수의학박사학위논문

Comparative Efficacy of Two PRRSV and PCV2 Vaccines

두 가지 PRRSV와 PCV2 백신의 효능 비교 평가

2017년 8월

서울대학교 대학원

수의과대학 수의병인생물학 및 예방수의학 전공

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Comparative Efficacy of Two PRRSV and PCV2 Vaccines

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A dissertation submitted in partial fulfillment of

The requirements for the degree of

DOCTOR OF PHILOSOPHY

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May 2017

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ABSTRACT

Comparative Efficacy of Two PRRSV and PCV2 Vaccines

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Porcine reproductive and respiratory syndrome (PRRS) induced by PRRS virus (PRRSV) has led to the most devastating economic losses in the global swine industry because PRRSV causes reproductive failure in breeding herds and respiratory disorder in growing/finishing pigs. Vaccination has become the most common strategic method for the prevention and control of PPRSV infection. Since simultaneous immunization is an efficient tool for controlling the co-challenge of PCV2 and PRRSV infection, the objective of the first study was to compare clinical, virological, immunological, and pathological indicators in pigs each treated simultaneously with both PRRSV and porcine circovirus type 2

(PCV2) vaccines from one of two commercial products and then later exposed to field strains of both viruses. Pigs in one group vaccinated with Fostera PCV® and Fostera PRRS® simultaneously and pigs in another group vaccinated with Ingelvac CircoFLEX® and Ingelvac PRRS MLV® simultaneously on study day -28 (21 days of age) were exposed to both viruses at study day 0 (49 days of age). No significant differences concerning transmission were seen between the two immunized groups in clinical, virological (except PCV2 viremia on day 14), immunological, or pathological examinations. Under these study conditions, there was no difference in protection whether PCV2 and PRRSV vaccines were administered simultaneously or not. The objective of the second study was to compare the efficacy of two modified live virus (MLV) PRRSV vaccines under field conditions. The clinical trial at the site was performed on a 1,000-sow herd with two-site generation: farrowing nursery and growing/finishing system. The farm had had difficult times because of losing animals due to respiratory disease brought on by PRRSV-2 in postweaning and late growing pigs at the same time of study. Via intramuscular injections, pigs in Group 1 were administered with 2.0 mL of the Fostera PRRS® vaccine (Zoetis, Lot No. A405013B), pigs in Group 2 were administered with 2.0 mL of the Ingelvac PRRS MLV® (Boehringer Ingelheim Vetmedica Inc., Lot No. 245-659A) and pigs in Group 3 were administered with 2.0 mL of phosphate buffered saline (0.01M, pH 7.4). This study indicated that pigs immunized with MLV vaccines Fostera PRRS® (Zoetis,

Florham New Jersey) and Ingelvac PRRS MLV® (Boehringer Ingelheim Vetmedica Inc., St Joseph Missouri) showed better growth performance and less lung lesions than unvaccinated controls under wild conditions. Moreover, no significant differences were detected between the MLV PRRSV vaccines in this study based on clinical (average daily weight gain), immunologic (antibodies), virologic (PCR testing), and pathologic (lesions and viral antigen) measurements.

Keywords:

Fostera®

Ingelvec®

Porcine circovirus type 2 (PCV2)

Porcine reproductive and respiratory syndrome virus (PRRSV)

Vaccine

Student number: 2015-31176

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LIST OF ABBREVIATION

ADG	Average daily gain
CMI	Cell mediated immunity
ELISA	Enzyme-linked immune-sorbent assay
IFN-γ-SCs	Interferon gamma secreting cells
IHC	Immunohistochemistry
IL	Interleukin
ISH	*in situ* hybridization
KV	Killed virus
NAb	Neutralizing antibody
MLV	Modified live virus
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PCV	Porcine circovirus
PMWS	Postweaning multisystemic wasting syndrome
PRDC	Porcine respiratory disease complex
PRRS	Porcine reproductive and respiratory syndrome
PRRSV	Porcine reproductive and respiratory syndrome virus
PRV	Pseudorabies virus
$TCID_{50}$	Median tissue culture infective dose

GENERAL INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) is reported as the main hazard to the lucrative global pork business. As the name indicates, porcine reproductive and respiratory syndrome (PRRS) has two pathological characteristics: reproductive disorders in gilts and pregnant sows, and respiratory disorders in growing pigs (pneumonia, performance handicap, and immunosuppression). Exposure to wild-type strains of PRRS can damage the fertility of pregnant sows and breeding herds since they may miscarry their litters or end up with a high percentage of stillborn piglets and mummies, and the lives of live-born piglets may be in danger (35).

Since the initial appearance of PRRS in the late 1990's in Western Europe (153) and North America (59), it has induced a substantial economic downturn in the international swine industry (98). The etiological background of PRRS is that is caused by an RNA virus (PRRSV) of the order *Nidovirales*, family *Arteriviridae*, genus *Arterivirus*. The strains of PRRSV are separated into two genotypes: the Type I or European (EU)-type (PRRSV-1) and the Type II or North American (NA)-type (PRRSV-2). NA- (44) and EU-type (39) genotype strains are antigenically and genetically different. PRRSV-1 with the prototype Lelystad Virus (LV), is prevalently endemic in Europe, while PRRSV-2 with the prototype

ATCC VR2332 typifies strains common on the American continent and in Asia (153). The latest reports on PRRSV categorization indicates that PRRSV-1 has been separated into 2 subtypes: pan-European subtype I and East European subtype 2 (150), and moreover, has been categorized into 12 different classes (142).

In Korea, PRRSV-2 was initially identified in 1994 (71), and up to 2005, was the only PRRSV found in Korean pig herds (64) until PRRSV-1 (pan-European subtype 1) was discovered (65); from that time, both PPRSV-1 and PRRSV-2 have been spread out in the field condition. Transmission of PRRSV-2 is only 54.4% (37/68 pig herds) and is the most common, followed by PRRSV-1 at only 29.4% (20/68 pig herds) and co-transmission of PRRSV-1 and PRRSV-2 16.2% (11/68 pig herds) (73). The Ingelvac PRRS® modified live virus (MLV) vaccine against PRRSV-2 was introduced for the first time to handle PRRS in Korea in 1996. Later, two commercial PRRSV-1 MLV vaccines, such as Porcilis PRRS (MSD Animal Health, Summit, NJ) and Unistrain PRRS (Hipra, Amer, Spain) were first permitted in Korea in 2014 for handling of PRRSV-1 transmission (71).

Two types of commercial PRRSV vaccines are accessible in Europe: killed virus (KV) or inactivated virus vaccines and MLV or attenuated vaccines. Commercial EU-type KV vaccines have shown insufficient efficacy because of low activation

of the immune system and no efficacious production of neutralizing antibodies. Nevertheless, KV vaccines can cause powerful cell mediated immunity (CMI). In contrast, commercial EU-type MLV vaccines show efficacious strain-specific defensive action but only low defensive capability against genetically heterologous PPRSV and draw rather late humoral and CMI reactions, which results in delayed defensive action (108).

In Europe, KV vaccination has demonstrated a decrease in the negative effects of PRRSV in breeding herds, such as better reproductive performance (elevation of farrowing rates in the number of live or weaned pigs) and decreases in premature farrowing rate, abortion rate, and the number of mummified and stillborn piglets. Moreover, the usage of commercial MLV vaccines for PRRSV-infected breeding herds has resulted in (a) better reproductive performance (decreased abortion and rebound estrus rate, and increased farrowing rate in the number of weaners), (b) lower he viremia, morbidity and mortality rate of piglets, and (c) better developmental activity in the vaccinated pigs. In consequence, these days the usage of MLV and KV vaccines in Europe has been the most effective way to handle the economic crisis caused by PRRSV transmission. On the other hand, the research and development of novel PRRSV vaccines is a very important future goal for PRRSV vaccinology (108).

