-2 challenged groups from the Vac1A/Ch2 (●), Vac1B/Ch2 (●), Vac2A/Ch2 (●), Vac2B/Ch2 (●), UnVac/Ch2 (●), and UnVac/UnCh (▲). Variation is expressed as the standard deviation. Significant difference between vaccinated challenged and unvaccinated challenged groups within the same PRRSV type challenge is indicated as P value <0.05*.

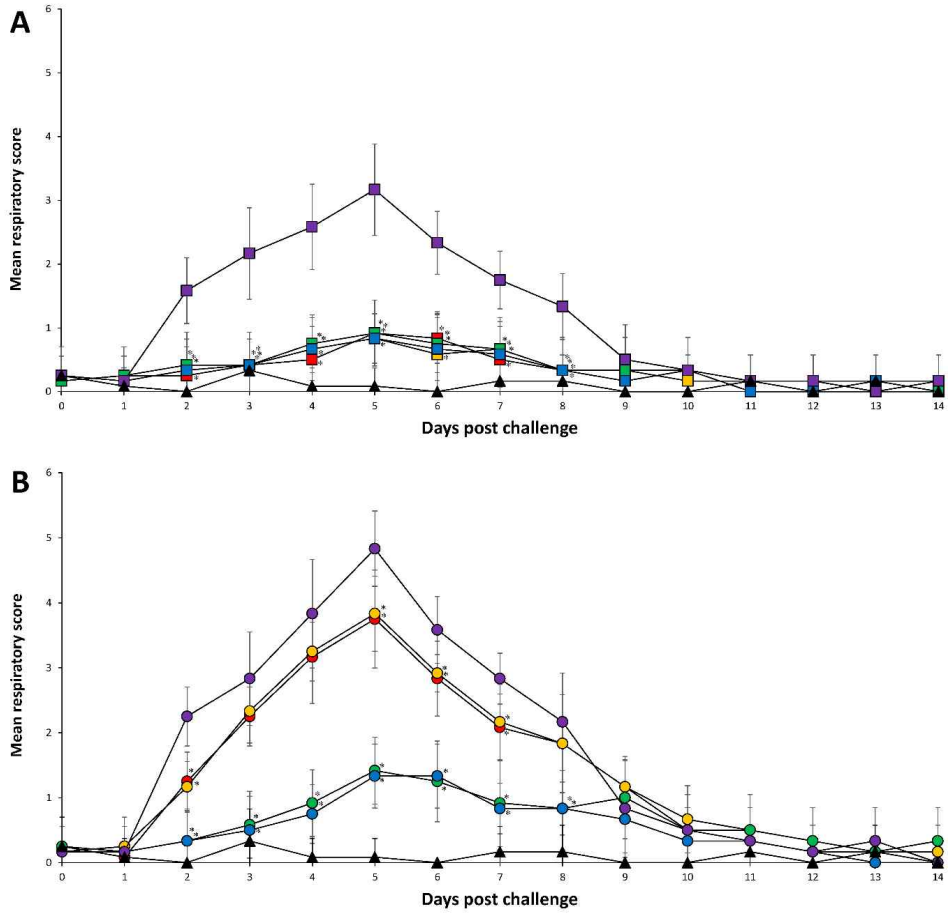

Figure 5. Frequency of PRRSV specific IFN-g-SC/106 PBMC. (A) PRRSV-1 challenged groups from the Vac1A/Ch1 (■), Vac1B/Ch1 (■), Vac2A/Ch1 (■), Vac2B/Ch1 (■), UnVac/Ch1 (■), and UnVac/UnCh (▲) (B) PRRSV-2 challenged groups from the Vac1A/Ch2 (●), Vac1B/Ch2 (●), Vac2A/Ch2 (●), Vac2B/Ch2 (●), UnVac/Ch2 (●), and UnVac/UnCh (▲). Variation is expressed as the standard deviation. Significant difference between vaccinated challenged and unvaccinated challenged groups within the same PRRSV type challenge is indicated as P value <0.05*.

Pathology

In PRRSV-1 challenged groups, pigs from the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, Vac2B/Ch1, and UnVac/UnCh groups exhibited significantly ($P < 0.05$) lower mean macroscopic lung lesion scores at 7 and 14 dpc compared to pigs from the UnVac/Ch1 group. Pigs from the UnVac/UnCh group had significantly ($P < 0.05$) lower mean macroscopic lung lesion scores at 7 dpc compared to pigs from the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, and Vac2B/Ch1 groups. Pigs from the Vac2A/Ch1, Vac2B/Ch1, and UnVac/UnCh groups had significantly ($P < 0.05$) lower mean microscopic lung lesion scores at 7 and 14 dpc compared to pigs from the UnVac/Ch1 group. Pigs from the Vac1A/Ch1, Vac1B/Ch1, Vac2A/Ch1, Vac2B/Ch1 groups had significantly ($P < 0.05$) less number of PRRSV-1 positive cells per area unit of lung at 7 and 14 dpc compared to pigs from the UnVac/Ch1 group. Pigs from the Vac2A/Ch1 and Vac2B/Ch1 groups had significantly ($P < 0.05$) less number of PRRSV-1 positive cells per area unit of lung at 7 dpc compared to pigs from the Vac1A/Ch1 and Vac1B/Ch1 groups (Table 2).

In PRRSV-2 challenged groups, pigs from the Vac2A/Ch2, Vac2B/Ch2, and UnVac/UnCh groups showed significantly ($P < 0.05$) lower mean macroscopic and microscopic lung lesion scores at 7 and 14 dpc compared to pigs from the Vac1A/Ch2, Vac1B/Ch2, and UnVac/Ch2 groups. Pigs from the Vac2B/Ch2 group also had significantly ($P < 0.05$) less number of

PRRSV-2 positive cells per area unit of lung at 14 dpc compared to pigs from the UnVac/Ch2 group. No PRRSV of any genotype was detected in the lung of pigs from the UnVac/UnCh group (Table 2).

Discussion

In this study, we compared the efficacy of two PRRSV-1 MLV vaccines and two PRRSV-2 MLV vaccines against heterologous challenge with PRRSV-1 and PRRSV-2. There was no significant difference between the two PRRSV-1 MLV vaccines as they both can provide partial protection against a PRRSV-1 strain but only limited protection against a PRRSV-2 strain, during the acute phase. In contrast, two commercial PRRSV-2 MLV vaccines can provide partial protection against both PRRSV-1 and -2 strains. Our conclusions are based on clinical, virological, immunological, and pathological comparisons. These results are consistent with previous studies, in which PRRSV-1 MLV vaccines provide partial protection against respiratory disease caused by heterologous type 1 PRRSV challenge but confer no protection against heterologous type 2 PRRSV challenge in pigs (18,22,23). Similar to our results, previous studies have also shown that vaccination of pigs with a PRRSV-2 vaccine can protect pigs against both heterologous PRRSV-1 and PRRSV-2 challenge (17,24). However, our results should be interpreted cautiously because only one strain for each genotype was used as challenge. The type of strain used as challenge can have a

significant impact on the efficacy of a vaccine. Our results do contrast with other studies in which vaccination of pigs with the same PRRSV-1 MLV vaccine provided partial protection against heterologous PRRSV-2 challenge (25,26). However, this study used a different PRRSV-2 strain suggesting that perhaps antigenicity plays a more important role on the efficacy of the PRRS MLV vaccine than genetic similarity between the vaccine and challenge strains.

PRRSV viremia plays a critical role in the development of respiratory disease. The levels of viremia are well correlated with the severity of interstitial pneumonia (12). Therefore, the reduction of PRRSV viremia could be essential in preventing respiratory disease and an important indicator of the efficacy of a PRRSV vaccine (22,27). Vaccination of pigs with either of the PRRSV-2 MLV vaccines resulted in a significant reduction both of PRRSV-1 and PRRSV-2 viremia. Vaccination of pigs with the PRRSV-1 MLV vaccines could only significantly reduce PRRSV-1 viremia. In addition, duration of PRRSV-1 viremia in vaccinated and PRRSV-1-challenged (Vac1A/Ch1 and Vac2A/Ch1) groups in the present study is similar to that in a previous study (28). However, duration of PRRSV-2 viremia in vaccinated and PRRSV-2-challenged (Vac1A/Ch2 and Vac2A/Ch2) groups is longer in present study compared to a previous study (28). Altogether, these data suggest that PRRSV-2 (strain SNUVR090851) challenge virus used in

this study is more virulent than PRRSV-2 (strain 19407b) challenge virus used in a previous study.

The difference in protection between the PRRSV-1 and PRRSV-2 MLV vaccines may be due to the possibility that they elicit different cellular immune responses against the two PRRSV types. In our experimental conditions, vaccination of pigs with the PRRSV-2 MLV vaccines resulted in induction of equal levels of IFN-r-SC against PRRSV-1 and PRSV-2. Vaccination with the PRRSV-1 MLV vaccines induced higher levels of IFN-r-SC against PRRSV-1 compared to PRRSV-2. T cell cross reactivity has been previously shown with genetically distant PRRSVs (29,30). Evidence of correlation between the increase of PRRSV-1 specific IFN-r-SC levels and reduction of PRRSV-1 viremia further supports the important role of T cells in cross protection of PRRSV-2 vaccinated pigs after PRRSV-1 challenge. Therefore, T cells activated by PRRSV-2 MLV vaccines respond against PRRSV-1 infection, resulting in partial cross protection. Despite the fact that the increase of IFN-r-SC does not always correlate with protection (31,32), cell-mediated immunity seems to play an important role in cross protection against PRRSV infection.

Since, in general, the PRRSV MLV vaccine provides a good homologous

protection but a variable heterologous protection (9), the PRRSV challenge viruses used in this study should not originate from the vaccine virus. The PRRSV-1 (SNUVR090485) challenge virus was isolated from pigs in 2009 before two PRRSV-1 MLV vaccines were introduced in South Korea in 2014. The PRRSV-2 (SNUVR090851) challenge virus belongs to lineage 1 while two PRRSV-2 MLV vaccines belong to lineage 5 (Ingelvac PRRS MLV) and 8 (Fostera PRRS), respectively, based on the classification system (33). Therefore, the degree of heterologous protection by the PRRSV MLV vaccines is not influenced by the PRRSV challenge viruses, which are not derived from the vaccine virus.

To the best of our knowledge, this is the first comparative study evaluating four commercial PRRS MLV vaccines, currently available in the Korean market, under the same experimental conditions. The results of this study are important because they provide swine producers and practitioners with valuable clinical information in order to better select future PRRSV vaccines.

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Chapter II

Comparison of four commercial PRRSV MLV vaccines in herds with co-circulation of PRRSV-1 and PRRSV-2

Abstract

The efficacy of four commercial porcine reproductive and respiratory syndrome virus (PRRSV) modified-live virus (MLV) vaccines against respiratory disease was evaluated and compared in pig farms suffering from co-infection with PRRSV-1 and PRRSV-2. All vaccinated groups on average exhibited improved growth rate compared to the unvaccinated pigs. Interestingly, the two groups vaccinated with either of the PRRSV-2 MLV vaccines had a better overall growth rate compared to the pigs vaccinated with either of the PRRSV-1 MLV vaccines. Vaccination of pigs with either of the PRRSV-1 MLV vaccines did not result in reduction of PRRSV-1 or PRRSV-2 viremia whereas vaccination of pigs with either of the PRRSV-2 MLV vaccines resulted in the reduction of PRRSV-2 viremia only. Taken together, the results of this field study demonstrate that a PRRSV-2 MLV vaccine can be efficacious against respiratory disease caused by co-infection with PRRSV-1 and PRRSV-2.

Keywords: Co-infection, Porcine reproductive and respiratory syndrome virus, Vaccine

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically devastating diseases to the global swine industry. The causative agent for PRRS is the PRRS virus (PRRSV), which belongs to the newly reclassified genus Porartevirus, family Arteriviridae, and order Nidovirales. PRRSV has two distinct species: PRRSV-1 (European-like) and PRRSV-2 (North American-like) which are genetically, antigenically, and pathogenically distinct (1,2,3) and were recently reclassified as two separate species, PRRSV-1 and PRRSV-2 in the new taxonomy (4). In Europe, PRRSV-1 is the prevailing virus with reproductive failure as the major clinical manifestation. In North America, PRRSV-2 is the predominant virus, and the symptoms include both reproductive failure in sows and respiratory disease in growing pigs.

In Korea, the current situation with PRRS is somewhat different compared to Europe and North America. PRRSV-2 is the more predominant virus, but both viruses are prevalent and cause reproductive failure in sows and respiratory disease in growing pigs. It is generally accepted that Korean PRRSV-2 induces a more severe respiratory disease compared to Korean PRRSV-1 (5) while both viruses have similar virulence in female reproductive failure (6). A recent diagnostic analysis of cases submitted between January 2017 and June 2018 determined that out of 167 PRRSV positive serum samples collected from growing pigs, 46 were positive for PRRSV-1, 67 were positive for PRRSV-2, and 54 were positive for both

PRRSV-1 and PRRSV-2. These cases underscore the need to control the respiratory disease caused by both PRRSV-1 and PRRSV-2 infection in growing pigs.

Currently, there are four modified-live virus (MLV) vaccines which are commercially available in Korea. Two are PRRSV-1 specific and the other two are PRRSV-2 specific. Only one of the PRRSV-1 MLV vaccines (www.hipra.com) and one of the PRRSV-2 MLV vaccines (www.boehringer-ingelheim.com) claims cross-protection against both species. The other PRRSV-2 MLV vaccine (www.zoetis.com) claims protection of pigs against PRRSV-2 but can cross-protect against PRRSV-1 under experimental conditions (7). To date, no comparative evaluation of the efficacy of these vaccines has been performed under the same field conditions. The objective of the current study was to compare the efficacy of these four commercial PRRSV MLV vaccines in herds suffering from co-circulation of PRRSV-1 and PRRSV-2.

2. Material and methods

2.1. History of farm

The pig farm used for this study is a one-site 1000-sow herd with continuously farrowing units but with all-in-all-out nurseries and finishing barns. Pigs were vaccinated for Mycoplasma hyopneumoniae at 7 days of age and porcine circovirus type 2 (PCV2) at 21 days of age. No clinical signs related to porcine circovirus associated disease were observed in any

of the growing pigs. However, no PRRSV MLV vaccine was administered for at least one year. The farm experienced an epizootic of respiratory symptoms in 7-11 week-old growing pigs between September and November 2015. The morbidity was 20-25%, while the mortality in pigs with respiratory symptoms was 5-8%. Interestingly, no reproductive failure symptoms such as abortion, premature farrowing, stillborn, or weak-born piglets were observed in breeding females during this period. Four growing pigs with respiratory symptoms were submitted to the Department of Veterinary Pathology at the Seoul National University. At necropsy, diffuse grayish-yellow fibrinopurulent exudates overlied the pleural, pericardial, and peritoneal surface in 3 of the growing pigs at 88 days of age. Fresh lung samples from all four pigs were collected for virus isolation. Some lung samples were also fixed in 10% neutral buffered formalin for histopathology and in situ hybridization. PRRSV-1 (SNUVR150266, GenBank MG271757) was isolated in lung tissue from pig A. PRRSV-2 (SNUVR150267, GenBank MG385131) was isolated in lung tissue from pig B. A phylogenetic analysis was performed to compare the field isolates with the vaccine viruses based on the open reading frame 5 (ORF5) nucleotide sequence (Fig. 1). Histopathological lesions were characterized by typical interstitial pneumonia with increased numbers of interstitial and alveolar macrophages present. In situ hybridization indicated PRRSV-1 and PRRSV-2 infection of interstitial and alveolar macrophages.

2.2. Clinical field study design

The field study was performed in December 2015 according protocols that follow the guidelines of the Republic of Korea's Animal, Plant & Fisheries Quarantine & Inspection Agency (QIA, http://qia.go.kr). QIA guidelines require that 40 pigs are assigned to each vaccinated and control groups. Forty sows were selected and 5 piglets were collected from each sow (200 total piglets) with each of the five piglets from an individual sow randomly assigned to each of the five groups using the random number generation function (Excel, Microsoft Corporation, Redmond, WA, USA). To minimize sow variation, we selected piglets with minimal variation in weight and an equal number of male and female. At 28 days of age (0 days post vaccination, dpv), pigs from the Vac1A group were injected intramuscularly on the right side of the neck with 2 mL of Porcilis PRRS (MSD Animal Health, Lot No. D353A07), pigs from the Vac1B group with UNISTRAIN PRRS (Hipra, Lot No. 61WK-B), pigs from the Vac2A group with Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica, Lot No. 245-659A), and pigs from the Vac2B group with Fostera PRRS (Zoetis, Lot No. A405013B) according to each of the manufacturer's instructions. Pigs from the UnVac group were administered an equal volume of phosphate buffered saline (PBS, 0.01M, pH 7.4) (Table 1).

Fig. 1. Phylogenetic analysis. Open reading frame 5 genome from the field and the vaccine viruses. An unrooted neighbor-joining tree was constructed from aligned nucleotide sequences.

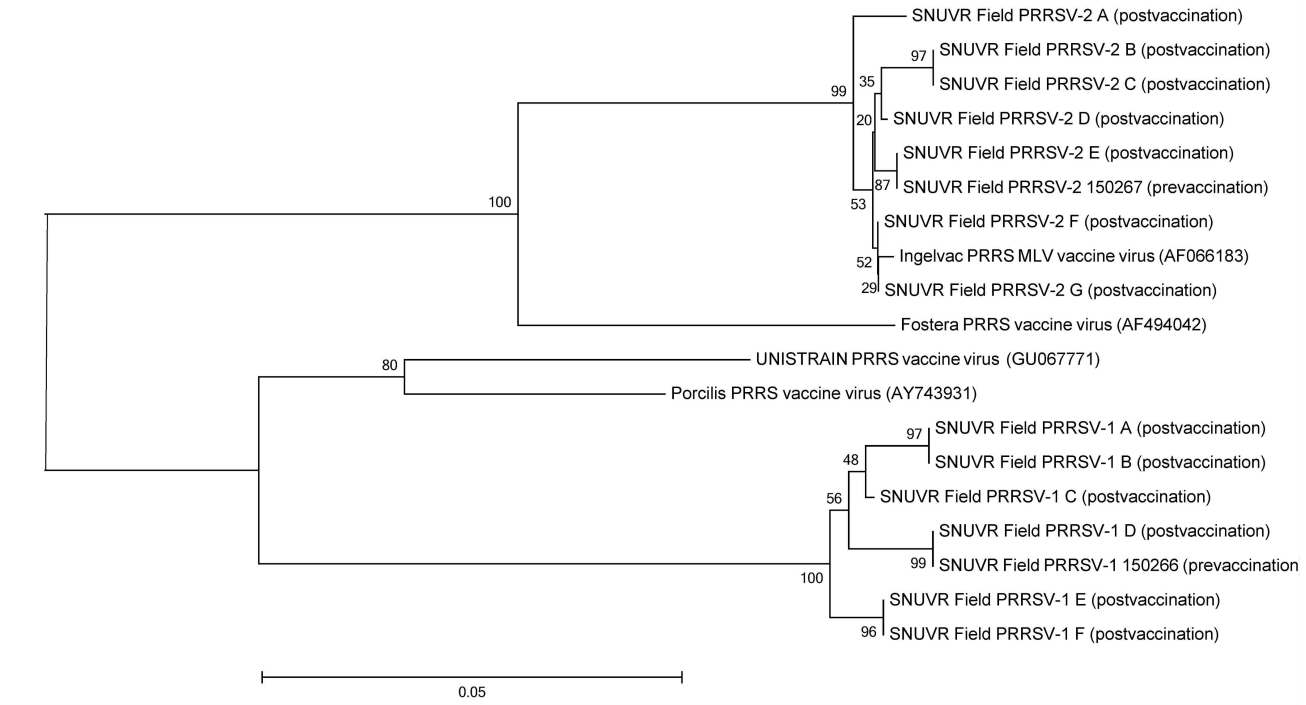

Table 1. Experimental design, and clinical and pathological results (mean ± standard error) among vaccinated and unvaccinated pigs under field conditions.