Ingelvac PRRS MLV® is an MLV vaccine proven safe for pigs, especially gilts and sows, at any time of their reproductive stage in PRRSV-positive herds. It allows cross protection against heterologous strains including the Lelystad virus, and has been shown to significantly lower reproductive dysfunction and respiratory disorders induced by PRRSV infection (8). Administering Ingelvac PRRS MLV® is recommended via the intramuscular (IM) route with a 2 mL dose size for pigs of adult breeding age or pregnant females. Except for boars, immunity requires at least a 4 month post-immunization period, and revaccination of a herd may be needed every 3 to 4 months or as guided by a veterinarian (8).

Fostera PRRS® from Zoetis is the only PRRSV vaccine, which 1) prohibits reproductive dysfunction when used in pregnant females with a period of immunity of at least 19 weeks, and 2) also prevents respiratory disorders related to PRRSV with a period of immunity of at least 26 weeks. Fostera PRRS® was registered for the immunization of healthy swine at 1 day of age or older in PRRSV-positive herds. Administering Fostera PRRS® is recommended as a single 2 mL intramuscular (IM) dose, consisting of the antiseptic rehydrated freeze-dried vaccine along with sterile diluent (35).

Postweaning multisystemic wasting syndrome (PMWS) was first reported in 1991 in Saskatchewan (Canada) (50). At that time, it was definitely a new disease

characterized by wasting, paleness of the skin and jaundice in nursery and fattening pigs. Infected animals show typical lesions in multiple tissues (multisystemic), mostly in the lymphoid organs (53, 120).

Porcine circovirus (PCV) is a member of the family *Circoviridae*, genus *Circovirus*, which consists of small (17 nm in diameter) non-enveloped DNA viruses with a unique single-stranded circular genome. PCVs replicate in the nucleus of infected cells, utilizing the host's polymerase for genome amplification. Two strains have been reported: type 1 (PCV1) and type 2 (PCV2). PCV1 (first identified in 1974) instantly infects a host but is not known to induce disease in swine; however, PCV2 (first identified in 1997) has created problems in recent years along with elevated cases of PMWS, and leads to significant depletion of lymphocytes, enlarged lymph nodes and abnormal lung tissue (89).

Infection by PCV2 has been seen both horizontally and vertically and is shed through feces and urine, as well as nasal and oral secretions (131). The existence of PCV2 in such secretions and excretions has been proven to enable horizontal infection when naïve pigs are exposed to PCV2-infected pigs (112), and PCV2 can be transmitted to naïve pigs between 1 and 42 days after a challenge (9). Moreover, the capability of PCV2 to traverse the placenta and be transmitted to fetuses (83), and the capability of PCV2 to disseminate intrauterinely and be

transmitted to fetuses facilitate vertical transmission. Earlier studies have corroborated the evidence that PCV2 is not only widespread, but can be transmitted through various routes as well (114).

Ingelvac CircoFLEX® is provided as a suspension for injection. It is intended to diminish mortality and clinical signs including weight loss and lesions in lymphoid tissues related to PCV2-caused diseases. In addition, immunization has also been revealed to decrease PCV2 nasal shedding, viral load in the blood and lymphoid tissues, and the duration of viremia. Onset of protection occurs as early as 2 weeks post-vaccination and persists for at least 17 weeks. The recommendation for pigs is to immunize them from 2 weeks of age by a single 1.0 mL intramuscular injection of one dose, regardless of body weight (34).

Zoetis Fostera PCV® PCV vaccine is a KV vaccine for use in healthy pigs 3 weeks of age or older as an aid in preventing viremia and controlling lymphoid depletion caused by PCV2. It provides protection against PCV2 infection in lymphoid tissues while helping to reduce lymphoid depletion in pigs challenged with virulent PCV2. This swine vaccine has demonstrated 4 months duration of immunity; dosage is a single 2 mL injection and intramuscular (IM) administration is required. This product may be suitable for use on organic farms (17, 104).

LITERATURE REVIEW

1. PRRS

Since the initial appearance of PRRS in the late 1990's in Western Europe (153) and North America (59), it has induced a substantial economic crisis in the international swine industry (98). In the wild environment, vaccination is one of the most effective ways of inhibiting and handling PRRS (108). PRRSV is globally acknowledged as a major hazard to the lucrative pork industry. According to an economic analysis study, scientists estimate that PRRS costs the US pork industry $664 million every year in reproduction-related deaths, and the combined effect of PRRSV totals more than $1 billion per year when other disease handling costs are included. Approximately half of the direct economic loss comes from breeding herds with PRRS and most of this ($300 million) is caused by 8.3 million less weaned pigs (57).

PRRS is a disease caused by a virus that impairs the pig's immune systems and makes them vulnerable to bacteria and other viruses. There is a reason that infections with disorders such as CSF and PCV2 may outbreak simultaneously, allowing more severe problems in treated farms. PRRS is also known as a mystery swine disease causing blue ear disease, porcine endemic abortion and respiratory syndrome and swine infertility respiratory syndrome (46).

2. PRRSV

The etiological background of PRRS is that its vector is an RNA virus (PRRSV) of the order *Nidovirales*, family *Arteriviridae*, genus *Arterivirus*. The strains of PRRSV are separated into two genotypes: Type I or EU-type and Type II or NA-type. The virus contains enveloped positive-sense single-stranded RNA genome of nearly 15 kb in size with nine open-reading frames (ORFs). These genotypes of PRRSV have a proportion of nearly 60% nucleotide sequence homology with each other (79). In each genotype, the virus extracts can show up to 20% differences in nucleotide sequences, which assemble in a diversity of heterogeneous clusters or subpopulations (137). Moreover, the concurrent existence of the two genotypes has been addressed in Europe, North America, and Asia, perplexing the diagnosis, prevention, and handling of the PRRS disorder (5).

PRRSV treated pigs normally suffer from slow growth and are most likely to catch co- or secondary bacterial and viral infections (79) caused by the followings (46):

- *Streptococcus suis*
- *Haemophilus parasuis*
- *Mycoplasma hyopneumoniae*
- *Pasteurella multocida*

- *Actinobacillus pleuropneumoniae*
- *Psudorabies* virus (Aujesky's disease)
- Classical swine fever (CSF)
- PCV2
- Swine influenza virus

3. Clinical Signs of PRRS

The clinical signs may vary between herds depend on the PRRSV strain that induced the disorder, the health status of the pigs (presence or absence of other infections), and the farm management system. With regard to reproductive and respiratory dysfunctions, younger pigs are more often contagious than older animals, with unbred boars and sows often exhibiting no signs of the disease. The incubation time persists from 3 to 37 days (7, 94), and the clinical signs are comparable to other viral or bacterial pig disorders, thus the diagnosis must not be dependent only on clinical signs and postmortem observations (46)

4. Infectious Activity of PRRSV

PRRSV is highly contagious and remains in treated pigs, which can spread the virus for a long period of time. Adult pigs can spread the virus for 14 days, and

growing pigs for 1 to 2 months (116). Most treated pigs ultimately become immune, but transmission will persist in a farm as long as non-infected or naïve animals are placed with infected pigs because the virus can spread to them. When this occurs, the cycle of transmission to responsive animals will be longer and PRRSV may stay active for a long time. To inhibit this occurrence, some breeding farms 'stabilize' the transmission by encouraging immunity in all breeding stock or exercise separated breeding of offspring and acclimatization of switch breeding stock or, a combination of these approaches (58).

4.1 Breeding Herd

From 3 to 6 weeks, infected pigs show the initial phase of inappetence and fever which spreads among the breeding herd. Cyanosis and blue colorization of ears is not usual and less than 5% of sows exhibit such a sign. Coughing happens in some sows and a few cases of pneumonia may be reported. This acute stage goes on for up to 6 weeks and is featured by early farrowing, elevated stillbirths, weak pigs, and an elevated number of large mummified pigs that have become decreased in the last three weeks of pregnancy. In some herds, this evidence may be found in up to 30% of pigs born. Stillbirth, abortions, and piglet death climaxes at 70% in 3 or 4 weeks. Reproduction and fertility rates revert to pre-infection levels after 8 to 12 weeks although reproductive dysfunction may continue for 4 to 8 months before being restored to normal again (46).

4.2 Piglets

Sows exposed to PRRSV while pregnant can miscarry or carry to full term at which point the piglets can be mummified, stillborn, or very weak, and large numbers may not be well-nourished. Splay leg, diarrhea, pneumonia, and coughing are frequently seen. Some newborn piglets may show adhesive brown material over the eyelids, and sometimes blisters are observed on the skin. As time goes by, health improves and more piglets survive (46).

4.3 Weaned and Growing Pigs

When a naïve herd is exposed to PRRSV for the first time, very few clinical symptoms (only decreased feed intake and mild coughing) are observed. However, the clinical symptoms become more serious when other disorders such as enzootic pneumonia concurrently breakout in the herd. Pigs may become contagious as maternal antibodies vanish and then remain viremic for 3 to 4 weeks, continuously producing the virus. The disorder can be observed from 4 to 12 weeks of age and presents as inappetence, wasting, coughing and pneumonia. Bacterial exposure is apparent in pigs from 12 to 16 weeks of age when large numbers exhibit lameness and fatality rates increase to between 12 and 15% (46).

5. PRRSV Immunity

The goal of immunization is to develop immunity that will defend humans or

animals against clinical disease, but immunization will not halt transmission. It is significant to establish farm-specific immunization programs derived from individualized farm's diagnostic data rather than advanced standard protocols. Because of the necessity of CMI to handle PRRS, MLV vaccines seem to be more effective than ones with an inactive virus. On the other hand, MLV vaccines must not be administered to PRRSV-negative herds, pregnant females, or breeding age boars. Moreover, multi-vaccine products have been recently released to the market such as ones against PRRSV, *Haemophilus parasuis*, *Erysipelothrix rhusiopathiae*, parvovirus, and *Leptospira interrogans spp.* (46).

However, commercial PRRS MLV vaccines of either the NA-type or EU-type genotype invoke relatively weak humoral and CMI responses. PRRSV-specific antibodies show at nearly 2 weeks and reach a maximum at 4 weeks after vaccination. Most of the antibodies are against viral nucleocapsid proteins but have no neutralizing action although they do present some clinical defensive action that is as yet undiscovered. PRRSV-specific neutralizing antibodies show nearly 4 weeks after immunization and have relatively low titers (nearly 2^3-2^5) during the entire course of immunization. (26), the cause of which is explicitly related to the presence of decoy neutralizing epitopes and the heavy glycosylation of the major and minor neutralizing epitopes (36).

A PRRSV-specific CMI response presents nearly 2-4 weeks after immunization and is affected by lymphocyte blastogenesis and interferon (IFN) secretion in a memory reaction. Most of the T cell subsets that react with PRRSV are $CD4^{+}CD8^{lo}$ and $CD4^{-}CD8^{+}$ (93), which are recognized as porcine memory T cells and cytotoxic T cells, respectively (19). PRRSV-specific T cells secreting IFN-γ elevate steadily with age, reaching a maximum at nearly 32 weeks after immunization. This is a very slow activity compared to the T cell response to the pseudorabies virus (PRV) MLV vaccine, which presents within 1 week of immunization and reaches a near maximum at 4 weeks after immunization (93). It has been acknowledged that the rationale for slow and weak CMI reactions to PRRSV is due to the virus-mediated suppression of type I IFN and other pro-inflammatory cytokines, such as interleukin (IL)-1, IL-12, and tumor-necrosis factor α (TNF-α) (157). The unsatisfactory CMI reaction could be caused by the virus's capacity to upregulate anti-inflammatory cytokine secretion (for example interleukin (IL)-10), modify growth factor-β in treated cells, and cause a regulatory T cell reaction (18).

Classical immunology claims that virus-neutralizing antibodies are the first line of defense against free virus particles, and a virus neutralization test provides an important indicator for the humoral protection index in a virus-infected host. However, PRRSV is different from other common virus infection as either

infected or vaccinated pigs respond to the PRRSV proteins by producing virus specific antibodies, but the early antibodies do not show virus-neutralizing activities (72). Viremia lasts a long time in infected pigs but gradually diminishes, and the total antibody response is similar to that against other viral infections although the appearance of serum neutralizing antibodies is independent of viral clearance (78). This indicates that virus clearance is not directly responsible for the protection even though neutralizing antibodies are an important factor in the humoral protection mechanism (80).

The PRRSV infected host either does not produce or show significantly reduced production of IFN, which is an important host defense mechanism in virus-infected cells that eventually prevents virus spread to adjacent cells. Viral clearance in the blood and viral load in the lymphoid tissue does not co-relate with CMI, and an IFN-γ assay is neither an absolute nor the only indicator in the evaluation of CMI although it is commonly used to do so. Highly variable numbers of T cells in either virally acute or persistent animals have been detected and showed no close correlation to the level of the virus in lymphoid tissues. No significant changes in $CD4^+$ and $CD8^+$ T-cell frequencies after PRRSV infection have been observed, although a decrease in the number of gamma and delta T cells has been recorded (156). This result supports that there is no or very little contribution of CMI toward fighting PRRSV infection and suggests that PRRSV

suppresses T-cell recognition of infected macrophages (80).

6. PRRSV MLV (Attenuated) Vaccine

The severe economic crisis induced by PRRSV in breeding herds has led the swine industry to pay attention to the usage of MLV PRRSV vaccines in gilts and sows. For instance, the latest study has demonstrated that breeding herds immunized with MLV vaccines in a PRRSV control program had their reproductive function immediately restored and incurred fewer total deaths than herds where a live-resident virus immunization method was adopted. Hence, it is meaningful if PRRSV vaccines can assist with inhibiting the negative reproductive effects of PRRS (e.g. miscarried litters, dead/low survival rate of piglets, etc.) when applied to gilts and sows, and to enhance respiratory wellbeing when applied to growing pigs (74).

PRRS MLV vaccines are registered for use in many countries worldwide. The MLV vaccines registered for use in the US originate from the NA-type PRRSV and, comprise Ingelvac PRRS MLV® and ReproCyc PRRS-PLE (both from VR-2332; Boehringer Ingelheim), and Ingelvac PRRS® ATP (from JA-142; Boehringer Ingelheim). Moreover, the MLV vaccines registered for use in the EU countries originate from the EU-type PRRSV and include Porcilis PRRS (from DV; Merck), Amervac PRRS (from VP046; Hipra), and Pyrsvac-183 (from All-

183, Syva). The MLV vaccines registered for use in other countries may not be limited to using either virus genotype and may be usable for both PRRSV genotypes (20).

6.1 Protective Efficacy

The usage of commercial MLV vaccines in PRRSV-infected breeding herds has imbued advantageous effect on their health and activity, lowered the abortion and rebound to estrus levels, and elevated farrowing levels and the number of weaners (1). Moreover, MLV immunization of gilts defends them from viremia and lowers the numbers of pre- and post-natal deaths and congenitally infected piglets (130). During birth, piglets from immunized gilts have high body weights and survival level during the weaning period than those observed from non-immunized gilts (123). The latest field studies in endemic PRRSV-infected farms experiencing severe reproductive dysfunction show that the MLV immunization of breeding stock can (a) enhance the reproductive function of the gilts and sows, (b) lower viremia, (c) reduce morbidity and mortality, and (d) improve the growth activity of the piglets (107).

Based on a number of studies, the usage of MLV commercial vaccines has advantageous effects on clinical disease outbreak and severity as well as the period of viremia and virus spread (68, 130, 143). MLV immunization can cause

virus neutralizing (VN) antibodies and defend against viremia, virus replication in the lungs, and virus caused respiratory and reproductive disorders (129, 159). Specifically, the usage of MLV commercial vaccines in piglets has ended up decreasing viremia and the severity of respiratory clinical symptoms, and enhancing their growth activity (12). In dual-infected farms by both PRRSV and PCV2, the MLV immunization of piglets (at approximately 5 weeks old) enhances their growth activity (70).