	Age (day)	Vac1A	Vac1B	Vac2A	Vac2B	UnVac
Vaccine		Porcilis PRRS	UNISTRAIN PRRS	Ingelvac PRRS MLV	Fostera PRRS	None
Vaccine type		PRRSV-1	PRRSV-1	PRRSV-2	PRRSV-2	None
No. of pigs		40	40	40	40	40
Mortality		2/40	2/40	2/40	1/20	4/40
Body weight (Kg)	28	6.32 ± 0.10	6.43 ± 0.08	6.41 ± 0.10	6.40 ± 0.10	6.38 ± 0.10
ADWG (gram/pig/day)	28 - 49	358.5 ± 12.5	358.6 ± 13.2	364.9 ± 14.8	372.0 ± 15.1	335.6 ± 15.8
	49 - 70	370.9 ± 15.7†	384.7 ± 15.9†	473.7 ± 12.9*	479.9 ± 10.6*	281.7 ± 12.1‡
	70 - 112	706.1 ± 12.9†	702.4 ± 13.9†	770.7 ± 17.5*	772.9 ± 14.9*	695.1 ± 17.0†
	112 - 168	746.7 ± 13.2*	750.0 ± 13.7*	749.1 ± 12.4*	781.1 ± 13.1*	694.9 ± 10.0†
	28 - 168	619.9 ± 5.5†	622.2 ± 5.1†	656.6 ± 5.3*	672.1 ± 6.7*	579.1 ± 5.6‡
Lung lesion score						
Macroscopic	168	32.9 ± 3.9†	32.6 ± 4.1†	24.2 ± 2.9†	23.3 ± 2.9†	50.8 ± 3.6*
Microscopic	168	1.34 ± 0.16†	1.34 ± 0.15†	1.05 ± 0.11†	1.05 ± 0.11†	2.14 ± 0.12*

Significant difference is indicated at P value <0.05*,† ,‡ .

Upon vaccination, pigs from each of 5 groups were housed by treatment, with a minimum of four pens per treatment and 10 pigs per pen. Pens were randomly assigned to litters/treatments with an empty pen between each occupied pen to minimize the shedding of the vaccine virus to the individual pigs in the control group. All animals were housed within the same building in similar conditions, receiving the same feed and subjected to the same management practices. At 90 days of age (62 dpv), since they do not shed the virus anymore, pigs were allowed to commingle to minimize pen variation. They were randomly reassigned into 20 pens (10 pigs per pen) within the same building for the remainder of the study. The full length of the study was 140 days, from 28 (0 dpv) to 160 (132 dpv) days of age.

Blood samples from each piglet were collected by jugular venipuncture at 28 (0 dpv), 49 (21 dpv), 84 (56 dpv), and 112 (84 dpv) days old. Necropsies were performed on piglets that had died of natural causes and lung tissue samples were collected for bacteria isolation (Actinobacillus pleuropneumoniae, Haemophilus papasuis, Pasteurella multocida, Streptococcus suis, and Trueperella pyogenes), histopathology, and in situ hybridization. RNA was extracted from lung homogenates. And ORF5 sequence was amplified from cDNA and sequenced as previously described (8,9). All protocols were previously approved by the Seoul National University Institutional Animal Care and Use Committee.

2.3. Clinical observation

Clinical observation for respiratory symptoms was performed twice per week using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (10). Observers were blinded to vaccination and type of vaccine status. Mortality rate was calculated as the number of pigs that died divided by the number of pigs initially assigned to that group within batch. Pigs that died throughout the study were necropsied.

The live weight of each pig was measured at 28 (0 dpv), 49 (21 dpv), 70 (42 dpv), 112 (84 dpv), and 168 (140 dpv) days old. The average daily weight gain (ADWG; gram/pig/day) was analyzed over four time periods: (i) 28-49 days of age, (ii) 49-70 days of age, (iii) 70-112 days of age, and (iv) 112-168 days of age, ADWG during the different production stages was calculated as the difference between the starting and final weight divided by the duration of the stage. Calculation of the mean ADWG for each group was based only on the ADWG of the surviving pigs.

2.4. Sequencing of field viruses

Five serum samples among PCR positive samples from each group were randomly selected at 28 (0 dpv), 49 (21 dpv), 84 (56 dpv), and 112 (84 dpv) days old. These serum samples were used for sequence analysis of ORF5 by polymerase chain reaction (PCR) amplification (8,9). The PCR products were purified using a commercial kit (Wizard PCR Preps DNA Purification and PCR Clean-Up System, Promega, Madison, WI, USA), cloned with the

TOPcloner Blunt kit (Enzynomics, Daejeon, Korea), and propagated in DH5α competent cells (Enzynomics) according to the manufacturer's instructions. Plasmid DNA was purified with a plasmid purification kit (iNtRON Biotechnology, Sungnam, Kyeonggido, Korea) and sequenced by a commercial service (Sol Gent Co Ltd, Daejeon, Korea). Three clones of each PCR product were independently sequenced at least three times.

2.5. Quantification of PRRSV RNA

RNA was extracted from serum samples using a commercial kit (QIAamp Viral RNA Mini Kit, Qiagen, Seoul, Korea). Genomic cDNA copy numbers were quantified using real-time PCR (11). Two different real-time PCRs were performed for the field and the vaccine viruses, respectively. For the detection of field strains, PRRSV-1 forward and reverse primers were 5′-TGGCCAGTCAGTCAATCAAC-3′ and 5′-AATCGATTGCAA GCAGAGGGAA-3′, respectively. PRRSV-2 forward and reverse primers were 5′-TGGCCAGTCAGTCAATCAAC-3′ and 5′-AATCGATTGCAAGCAGAGGGAA-3′, respectively.11 Real-time PCR for the quantification of genomic cDNA from the vaccine viruses, was performed as previously described (12, 13, 14).

2.6. Serology

The serum samples were tested using the commercially available PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS X3 Ab test,

IDEXX Laboratories Inc). Serum samples were considered positive for PRRSV antibody if the sample/positive (S/P) ratio was ≥0.4, according to the manufacturer's instructions.

2.7. Enzyme-linked immunospot assay

The numbers of PRRSV-specific interferon-r secreting cells (IFN-r-SC) stimulated with the field viruses isolated from farm were determined in peripheral blood mononuclear cells (PBMC) as previously described (13,15,16). PBMC seeded at (5 × 105 PBMCs per well) were stimulated with MARC-145 cell lysate (multiplicity of infection equivalent of 0.01) as the recall antigen for 20 hours, incubating at 37oC in a 5% CO2 atmosphere. The IFN-r positive spots on the membranes were imaged, analyzed and counted using an automated enzyme-linked immunospot (ELIPOT) assay ELISPOT Reader (AID ELISPOT Reader, AID GmbH, Strassberg, Germany). The results were expressed as the numbers of IFN-r-SC per million PBMC. ELISPOT assay was repeated twice.

2.8. Pathology

The estimation of macroscopic lung lesions (ranging from 0 to 100% of the affected lung) was based on the percentage of the volume of the entire lung and the percentage volume from each lobe added to the entire lung score (10). The total amount of microscopic lung lesions was scored blindly for each lung sections ranging from 0 (normal) to 4 (severe) by two

independent pathologists and analyzed morphometrically with the NIH Image J 1.51r Program (http://imagej.nih.gov/ij/download.html) (5,10).

2.9. Statistical analysis

The number of genomic copies of PRRSV data was log transformed prior to analysis. A generalized linear mixed model was used for all statistical comparisons with SAS version 9.3 (SAS Institute, Cary, NC) where group, time and their interaction were fixed effects while pigs were a random effect. A value of $P < 0.05$ was considered significant. The difference in mean response was assessed between groups.

3. Results

3.1. Clinical observation

The mean respiratory scores were significantly lower ($P < 0.05$) in pigs from all four vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B) at 56 (28), 63 (35), 70 (42), and 84 (56) days old compared to the unvaccinated group (UnVac). Only pigs from the Vac1A and Vac1B groups had significantly lower ($P < 0.05$) mean respiratory scores compared to the UnVac group at 77 (49 dpv) days old (Fig. 2).

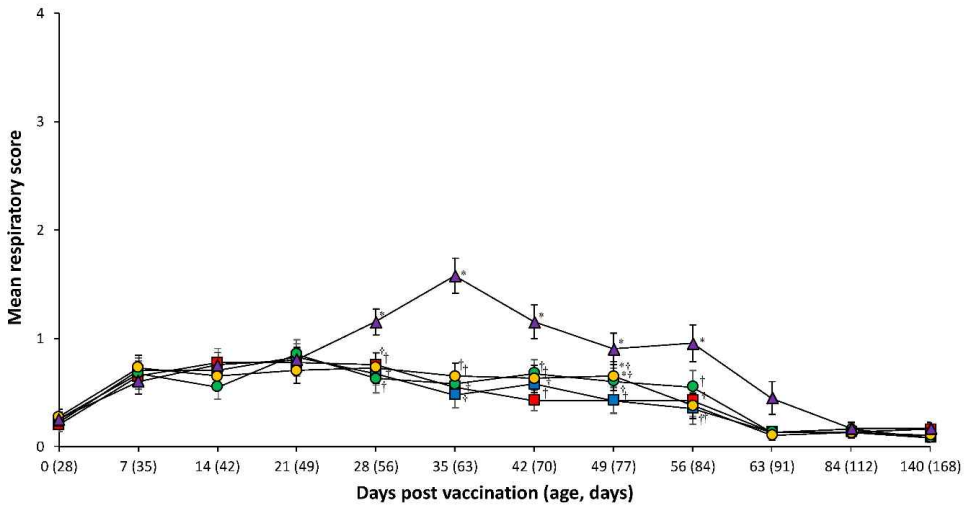

Fig. 2. Mean respiratory score in pigs from the Vac1A (■), Vac1B (■), Vac2A (●), Vac2B (●), and UnVac (▲) groups. Variation is expressed as the standard error. Significant difference is indicated at P value <0.05*,† .

There was no significant difference in body weight among the Vac1A (average weight 6.32 kg ± 0.65), Vac1B (average weight 6.43 kg ± 0.51), Vac2A (average weight 6.41 kg ± 0.62), Vac2B (average weight 6.40 kg ± 0.61), and UnVacA (average weight 6.38 kg ± 0.61) groups at 28 days old. The ADWG was significantly higher ($P < 0.05$) in pigs from the Vac2A and Vac2B groups compared to pigs from the Vac1A, Vac1B, and UnVac groups during the 49-70 (21-42 dpv) and 70-112 (42-84 dpv) day period. The ADWG was significantly higher ($P < 0.05$) in pigs from the Vac1A, Vac1B, Vac2A, and Vac2B groups compared to pigs from the UnVac group during the 112-168 (84-140 dpv) day period. The ADWG was significantly higher ($P < 0.05$) in pigs from the Vac1A and Vac1B groups compared to pigs from the UnVac group during the 49-70 day period. The overall growth rate (28 to 168 days of age) of pigs from the Vac2A and Vac2B groups was significantly higher ($P < 0.05$) compared to pigs from the Vac1A, Vac1B, and UnVac groups. The overall growth rate (28 to 168 days of age) was significantly higher ($P < 0.05$) in pigs from the Vac1A and Vac1B groups compared to pigs from the UnVac group (Table 1).

3.2. Diagnosis of dead pigs

In the Vac1A group there were two pigs that died at 85 days old (57 dpv) and both exhibited symptoms of severe bronchointerstitial pneumonia. Both PRRSV-1 and PRRSV-2 were detected in interstitial and alveolar macrophages within the lung lesions by in situ hybridization. P. multocida

was isolated in the pneumonic lung of one of the individual pigs. H. parasuis was isolated in diffuse grayish-yellow fibrinopurulent exudates overlining the pleural surface of the other individual pig. Vac1B group also had two pigs that died one at 85 (57 dpv) the other at 90 (66 dpv) days old. Both pigs had severe interstitial pneumonia with fibrinopurulent pleuritis. Interstitial and alveolar macrophages within lung lesions were positive for PRRSV-1 (Fig. 3A) and PRRSV-2 (Fig. 3B) as detected by in situ hybridization. H. parasuis was isolated in fibrinopurulent exudates overlining the pleural surface of both dead pigs. Two pigs from the Vac2A group died at 87 (59 dpv) and 88 (60 dpv) days old. The individual pig that died at 87 (59 dpv) days of age had severe interstitial pneumonia and H. parasuis was present in fibrinopurulent exudates overlining the pleural surface. The pig that died at 88 (60 dpv) days old had severe bronchointerstitial pneumonia and interstitial and alveolar macrophages within lung lesions were positive for PRRSV-1 and PRRSV-2 by in situ hybridization. P. multocida was also isolated from the pneumonic lung. The Vac2B group had only one pig that died at 90 (66 dpv) days old with severe interstitial pneumonia. Only PRRSV-2 could be detected in alveolar macrophages within lung lesions by in situ hybridization. H. parasuis was present in fibrinopurulent exudates overlining the pleural surface. The UnVac group had a total of four pigs die between 85 (57 dpv) to 95 (71 dpv) days of age. One pig died at 85 (57 dpv) days of age with severe pleuropneumonia and had PRRSV-1 positive macrophages within lung lesions. A second pig died at 86 (58 dpv) days of

age with severe pleuropneumonia and had macrophages within lung lesions that were positive for both PRRSV-1 and PRRSV-2. Additionally, A. pleuropneumoniae was isolated from its pleuropneumonic lung. The last two pigs died at 92 (68 dpv) and 95 (71 dpv) days of age respectively and both had severe bronchointerstitial pneumonia. However, only the pig that died at 95 (71 dpv) days of age had PRRSV-2 positive interstitial and alveolar macrophages and had H. parasuis in fibrinopurulent exudates overlining the pleural surface.

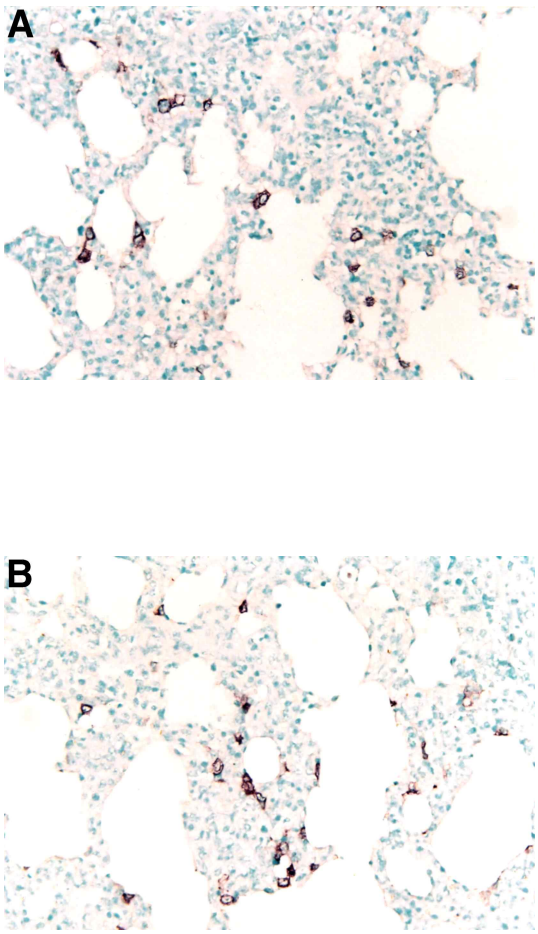

Fig. 3. In situ hybridization of PRRSV. PRRSV-1 nucleic acid was detected in interstitial macrophages in interstitial pneumonia (A). PRRSV-2 nucleic acid was detected in interstitial macrophages in interstitial pneumonia (B).

3.3. Sequence analysis

Lung samples from 11 total pigs that died during the field study from all 5 groups were collected and screened by PCR. Six of the samples were positive for PRRSV-1 and seven were positive for PRRSV-2. The PRRSV strains isolated from the farm prior to vaccination were PRRSV-1 (SNUVR150266, GenBank MG271757) and PRRSV-2 (SNUVR150267, GenBank MG385131). A comparison of the ORF5 sequences of PRRSV-1 and PRRSV-2 isolates prior to vaccination with the vaccine strains, revealed only a 98.2-100% and 97.2-100% identity with PRRSV-1 (SNUVR150266, GenBank MG271757) and PRRSV-2 (SNUVR150267, GenBank MG385131) strains respectively (Fig. 1).

Sequence analysis of the 6 PRRSV-1 strains isolated in this field study revealed that they were highly homologous (97.8% to 100%) with the PRRSV-1 strain (SNUVR150266, GenBank MG271757), isolated from the same farm prior to PRRSV vaccination. Similarly, the 7 PRRSV-2 strains isolated during this study were highly homologous (98.8% to 100%) with the PRRSV-2 strain (SNUVR150267, GenBank MG385131) isolated from the same farm prior to PRRSV vaccination (Fig. 1).

The vaccine virus from the Vac1B group was detected in the serum sample of only one pig at 47 (21 dpv) days old. Vaccine virus from the Vac2A group was detected in serum samples at 49 (21 dpv, three pigs) and 84 (56 dpv, two pigs) days old. In Vac2B group, the vaccine virus was detected at 49 (21 dpv, one pig) and 28 (56 dpv, one pig) days old. Based on ORF5

sequencing following vacccination, no vaccine strain cross-contamination was observed among any of the vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B). None of the vaccine virus strains were detected in pigs from the UnVac group.

3.4. Quantification of PRRSV RNA in sera

No genomic copies of PRRSV were detected in the serum samples of any of the individual pigs at the time of vaccination (0 dpv, 28 days old). There were no significant differences in the number of genomic copies of PRRSV-1 RNA among the five groups throughout the study (Fig. 4A). However, pigs from the Vac2A and Vac2B groups had significantly lower ($P < 0.05$) number of genomic copies of PRRSV-2 RNA in their sera at 28 (56 dpv) days old compared to the Vac1A, Vac1B, and UnVac groups (Fig. 4B).

3.5. Serology

PRRSV ELISA was used to measure the presence of antibodies in serum samples. At the time of PRRSV vaccination (0 dpv, 28 days old), pigs in all five groups were seronegative for PRRSV. At 49 (21 dpv) and 84 (56 dpv) days old, pigs from all 4 vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B) had significantly higher ($P < 0.05$) PRRSV antibodies compared to the unvaccinated group (UnVac). At 56 dpv, pigs from the Vac2A and Vac2B groups had significantly higher ($P < 0.05$) PRRSV antibodies compared to the Vac1A and Vac1B groups (Fig. 5).

3.6. Interferon-r secreting cells

Pigs from all four of the vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B) had a significantly ($P < 0.05$) higher numbers of PRRSV-1 and PRRSV-2 specific IFN-r-SC in PBMC compared to the unvaccinated group (UnVac) at 49 (21 dpv), 84 (56 dpv), and 112 (84 dpv) days old. At 28 (56 dpv) and 112 (84 dpv) days old, pigs from the Vac1A and Vac1B groups had a significantly higher ($P < 0.05$) numbers of PRRSV-1 specific IFN-r-SC in PBMC compared to the Vac2A and Vac2B groups (Fig. 6A). Pigs from the Vac2A and Vac2B groups had a significantly higher ($P < 0.05$) number of PRRSV-2 specific IFN-r-SC in PBMC compared to the Vac1A and Vac1B groups at 49 (21 dpv), 84 (56 dpv), and 112 (84 dpv) days old. Lastly, pigs from the Vac2B group had significantly higher ($P < 0.05$) numbers of PRRSV-2 specific IFN-r-SC in PBMC compared to pigs from the Vac2A group at 84 (56 dpv) days old (Fig. 6B).