A number of studies have emerged the efficacy of MLV vaccines. The defensive immune reaction induced by commercial MLV vaccines are affected by genetic differences, as these vaccines do not adequately defend (or only to some degree) against re-transmission and transplacental transmissions caused by heterologous PRRSV strains at the time (68, 130, 143). Incidentally, the latest study has reported that immunization of piglets at 5 weeks of age with a commercial MLV vaccine induced a clinical defensive action related to an effective CMI reaction, when the immunized pigs were exposed to a heterologous wild-type strain (90). It may happen that farmers adopting an MLV vaccine experience reduced herd fertility for the first time, and experiments with MLV immunizations of breeding stock have indicated the occurrence of acute PRRS-specific clinical symptoms, associated with the elevation of late period abortions, increased numbers of stillborn and mummified piglets, and decreased numbers of live born and weaned

piglets (10, 29).

6.2 Safety

The main interest in MLV vaccines is on safety and primarily the feasibility of inversion of the impaired virus to virulence because of genetic mutations in the vaccine virus and/or recombination with wild-type virulent PRRSV strains (95). Lab and field studies have indicated that MLV strains may induce viremia or become restored to virulence and be spread transplacentally and horizontally within immunized herds (infection of non-immunized pigs) and to the closest non-immunized herds (10, 68). Viruses in a restored-to-virulence MLV vaccine may possibly transverse the placenta during the late period of gestation and induce elevated numbers of mummified and stillborn piglets, and piglets born to MLV-vaccinated sows can become conveyers of PRRSV by spreading the MLV vaccine virus to other new pigs (123).

In addition, an MLV vaccine can induce clinical respiratory symptoms and cause poor growth activity of immunized piglets (123), and it can cause viremia with the possibility of transmitting the MLV vaccine virus to new animals for at least 4 weeks (146). Therefore, farmers employing an MLV vaccine for the first time may have the experience of reduced herd fertility, the elevation of delayed abortions, and an increased number of stillborn and mummified piglets (10, 29).

Furthermore, according to the latest studies, there is the additional point that the MLV immunization could prohibit the defensive efficacy action of *Mycoplasma hyopneumoniae* vaccines (119).

Commercial EU-type MLV vaccines offer efficient genotype/strain-specific defensive action with only a small proportion of defensive action against genetically heterologous PRRSVs and induce rather late humoral and CMI reactions, which lead to delayed defensive action (90, 130). Incidentally, the MLV vaccine virus has the potential risk virulence being restored and inducing clinical disorder (95). In contrast, the EU-type KV vaccines contribute insufficient efficacy because of the low activation of the immune system and no effective transformation of VN antibodies, which could have an important role in the defense against either homologous or heterologous PRRSVs. Nevertheless, KV vaccines can cause a powerful CMI reaction, which can be related to defensive action when administered to PRRSV-infected pigs (129, 159).

6.3 Commercial Product

6.3.1 Ingelvac PRRS MLV®

Ingelvac PRRS MLV® vaccine, originating from the VR2332 isolate after the fourth sequential passage of the virus in a monkey kidney cell line (CL2621) was launched onto the US market in 1996 (the fourth sequential passage in the cell culture attenuates the vaccine virus). The vaccine viruses grew much better in MARC-145 cells (a clone of African green monkey kidney cell line MA104 (60)) than their maternal wild-type viruses (67).

Ingelvac PRRS MLV® is a MLV PRRS vaccine proven safe in pigs and also in gilts and sows at any stage of the reproductive cycle in PRRSV-positive herds. It allows cross protection against heterologous strains (including the Lelystad virus) and has been shown to significantly lower reproductive dysfunction and respiratory disorder induced by PRRSV (8).

The manufacturer recommends administering Ingelvac PRRS MLV® via the intramuscular (IM) route with a single 2 mL dose for pigs of adult breeding-age or pregnant females. The time to immunity requires at least a 4 months post-immunization period. Revaccination of the herd except for boars may be needed every 3 to 4 months or as guided by a veterinarian. Moreover, immunized pigs,

sows, and gilts should not be processed for human intake before 21 days post-immunization (8).

However, there are disadvantageous aspects of MVL vaccines. Since the vaccines use live viruses even if attenuated, inversion of the vaccine virus to virulence has become a crucial problem and was reported by Danish pig growers after the MLV vaccine was launched in 1995 onto the Danish market to control PRRSV. After the launch in 1996, viruses closely associated with the MLV vaccine virus were extracted from severely infected pigs (81). Because there was no NA-type PRRSV detected in Denmark or other European countries until 1996, it was determined that these field isolates came from the vaccine virus (145). A number of field isolates, which are genetically similar to both the vaccine virus and its parental strain, are continually being discovered in clinical PRRS cases (155), boosting the necessity for a proper way to distinguish wild-type and vaccine viruses (66).

6.3.2 Fostera PRRS®

Fostera PRRS® from Zoetis, is the only PRRSV vaccine, which 1) prohibits reproductive dysfunction when used in pregnant females with a period at least 19 weeks of immunity, and 2) also prevents respiratory disorders related to PRRSV with a period at least 26 weeks of immunity. Introduced in 2012, Fostera PRRS® was developed based upon innovative research by Zoetis® scientists who

discovered an important cellular receptor protein for PRRSV, thus permitting the generation of useful cell lines. This vaccine virus was developed based on the virulent NA-type PRRSV isolate (P129) and was attenuated using CD163-expressing cell lines (35).

Fostera PRRS® was registered for the immunization of healthy swine 1 day of age or older in PRRSV-positive herds as an aid in preventing respiratory disorders induced by PRRSV. A 26-week immunity period has been demonstrated against respiratory disorders. Administration is recommended as a single 2 mL intramuscular (IM) dose consisting of the antiseptic rehydrated freeze-dried vaccine along with sterile diluent. The safety of this product has been demonstrated when gilts were vaccinated 6 weeks before breeding or sows at any stage of pregnancy (35).

7. PMWS

PMWS was first reported in Saskatchewan (Canada) 1991 (50). At that time, it was definitely identified as a new disease and is characterized by wasting, paleness of the skin and jaundice in nursery and fattening pigs. Infected animals typically show lesions in multiple tissues (i.e. multisystemic), mostly in the lymphoid organs (53, 120). In 1997, the presence of PCV antigen was proven to be associated with lesions of animals showing PMWS (23). Nucleotide sequence

analysis of the PCV related to PMWS exhibited significant discrepancies compared to the earlier discovered PCV originating from porcine kidney cell line PK-15 cells (ATCC CCL-33) (48), identified as PCV1 for the cell culture-derived virus, and PCV2 for the virus associated with the new disease (3).

After 1997, studies on PCV2 have primarily concentrated on reproducing PMWS symptoms, and even though PCV2 was not thought to be the main element causing PMWS, little skepticism existed on the linkage between the virus and the wasting syndrome (60). Furthermore, the clinical and pathological aspect of PCV2 infection has been broadly studied since 1991. Even though PCV2 was a virus mainly related to PMWS, it is also thought to be involved in other conditions. PCV2 has been reported to cause reproductive disorders (99), porcine dermatitis and nephropathy syndrome (PDNS) (4), porcine respiratory disease complex (PRDC) (52), proliferative and necrotizing pneumonia (115), and congenital tremors (144).

However, PMWS is not induced by PCV2 infection alone, but also by utilizing infectious DNA clones of the virus or a pure form of PCV2 originating from infectious DNA clones. Hence, it has been predicted that PMWS is a multifactor-based disease. PCV2 is required but is not enough for the development of PMWS since the viral infection by itself seems to cause only mild disease, and co-factors

such as other infections or immunostimulation have been implicated in the development of severe diseases. For example, co-infection with porcine parvovirus or PRRSV, or immunostimulation has been shown to lead to elevated replication of PCV2 and more severe disease in PCV2-infected pigs (33). Furthermore, there was no significant relationship between the disease and virus sequence variation in infected or control pigs.

Many pigs infected with PCV appear to develop secondary bacterial infections, such as Glässer disease (*Haemophilus parasuis*), pulmonary pasteurellosis, colibacilosis, and salmonellosis, among others. Postmortem lesions might be found in multiple organs, especially in lymphoid tissues and lung, evoking the term 'multisystemic'. Lesions can infect the skin, kidney, reproductive tissue, brain, and/or blood vessels (101). Wasting pigs have shown the most widely known signs of PMWS infection, elevating the mortality rate significantly. Nowadays, PCV disease (PCVD), as named by the European Industry and PCV associated disease (PCVAD), as named by the North American Industry (both are other acronyms of PMWS) have been found in domestic pigs. These disorders induce symptoms in piglets including severe loss of body condition, enlarged lymph nodes, difficulty in breathing, and sometimes diarrhea, pale skin, and jaundice (33). PCVD has had a devastating effect on the swine industry and has been reported worldwide.

Since the early 1990's, PCV2 has been associated with a number of disease manifestations including PMWS, respiratory disease, PDNS, reproductive failure, enteritis, and neuropathy (13). The combination of all the described disease manifestations is today known as PCVAD.

8. PCV2

PCV was initially discovered in 1974 as a contaminant in the continuous PK-15 ATCC CCL-33 (147) and was proven to be non-pathogenic in swine after a while (149). In 1997, a new genotype of PCV was discovered in swine living in North America and Europe which caused the wasting disease; it is referred to as PCV2 as opposed to the original genotype, which is now called PCV1 (2).

PCV2 is a member of the family *Circoviridae*, genus *Circovirus*, which consists of small (17 nm in diameter), non-enveloped DNA viruses with a unique single-stranded circular genome. PCVs replicate in the nucleus of infected cells by utilizing host polymerase for genome amplification. PCV1 (first identified in 1974) instantly infects but is known to not induce disease in swine, but PCV2 (first identified in 1997) has created problems in recent years because of elevated cases of PMWS, and leads to significant depletion of lymphocytes, enlarged lymph nodes, and abnormal lung tissues (89)..

PCV's genome has one of the simplest structures of all viruses and consists of only two open reading frames (ORFs): a capsid protein (ORF2) and two replicase proteins (ORF1) to generate and replicate the functional virus. Due to the simple components of PCV, it must depend tremendously on the host's cellular mechanism to replicate. The ‘Cap’ site in the ORF2 region is slightly dissimilar between PCV1 and PCV2 and may clarify why PCV1 is non-pathogenic, whereas PCV2 is pathogenic (54). The PCV2 genome has three main ORFs: ORF1 encodes viral, replication-associated proteins (21, 22), ORF2 encodes the viral immunogenic protein, which has been the research area for creating a recombinant vaccines (41), and ORF3 encodes an apoptosis-associated protein that has a significant roles in the pathogenesis of PCV2 (76).

The genomic structures of the pathogenic PCV2 and the non-pathogenic PCV1 are slightly different; they share about 68–76% nucleotide sequence identity in the genome (48), and dissimilarities in the transcriptional patterns and antigenic profile of the capsid protein have been revealed (21, 22). The two primary genes encoded by the viral genome involve the 942-base-pair ‘Rep’ gene (88) and the 702-base-pair ‘Cap’ gene. The ‘Rep’ gene is preserved between PCV1 and PCV2 at approximately 83% nucleotide sequence identity, while the ‘Capsid’ gene is less so at only 67–70% identity (87).

So far, at least three PCV2 subtypes have been found worldwide: PCV2a, PCV2b, and PCV2c (31). PCV2a and PCV2b have both been associated with PCVAD with various levels of severity (103). Before 2005, only PCV2a had been identified in the United States and Canada, while both PCV2a and PCV2b had been observed in Europe and China. After 2005, PCV2b strains have been found in the United States, thus a global change in subtype leading to a predominance of PCV2b with concurrent elevated severity of PCVAD has been in progress in swine populations. The pathogenicity of PCV2c is unknown, as it has only found in non-diseased herds in Denmark (31).

PCV infects a wide variety of cell types, including hepatocytes, cardiomyocytes, and macrophages. A recent study has indicated that PCV uses clathrin-mediated endocytosis to enter the cell, although there may be other elements that have not yet been discovered. When endocytosis occurs, endosome and lysosome formation induces an acidic pH change, which permits ATP-driven un-coating of the virus that are then able to exit from the endosomes and lysosomes. After this, the virus moves toward the nucleus through unidentified measures (75).

9. Clinical Signs of PCV2

PCV2 associated reproductive failure is typically featured by elevated numbers of abortions, mummified and stillborn fetuses, and piglets born that are weak (99).

Identification of PCV2 as the causative agent of reproductive failure has also been linked to myocardial fibrosis and lymphoplasmacytic myocarditis, and the relationship between the PCV2 antigen and fetal heart lesions has been investigated by immunohistochemistry (IHC). Newborn piglet serum or fetal thoracic fluid has also been proven to be positive for PCV2 DNA or PCV2 specific antibodies (86).

PCV2 can shed through oral and nasal secretions, and fecal excretions (113), and has been observed in boar semen without destroying sperm morphology (85). It has also been found that insemination of naïve gilts or sows with semen having low levels of PCV2 DNA did not result in virus transmission, or reproductive failure, but insemination with semen spiked with high levels of PCV2 DNA was able to induce reproductive failure in naïve gilts and sows (83).

The signs and symptoms of PCV2-induced PMWS included wasting, paleness of the skin, respiratory distress, diarrhea, and sometimes icterus (32). Other signs and symptoms are pseudorabies, PRRS, porcine parvovirosis, Glasser's disease, *streptococcal meningitis*, salmonellosis, postweaning colibacillosis, non-specific diarrhea, dietetic hepatosis, and suppurative bronchopneumonia (*Pasteurella multocida*, *Bordetella bronchiseptica,* and *Streptococcus suis* are the major bacteria involved) (141).

During necropsy, the most noticeable lesions found have been non-collapsed lungs and enlargement of the lymph nodes (mainly the superficial inguinal, submandibular, mesenteric and mediastinal ones) (120). At a low level, lymph nodes have shown multifocal areas of necrosis that can be seen macroscopically (134). Non-collapsed, tan-mottled lungs, sometimes with interstitial edema, has been a rather common feature of PMWS. PMWS-affected animals also showed atrophic, discolored livers (icterus is an apparent finding usually in these cases), and/or multifocal white foci in the kidney cortices. Relatively high numbers of PMWS-affected pigs have shown bronchopneumonia and gastric ulceration of the pars oesophagea which could not be associated with the direct effect of PCV2; for example, bronchopneumonia has been linked to bacterial infections, while gastric ulceration has a multifactor-based origin. However, the stomach lesions have induced internal hemorrhaging and has been the cause of death for a number of pigs with PMWS (134). At the end phase of the disease, cachexia may occur.

The most typical microscopic lesions in PMWS-affected pigs have been observed in lymphoid tissues. Different levels of lymphocyte depletion with loss of follicles have been shown in almost all pigs having PMWS. Usually these findings have been consolidated with multifocal to diffuse, slight to very intense histiocytic and/or multinucleated giant cell infiltration. Another major finding has been the appearance of sharply detached spherical basophilic cytoplasmic inclusions of

PCV2 in histiocytic cells. Subacute interstitial pneumonia has caused the most typical lung lesions in PMWS-affected pigs (120). In some instances, large histiocytic and multinucleate giant cells have appeared in the thickened interalveolar walls and/or within the alveoli, and on chronic occasions, bronchiolitis fibrosa obliterans might appear. Hepatic lesions have been depicted as lymphocytic-histiocytic inflammatory infiltration in portal zones, single cell necrosis of hepatocytes, swelling and vacuolation of hepatocyte cytoplasm, and karyomegaly (23).