3.7. Pathology

Pigs from all four vaccinated groups (Vac1A, Vac1B, Vac2A, and Vac2B) had significantly lower ($P < 0.05$) macroscopic and microscopic lung lesion scores compared to pigs from the unvaccinated group (UnVac) at 168 (140 dpv) days old. There were no significant differences among the vaccinated groups (Table 1).

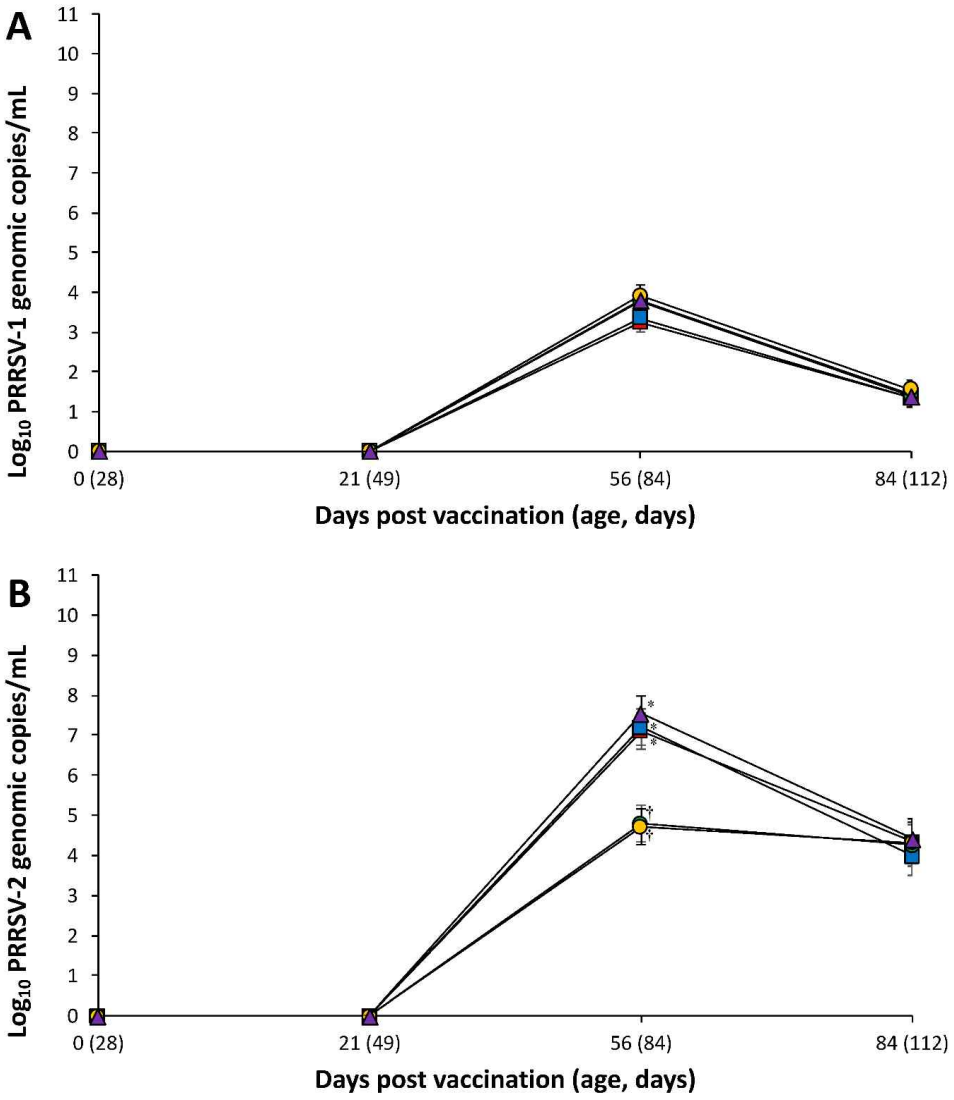

Fig. 4. Mean values of the genomic copy number of PRRSV-1 (A) and PRRSV-2 (B) PRRSV RNA in serum of pigs from the Vac1A (■), Vac1B (■), Vac2A (●), Vac2B (●), and UnVac (▲) groups. Variation is expressed as the standard error. Significant difference is indicated at P value <0.05*,† .

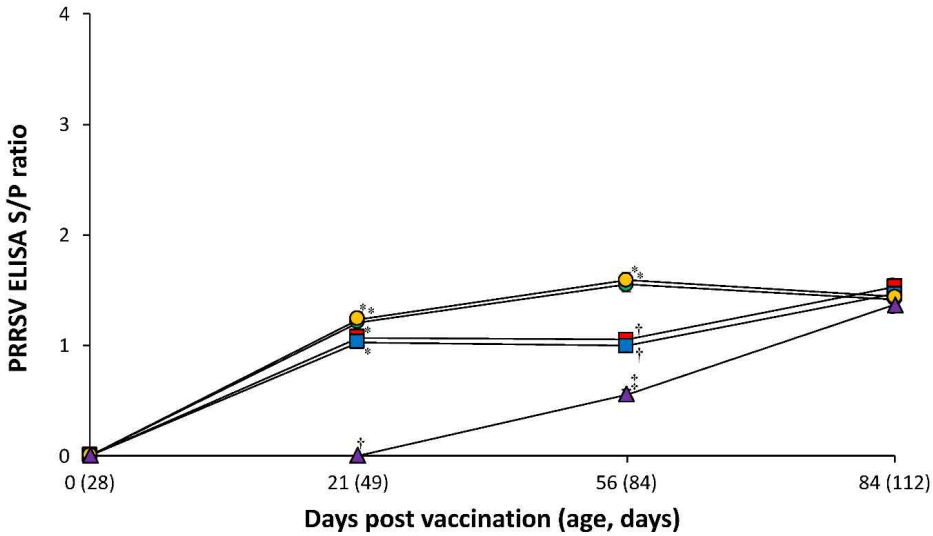

Fig. 5. Mean values of the PRRSV ELISA S/P ratio in serum of pigs from the Vac1A (■), Vac1B (■), Vac2A (●), Vac2B (●), and UnVac (▲) groups. Variation is expressed as the standard error. Significant difference is indicated at P value <0.05*,† ,‡ .

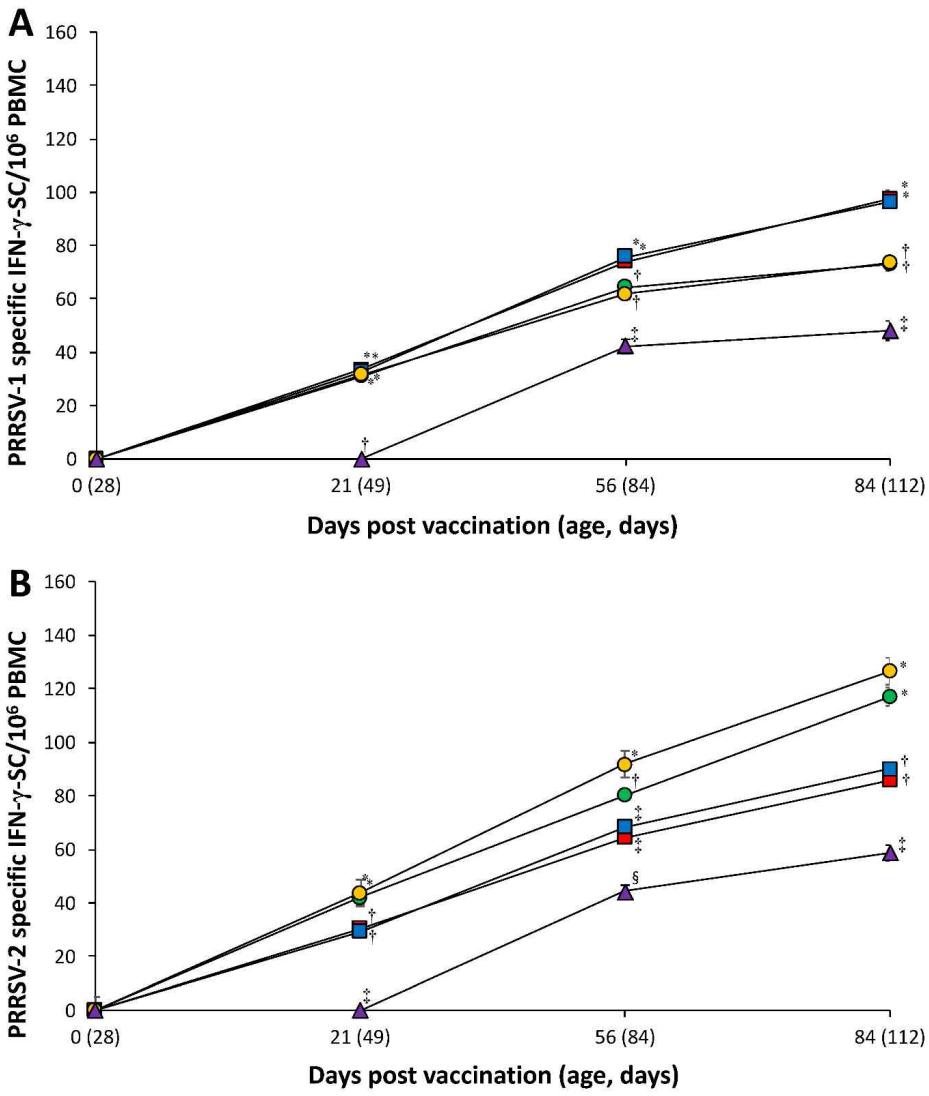

Fig. 6. Frequency of PRRSV-1 (A) and PRRSV-2 (B) specific IFN-r-SC/106 PBMC in pigs from the Vac1A (■), Vac1B (■), Vac2A (●), Vac2B (●), and UnVac (▲) groups. Variation is expressed as the standard error. Significant difference is indicated at P value <0.05*,† ,‡ ,§.

4. Discussion

In this field study we evaluated the efficacy of four commercially available PRRSV MLV vaccines. Of the four vaccines, two were based on PRRSV-1 and two on PRRSV-2. The efficacies were evaluated under field conditions instead of a more controlled study because, they represent real life conditions where the vaccinated pigs are continuously exposed to field viruses circulating in the farm which can ultimately significantly affect the efficacy of a vaccine. Therefore, field trial results may not always agree with controlled studies but they are the ultimate "real world" data providing swine practitioners and producers with valuable data in selecting the right PRRSV vaccine for their farm. In this study, pigs were housed according to treatment groups until 88 days of age (60 days post vaccination) to avoid possible transmission of vaccine viruses between the different vaccinated groups within the same building. All conditions within the housing facility including air handling were the same for all groups ensuring that all individual pigs are exposed to similar field conditions at the same time. This field trial design combined with the broad sampling interval allows us to draw the conclusion that vaccination results in improved growth rate compared to unvaccinated groups.

Growth rate is one of the most important parameters in evaluating vaccine efficacy under field conditions because respiratory disease caused by PRRSV typically results in weight loss. Regardless of which vaccine or the vaccine type, vaccinated pigs showed better overall growth rate compared to

unvaccinated pigs. There was also no significant difference in growth rate between each of the PRRSV-1 or PRRSV-2 based vaccines respectively. However, pigs vaccinated with PRRSV-2 MLV vaccines (both groups combined) exhibited a better growth rate compared to PRRSV-1 MLV vaccinated pigs (both groups combined). The differences in growth rate between PRRSV-1 MLV- and PRRSV-2 MLV-vaccinated groups may be due to genetic similarity between vaccine and field viruses. The identity between the field isolates and PRRSV-2 vaccine strains is 91.2-99.8% compared to 88.4-89.1% identity with the PRRSV-1 vaccine strains. There is also some evidence that the genetic similarity within field PRRSV strains may affect the efficacy of the same PRRSV MLV vaccine used in this study (17). However, genetic similarity between vaccine virus and field virus does not always predict vaccine efficacy (18, 19). Further studies are needed to elucidate the relationship between vaccine efficacy and genetic similarities between vaccine and field strains. Another reason for the difference in growth rate observed between the two types of vaccines could be due to the difference in virulence between PRRSV-1 and PRRSV-2. In general, Korean PRRSV-2 is more virulent than Korean PRRSV-1 (5). Therefore, in farms where both PRRSV types are circulating, protection against PRRSV-2 field strains by PRRSV-2 MLV vaccines can lead to a better growth rate compared to the protection against PRRSV-1 strains by the PRRSV-1-MLV vaccines.

Interestingly, even though vaccination with PRRSV-1 MLV vaccines did not result in reduction of PRRSV-1 viremia it did result in an improved the growth rate compared to unvaccinated pigs. Moreover, the same PRRSV-1 MLV vaccine used in this study also improved the growth rate and clinical signs in spite of not decreasing PRRSV-1 viremia in pig farms circulating PRRSV-1 only (20,21). These results clearly suggest that PRRSV-1 infection can significantly hinder growth rate and that vaccination with PRRSV-1 MLV is highly beneficial.

Vaccination against PRRSV has vast economic benefits. The average market weight of PRRSV-1 MLV vaccinated pigs (both groups combined) increased by 5.87 Kg/pig compared to unvaccinated pigs (93.31 Kg in PRRSV-1 MLV vaccinated group vs. 87.44 Kg in unvaccinated group; $P < 0.05$). The improved market weight of 5.87 kilograms/pig increased revenue by approximately 13.80 US$ (exchange rate; US $1.00 = 1,141 Korean Won) per pig. In PRRSV-2 MLV vaccinated pigs (both groups combined), the average market weight increased by 11.98 Kg/pig compared to unvaccinated pigs (99.42 Kg in PRRSV-2 MLV vaccinated group vs. 87.44 Kg in unvaccinated group; $P < 0.05$). The improved market weight of 11.98 kilograms/pig increased revenue by approximately 28.15 US$ (exchange rate; US $1.00 = 1,141 Korean Won) per pig. Thus, this growth improvement had a clear economic impact on the pig farmers.

Another way that PRRSV can affect pigs is through an increased incidence of secondary bacterial infection, including H. parasuis, P. multocida, and A.

pleuropneumoniae (22). Infection of pigs with PRRSV-1 and PRRSV-2 followed by secondary bacterial infection can cause respiratory disease, leading to growth retardation and even death. In our study, several individual pigs that died, in addition to PRRSV infection were also infected with bacteria such as P. multocida, H. parasuis, and A. pleuropneumoniae. Vaccination of pigs in our study improved significantly the growth rate as well as the severity of respiratory disease, mortality rate and severity of lung lesions, compared to the unvaccinated group.

Cell-mediated immunity seems to play an important role in the protection against respiratory disease caused by PRRSV infection (23,24,25). In a previous study with a controlled dual challenge, activation of T cell correlated with a reduction of PRRSV viremia (26,27). In this field study, viral lysates of field PRRSV isolates from the same farm were used for the quantification of IFN-r-SC. All four commercial vaccines were able to activate T cell responses against field viruses. As expected, PRRSV-1 MLV vaccines induced a stronger PRRSV-1 specific IFN-r-SC response while PRRSV-2 MLV vaccines induced a stronger PRRSV-2 specific IFN-r-SC response. Nonetheless, neither PRRSV-1 nor PRRSV-2 MLV vaccines were able to reduce PRRSV-1 viremia. In contrast, vaccination with either of the PRRSV-2 based MLV vaccines resulted in reduction of PRRSV-2 viremia. This suggests that induction of IFN-r-SC does not always correlate with protection as reported in previous studies (28,29). Alternatively, sequence variation between the four vaccine viruses and field PRRSV-1 isolates could

definitely have an effect on immunodomainace especially in PRRSV-T cell responses suggesting they may not be antigenically related. Further studies are needed to understand the difference in reduction of viremia between PRRSV-1 and PRRSV-2 based MLV vaccines.

We examined whether or not new viruses were introduced to the farm after vaccination. This is important because this could affect the efficacy of the PRRSV MLV vaccine. There were 11 pigs that died during this study between 85-96 days of age. In 5 out of the 11, both PRRSV-1 and PRRSV-2 were detected, indicating that both species were co-circulating in the population as early as 85 days of age (57 days post vaccination). Sequence analysis of ORF5 confirmed that field PRRSV-1 isolated post vaccination had a 97.8-100% identity with PRRSV-1 isolated prior to vaccination and PRRSV-2 isolated post vaccination had a 98.8-100% identity with PRRSV-2 isolated prior to vaccination. According to interpretation of the sequence analysis, 97-98% sequence identity indicates close relatedness of two viruses (30). This suggests that no new PRRSV strains were introduced to the herd after vaccination.

Since transplacental infection is more than likely a main route of virus transmission in a herd it is important to note that PRRSV was not detected in the blood of 28-day-old pigs at the time of vaccination. All of the pigs in each of the vaccinated groups were exposed to the circulating viruses from the growing pig population, especially during the fattening period. Even though respiratory disease symptoms were recorded in vaccinated

individuals, they did not correlate with the peak of the respiratory symptoms observed in unvaccinated animals around 63 days of age (35 days post vaccination). Those clinical signs could be attributed to other pathogens such as M. hyopneumoniae circulating in the population apart from PRRSV. After completion of our field study, the swine farmer did vaccinate with a mycoplasma vaccine at 21 days of age instead of 7 days of age and respiratory symptoms in pigs around 63 days of age were no longer observable.

In general, PRRSV-2 is more virulent and causes more severe respiratory disease in growing pigs compared to PRRSV-1 (5,31,32). However, virulence and damage by the highly virulent PRRSV-1 Lena strain (subtype 3) in Europe is comparable to some Korean PRRSV-2 field strains (33). In addition, Korean swine producers have recently observed unusual severe respiratory disease caused by highly virulent PRRSV-1 in their farms (34). In this study the efficacy of the commercial vaccines was tested in a farm co-infected with regular virulence PRRSV-1 and PRRSV-2. Therefore, the conclusions drawn from the results in this study would be difficult to predict the level of efficacy in the case of a co-infection with a high virulent PRRSV-1 and typically virulent PRRSV-2. Our results however suggest that it is effective to use a PRRSV-2 MLV vaccine to prevent respiratory disease against co-infection with PRRSV-1 and PRRSV-2 under field conditions.

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Chapter III

A comparative study of the efficacy of a porcine reproductive and respiratory syndrome subunit and a modified-live virus vaccine against respiratory diseases in endemic farms

Abstract

We evaluated the efficacy of a porcine reproductive and respiratory syndrome (PRRS) subunit vaccine and compared it with a modified-live virus (MLV) vaccine under field conditions. Three farms were selected based on their history of respiratory diseases caused by co-infection with both PRRSV-1 and PRRSV-2. In each farm, 60 pigs were randomly allocated to two vaccinated and one unvaccinated groups (20 pigs per group). One group of pigs were administered the PRRS subunit vaccine at 21 and 42 days of age and another group administered the PRRS MLV vaccine at 21 days of age. The subunit vaccine had a similar efficacy and, in some instances, performed even better than the MLV vaccine. Vaccination of pigs with either of the PRRS vaccines, resulted in significantly improved growth performance in Farm B but not in Farm C. Interestingly, in Farm A, pigs vaccinated with the PRRS subunit vaccine had a better growth performance statistically compared to those vaccinated with the PRRS MLV vaccine. At the peak of PRRSV-1 and PRRSV-2 viremia, neutralizing antibodies and T cell responses against PRRSV-1 and PRRSV-2 were at low levels suggesting that either vaccine is only able to provide a partial protection against co-circulating PRRSV-1 and PRRSV-2.