However, on some occasions, it is feasible to identify very severe lesions displaying perilobular fibrosis with derangement of hepatic plates leading to a huge loss of hepatocytes. These lesions can be related to icterus and macroscopic lesions in the liver (121). Other microscopic lesions observed in PMWS-affected pigs sometimes include lympho-histiocytic inflammatory infiltrates in the kidneys, pancreas, intestines, and myocardium (120), and moderate to severe granulomatous enteritis with blunting of villi has been identified from time to time.

10. Infectious Activity of PCV2

Infection with PCV2 has been seen by both horizontal and vertical routes. The virus has been found in feces and urine as well as nasal and oral secretions (131), and its existence in such secretions and excretions has been proven to allow

horizontal infection when PCV2-infected pigs have been reared with naïve pigs (112). PCV2 can be transmitted to naïve pigs between 1 and 42 days after challenge (9). Moreover, the capability of PCV2 to traverse the zona pellucida and be transmitted to oocytes (91) or the placenta and be transmitted fetuses, or else to disseminate intrauterinely and be transmitted to fetuses (114) permits vertical transmission, and previous experimental vaccination studies on pregnant breeding animals have demonstrated this (83). These studies have witnessed the fact that PCV2 is not only widespread, but can infect through various routes as well.

10.1 Sows

PCV2 can be transmitted to breeding females leading to subclinical infections (82). Sows sometimes do not exhibit clinical signs of disease, but PCV2 viremia has been shown during pregnancy, although PCV2 can traverse the placenta and be transmitted to fetuses. In field situations, the natural transmission of PCV2 to pregnant sows has led to late-term abortions featured by elevated numbers of mummified fetuses having sufficient amounts of PCV2 antigen in the myocardium (154) as well as elevated mortality rates in piglets. Pregnant sows that have been intranasally challenged with PCV2 have shown elevated numbers of stillborn and mummified fetuses (111). Furthermore, reproductive dysfunction has been shown to occur by spiking semen with PCV2 and manually fertilizing naïve sows (83).

10.2 Fetuses

After intranasal infection of sows, PCV2 antigen and DNA have been detected in lymphoid tissues and other organs expressing PCV2 replication in fetuses (111). PCV2 aims for the myocardial tissues for replication resulting in vasculitis and cardiac failure in fetuses in some cases. Furthermore, PCV2 antigen has been observed in the myocardial tissues and tonsils of stillborn and mummified fetuses (84). In one study, healthy piglets in the US and Mexico were examined for the occurrence of PCV2 viremia and PCV2-specific antibodies before colostrum uptake, and 39.9% and 21.4% of the animals, respectively, were shown to be positive (136). Such an outcome indicates that vertical transmission of PCV2 has become frequent.

11. PCV2 Immunity

Previous studies have exhibited that lymphoid depletion impacts B, T, and natural killer (NK) cells (97, 138), and it has commonly been found that the relative levels of neutrophils and lymphocytes in peripheral blood change in animals with PMWS compared to healthy ones (28). Several research outcomes have found that PCV2 can be detected in the thymus of infected animals; this organ has also shown severe lymphocyte depletion (60, 158), and the presence of thymic lesions was concurrent to the occurrence of lymphocyte depletion in other tissues (125). After 10 days post-inoculation, it has been observed that PCV2 induced

significant amounts of $CD4^+$, $CD8^+$, and IgM^+ cells in piglets inoculated at 1 day of age. Later, Yu et al. (158) indicated that concanavalin A elevated replication of PCV2 in peripheral blood mononuclear cells (PMBCs) and that particularly, activated T lymphocytes sustained replication of the virus.

Some studies have shown significant alterations in the PBMCs of infected pigs. In one study, pigs with PMWS exhibited elevation of circulating monocytes, a decrease in T cells (mainly $CD4^+$) and B lymphocytes, and the appearance of immature granulocytes when contrasted with clinically healthy, non-PCV2 infected pigs (96). In another study, a significant diminution on T cells (mainly $CD8^+$ and $CD4^+/CD8^+$) and B lymphocytes was identified when contrasting PCV2 infected pigs with diseased, non-PCV2 infected or healthy pigs. Moreover, significantly lower numbers of T $CD8^+$ and B lymphocytes were observed in pigs with moderate to severe lesions and a moderate to high quantity of PCV2 in tissues (typical PMWS cases) when contrasted to pigs with slight lesions and a low quantity of virus in tissues (28). All together, these results indicate an incapacity in pigs seriously ill with PMWS to stimulate an effective immune response.

In general, IFN-γ, which is generated by PCV2-specific IFN-γ secreting cells (SCs), is a major immunoregulatory cytokine that regulates the differentiation of

naïve $CD4^+$ into stimulated $CD4^+$ cells and modulates CMI against viral infections (128). The outcome of one study indicated that increased numbers of $CD4^+$ cells were found in immunized animals only, and lymphocyte subset analysis showed that the numbers of $CD3^+$ and $CD4^+$ cells were elevated in immunized animals but the numbers of $CD4^+$ cells reduced temporally in non-immunized animals. This particular deficiency of $CD3^+$ and $CD4^+$ cells observed in pigs with PMWS (97) might indicate damage to the immune system and lead to co-infections by other viral and bacterial pathogens since co-infections often occur in pigs with PMWS under field conditions (63).

Because $CD4^+$ cells stimulate a delayed-type hypersensitivity (DTH) response seen in immunized animals only, this advocates a $CD4^+$ cell-related protective cellular immune response caused by a reformulated inactivated chimeric PCV1-2 vaccine. PCV2-specific memory T lymphocytes led by the chimeric PCV1-2 vaccine invoked DTH reactions after intradermal injection of the PCV2 antigen. Therefore, PCV2-specific neutralizing antibodies (NAbs) and IFN-γ-SCs by the chimeric PCV1-2 vaccine induced an important protective immune response that led to decreased PCV2 viremia and handled the PCV2 infection (126).

Previous studies dealing with the cytokine schemes in the tissues or PBMCs of pigs with PMWS (25, 62) proposed that a frequent observation in lymphoid tissues was

a high level of IL-10 expression. It was also addressed that the elevated IL-10 levels in mandibular lymph node, spleen and tonsil of PCV2 infected pigs. Such an IL-10 expression was primarily located in T-cell rich areas and scarcely in B cell or macrophage rich areas (30). Crisci et al. (24) also addressed that IL-10 was elevated in PMWS-affected pigs and this generation was related to $CD163^+$, $CD4^+$, and $CD8^+$ cell subpopulations in the spleen. Ex vivo data proposed that PMWS-affected animals showed high IL-10 in serum (54). Interestingly, the stimulation of PBMC in PMWS-affected pigs with PCV2 led to high levels of IL-10. In addition, PCV2 could induce strong IL-1α and IL-8 reactions in the PBMCs of both naive and PCV2-infected pigs (25, 27) associated with the chronic inflammatory nature of PMWS. For instance, in the lungs of pigs experiencing PCV2-associated respiratory disease, increased levels of IL-1α and IL-8 mRNA were seen whereas IL-10 was not found, which might be the phenomena to predict in a pig with interstitial pneumonia (16). Alveolar macrophages inoculated with PCV2 generated significant amounts of TNF-α and IL-8 as well as exhibited the upregulation of neutrophil chemotactic factor-II, granulocyte colony-stimulating factor, and monocyte chemotactic protein-1 levels (17).

In the events of PCV2 infection, viral clearance was believed to be regulated by the collaboration of NAbs and CMI. A previous study (117) addressed that NAbs were formed about the fourth week post-inoculation in the case of PCV2 infection.

Later, it was found that levels of viral replication associated with the presence of various schemes of adaptive responses to PCV2 infection. Thus, pigs with elevated NAbs and IFN-γ reactions induced the minimum levels of viral replication whereas pigs with weak or no responses of NAbs and IFN-γ induced the maximum levels of viral replication (92). Moreover, the natural events of PMWS were investigated with subclinically PCV2-infected pigs. It was shown that the levels of NAbs might be related to the clinical status of the pigs and viral loads became a new indication of the significant role of NAbs in the defensive action against PCV2 (40). In sows, the role of NAbs needs to be clearly defined because contagious PCV2 can be found in the milk and colostrum of naturally infected sows even in the presence of high anti-PCV2 IgA antibodies and NAbs levels in serum and colostrum (43).

Virus- or cytokine-derived apoptosis has also been reported as a mechanism of action for illustrating lymphoid depletion in PMWS. Shibahara et al. (138) proposed that PCV2 could lead to the apoptosis of B-lymphocytes, while Kiupel et al. (69) also proposed the characterization of apoptosis in lymphocyte depletion in relation to PCV2 in a mouse model. In addition, PCV2 is thought to trigger the nuclear factor kappa-light-chain-enhancer (NF-κB) pathway in PK15 cells (this pathway has been implicated in the secretion of various cytokines inducing apoptosis). However, Vincent et al. (151) failed to display apoptosis in lymphocytes dually

cultured with contagious dendritic cells. Briefly, lymphoid depletion in PMWS might be caused by the synergy of apoptosis, viral-induced lysis, the destruction of the lymphoid organization, and other as yet unknown pathways.

In addition other laboratory outcomes and field findings have advocated the hypothesis of immunosuppression in PMWS-affected pigs: 1) the appearance of lymphocellular depletion in both follicle center and parafollicular areas along with histiocytic and multinucleate giant cell infiltration in infected lymphoid tissues (23); 2) PCV2-induced apoptosis obviously occurred in lymphocytes in pigs with PMWS (138); 3) alterations in cell subpopulations of lymphoid tissues (127) featured by the reduction of B and T lymphocytes; and elevation in the expression of swine leukocyte antigen class II molecules; 4) appearance of *Pneumocystis carinii*, *Aspergillus spp*. and *Chlamydia spp*. (opportunistic pathogens usually related to immunosuppression) have been observed in the lungs and intestine of pigs with PMWS (23); 5) many pigs infected with PMWS had also contracted pulmonary and/or septicaemic infections caused by bacteria such as *Pasteurella multocida* or *Haemophilus parasuis* (134); and 6) typical lesions related to PRV have been described in pigs with PMWS (118). Therefore, it has been proposed that the immune systems of pigs with PMWS were immunosuppressed, and it is considered that immunostimulation could be a triggering factor for the progression of the syndrome, while immunosuppression is deemed to be the

outcome of seriously infected pigs (132).

12. PCV2 Vaccine

PCV2 vaccines were initially developed to control PMWS, but they are now also used against other PCVADs. To identify an effective vaccine, it is important for veterinarians to focus on the types of commercial vaccines; the criteria of vaccine efficacy; the clinical, virological, immunological, and pathological efficacy; and the use of PCV2 vaccines against different clinical manifestations of PCVAD in the farm (14).

Quantitation of PCV2 viremia could help interpret the extent of the PCV2 infectious condition. A number of studies have already indicated that the PCV2 DNA quantity in serum is higher in pigs with PMWS than in subclinically affected pigs (77, 120). Hence, the decrease of PCV2 viremia induced by the PCV2 vaccine performs an important role in handling PCV2 infection. In a recent study, it was demonstrated that the reformulated inactivated chimeric PCV1-2 vaccine was able to induce PCV2-specific NAbs and IFN-γ-SCs in vaccinated animals. However, it has been proposed that the appearance of anti-PCV2 IgG antibodies is not associated with the decrease in PCV2 viremia. The reformulated inactivated chimeric PCV1-2 vaccine also diminished the PCV2 load in nasal shedding in immunized animals, thus reducing the chance of infection of other

pigs via the nasal route and decreasing the circulation level of PCV2 among the pigs (105).

Seo et al. (135) proposed the efficacy of the reformulated inactivated chimeric PCV1-2 vaccine under field conditions. Clinical examination showed that vaccinated animals exhibited an improved average daily gain (ADG), and virological evaluation showed that vaccinated animals exhibited a decreased PCV2 load in the blood and nasal swabs compared to non-vaccinated animals. Moreover, pathological examinations indicated that the vaccination of pigs against PCV2 effectively decreased the number of PMWS-associated lesions and the PCV2 load in lymphoid tissues compared to non-vaccinated animals, and immunological evaluation exhibited that vaccinated animals produced PCV2-specific NAbs and IFN-γ-SCs. To be specific, a decrease in the PCV2 load in the blood coincided with the presence of both PCV2-specific NAbs and IFN-γ-SCs in the vaccinated animals, the number of $CD4^+$ cells was reduced in non-vaccinated animals compared to vaccinated animals, and the reformulated inactivated chimeric PCV1-2 vaccine appeared to be very efficient in handling PCV2 infection based on clinical, virological, pathological, and immunological evaluations under field conditions (135).

Currently, various types of commercial vaccine products are available and they

have differences in antigen types. One vaccine has been developed on the basis of inactivated PCV2a viruses (6), while two subgroup vaccines have been made from capsid protein expressed in the baculovirus composition. Another chimeric PCV1-2a vaccine has been structured on the genomic backbone of PCV1 with the capsid gene substituted by that of PCV2a (38). Furthermore, it has been found that pigs immunized with $10^{3.5}$ or $10^{4.0}$ 50 % tissue culture infective dose ($TCID_{50}$)/ml doses of live-attenuated chimeric PCV1-2 vaccine formed high levels of antibodies and the immunized pigs were totally defended against challenges with PCV2. However, there has been a parallel comparison performed between the efficacy of inactivated and live-attenuated PCV1-2b vaccines with different doses ($2 \times 10^{3.5}$ or $2 \times 10^{4.0}$ $TCID_{50}$ dose) in growing pigs. In this study, it was demonstrated that pigs might be efficiently protected against a PCV2b challenge by immunization with inactivated or live-attenuated PCV1-2b vaccines. Moreover, Opriessnig et al. (101) showed that PCV1-2b vaccination significantly reduced the prevalence and amount of PCV2b viremia compared with PCV1-2a vaccination.

There is one concern with any live-attenuated vaccine that vaccine-induced viremia in immunized pigs can be transmitted among pigs and herds. Interestingly, a chimeric PCV1-2 virus was recovered from clinically healthy pigs on Canadian farms with no signs of PCVAD (42). Another concern when using live-attenuated chimeric vaccines with the PCV1 backbone was possibly causing disease

associated with PCV1. Earlier studies have indicated that PCV1 was implicated in congenital tremors in newborn fetuses (56), and recently, PCV1 has been found to induce hemorrhages in lung tissues of fetuses experimentally inoculated with the PK-15 cell-derived PCV1 isolate but not a field isolate of PCV1 (124).