Keywords inactivated vaccine, modified-live virus vaccine, porcine reproductive and respiratory syndrome virus, subunit vaccine

Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first discovered in 1987 in North America. Since then, the disease has become endemic and is one of the most important infectious diseases to the swine industry, resulting in tremendous economic losses worldwide. Infection with PRRS virus (PRRSV) causes reproductive failures in pregnant sows, and results in high preweaning mortality in piglets infected in utero, and respiratory distress in growers and finishers (1). PRRSV is an enveloped positive-sense, single-stranded RNA virus recently reclassified into the new genus Porartevirus, in the family Arteriviridae within the order Nidovirales (2). The viral genome is about 15 kb in length and includes at least ten open reading frames (ORFs), ORF1a, ORF1b, and ORFs 2-7 (3). ORFs 2-5 mainly encode viral structural glycoproteins (GP2a, GP2b, GP3, GP4, GP5, and GP5a, respectively), while ORFs 6 and 7 encode the matrix (M) and nucleocapsid (N) proteins respectively (4-6). PRRSV isolates are further classified into two major species: PRRSV-1 (European type) and PRRSV-2 (North American type) based on marked genetic and antigenic differences (7-9).

In Korea, a commercial PRRS modified-live virus (MLV) vaccine has been widely used to control epidemic and endemic PRRSV infection since its first introduction in 1996. Despite the fact that the PRRS MLV vaccine has been efficacious in controlling PRRSV infection, there are increased concerns

about its safety because of the possible risk of reversion to virulence (10). In addition, a new virulent PRRSV has emerged even in PRRS MLV-vaccinated farms (11). Currently, most of the pig farms in Korea are endemic. As a result, many swine producers and practitioners are increasingly interested in an inactivated PRRS vaccine, particularly those in endemic PRRS farms. Several inactivated PRRS vaccines are currently available worldwide (12). However, the majority have not been evaluated scientifically by peer-reviewed publications (12). A PRRS subunit vaccine (PRRSFREETM PRRS subunit vaccine, Reber Genetics Co. Ltd., Taiwan, Republic of China) based on a plasmid containing a detoxified Pseudomonas exotoxin- and expressing ORF7, ORF1b, and ORF6 & 5 chimeric subunits of PRRSV-1 and PRRSV-2 (13), claims protection against both species and was introduced in the market in 2012. This PRRS subunit vaccine was shown to be efficacious in protecting growing pigs from respiratory disease against PRRSV-1 and PRRSV-2 under experimental conditions (14). Although a PRRS MLV has been regarded as more efficacious compared to an inactivated vaccine (12,15), no comparative field studies have been performed so far. The objective of this study was to evaluate and compare the efficacy of the PRRS subunit vaccine with a PRRS MLV vaccine in endemic PRRS farms for the purpose of its registration in accordance with the registration guidelines and protocols of the Republic of Korea's Animal, Plant & Fisheries Quarantine & Inspection Agency (QIA, http://www.qia.go.kr).

Materials and methods

Farms

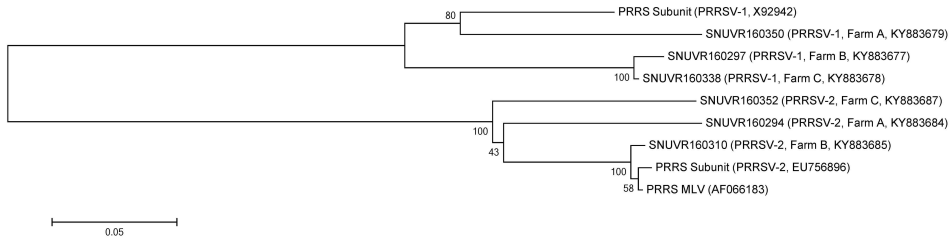
The clinical field trial was conducted on three separate farms. Farms A and B housed 1,000-sow herds and 2 site (farrow-to-nursery and nursery-to finish) production with all-in-all-out system. Farm C housed a 1,000-sow herd and 1 site (farrow-to-finish) production with all-in-all-out system.

Farm A reported endemic respiratory symptoms in growing pigs between the ages of 7-11weeks old for at least 1 years, with 15-20% morbidity and 3-7% mortality. No PRRSV vaccination was administered to the herd for at least two years. Farm B reported endemic respiratory symptoms for at least two years in growing pigs ranging from 6-12 weeks old with 14-18% morbidity and 5-8% mortality. A PRRSV vaccination had not been administered for at least 1 year. Farm C reported endemic respiratory symptoms in growing pigs between 7-12 weeks old for at least 2 years. Morbidity ranged between 12-15%, with 3-9% mortality rate. Farm C also had not vaccinated against PRRSV for at least two years. At the time of the diagnosis, there were no reports of reproductive failures in breeding females in any of the three farms.

Prior to the beginning of this study, a random number of growing pigs at 8

weeks of age were collected from each individual farm. The pigs were submitted to the Department of Veterinary Pathology in Seoul National University for strain isolation. Lungs and tonsils were collected, pooled separately for each individual farm, and homogenized. The homogenate material was cultured in MARC-145 cells and porcine alveolar macrophages. PRRSV-1 isolates were, SNUVR160350 from Farm A, SNUVR160297 from Farm B, and SNUVR160338 from Farm C. PRRSV-2 isolates were SNUVR160294 from Farm A, SNUVR160310 from Farm B, and SNUVR160352 from Farm C. A sequence analysis was performed based on deduced amino acid sequence of ORF5 for all field viruses. A phylogenetic tree was built comparing all the field isolates and the vaccine strains (Figure 1).

Figure 1. Phylogenetic analysis of ORF5 from the vaccine viruses and the field viruses isolated from 3 farms. An unrooted neighbor-joining was constructed from aligned nucleotide sequences.



Experimental design

Since the ultimate purpose of this field study is registration, we followed strict QIA guidelines and protocols. This study used a randomized, blinded, weight- and sex-matched, and controlled clinical trial design was used. To minimize sow variation, six 7-day-old piglets were randomly selected from each sow and assigned evenly to one of the three groups (two vaccinated and one unvaccinated). QIA protocols require a total of 20 pigs assigned to each group (10 male and 10 female) using the random number generation function (Excel, Microsoft Corporation, Redmond, Washington, USA).

Pigs in the VacA/Subunit, VacB/Subunit, and VacC/Subunit groups from Farms A, B, and C respectively, were injected intramuscularly on the right side of the neck with 2.0 mL of the PRRS subunit vaccine (PRRSFREETM PRRS subunit vaccine, Reber Genetics Co. Ltd., Lot No. F5002) at 21 and 42 days of age according to the manufacturer's instructions. Pigs in the VacA/MLV, VacB/MLV, and VacC/MLV groups from Farms A, B, and C respectively were administered intramuscularly on the right side of the neck, a 2 mL dose of the PRRS MLV vaccine (Ingelvac PRRS MLV, Boehringer Ingelheim Vetmedica, Lot No. 245-G28B) at 21 days of age, according to the manufacturer's instructions. An equal volume of phosphate buffered saline

(PBS, 0.01M, pH 7.4, 2.0 ml) was injected in the same anatomic location to pigs from the UnVacA, UnVacB, and UnVacC groups of pigs from Farms A, B, and C, respectively, at 21 and 42 days of age.

Pigs were allocated according to treatment with each treatment housed in a separate pen. Pigs were randomly assigned to one of four pens using the random number generation function (Excel, Microsoft Corporation). Pens were randomly assigned to treatments with an empty pen between each occupied pen to minimize the shedding of the vaccine virus to the controls. Blood samples were collected at 0 (21 days of age), 21, 49, and 91 (168 days of age) days post vaccination (dpv). All protocols used in this study were approved by the Seoul National University Institutional Animal Care and Use Committee.

Clinical observations

Following vaccination and PRRSV challenge, the pigs were monitored daily for changes in physical conditions and the observations were recorded bi-weekly for clinical respiratory disease severity using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (16). Same observers were blinded to vaccination status. Dead pigs were not included in the data analyses.

The live weight of each pig was measured at 21 (0 dpv), 70, 112, and 168 (147 dpv) days of age. The average daily weight gain (ADWG; gram/pig/day) was analyzed over three time periods: (1) between 21 and 70 days of age, (2) between 70 and 112 days of age, and (3) between 112 and 168 days of age. ADWG during the different production stages was calculated as the difference between the starting and final weight divided by the duration of the stage. Data for dead or removed pigs were included in the calculation.

Quantification of PRRSV RNA

RNA was extracted from serum samples using the QIAamp Viral RNA Mini Kit (QIAGEN Ltd, Crawley, UK). Within the highly conserved ORF 7 region and 3′ untranslated region (UTR) of the genome of both genotypes, forward primer for PRRSV-1 5'-GTGAATGGCCGCGATTG-3' (nucleotide no. 14997-15013) and reverse primer 5'-CGGTCACATGGTTCCTGC-3' (nucleotide no. 15093-15110) were selected. The forward primer for PRRSV-2 is 5'-GTGGTGAATGGCACTGATTG-3' (nucleotide no. 15308-15327) and reverse primer is 5'-CCCCACACGGTCGCC'-3' (nucleotide no. 15358-15372) (17,18). For the vaccine virus, the forward and reverse primers were 5′-CTAACAAATTTGATTGGGCAG-3′ and

5′-AGGACATGCAATTCTTTGCAA-3′, respectively (7). Real-time PCR was performed as previously described (19,20).

Sequencing of field viruses

Lung and lymph nodes from dead pigs were used to analyze sequence of ORF5 by PCR (21,22). The PCR products were purified using a commercial kit (Wizard PCR Preps DNA Purification and PCR Clean-Up System, Promega, Madison, Wisconsin), cloned with the TOPcloner Blunt kit (Enzynomics, Daejeon, Korea), and propagated in DH5α competent cells (Enzynomics) according to the manufacturer's instructions. Plasmid DNA was purified with a plasmid purification kit (iNtRON Biotechnology, Sungnam, Gyeonggi-do, Korea) and sequenced by a commercial service (Sol Gent Co Ltd, Daejeon, Korea). Three clones of each PCR product were independently sequenced at least three times.

Serology

The serum samples were tested using the commercially available PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS 3XRTM, IDEXX Laboratories Inc., Westbrook, Maine, USA). Serum samples were considered positive for PRRSV antibody when the S/P ratio $\geq$0.4, according to the manufacturer's instructions. Serum virus neutralization was also

performed on porcine alveolar macrophages using PRRS field viruses isolated from each of 3 farms (23). The neutralizing antibody (NA) titers of each serum were determined as the reciprocal of the highest dilution in which no evidence of virus growth was detected. Serum samples were considered to be positive for NA if the titer was greater than 2.0 (log2) (24).

Enzyme-linked immunospot (ELISPOT) assay

The numbers of PRRSV-specific interferon-r secreting cells (IFN-r-SC) were determined in vitro by stimulating peripheral blood mononuclear cells (PBMC) with field PRRS viruses isolated from each of 3 farms with slight modifications (20,25-27).

Pathology

The estimation of macroscopic lung lesions (ranging from 0 to 100% of the affected lung) was based on the percentage of the volume of the entire lung and the percentage volume from each lobe added to the entire lung score (16). Microscopic lung lesion and in situ hybridization (ISH) were performed on three blocks of lung tissues, which included eight pieces of lung: two piece from the right cranial lobe, two from the right middle lobe, one from the ventromedial part of the right caudal lobe, one from the dorsomedial part of the right caudal lobe, one from the midlateral part of the right caudal

lobe, and one from the accessory lobe of each pig. The choice of lung tissues was based on the presence of macroscopic lesions.

Microscopic lesions were scored on a scale from 0 to 4: 0 = no microscopic lesions; 1 = mild interstitial pneumonia; 2 = moderate multifocal interstitial pneumonia; 3 = moderate diffuse interstitial pneumonia; and 4 = severe diffuse interstitial pneumonia by two pathologists (28).

In situ hybridization (ISH) was performed to detect PRRSV-1 and PRRSV-2 in lung tissues as previously (29). Three sections were cut from each of three blocks of tissue from one entire pulmonary lobe of each pig. In each slide, 10 fields were randomly selected, and the number of positive cells per unit area (0.95 mm2) was analyzed with the NIH Image J 1.51r Program (http://imagej.nih.gov/ij/download.html) (30).

Statistical analyses

Statistical analyses were performed using SPSS software (version 21; IBM, Armonk, New York). Continuous data included ADWG determined by the difference between the starting and final weights divided by the duration of the stage; PRRSV RNA (log10 of the number of PRRSV genomic copies per

mL) was quantified by real-time PCR; PRRSV antibody titer; and numbers of IFN-r-SC were measured by ELISPOT assay. Continuous data were analyzed using Tukey's multiple comparisons test for comparison between groups in order to estimate the difference at each time point. Discrete data (clinical signs, lung lesion scores, and ISH scores) were analyzed with the Kruskal-Wallis test. When the Kruskal-Wallis test was significant, the Mann-Whitney test was performed to determine the significant differences between the groups. A value of $P < 0.05$ was considered significant.

Results

Clinical observations

In Farm A, the mean respiratory scores were significantly lower ($P < 0.05$) in pigs from the VacA/Subunit and VacA/MLV groups compared to the UnVacA group at 56 dpv. In Farm B, the mean respiratory scores were significantly lower ($P < 0.05$) in pigs from the VacB/Subunit group compared to the UnVacB group at 14 and 28 dpv and compared to the VacB/MLV group at 28 and 70 dpv. Pigs from the VacB/Subunit and VacB/MLV groups had significantly lower ($P < 0.05$) mean respiratory scores compared to the UnVacB group at 126 and 140 dpv. In Farm C, pigs from the VacC/Subunit and VacC/MLV groups had significantly lower ($P < 0.05$) mean respiratory scores compared to the UnVacC group at 28 and 56

dpv. Pigs from the VacC/Subunit group also had significantly lower ($P < 0.05$) mean respiratory scores compared to the UnVacC group at 42 dpv (Figure 2).

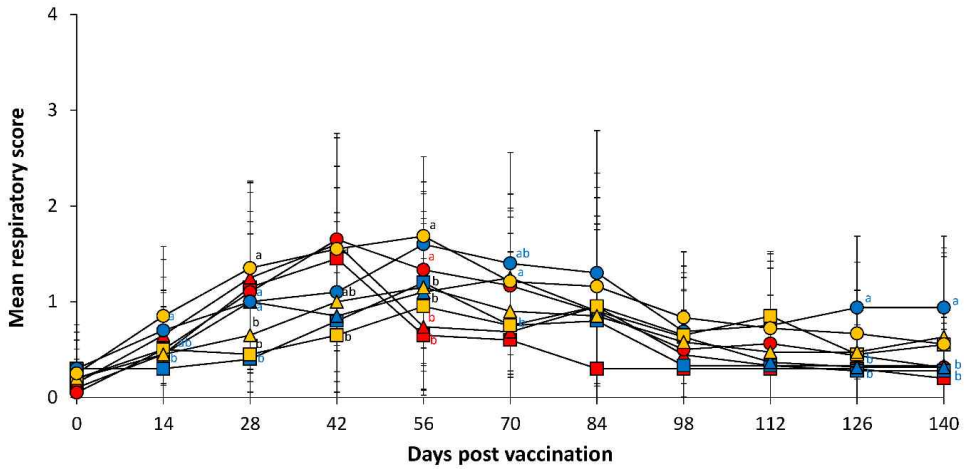


Figure 2. Mean respiratory score in pigs from the VacA/Subunit (■), VacA/MLV (▲), UnVacA (●), VacB/Subunit (■), VacB/MLV (▲), UnVacB (●), VacC/Subunit (■), VacC/MLV (▲) and UnVacC (●) groups. Variation is expressed as the standard deviation. Significant difference ($P < 0.05$) among subunit-vaccinated, MLV-vaccinated, and unvaccinated group within the Farm A (ab), Farm B (ab), and Farm C (ab).

Growth performance

In Farm A, the ADWG was significantly higher ($P < 0.05$) in pigs from the VacA/Subunit and UnVacA groups compared to the VacA/MLV group during the 112-168 days period. The overall growth performance (21 to 168 days of age) was also was significantly higher ($P < 0.05$) in pigs from the VacA/Subunit and UnVacA groups compared to the VacA/MLV group. In Farm B, the ADWG was significantly higher ($P < 0.05$) in pigs from the VacB/MLV group compared to the UnVacB group between 70-112 days of age. The overall growth performance (21 to 168 days of age) was significantly higher ($P < 0.05$) in the VacB/Subunit and VacB/MLV groups compared to the UnVacB group. In Farm C, pigs in the VacC/Subunit group had a significantly higher ($P < 0.05$) ADWG compared to the UnVacC group between 21-70 days of age. There was no significant difference on the overall growth performance (21 to 168 days of age) among the VacC/Subunit, VacC/MLV, and UnVacC groups (Table 1).

Table 1: Average daily weight gain (ADWG), mortality rate, pathology, and in situ hybridization (ISH) between vaccinated and unvaccinated animals on 3 farms

		Farm A			Farm B			Farm C		
		VacA/ Subunit	VacA/ MLV	UnVacA	VacB/ Subunit	VacB/ MLV	UnVacB	VacC/ Subunit	VacC/ MLV	UnVacC
ADWG (weeks of age)	3-10	392 ± 59	336 ± 81	386 ± 77	365 ± 46	344 ± 67	336 ± 72	486 ± 80^{a}	474 ± 58^{ab}	423 ± 64^{b}
	10-16	688 ± 147	671 ± 118	633 ± 150	751 ± 106^{ab}	767 ± 99^{a}	653 ± 144^{b}	769 ± 181	794 ± 174	712 ± 184
	16-24	906 ± 71^{a}	794 ± 90^{b}	878 ± 49^{a}	957 ± 124	984 ± 101	951 ± 146	853 ± 80	860 ± 66	856 ± 104
	3-24	672 ± 38^{a}	606 ± 47^{b}	644 ± 38^{a}	700 ± 47^{a}	709 ± 40^{a}	661 ± 47^{b}	707 ± 47	712 ± 52	670 ± 52
Market weight (Kg)		104.8 ± 5.4^{a}	95.0 ± 6.7^{b}	100.7 ± 5.1^{a}	108.4 ± 7.2^{a}	109.9 ± 6.0^{a}	102.8 ± 6.7^{b}	109.6 ± 6.7^{ab}	110.3 ± 7.6^{a}	104.2 ± 7.5^{b}
Mortality rate		0/20	2/20	4/20	2/20	1/20	4/20	1/20	2/20	3/20
Macroscopic lung lesion score		16.00 ± 5.83 ^{c}	33.00 ± 6.78 ^{b}	48.00±10.30 ^{a}	32.00 ±6.78 ^{b}	25.00 ±7.07 ^{b}	47.00 ± 9.27 ^{a}	26.00 ±10.20	27.00 ±10.30	42.00 ±13.27
Microscopic lung lesion score		0.34 ± 0.31 ^{b}	1.62 ± 0.71 ^{a}	1.34 ± 0.47 ^{a}	0.92 ± 0.38	0.80 ± 0.33	1.48 ± 0.57	0.74 ± 0.68	0.80 ± 0.63	1.34 ± 0.47
PRRSV-1 ISH		0.40 ± 0.49	1.00 ± 0.63	0.80 ± 0.75	0.80 ± 0.40	0.80 ± 0.75	1.20 ± 0.75	0.60 ± 0.80	0.60 ± 0.80	1.20 ± 0.75
PRRSV-2 ISH		0.40 ± 0.49	1.40 ± 1.02	1.60 ± 1.02	0.80 ± 0.75	1.40 ± 0.80	1.40 ± 1.02	0.80 ± 0.75	0.80 ± 0.75	2.20 ± 0.75

a,b,c Significant difference ($P < 0.05$) among subunit-vaccinated, modified-live virus (MLV)-vaccinated, and unvaccinated groups within the same farm.

Mortality

In Farm A, two pigs from the VacA/MLV group died of pneumonic pasteurellosis caused by Pasteurella multocida at 64 and 107 days of age. One of the lung samples was positive for both PRRSV-1 and PRRSV-2. In the UnVacA group two pigs died of pleuropneumonia caused by Actinobacillus pleuropneumoniae at 68 days of age and two pigs died of pneumonic pasteurellosis caused by P. multocida at 110 days of age. One lung sample was positive for PRRSV-1 and two lung samples were positive for PRRSV-2. ORF5 sequences of PRRSV-1 isolated from the lung samples were highly homologous (98.6% to 99.2%) with the PRRSV-1 strain (SNUVR160350), which was isolated from the same farm prior to PRRSV vaccination. ORF5 sequences of PRRSV-2 isolated from the lung samples were highly homologous (97.4% to 100%) with the PRRSV-2 strain (SNUVR160294), which was also isolated from the same farm prior to PRRSV vaccination.

In Farm B, two pigs from the VacB/Subunit group died of unknown etiology without any pneumonia or pathological lesions at 107 days of age. One lung sample was positive for PRRSV-2. One pig in the VacB/MLV group died of Glasser's disease caused by Haemophilus parasuis at 111 days of age. One lung sample was positive for PRRSV-2. Four pigs in the UnVacB group died of Glasser's disease caused by H. parasuis at 109 days

of age. One lung sample was positive for PRRSV-1 and two lung samples were positive for PRRSV-1 and PRRSV-2. ORF5 sequences of PRRSV-1 isolated from the lung samples were highly homologous (98.8%) with the PRRSV-1 strain (SNUVR160297), which was isolated from the same farm prior to PRRSV vaccination. ORF5 sequences of PRRSV-2 isolated from the lung samples were highly homologous (98.8% to 100%) with the PRRSV-2 field strain (SNUVR160310), which was isolated in the same farm prior to PRRSV vaccination.

In Farm C, one pig from the VacC/Subunit group died of pneumonic pasteurellosis caused by P. multocida at 140 days of age. No PRRSV was detected in lung sample. Two pigs from the VacC/MLV group died of pneumonic pasteurellosis caused by P. multocida at 110 and 140 days of age. One lung sample was positive for PRRSV-2. Three pigs from the UnVacC group died of pneumonic pasteurellosis caused by P. multocida at 68, 109, and 138 days of age, respectively. Two lung samples were positive for PRRSV-1 and PRRSV-2 at 68 and 109 days of age. ORF5 sequences of PRRSV-1 isolated from the lung samples were highly homologous (99.2% to 100%) with the PRRSV-1 strain (SNUVR160338), which was isolated in the same farm prior to PRRSV vaccination. ORF5 sequences of PRRSV-2 isolated from the lung samples were highly homologous (99.6% to 100%) with the PRRSV-2 field strain (SNUVR160352), which was isolated in the

same farm prior to PRRSV vaccination.

Quantification of PRRSV RNA in sera

There was no significant difference between vaccinated and unvaccinated pigs in all 3 farms, in the number of genomic copies of PRRSV-1 (Figure 3A) or PRRSV-2 (Figure 3B) detected in the blood samples collected. Vaccine virus was detected in the serum samples of 16/20 pigs at 42 dpv and 9/19 pigs at 70 dpv in the VacA/MLV group, 15/20 pigs at 42 dpv and 9/20 pigs at 70 dpv in VacB/MLV group, and 12/20 pigs at 42 dpv and 13/19 pigs at 70 dpv in VacC/MLV group. No vaccine virus was detected in the serum of pigs from the VacA/Subunit, VacB/Subunit, VacC/Subunit, UnVacA, UnVacB, and UnVacC groups throughout the experiment. The prevalence rates of genomic copies of PRRSV-1 and PRRSV-2 positive pigs were summarized in Table 2.

Table 2: Number of positive cells in PRRSV viremia, enzyme-linked immunosorbent assay (ELISA), and interferon-r secreting cells (IFN-r-SC) between vaccinated and unvaccinated animals on 3 farms at different days post vaccination (dpv)

No. of positive pigs	dpv	Farm A			Farm B			Farm C		
		VacA/Subunit	VacA/MLV	UnVacA	VacB/Subunit	VacB/MLV	UnVacB	VacC/Subunit	VacC/MLV	UnVacC
PRRSV-1 viremia	0	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
	21	16/20	16/20	17/20	5/20	7/20	5/20	6/20	5/20	4/19
	49	12/20	12/19	13/18	5/20	8/20	3/20	5/20	6/19	5/18
	91	1/20	2/18	4/16	1/18	2/19	3/16	0/19	0/18	0/17
PRRSV-2 viremia	0	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
	21	0/20	2/20	2/20	2/20	2/20	1/20	2/20	6/20	3/19
	49	5/20	3/19	2/18	0/20	1/20	1/20	11/20	4/19	11/18
	91	0/20	0/18	0/16	0/18	0/19	0/16	4/19	6/18	4/17
PRRSV ELISA	0	4/20	6/20	3/20	1/20	3/20	1/20	5/20	5/20	4/20
	21	20/20	19/20	17/20	3/20	18/20	1/20	0/20	13/20	2/19
	49	20/20	19/19	18/18	20/20	20/20	20/20	20/20	19/19	18/18
	91	20/20	18/18	16/16	18/18	19/19	16/16	18/19	18/18	17/17
PRRSV-1 IFN-γ-SC	0	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
	21	5/20	5/20	1/20	6/20	5/20	3/20	4/20	3/20	1/19
	49	20/20	15/19	14/18	17/20	15/20	13/20	14/20	9/19	9/18
	91	20/20	18/18	12/16	18/18	19/19	7/16	19/19	18/18	15/17
PRRSV-2 IFN-γ-SC	0	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
	21	6/20	10/20	4/20	7/20	9/20	3/20	6/20	7/20	4/19
	49	20/20	19/19	18/18	20/20	20/20	14/20	20/20	19/19	10/18
	91	20/20	18/18	16/16	18/18	19/19	12/16	19/19	18/18	10/17

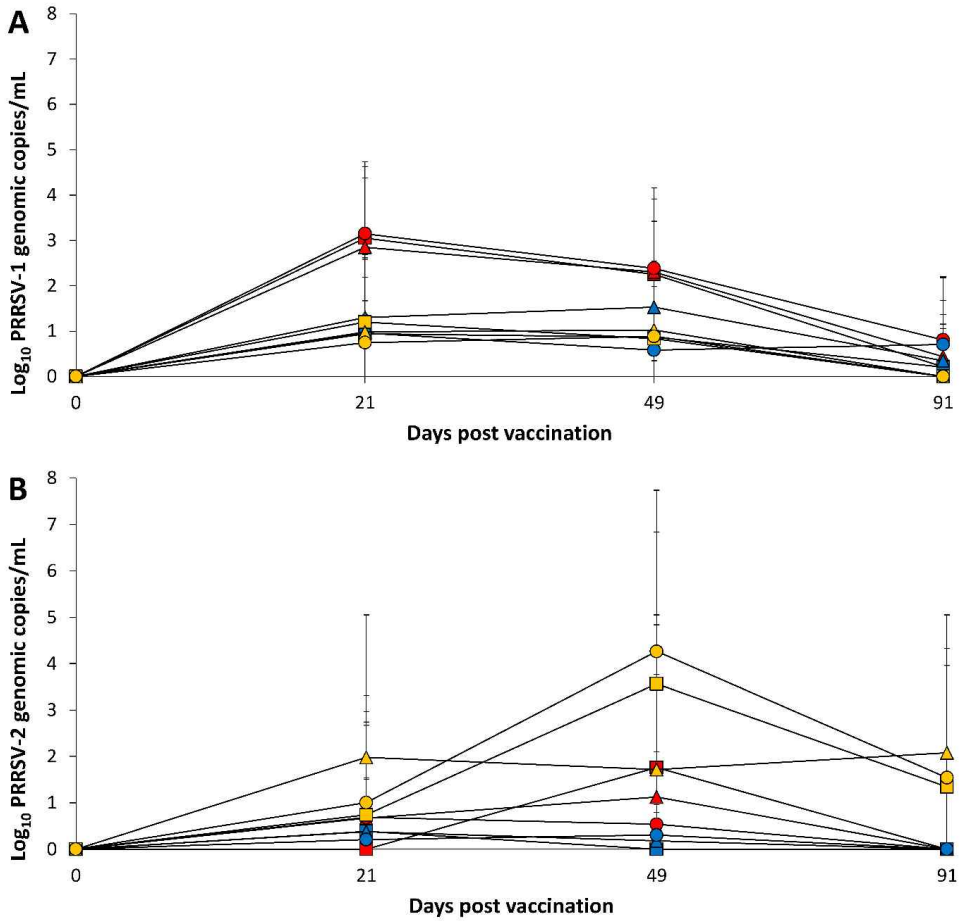


Figure 3. Mean values of the genomic copy number of PRRSV-1 (A) and PRRSV-2 (B) RNA in serum of pigs from the VacA/Subunit (■), VacA/MLV (▲), UnVacA (●), VacB/Subunit (■), VacB/MLV (▲), UnVacB (●), VacC/Subunit (■), VacC/MLV (▲) and UnVacC (●) groups.

Serology

In Farm A, anti-PRRSV antibody titers were significantly ($P < 0.05$) higher in pigs from the VacA/MLV group compared to the UnVacA group at 49 dpv. In Farm B, anti-PRRSV antibody titers were significantly ($P < 0.05$) higher in pigs from the VacB/MLV group compared to the VacB/Subunit and UnVacB groups at 21 dpv. ELISA PRRSV S/P ratio was significantly ($P < 0.05$) higher in pigs from the VacB/MLV group compared to the UnVacB group at 49 dpv. In Farm C, ELISA PRRSV S/P ratio was significantly ($P < 0.05$) higher in pigs from the VacC/MLV group compared to the VacC/Subunit and UnVacC groups at 21 dpv. ELISA PRRSV S/P ratio was significantly ($P < 0.05$) higher in pigs from the VacC/Subunit group compared to the UnVacC group at 49 dpv (Figure 4).

In Farm A, neutralizing antibodies against PRRSV-1 were significantly ($P < 0.05$) higher in pigs from the VacA/Subunit group compared to the UnVacA group at 91 dpv. In Farm B, neutralizing antibodies against PRRSV-1 were significantly ($P < 0.05$) higher in pigs from the VacB/Subunit group compared to the UnVacB group at 49 and 91 dpv. In Farm C, neutralizing antibodies against PRRSV-1 were significantly ($P < 0.05$) higher in pigs from the VacC/Subunit and VacC/MLV groups compared to the UnVacC group at 49 dpv. Neutralizing antibodies against PRRSV-1 were significantly ($P < 0.05$) higher in pigs from the VacC/Subunit group compared to the

UnVacC group at 91 dpv (Figure 5A).

In Farm A, neutralizing antibodies against PRRSV-2 were significantly ($P < 0.05$) higher in pigs from the VacA/Subunit and VacA/MLV groups compared to the UnVacA group at 49 and 91 dpv. In Farm B, neutralizing antibodies against PRRSV-2 were significantly ($P < 0.05$) higher in pigs from the VacB/Subunit and VacB/MLV groups compared to the UnVacB group at 49 and 91 dpv. In Farm C, neutralizing antibodies against PRRSV-2 were significantly ($P < 0.05$) higher in pigs from the VacC/MLV group compared to the UnVacC group at 21 and 49 dpv. Neutralizing antibodies against PRRSV-2 were significantly ($P < 0.05$) higher in pigs from the VacC/MLV group compared to the VacC/Subunit and UnVacC groups at 91 dpv. Neutralizing antibodies against PRRSV-2 were significantly ($P < 0.05$) higher in pigs from the VacC/Subunit group compared to pigs in the UnVacC group at 91 dpv (Figure 5B).

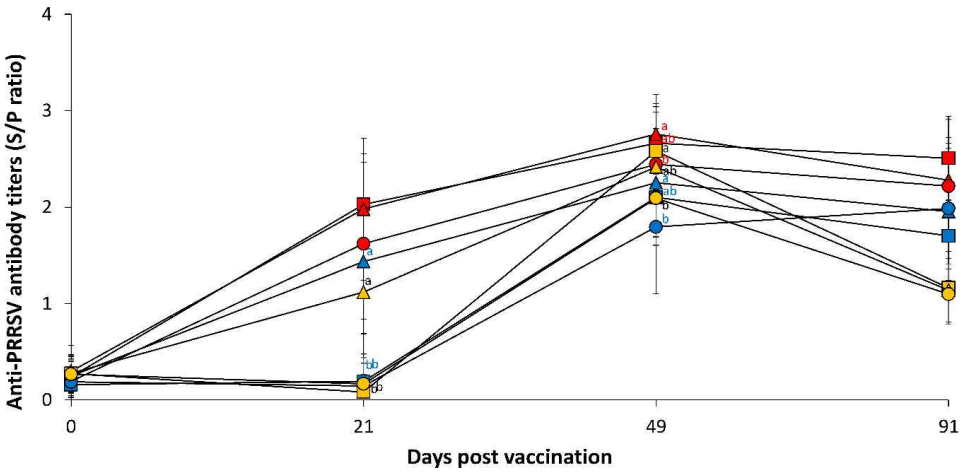


Figure 4. Mean values of the anti-PRRSV ELISA antibodies in pigs from the the VacA/Subunit (■), VacA/MLV (▲), UnVacA (●), VacB/Subunit (■), VacB/MLV (▲), UnVacB (●), VacC/Subunit (■), VacC/MLV (▲) and UnVacC (●) groups. Variation is expressed as the standard deviation. Significant difference ($P < 0.05$) among subunit-vaccinated, MLV-vaccinated, and unvaccinated group within the Farm A (ab), Farm B (ab), and Farm C (ab).

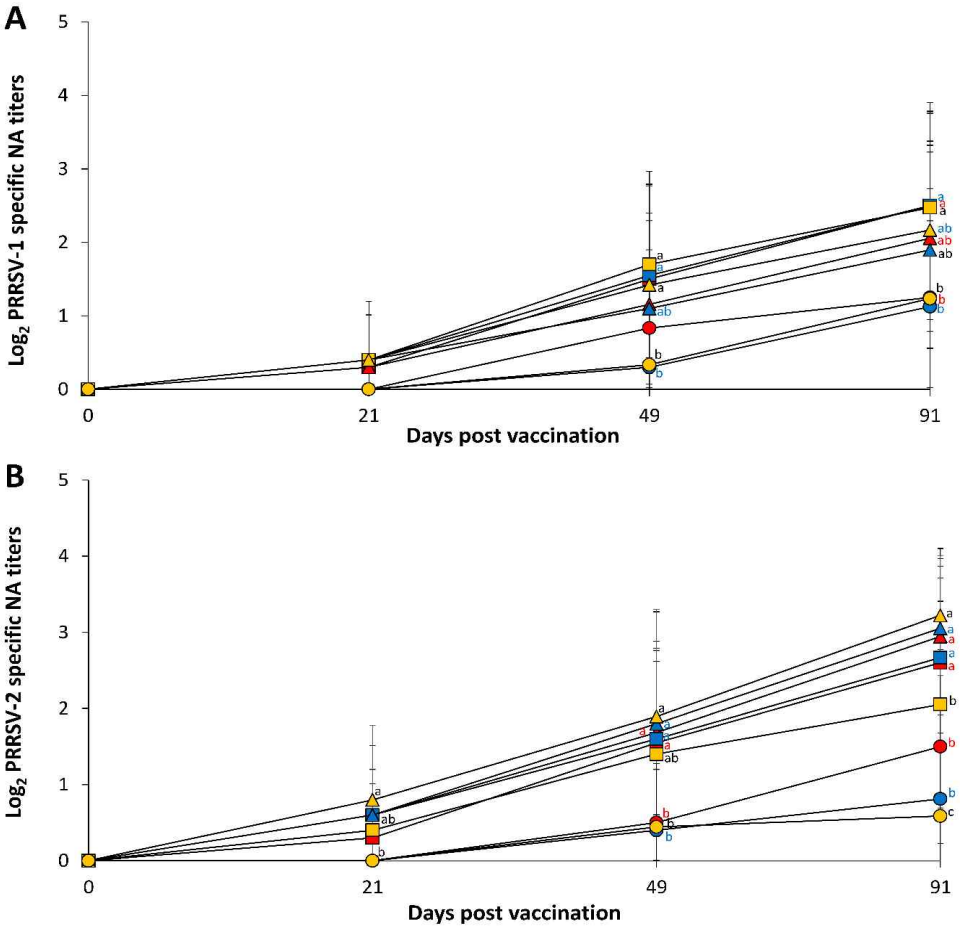


Figure 5. Mean values of PRRSV-1 (A) and PRRSV-2 (B) specific neutralizing antibody (NA) titers in pigs from the the VacA/Subunit (■), VacA/MLV (▲), UnVacA (●), VacB/Subunit (■), VacB/MLV (▲), UnVacB (●), VacC/Subunit (■), VacC/MLV (▲) and UnVacC (●) groups. Variation is expressed as the standard deviation. Significant difference ($P < 0.05$) among subunit-vaccinated, MLV-vaccinated, and unvaccinated group within the Farm A (ab), Farm B (ab), and Farm C (ab).

Interferon-r secreting cells

In Farm A, pigs from the VacA/Subunit group had significantly ($P < 0.05$) higher numbers of PRRSV-1 specific IFN-g-SCs in the PBMC compared to the VacA/MLV and UnVacA groups at 49 dpv. Pigs from the VacA/Subunit group had significantly ($P < 0.05$) higher numbers of PRRSV-1 specific IFN-g-SCs in the PBMC compared to pigs from the UnVacA group at 91 dpv. In Farm B, pigs from the VacB/Subunit and VacB/MLV groups had significantly ($P < 0.05$) higher numbers of PRRSV-1 specific IFN-g-SCs in the PBMC compared to the UnVacB group at 91 dpv. In Farm C, pigs from the VacC/Subunit group had significantly ($P < 0.05$) higher numbers of PRRSV-1 specific IFN-g-SCs in the PBMC compared to the UnVacC group at 91 dpv (Figure 6A).

In Farm A, pigs from the VacA/MLV group had significantly ($P < 0.05$) higher numbers of PRRSV-2 specific IFN-g-SC in PBMC compared to the UnVacA group at 49 dpv. Pigs from the VacA/MLV group had significantly ($P < 0.05$) higher numbers of PRRSV-2 specific IFN-g-SC in PBMC compared to the VacA/Subunit and UnVacA groups at 91 dpv. In Farm B, pigs from the VacB/MLV group had significantly ($P < 0.05$) higher numbers of PRRSV-2 specific IFN-g-SC in PBMC compared to the UnVacB group at 21 dpv. Pigs from the VacB/Subunit and VacB/MLV groups had significantly ($P < 0.05$) higher numbers of PRRSV-2 specific IFN-g-SC in

PBMC compared to the UnVacB group at 49 and 91 dpv. In Farm C, pigs from the VacC/Subunit and VacC/MLV groups had significantly ($P < 0.05$) higher numbers of PRRSV-2 specific IFN-g-SC in PBMC compared to the UnVacC group at 49 and 91 dpv (Figure 6B).

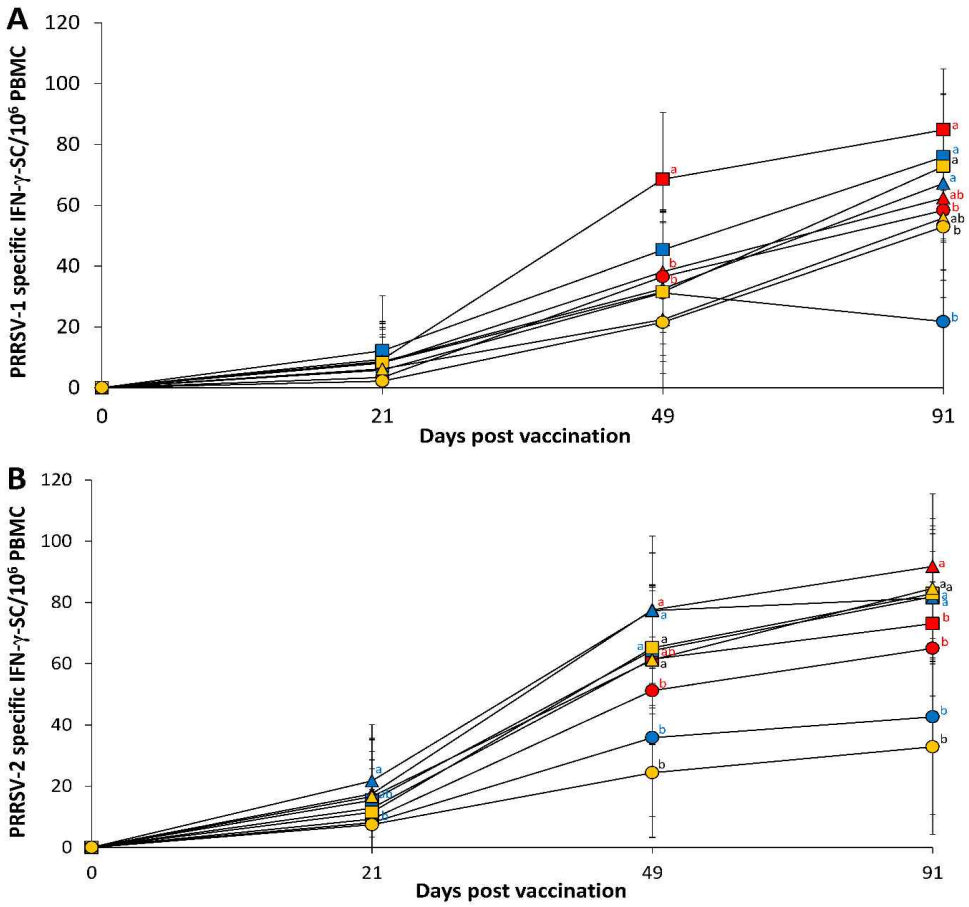


Figure 6. Mean values of the PRRSV-1 (A) and PRRSV-2 (B) specific IFN-g-SC/106 PBMC in pigs from the the VacA/Subunit (¢), VacA/MLV (▲), UnVacA (●), VacB/Subunit (■), VacB/MLV (▲), UnVacB (●), VacC/Subunit (■), VacC/MLV (▲) and UnVacC (●) groups. Variation is expressed as the standard deviation. Significant difference ($P < 0.05$) among subunit-vaccinated, MLV-vaccinated, and unvaccinated group within the Farm A (ab), Farm B (ab), and Farm C (ab).

Pathology

In Farm A, pigs from the VacA/Subunit and VacA/MLV groups had significantly lower ($P < 0.05$) macroscopic lung lesion scores compared to the UnVacA group at 147 dpv. Pigs from the VacA/Subunit group had significantly ($P < 0.05$) lower macroscopic lung lesion scores compared to the VacA/MLV group at 147 dpv. Pigs from the VacA/Subunit group had significantly ($P < 0.05$) lower microscopic lung lesion scores compared to the VacA/MLV and UnVacA groups at 147 dpv. In Farm B, pigs from the VacB/Subunit and VacB/MLV groups had significantly ($P < 0.05$) lower macroscopic lung lesion scores compared to the UnVacB group at 147 dpv. There was no significant difference in PRRSV-1 and PRRSV-2 positive cells scores between vaccinated and unvaccinated pigs in all three farms at 147 dpv (Table 1).

Discussion

In this study we have compared the efficacy of a PRRS subunit vaccine with that of a widely used PRRS MLV vaccine against respiratory disease in farms that are endemic with both PRRSV-1 and PRRSV-2. One of the most important criteria for assessing the efficacy of a PRRS vaccine is the growth performance under field conditions. In our study, vaccination of pigs with either of the PRRS vaccines improved the growth performance

significantly in Farm B. We did not see an improvement in growth performance by either of the vaccines in Farm C. Interestingly, in Farm A, vaccination of pigs with the PRRS subunit vaccine resulted in significantly better growth performance compared to the MLV vaccine. Therefore, the subunit vaccine had similar efficacy and sometimes even better compared to the MLV vaccine under field conditions. However, it is premature to conclude that the subunit vaccine is more effective than the MLV vaccine based solely on the improved growth performance based on 3 farms. It is important to note the efficacy of both vaccines varies between the three farms and these results cannot be easily explained. The inconsistency of the PRRS MLV vaccine against heterologous challenge has been shown before (12). Whether this inconsistency is also present in the subunit vaccine is not known. Differences in protection by the subunit and MLV vaccines may be due to the genetic, antigenic and biological diversity of field viruses (12). Alternatively, the PRRS MLV vaccine used in this study is based on PRRSV-2 and may not be able to effectively control PRRSV-1 in farms where PRRSV-1 and PRRSV-2 are co-circulating, which may result in inconsistent growth performance. The PRRS subunit vaccine is based on PRRSV-1 and PRRSV-2 therefore it has the potential to be effective against both species. In general, the PRRS MLV provides better protection against a homologous virus (20,31,32). In contrast, the PRRS subunit vaccine has been shown to provide good protection against heterologous PRRSV-1 and PRRSV-2 challenge under experimental conditions (14).

The farms used in this field trial exhibited the typical pattern of PRRSV infection where growing pigs at 42-84 days of age, were infected in field situations. Pigs in the nursery and grower were the major reservoir for PRRSV in these farms. Pigs became infected with PRRSV in the nursery through contact with older infected pigs, rather than by in utero or postpartum exposure to sows infected with PRRSV. Reduction of PRRSV viremia is an important parameter to evaluate the vaccine efficacy. In our study, vaccinated and unvaccinated pigs were housed in the same barn, increasing the risk that vaccinated pigs face compared to normal field conditions where all the pigs are vaccinated. Thus, the circulation of PRRSV in the nursery and continuous exposure to the virus may explain why the vaccinated pigs did not exhibit a significantly reduced PRRSV viremia as previous field studies (33-35).

Another important parameter for PRRS vaccine efficacy is the severity of respiratory disease. In our study we excluded dead pigs from clinical observations analyses. However, necropsies were performed indicating that most deaths were caused by PRRSV infection followed by bacterial infection with severe respiratory signs scored between 3 and 5. Overall, respiratory symptoms were mild in both vaccinated and unvaccinated groups. Nevertheless, in Farms A and C, pigs vaccinated with either the PRRS

subunit or MLV had significantly lower respiratory scores compared to unvaccinated pigs. Severe respiratory scores correlated with peak levels of PRRSV-1 viremia in Farm A and PRRSV-2 viremia in Farm C. Despite the fact that neither PRRS vaccine significantly reduced PRRSV viremia, the observed reduction in respiratory disease severity believed is due to vaccination.

Neutralizing antibodies and cell-mediated immunity play the most important role in controlling PRRSV infection in vaccinated pigs (12,15). In general, neutralizing antibodies are very strain specific while cell-mediated immunity such as T cell responses has a broad specificity (36,37). Neutralizing antibody responses following vaccination and infection can also sometimes be weak and delayed (15). In our study, neutralizing antibodies against PRRSV-1 and PRRSV-2 in the subunit- and MLV-vaccinated pigs, rarely reached above 1:8 titers, which are considered protective titers levels (38) during PRRSV-1 and PRRSV-2 peak viremia. In contrast, regardless of the vaccine type, vaccinated pigs exhibited a strong T cell response as measured by IFN-g secreting PBMC. This suggests that both vaccines are able to elicit T cell responses resulting in protection against respiratory disease caused by PRRSV-1 and PRRSV-2 infection under field conditions. It is important to note that the frequency of PRRSV-specific IFN-g secreting PBMC in the subunit- and MLV-vaccinated pigs is also relatively

lower compared with previous studies using the same vaccine under experimental conditions (14,39). This suggests that the PRRS subunit and MLV vaccines are only able to provide a partial protection against respiratory disease in farm where both PRRSV-1 and PRRSV-2 are co-circulating.

To the best of our knowledge, this is the first comparative field study between a subunit and an MLV vaccine. Comparison of these two commercial PRRS vaccines provides swine practitioners and producers with relevant clinical information in order to control PRRSV infection. Typically, inactivated vaccines are considered to be less effective than MLV vaccines. However, in this field study, the subunit vaccine was similar or even better than the MLV vaccine in our assessment of growth performance. In Asian countries where both PRRSV types are prevalent, an MLV vaccine based on one species is often less successful (31). A PRRS subunit vaccine based on both PRRSV-1 and PRRSV-2 can provide adequate protection for growing pigs against respiratory disease in pig farms where both PRRSV species are prevalent.

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Chapter IV

Evaluation of a commercial porcine reproductive and respiratory syndrome virus (PRRSV) subunit vaccine against heterologous PRRSV-1 and PRRSV-2 challenge in late term pregnant gilts

Abstract

The objective of the present study was to evaluate the efficacy of a commercial porcine reproductive and respiratory syndrome virus (PRRSV) subunit vaccine against heterologous PRRSV-1 and PRRSV-2 challenge in late term pregnant gilts. Pregnant gilts were immunized intramuscularly at 56 and 35 days antepartum (58 and 79 days of gestation) and challenged intranasally with PRRSV-1 or PRRSV-2 at 21 days antepartum (93 days of gestation). Regardless of the challenge strain genotype, pregnant gilts that were vaccinated carried their pregnancies to full term and farrowed between 114 and 115 days of gestation with 0% abortion rate. Unvaccinated pregnant gilts aborted between 105 and 110 days of gestation with a 100% abortion rate. Vaccinated gilts had a significantly ($P < 0.05$) lower PRRSV viremia and exhibited significantly ($P < 0.05$) higher levels of virus neutralizing antibodies and interferon-r secreting cells compared to unvaccinated gilts. When fetal tissues were examined, the score for the mean number of PRRSV-positive cells per tissue area unit did differ significantly between the litters from vaccinated and unvaccinated groups. The data presented here indicate that vaccination of late term pregnancy gilts with PRRS subunit vaccine is efficacious against reproductive failure due to heterologous PRRSV-1 and PRRSV-2 infection.

Keywords: Inactivated vaccine, Porcine reproductive and respiratory syndrome virus vaccine, Porcine reproductive and respiratory syndrome virus, Sow

Introduction

Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) is a small, enveloped, single-strand, positive sense, RNA virus that belongs to the family Arteriviridae in the order Nidovirales (1). PRRSV is further divided into two genotypes based on where they originated from, PRRSV-1 is of European origin and PRRSV-2 of North American origin (2,3). Infection with PRRSV as indicated by the name causes reproductive failure in sows and respiratory disorders particularly in young pigs. The hallmarks of reproductive failure are mummified fetuses, stillborn and weak piglets at birth, and a decrease in the number of live born and weaned piglets. Respiratory disorders are characterized by retarded growth and increased mortality (4).

Even though each genotype has historically been more prevalent in different parts of the world, recently co-circulation of both genotypes is becoming more of an issue in many parts of the world most notably in East Asia, including Korea where both genotypes cause serious clinical reproductive problems (5). Typically, PRRSV-2 causes a more severe respiratory disease compared to PRRSV-1 in growing pigs (6). The severity of reproductive failure in pregnant sows is similar for both genotypes (5). Currently, attempts to control PRRSV infection are through vaccination and co-circulation of both genotypes in Asia underscores the importance of vaccines that can cross-protect. PRRSV-2-based modified live vaccines have

shown promising cross-protection against respiratory disease caused by PRRSV-1 in growing pigs but have been largely ineffective against reproductive failure by PRRSV-1 in adults (7,8). Consequently, control of PRRSV infection of both genotypes is a major clinical issue and there is increased demand for an efficacious PRRS vaccine that protects gilts and sows against PRRSV-1 and PRRSV-2 infection.

A commercially available PRRS subunit vaccine (PRRSFREE subunit vaccine, Reber Genetics Co. Ltd., Taiwan, Republic of China) claims protection against both PRRSV genotypes. In a prior study PRRSFREE was able to improve the reproductive performance of sows in farms that were endemically infected with either PRRSV-1 or PRRSV-2 (9). In farms that are endemically infected with PRRSV, vaccinated sows are continuously exposed to circulating field viruses, therefore the immunological and virological response to a vaccine can be difficult to evaluate. No controlled challenge efficacy study has been performed yet under experimental conditions. The objective of this study was to evaluate the efficacy of PRRSFREE against heterologous PRRSV-1 and PRRSV-2 challenge in gilts in terms of reproductive failure based on clinical, immunological, virological, and pathological outcomes.

Materials and methods

Commercial PRRS subunit vaccine

The commercial PRRS subunit vaccine (PRRSFREETM PRRS subunit vaccine, Reber Genetics Co. Ltd.) used in this study consists of a plasmid containing a detoxified Pseudomonas exotoxin-expressing ORF7, ORF1b, and ORF6 & 5 chimeric subunits of PRRSV-1 and PRRSV-2 (10). The ORF1b gene encodes the key enzymes for PRRSV RNA synthesis, which are essential for genome replication and synthesis (11). The PRRS viral subunit antigens encoded by ORF5 through 7 have been reported to confer immunogenicity (12, 13). Among those, the GP5 protein encoded by ORF5 gene and M protein encoded by ORF6 gene have been previously shown to induce neutralizing antibodies (14,15).

PRRSV isolates

PRRSV-1 (SNUVR090485, pan-European subtype 1, GenBank JN315686) and PRRSV-2 (SNUVR090851, lineage 1, GenBank JN315685) strains were used as inocula for this study (6,16). The SNUVR090485 challenge strain shares 88.7% and 61.3% amino acid identity for ORF5 with PRRSV-1 (GenBank CAA63493.1) and PRRSV-2 (GenBank ACG52416.1) PRRSV vaccine strains, respectively. The SNUVR090851 challenge strain shares 55.8% and 85.2% amino acid identity for ORF5 with PRRSV-1 and PRRSV-2 vaccine strains,

respectively.

Experimental design

Thirty six clinically healthy, cross-bred, pregnant gilts were purchased at 63 days antepartum (51 days of gestation) from a commercial PRRSV free farm that had not vaccinated against PRRSV. All gilts were tested for PRRSV and porcine circovirus type 2 (PCV2) by antibody ELISA and real-time reverse transcription polymerase chain reaction (RT-PCR) and were negative (17,18).

Two studies were carried out using gilts from the same herd. In each study, eighteen pregnant gilts were randomly assigned into 6 groups (3 gilts in each group) using the random number generation function (Excel, Microsoft Corporation, Redmond, Washington, USA). Each group was housed in a separate room (Table 1) which contained 3 pens and each individual pregnant gilt was randomly assigned to an individual pen.

At -35 and -14 days post challenge (dpc, 56 and 35 days antepartum; 58 and 79 days of gestation), pregnant gilts in the vaccinated groups, Vac/Ch1, Vac/Ch2, and Vac/UnCh were administered a 2.0 mL dose of the PRRSFREE PRRS subunit vaccine (Lot No. F4001) intramuscularly on the right side of the neck. Pregnant gilts in the unvaccinated groups, UnVac/Ch1, UnVac/Ch2, and UnVac/UnCh were administered an equal

volume of phosphate buffered saline (PBS, 0.01M, pH 7.4, 2.0 mL) at the same time.

Gilts in the Vac/Ch1 and UnVac/Ch1 groups were challenged intranasally with 6 mL of tissue culture supernatant containing 104 TCID50/mL of PRRSV-1 (strain SNUVR090485, 2nd passage in alveolar macrophages) at 0 dpc (21 days antepartum; 93 days of gestation). Pregnant gilts in the Vac/Ch2 and UnVac/Ch2 groups were inoculated the same route with 6 mL of tissue culture supernatant containing 104 TCID50/mL of PRRSV-2 (strain SNUVR090851, 2nd passage in MARC-145 cells) at the same time. Pregnant gilts in the control groups (Vac/UnCh and UnVac/UnCh) were similarly inoculated with PBS.

Blood samples were collected from all pregnant gilts by jugular venipuncture at -35, -14, 0, 7, and 21 dpc. Up to two live born piglets from each of the gilts in all 6 groups were selected using the random number generation function (Excel, Microsoft Corporation). The piglets were euthanized by an intravenous overdose of pentobarbital and different tissues (lung, inguinal lymph node, heart, tonsil, and thymus) were collected for pathological evaluation. All of the above methods were approved by the Seoul National University Institutional Animal Care and Use Committee.

Reproductive performance

The rectal temperature of each individual gilt was monitored daily following challenge by the same personnel. The following farrowing data were also recorded, at birth and at the time of weaning (21days old); litter size, total number of piglets, number of live births, stillborn, mummified, and lightweight (<1 Kg body weight) per litter.

Quantification of PRRSV RNA in blood

Serum samples from pregnant gilts were collected and RNA was extracted as previously described (17,19). The primers for both virus types were designed based on the highly conserved ORF7 region. For PRRSV-1, the forward and reverse primers were 5′- TGGCCAGTCAGTCAATCAAC-3′ and 5′-AATCGATTGCAAGCAGAGGGAA-3′, respectively. For PRRSV-2, the forward and reverse primers were 5′-TGGCCAGTCAGTCAATCAAC-3′ and 5′-AATCGATTGCAAGCAGAGGGAA-3′, respectively (17). Real-time RT-PCR for PRRSV-1 and PRRSV-2 was used to quantify PRRSV genomic cDNA copy numbers using RNA extracted from serum samples as previously described (8,17,20).

Serology

Serum samples were also tested for total PRRSV-specific antibodies using a commercially available PRRSV ELISA (HerdCheck PRRS X3 Ab test,

IDEXX Laboratories Inc., Westbrook, ME, USA). Serum virus neutralization tests were also performed with either the PRRSV-1 or PRRSV-2 challenge strains, as previously described (21). Serum samples were considered to be positive for virus neutralizing antibodies (NA) if the titer was greater than 2.0 (log2) (22).

Enzyme-linked immunospot (ELISPOT) assay

The numbers of PRRSV specific interferon-r secreting cells (IFN-r-SC) were determined in peripheral blood mononuclear cells (PBMC) as previously described (23,24).

In situ hybridization

In situ hybridization (ISH) was performed to detect PRRSV-1 and PRRSV-2 nucleic acid in tissues collected from stillborn and live born piglets. Morphometrical analysis was performed as previously described (6,25).

Statistical analysis

Prior to statistical analysis, RT-PCR and neutralizing antibody data were transformed to log10 and log2 values, respectively. Statistical analysis was

performed on the data from duplicate studies with 6 gilts in each treatment group. A general linear mixed model for repeated measurement was used to compare the normal distribution of the data (PRRSV RNA quantification, serology, and ELISPOT) and the ranks of the Poisson or negative binomial distribution of the data (ISH scores) among gilts in the 6 groups. A value of $P < 0.05$ was considered statistically significant.

Results

Reproductive performance

Two pregnant gilts from the UnVac/Ch1 group were anorectic at 2 and 3 dpc, and three pregnant gilts from the UnVac/Ch2 group were anorectic at 3 dpc. Pregnant gilts from the vaccinated Vac/Ch1, Vac/Ch2, and Vac/UnCh groups carried 100% of their pregnancies to full term and had a normal farrowing between 114 and 115 days of gestation. In contrast, pregnant gits from the UnVac/Ch1 and UnVac/Ch2 groups had 100% abortions between 105 and 110 days of gestation. Pregnant gilts in the control UnVac/UnCh group carried the pregnancy to full term and farrowed between 114 and 115 days of gestation. The number of litters from the sows in all 6 groups is summarized in Table 1.

Table 1 Reproductive and pathological evaluation of pregnant gilts in six different groups

Parameters	Groups					
	Vac/Ch1	Vac/Ch2	Vac/UnCh	UnVac/Ch1	UnVac/Ch2	UnVac/UnCh
Vaccination[a]	Yes	Yes	Yes	None	None	None
Challenge[b]	PRRSV-1	PRRSV-2	None	PRRSV-1	PRRSV-2	None
Piglets/litter[c]						
Totally born	11.17 ± 1.34	11.83 ± 1.34	11 ± 1.29	11.17 ± 1.34	11 ± 1.29	11.5 ± 1.71
Live born	10 ± 1.29*	10.33 ± 1.80†	10.67 ± 1.11	1.67 ± 0.94*	2 ± 0.58†	11.17 ± 1.46
Stillborn	1.17 ± 0.37*	1.5 ± 0.76†	0.33 ± 0.47	9 ± 1.15*	8.5 ± 0.96†	0.33 ± 0.47
Mummified	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0.76	0.33 ± 0.47	0 ± 0
Light (< 1Kg)	0.5 ± 0.5	0.67 ± 0.47	0.17 ± 0.37	0 ± 0	0.17 ± 0.37	0.17 ± 0.37
Splay-legs	0.5 ± 0.76	0.17 ± 0.37	0.17 ± 0.37	0 ± 0	0 ± 0	0.33 ± 0.47
Piglet weaned	9 ± 1.63*	9 ± 1.41†	10.17 ± 1.07	1.5 ± 0.76*	2 ± 0.58†	10.33 ± 0.94
Lung lesion scores[d]	1.17 ± 0.37*	1.25 ± 0.60†	0 ± 0	2.25 ± 0.72*	2.17 ± 0.55†	0 ± 0
PRRSV-1 scores[e]						
Lymph node	13.75 ± 2.38*	0 ± 0	0 ± 0	18.33 ± 3.70*	0 ± 0	0 ± 0
Thymus	26.58 ± 3.68*	0 ± 0	0 ± 0	42.58 ± 6.49*	0 ± 0	0 ± 0
Lung	5.33 ± 1.31*	0 ± 0	0 ± 0	8.58 ± 2.78*	0 ± 0	0 ± 0
Tonsil	11.25 ± 2.20*	0 ± 0	0 ± 0	18.83 ± 3.89*	0 ± 0	0 ± 0
Heart	1.83 ± 0.99*	0 ± 0	0 ± 0	4.83 ± 2.03*	0 ± 0	0 ± 0
PRRSV-2 scores[e]						
Lymph node	0 ± 0	14.5 ± 2.66†	0 ± 0	0 ± 0	19.25 ± 4.49†	0 ± 0
Thymus	0 ± 0	27.83 ± 3.60†	0 ± 0	0 ± 0	37.42 ± 3.35†	0 ± 0
Lung	0 ± 0	5.58 ± 1.44†	0 ± 0	0 ± 0	7.92 ± 1.32†	0 ± 0
Tonsil	0 ± 0	11.75 ± 3.68†	0 ± 0	0 ± 0	16 ± 3.44†	0 ± 0
Heart	0 ± 0	1.92 ± 0.95†	0 ± 0	0 ± 0	4.08 ± 1.26†	0 ± 0

[a] Pregnant gilts were administered intramuscularly with 2.0 mL of PRRSFREE PRRS subunit vaccine at 56 and 35 days antepartum.

[b] Pregnant gilts were inoculated intranasally with PRRSV at 14 days antepartum.

[c] Mean number of piglets per litter in each category on day of farrowing (mean ± standard deviation).

[d] Mean lung lesion scores (mean ± standard deviation): 0 = no microscopic lesion, 1 = mild interstitial pneumonia, 2 = moderate multifocal interstitial pneumonia, 3 = moderate diffuse interstitial pneumonia, and 4 = severe interstitial pneumonia.

[e] Mean number of pulmonary cell positive cells (mean ± standard deviation) for PRRSV nucleic acid per unit area (0.25 mm^2) of lung from formalin-fixed paraffin-embedded tissue by in situ hybridization.

[*] Significant (P <0.05) differences between Vac/Ch1 and UnVac/Ch1.

[†] Significant (P <0.05) differences between Vac/Ch2 and UnVac/Ch2.

Different letters ([*] and [†]) indicate that the groups are significantly (P <0.05) different between vaccinated challenged and unvaccinated challenged groups.

Quantification of PRRSV RNA in sera

The serum of all individual gilts was tested with real-time RT-PCR for the presence of PRRSV RNA. No genomic copies of PRRSV were detected in the serum of any pregnant gilts at the time of vaccination (56 and 35 days antepartum; -35 and -14 dpc). Pregnant gilts from the Vac/Ch1 group had significantly ($P < 0.05$) less copies of PRRSV-1 RNA in their sera compared to the UnVac/Ch1 group at 7 and 21 dpc (Fig. 1A). Similarly pregnant gilts from the Vac/Ch2 group had significantly ($P < 0.05$) less copies of PRRSV-2 RNA in their sera compared to the UnVac/Ch2 group at 7 and 21 dpc (Fig. 1B). No PRRSV-1 was detected in the sera of pregnant gilts from the Vac/Ch2 and UnVac/Ch2 groups and vice versa. No PRRSV genomes regardless of genotype were detected in the sera of pregnant gilts from the Vac/UnCh and UnVac/UnCh groups throughout the experiment.

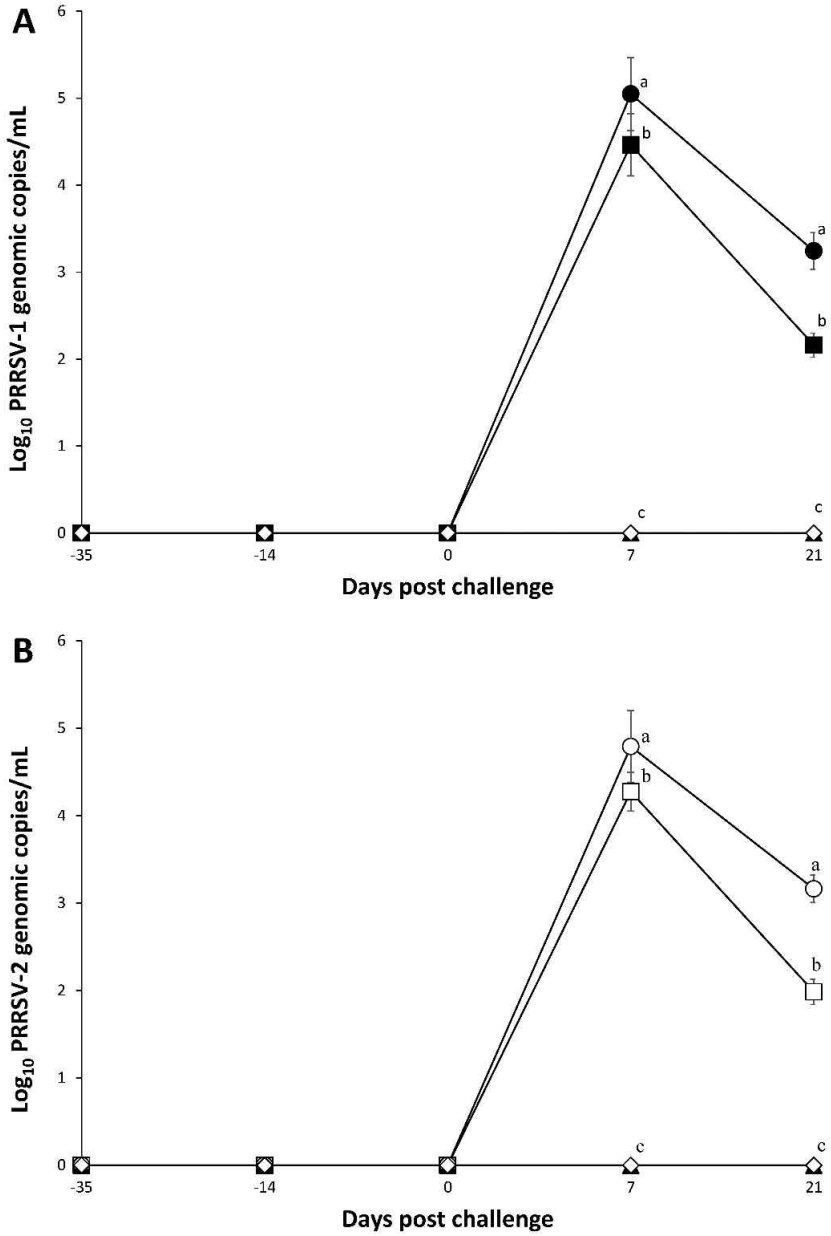

Fig. 1 A. Mean values of log10 transformed genomic copy numbers of PRRSV-1 RNA in the serum samples from Vac/Ch1 (■), Vac/UnCh (▲), UnVac/Ch1 (●), and UnVac/UnCh (◇) groups. B. Mean values of log10 transformed genomic copy numbers of PRRSV-2 RNA in the serum samples from Vac/Ch2 (□), Vac/UnCh (▲), UnVac/Ch2 (○), and UnVac/UnCh (◇) groups. Different letters (a, b, and c) indicate significant ($P < 0.05$) difference among groups.

Serology

PRRSV antibody titers were also measured from the serum of each individual gilt using ELISA. Prior to vaccination (-35 and -14 dpc), pregnant gilts in all 6 groups were seronegative. Pigs from the Vac/Ch1 and Vac/Ch2 groups had significantly ($P<0.05$) higher anti-PRRSV antibody titers compared to the Vac/UnCh, UnVac/Ch1, and UnVac/Ch2 groups at 7 and 21 dpc. No anti-PRRSV antibodies were detected in pregnant gilts from the UnVac/UnCh group throughout the study.

Pregnant gilts from the Vac/Ch1 group consistently showed significantly ($P < 0.05$) higher PRRSV-1 specific NA titers compared to the UnVac/Ch1 and Vac/UnCh groups at 7 and 21 dpc (Fig. 2A). Similarly, pregnant gilts from the Vac/Ch2 group exhibited significantly ($P < 0.05$) higher PRRSV-2 specific NA titers compared to the UnVac/Ch2 and Vac/UnCh groups at 7 and 21 dpc (Fig. 2B). We could not detect any neutralizing antibodies in pigs from the control group (UnVac/UnCh) throughout the study.

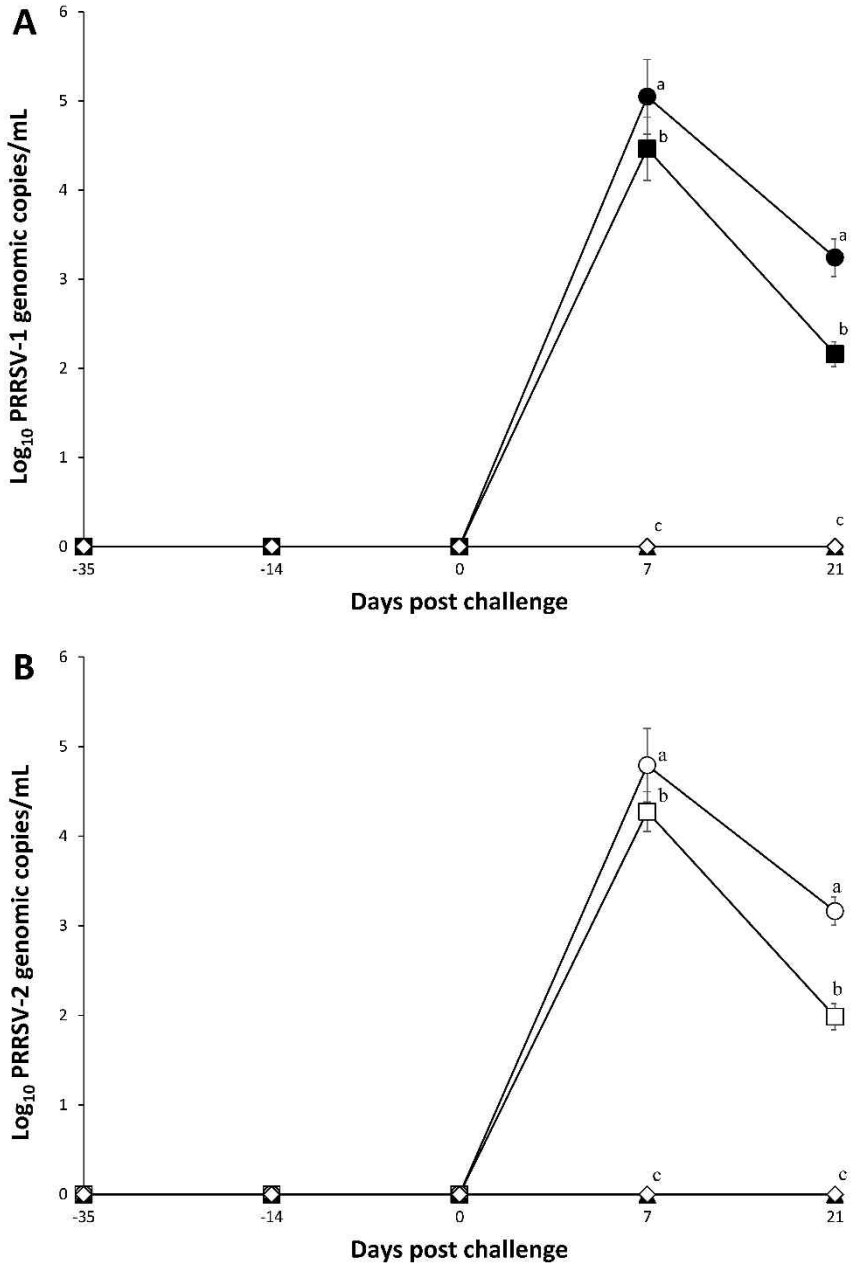

Fig. 2 A. Mean values of log2 transformed PRRSV-1 specific neutralizing antibody (NA) titers in the serum samples from Vac/Ch1 (■), Vac/UnCh (▲), UnVac/Ch1 (●), and UnVac/UnCh (◇) groups. B. Mean values of log2 transformed PRRSV-2 specific neutralizing antibody (NA) titers in the serum samples from Vac/Ch2 (□), Vac/UnCh (▲), UnVac/Ch2 (◯), and UnVac/UnCh (◇) groups. Different letters (a, b, and c) indicate significant ($P < 0.05$) difference among groups.

Interferon-r secreting cells

T cell response was quantified by measuring the number of PRRSV-specific IFN-r-SC. Pregnant gilts from the Vac/Ch1 and Vac/UnCh groups had a significantly ($P < 0.05$) higher numbers of PRRSV-1 specific IFN-r-SC at -14, 0, and 7 dpc compared to the UnVac/Ch1 group. At 21 dpc pregnant gilts from the Vac/Ch1 group had a significantly ($P < 0.05$) higher number of PRRSV-1 specific IFN-r-SC compared to the Vac/UnCh and UnVac/Ch1 groups (Fig. 3A). The number of PRRSV-2 specific IFN-r-SC was significantly ($P<0.05$) higher in pregnant gilts from the Vac/Ch2 and Vac/UnCh groups at -14, 0, and 7 dpc compared to the UnVac/Ch2 group. At 21 dpc, pregnant gilts from the Vac/Ch2 group had a significantly higher ($P < 0.05$) number of PRRSV-2 specific IFN-r-SC compared to the Vac/UnCh and UnVac/Ch2 groups (Fig. 3B). The mean numbers of PRRSV-specific IFN-r-SC remained at basal levels (< 20 cells/106 PBMC) in pregnant gilts in the UnVac/UnCh group throughout the study.

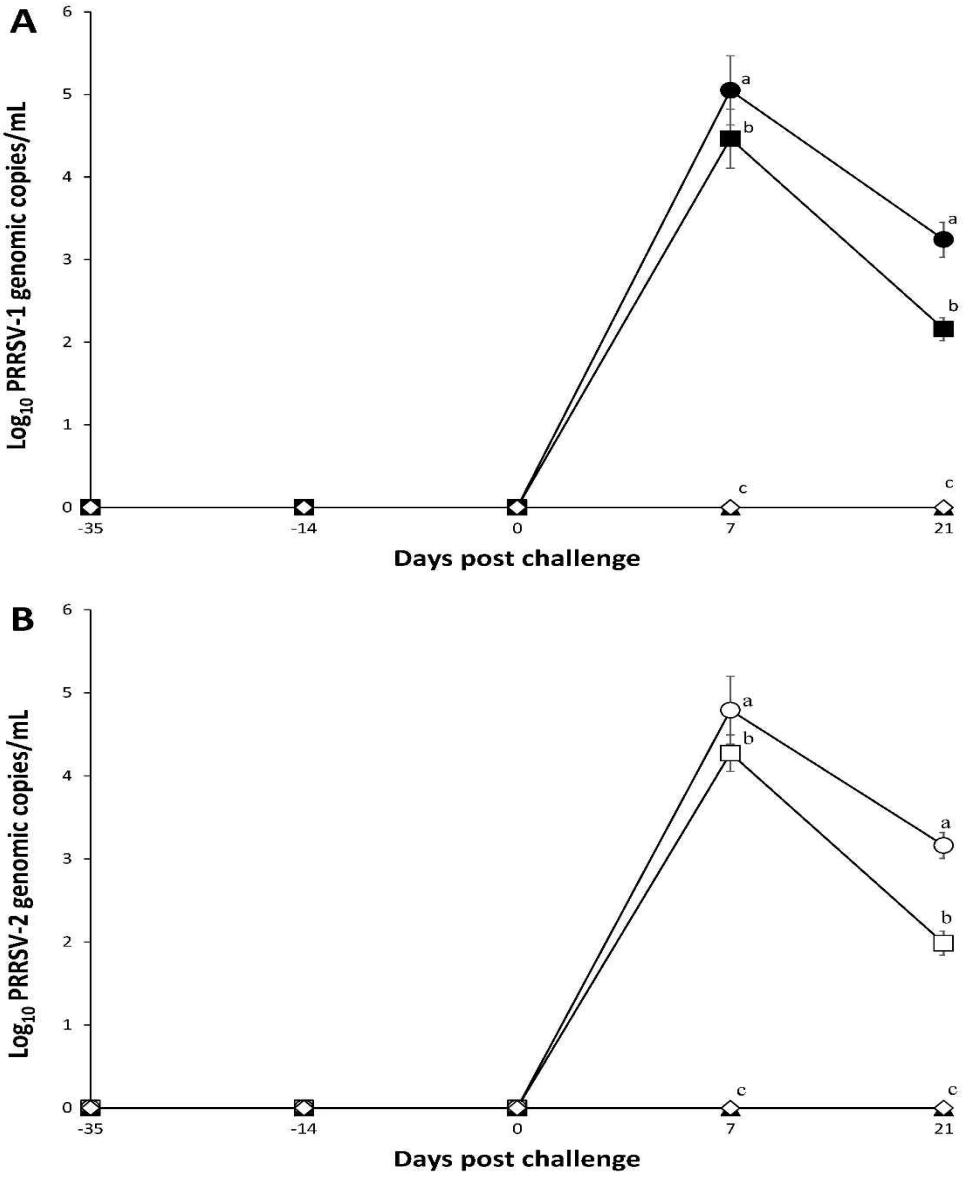

Fig. 3 A. Mean numbers of PRRSV-1 specific interferon-r secreting cells (IFN-r-SC) in peripheral blood mononuclear cells (PBMC) from Vac/Ch1 (■), Vac/UnCh (▲), UnVac/Ch1 (●), and UnVac/UnCh (◇) groups. B. Mean numbers of PRRSV-2 specific IFN-r-SC in PBMC from Vac/Ch2 (□), Vac/UnCh (▲), UnVac/Ch2 (○), and UnVac/UnCh (◇) groups. Different letters (a, b, and c) indicate significant ($P < 0.05$) difference among groups.

Pathology

Microscopic lung lesions were present in live born piglets from pregnant gilts in the UnVac/Ch1 and UnVac/Ch2 groups. The microscopic lung lesions were characterized by septal thickening with mononuclear cells and accumulation of macrophages in alveolar spaces. There were significant differences in microscopic lung lesion scores between Vac/Ch1 and UnVac/Ch1 ($P < 0.05$) groups, and between Vac/Ch2 and Unvac/Ch2 ($P < 0.05$) groups. The microscopic lung lesions in litters from the UnVac/Ch1 group were significantly ($P < 0.05$) more severe than those in litters from the Vac/Ch1 group. The microscopic lung lesions in litters from the UnVac/Ch2 group were significantly ($P < 0.05$) more severe than those in litters from the Vac/Ch2 group (Table 1). No microscopic lung lesions were observed in Vac/UnCh and UnVac/UnCh groups.

The mean number of PRRSV-positive cells per tissue area unit was also scored for lymph node, thymus, lung, tonsil, and heart. There was a significant difference ($P < 0.05$) between the litters from the Vac/Ch1 and Vac/Ch2 groups compared to the UnVac/Ch1 and UnVac/Ch2 groups (Table 1). Significantly more PRRSV-1 positive cells were detected in lymph node (Fig. 4), thymus, lung, tonsil, and heart of litters from the UnVac/Ch1 group than were detected in the same tissues of litters from the Vac/Ch1 group. Significantly more PRRSV-2 positive cells were detected in lymph node, thymus (Fig. 5), lung, tonsil, and heart of litters from the UnVac/Ch2 group than were detected in the same tissues of litters from the Vac/Ch2 group. As expected, PRRSV-1 positive cells were only detected in the litters from pregnant gilts in the Vac/Ch1 and UnVac/Ch1 groups, while PRRSV-2 positive cells were only detected in the litters from pregnant gilts in the Vac/Ch2 and UnVac/Ch2 groups. No PRRSV-1 or PRRSV-2 was detected in any litters from pregnant gilts in the Vac/UnCh and UnVac/UnCh groups (Table 1).

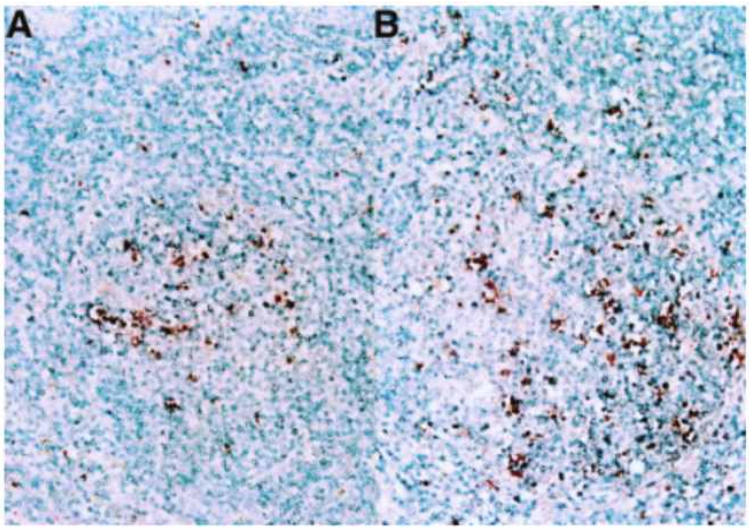

Fig. 4 A. Representative in situ hybridization for the detection of PRRSV-1 nucleic acid in lymph node of litters from the Vac/Ch1 group. B. Representative in situ hybridization for the detection of PRRSV-1 nucleic acid in lymph node of litters from the UnVac/Ch1 group. New born piglets from the Vac/Ch1 group (A) had significantly ($P < 0.05$) lower PRRSV-1 positive cells scores compared to newborn piglet from the UnVac/Ch1 group (B).

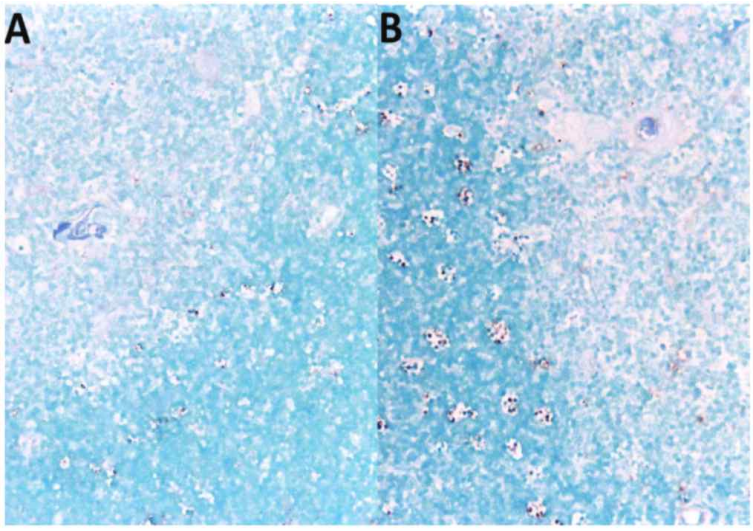

Fig. 5 A. Representative in situ hybridization for the detection of PRRSV-2 nucleic acid in thymus of litters from the Vac/Ch2 group. B. Representative in situ hybridization for the detection of PRRSV-2 nucleic acid in thymus of litters from the UnVac/Ch2 group. New born piglets from the Vac/Ch2 group (A) had significantly ($P < 0.05$) lower PRRSV-2 positive cells scores compared to newborn piglet from the UnVac/Ch2 group (B).

Discussion

The results presented in current study provide evidence that vaccination of pregnant gilts with a PRRS subunit vaccine is able to protect pregnant gilts from reproductive failure caused by PRRSV infection. Our results are in agreement with previous findings where an inactivated vaccine based on PRRSV-1 improved reproductive performance (e.g. increase in the number of live born) and provided partial protection against subsequent challenge of homologous PRRSV-1 challenge in pregnant sows (26). These observations are further supported by another study in which another PRRSV-1 inactivated vaccine (27) significantly lowered the preweaning mortality of live born piglets. However, this vaccine failed to prevent clinical signs and improve the reproductive failures commonly associated with PRRSV infection (27). Even though the PRRS subunit vaccine used in this study and the inactivated PRRS vaccine used in the previous study (27) on the same PRRSV-1 pan-European subtype 1 (Lelystad-like cluster strain), we have no clear explanation for this discrepancy. One reason could be due to antigenic variation between the two PRRSV-1 challenge strains. The ORF5 nucleotide and amino acid sequences of the vaccine strain and the challenge strain SNUVR 090485 used in this study and the challenge strain 2156 (GenBank no. PRU40896) used in the previous study, share 85.5% and 87.5% sequence identity, respectively. This genetic difference could be enough to result in a difference in antigenicity.

In our study, the PRRS subunit vaccine induced similar levels of NA and IFN-g responses to PRRSV-1 and PRRSV-2. This could explain why this PRRS subunit vaccine is effective against both genotypes. The PRRS subunit vaccine enhances anamnestic virus-specific NA and IFN-g responses following a wild-type virus challenge. These responses appear to be responsible for the viral clearance similar to other inactivated PRRS vaccines (22,28,29). PRRSV infection in pregnant gilts at late gestation consistently results in transplacental infection of fetuses and reproductive failure (30,31). Viral clearance plays an important role in preventing transplacental infection. In the present study, viremic reduction coincides with the appearance of PRRSV-specific NA and IFN-r responses following challenge in vaccinated gilts. It is important to note that, NA responses were positive following PRRSV-1 and

PRRSV-2 challenge (7 and 21 dpc), but negative on 0 dpc even after two doses of vaccination in all vaccinated groups. However, PRRSV-specific IFN-r responses were positive both before and after PRRSV challenge. The results were similar for both genotypes of PRRSV. This suggests that perhaps PRRSV specific IFN-r responses following vaccination with the recombinant chimeric PRRS vaccine play a more important role for protection against reproductive failure. Lack of protection despite high induction of neutralizing antibodies has been previously shown with some inactivated PRRS vaccines even suggesting that sometimes, a very high adaptive immune response is associated with more serious clinical symptoms after challenge infection (22,32).

Clinical analysis of reproductive performance is critical in evaluating the efficacy of a PRRS vaccine. In our study the abortion rate is significantly different between vaccinated (0%) and unvaccinated (100%) gilts. Pathological analysis by ISH also revealed significant differences in the number of PRRSV-positive cells in fetal tissues between vaccinated and unvaccinated gilts. Viral replication within fetuses and spread of PRRSV to adjacent fetuses are pivotal events in the pathogenesis of fetal death (33). Vaccinated pregnant gilts showed fewer numbers of PRRSV-1 and PRRSV-2 RNA-positive cells in the fetal thymus and other organs compared to the unvaccinated group. Taken together, the data suggest that the PRRS subunit vaccine is effective against both PRRSV genotypes. Therefore, the PRRS subunit vaccine is clinically useful in controlling reproductive failure against PRRSV-1 and PRRSV-2 in gilts and sows in many Asian countries where co-circulation of two PRRSV genotypes is a consistent problem.

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GENERAL CONCLUSION

As both PRRSV-1 and PRRSV-2 are prevalent together in Asian countries including Korea and caused more complicated disease, this thesis investigated the efficacy of each type of Modified Live vaccines against PRRSV-1 and PRRSV-2 and also compared the efficacy of PRRS subunit vaccine with MLV vaccine in various conditions.

This study compared the efficacy of two PRRSV-1 MLV vaccines and two PRRSV-2 MLV vaccines against heterologous challenge with PRRSV-1 and PRRSV-2. There was no significant difference between the two PRRSV-1 MLV vaccines as they both can provide partial protection against a PRRSV-1 strain but only limited protection against a PRRSV-2 strain, during the acute phase. In contrast, two commercial PRRSV-2 MLV vaccines can provide partial protection against both PRRSV-1 and -2 strains based on clinical, virological, immunological, and pathological comparisons. The results of this study are important because they provide swine producers and practitioners with valuable clinical information in order to better select future PRRSV vaccines. To help choosing practically more efficient MLV vaccines, we applied this experiment to PRRSV-1 and PRRSV-2 co-infected commercial farm where the vaccinated pigs are continuously exposed to field viruses circulating in the farm which can ultimately significantly affect the efficacy of a vaccine. Regardless of the type of MLV, all vaccinated groups generally exhibited improved growth rate compared to the unvaccinated pigs but as first study implied, either of the MLV 2 vaccines had a better overall growth rate compared to the pigs vaccinated with either of the MLV 1 vaccines. The differences in growth rate between PRRSV-1 MLV- and PRRSV-2 MLV-vaccinated groups may be due to genetic similarity between vaccine and field viruses. The identity between the field isolates and PRRSV-2 vaccine strains is 91.2-99.8% compared to 88.4-89.1% identity with the PRRSV-1 vaccine strains. Another reason for the difference in growth rate observed between the two types of vaccines could be due to the difference in virulence between PRRSV-1 and PRRSV-2. In general, Korean PRRSV-2 is more virulent than Korean PRRSV-1. Also, MLV 1 vaccination failed to reduce any type of PRRSV-1 or PRRSV-2

viremia while MLV 2 vaccines decreased PRRSV-2 viremia. Although this study used regular virulence PRRSV-1 and PRRSV-2, so was not able to predict the efficacy in highly virulent PRRSV-1 and typically virulent PRRSV-2, these results however suggest that it is effective to use a PRRSV-2 MLV vaccine to prevent respiratory disease against co-infection with PRRSV-1 and PRRSV-2 under field conditions.

Although right type MLV were selected, the drawback of safety in MLV vaccines are not ignorable but Killed vaccines are not an option because of poor efficacies. To meet this demand, relatively recently PRRS subunit vaccine was developed claiming comparable efficacies to MLV vaccines. In this study we have compared the efficacy of a PRRS subunit vaccine with that of a widely used PRRS MLV vaccine against respiratory disease in farms that are endemic with both PRRSV-1 and PRRSV-2. Three farms were selected based on their history of respiratory diseases caused by co-infection with both PRRSV-1 and PRRSV-2. Although there were inconsistent results among farms, pigs vaccinated with the PRRS subunit vaccine had similar or a better growth performance statistically compared to those vaccinated with the PRRS MLV vaccine. This can be explained by that the PRRS subunit vaccine is based on PRRSV-1 and PRRSV-2 therefore it has the potential to be effective against both species but the PRRS MLV vaccine used in this study is based on PRRSV-2 and may not be able to effectively control PRRSV-1 in farms where PRRSV-1 and PRRSV-2 are co-circulating. For respiratory disease, Farms A and C, pigs vaccinated with either the PRRS subunit or MLV had significantly lower respiratory scores compared to unvaccinated pigs. Severe respiratory scores correlated with peak levels of PRRSV-1 viremia in Farm A and PRRSV-2 viremia in Farm C. But the frequency of PRRSV-specific IFN-g secreting PBMC in the subunit- and MLV-vaccinated pigs is also relatively lower compared with previous studies using the same vaccine under experimental conditions. This suggests that the PRRS subunit and MLV vaccines are only able to provide a partial protection against respiratory disease in farm where both PRRSV-1 and PRRSV-2 are co-circulating.

Lastly, effectiveness of a commercial porcine reproductive and respiratory syndrome virus (PRRSV) subunit vaccine against heterologous PRRSV-1 and PRRSV-2 challenge in late-term pregnant gilts were investigated. Regardless of the challenge strain's genotype, the vaccinated gilts carried their pregnancies to term and farrowed between days 114 and 115 of gestation while unvaccinated gilts aborted between days 105 and 110 of gestation. The PRRS subunit vaccine induced similar levels of neutralizing-antibody and IFN-γ-SCs responses to PRRSV-1 and PRRSV-2, which could explain why this vaccine is effective against both genotypes. The vaccinated gilts had a significantly lower level of PRRSV viremia and significantly higher levels of virus-neutralizing antibodies and interferon-γ-secreting cells compared with the unvaccinated gilts. Pathological analysis by ISH also revealed significant differences in the number of PRRSV-positive cells in fetal tissues between vaccinated and unvaccinated gilts. These results revealed that vaccination in late-term pregnancy with PRRSV subunit vaccine was efficacious against reproductive failure due to heterologous PRRSV-1 and PRRSV-2 infection. Since PRRS live vaccines are rarely allowed in breeding farms because of possible viral spreading to commercial farms, the PRRS subunit vaccine could be vaccine of choice.

국문 논문 초록

돼지 생식기 호흡기 증후군 생독 및 사독 백신의 병리학적 및 면역학적 평가

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돼지 생식기 호흡기 증후군 (PRRS)은 번식 저하 및 호흡기 질환을 일으키는 현대 양돈 산업에서 경제적으로 가장 중요한 질병이다. PRRS 바이러스(PRRSV)는 크게 PRRSV-1 및 PRRSV-2 의 두 가지 균주로 나누어 지는데 PRRSV-1과 PRRSV-2 는 1991 년경 유럽과 북미에서 각각 처음 분리되었으며, 두 균주 모두 발견된 대륙에서 여전히 우세한 균주이다. 그러나 우리 나라를 비롯한 아시아 국가는 PRRSV-1과 PRRSV-2 가 함께 나타나 더 심한 합병증을 일으킨다.

동종 균주를 사용한 백신 접종은 이종 균주를 사용한 백신 접종보다 더 효과적이며 약독화 생백신은 생산 및 경제적 손실과 야외 바이러스 배출을 줄였다. 현재 국내에는 4 개의 약독화 생백신이 판매 중이며 2개는 PRRSV-1 유래 약독화 생백신이고 나머지 2개는 PRRSV-2 유래 약독화 생백신이다. 그러나 PRRSV-1 및 PRRSV-2 야외 이종 바이러스에 대한 4 가지 약독화 생백신의 효능을 평가한 연구는 아직 수행되지 않았다. 이러한 이유로, 본 연구는 육성돈에 PRRSV-1 및 PRRSV-2 를 공격접종하여 두 개의 MLV 1 상용 PRRS 백신과 두 개의 MLV 2 상용 PRRS 백신의 효능을 평가했다. 두 개의 MLV 1 백신은 동종 PRRSV-1 공격접종에서만 폐 병변과 PRRSV 감염 세포를 감소시켰지만, 두 개의 MLV 2 백신은 동종 PRRSV-2 뿐만 아니라 이종

PRRSV-1 공격접종에서도 교차면역을 나타냈다. 이러한 결과를 가지고 MLV 1과 MLV 2 백신 효능 비교 실험을 PRRSV-1 및 PRRSV-2 함께 감염된 일반 양돈장으로 확대하였다. MLV의 유형에 관계없이 모든 백신 접종 그룹은 일반적으로 백신을 접종하지 않은 돼지에 비해 향상된 성장률을 보였지만 선행 연구처럼 MLV 2 백신이 MLV 1 백신을 접종 한 돼지에 비해 전체 성장 속도가 더 좋았다. MLV 1 백신 접종은 PRRSV-1 과 PRRSV-2 어느 것도 바이러스 혈증을 감소시키지 못한 반면 MLV 2 백신은 PRRSV-2 바이러스 혈증을 감소 시켰다. 종합하면 MLV 2 백신은 PRRSV-1 및 PRRSV-2 혼합감염 농장에서 MLV 1 백신보다 더 효과적일 수 있다.

PRRS 생독백신이 사독백신보다 더 효과적이긴 하지만 생독백신의 안전성에 대한 우려가 커지고 있는데 종돈장의 경우 더욱 그러하다. PRRS 서브유닛 백신과 PRRS MLV 간의 야외임상 비교 연구는 수행되지 아니하여 돼지 생식기 및 호흡기 증후군을 보이는 농장에서 PRRS MLV 백신과 PRRS 서브유닛 백신의 효능을 비교하였다. PRRSV-1 및 PRRSV-2 와의 혼합감염으로 인한 호흡기 질환의 병력을 바탕으로 3 개의 농장이 선택 되었다. PRRS 서브유닛 백신을 접종 한 돼지는 MLV 백신을 접종 한 돼지에 비해 호흡기 질환에 대해 비슷하거나 더 나은 효능을 보였으며 성장률도 좋았다. 그러나 PRRSV-1 및 PRRSV-2 바이러스 혈증이 가장 높을 때, PRRS 서브유닛 백신과 MLV 모두 PRRSV-1 및 PRRSV-2 에 대한 중화항체와 T 세포 반응이 낮은 수준이었으며 이는 PRRSV-1 및 PRRSV-2 혼합감염에 대한 부분적 방어로 여겨진다.

마지막으로 임신 후기 후보돈에서 PRRS 서브유닛 백신의 PRRSV-1 과 PRRSV-2 이종 바이러스 공격접종에 대한 효과를 연구하였다. 공격 균주의 유전형에 관계없이, 백신 접종한 후보돈은 임신 114 일에서 115 일 사이에 정상적으로 분만을 했으나 백신 접종을 하지 않은 후보돈은 임신 105 일에서 110 일 사이에 유산하였다. 또한, 백신 접종한 후보돈은 백신을 접종하지 않은 후보돈에 비해 PRRSV 바이러스 혈증 수준이 상당히 낮았고 바이러스 중화 항체 및 인터페론 -γ 분비 세포 수준이 상당히 높았다. 이러한 결과는 임신돈에서 이종 PRRSV-1 및 PRRSV-2 감염으로 인한 번식 질환에 PRRSV 서브유닛 백신이 효과가 있음을 보여주었다.

주요어: 돼지 생식기 호흡기 증후군; 혼합감염; 생독 백신; 사독 백신; 서브유닛 백신

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