12.1 Protective Efficacy

Efficacy and potential risk (safety) from the application of the vaccine were investigated in two laboratories and three field studies (34). Several studies have been conducted to evaluate the efficacy of the vaccines against acute and chronic types of PCVD. In all of the studies, the dose volume of 1 ml was administered to each animal. Under the experimental conditions, the efficacy of one dose of a standard reference vaccine was shown to decrease the lymphoid depletion and inflammation caused by PCV2 infection. Moreover, supportive outcomes such as decreased virus load in lymphoid tissues and decreased mean time period of PCV2 nasal shedding was observed. Efficacy of the vaccine was observed from 2 weeks after immunization up to 4 months. Under field conditions, decreased viremia (the proportion of animals showing viremia, time period of viremia, and viral loads in the blood) was continuously recorded, as well as decreased weight losses up to the end of the fattening phase within the scope of PCVD. Moreover, reduction of mortality was continuously recorded within the scope of acute PCVD. Data were acquired which indicated the claimed time period of 17-week

protection. Active vaccination of pigs over 2 weeks of age against PCV2 diminished mortality and clinical signs including weight loss and lesions in lymphoid tissues related to PCV2-induced PCVD. Furthermore, vaccination was shown to decrease PCV2 nasal shedding, viral load in blood and lymphoid tissues, and the time period of viremia. In conclusion, the efficacy of Ingelvac CircoFLEX® was properly demonstrated associated with the given indications (34)

12.2 Safety

Laboratory studies were performed to examine the safety of Ingelvac CircoFLEX® (34). A very detailed safety test of a single dose and repeat dose of PCV2 vaccine in 2-week-old pigs was established. The vaccine was not associated with any significant adverse responses throughout the study. A temporal elevation of body temperature was recorded in field studies 4 hours after immunization, but the elevation in body temperature under laboratory conditions lasted no longer than 24 hours. A small number of unpredicted deaths of piglets in both the vaccine and control groups was observed, which seemed not to be associated with the treatment of test materials. None of the preferred parameters were affected by the immunization. There were no lesions observed at the injection sites during the study. Microscopic evaluation of selected animals necropsied on days 7, 14 and 21 exhibited only mild to moderate muscle inflammation (compared to the responses observed in control animals). The results certainly indicate that

Ingelvac CircoFLEX® was well tolerated by the immunized animals (34).

The safety of repeated treatment with one dose was evaluated. No significant difference was detected at any time between the immunized and control groups in terms of rectal temperature, abnormal clinical signs, weight gain, or injection site responses observed throughout the study. Again, a low number of deaths were observed in both groups but this was not associated with treatment with the vaccine (34).

Administering pigs with a single overdose injection volume (4 ml) of vaccine or a placebo injection volume (4 ml) of water was carried out (34). These materials were injected intramuscularly (IM) in the right side of the neck. Good tolerance of the vaccine was acknowledged when no injection site responses were detected or palpated in any of the immunized animals. With regard to statistical analysis, no significant differences in body temperature were seen in treatment groups during the evaluation period. In both groups, temperature elevated a little above the baseline after treatment. The results demonstrate that an intramuscular injection of a 4 ml dose of vaccine in piglets of the recommended age (ex. manufacturer's user guide) for immunization was well tolerated in reference to local and systemic safety (34).

12.3 Commercial Product

12.3.1 Ingelvac CircoFLEX®

Ingelvac CircoFLEX® is a biotechnology based inactivated immunological veterinary medicinal product (34). This vaccine was designed to be applied for the active immunization of pigs against acute and chronic types of PCVD. This product has been developed as a vaccine against PCV2 by applying the baculovirus system employed to express the ORF2 capsid protein of PCV2. The development of such a subgroup vaccine was inspired by the fact that ORF2 has been scientifically identified to be the main immunogen protein, making use of the special features of the baculovirus expression system. The active component of the vaccine is expressed in insect cells after inoculation with a baculovirus vector containing the ORF2 gene of PCV2. The ORF2 antigen protects piglets against a PCV2 challenge, in accordance with the promotion of an active immune response against the virus (34).

Ingelvac CircoFLEX® is provided as a suspension for injection (34). It is intended to diminish mortality, clinical signs including weight loss and lesions in lymphoid tissues related to PCV2-caused PCVDs. In addition, immunization has also been demonstrated to decrease PCV2 nasal shedding, viral load and virus persistence in blood and lymphoid tissues, and the duration of viremia. The onset of protection

happens as early as 2 weeks post-vaccination and persists for at least 17 weeks. It is recommended to immunize pigs from 2 weeks of age by a single 1.0 mL intramuscular injection, regardless of body weight (34).

12.3.2 Fostera PCV®

Fostera PCV® vaccine (Suvaxyn PCV2 One Dose, Pfizer Animal Health/Fort Dodge Animal Health) is made from a chimeric PCV1-2 virus having the genomic backbone of the non-pathogenic PCV1 with the ORF2 capsid gene substituted by that of PCV2 (38). In 2008, Pfizer Animal Health temporarily eliminated the inactivated chimeric PCV1-2 vaccine product from the markets due to the incidental detection of a chimeric PCV1-2 virus in the field based upon unfinished inactivation of the vaccine. In August 2011, a reformulated version of the chimeric PCV1-2 vaccine under a new brand name (Fostera PCV®, Pfizer Animal Health) was re-launched onto the market for PCV2 viremia (42).

Zoetis Fostera PCV® is a KV vaccine for use in healthy pigs 3 weeks of age or older as an aid to prevent viremia and to control lymphoid depletion caused by PCV2. It provides protection against PCV2 infection in lymphoid tissues while helping to reduce lymphoid depletion in pigs challenged with virulent PCV2. This swine vaccine has demonstrated a 4 month duration of immunity. Dosage is a single 2 mL and intramuscular (IM) injection is required. This product may be

suitable for use on organic farms (14, 104).

13. Interaction between PCV2 and PRRSV

PCV2, PRRSV, and *Mycoplasma hyopneumoniae*are are deemed to be the most clinically effective pathogens for PRDC, thus resulting in a huge impact on global swine industry (63, 106). PRDC is a multifactor-based, complicated disease induced by an alliance of infectious viral or bacterial pathogens, environmental factors, and differences in breeding and growing systems (49). Historically, PRRSV and *M. hyopneumoniae* used to be known as the most common causative agents for PRDC, but nowadays, PCV2 has been implicated as an etiological agent with several signs and symptoms collectively known as PCVAD (13). There has been an increasing evidence that PCV2 primarily cause PRDC as part of PCVAD (147).

Several studies have already indicated the correlation between PCV2 and PRRSV (102). The latter is well known to promote PCV2 inflicted lesions (53) by elevating the PCV2 DNA load in the sera of co-infected pigs (100) and the levels of PCV2 antigens in tissues (5), which leads to severe PCV2-inflicted lesions (53). On the other hand, PCV2 does not impact PRRSV replication or lesions (109). Irrespective of the PCV2 genotype (either PCV2a or PCV2b), co-infection with PRRSV-2 significantly elevated PCV2 viremia and deepened the severity of

PCV2-inflicted lesions compared to PRRSV-1 in a double PCV2-PRRSV infection model (110).

Interactions between PCV2 and PRRSV should be analyzed carefully because various outcomes can be obtained when applying various strains of PCV2 and PRRSV. Variation in the virulence of various PCV2 and PRRSV isolates with identical genotypes in pigs has been reported (47, 102). In theory, one way to reduce the impact of PRRSV increasing PCV2 replication may be the immunization of pre-weaned pigs against PRRSV in such double PCV2-PRRSV-infected farms.

It was unforeseen that the PRRSV vaccine would increase PCV2 replication, which could lead to the failure of the PRRSV vaccine in vaccinated co-infected pigs compared to non-vaccinated co-infected pigs (109). The increased impact in PCV2 viremia following MLV PRRSV vaccination in co-infected pigs implies lower efficacy of the PRRSV vaccine under experimental conditions (100).

In contrast to PRRSV vaccination, the efficacy of PCV2 vaccination was not affected by PRRSV infection (140). PCV2 vaccination alone was able to lower levels of PCV2 viremia and the related lymphoid lesions in pigs irrespective of their PRRSV infection condition (109), and it has been shown to be effective

under field conditions where PCV2 and PRRSV were epidemic in a swine population (37).

Simultaneous vaccination of pigs with PRRSV and PCV2 significantly decreased levels of PRRSV viremia (even in co-infected pigs) compared to a single challenge of PRRSV alone. Consequently, the vaccination regime that the double challenge model proposed to control PCV2 infections by PCV2 vaccination surely affected the efficacy of the PRRSV vaccine in co-infected pigs. It is absolutely necessary to ascertain the interaction between the vaccines and the major respiratory pathogens to effectively control PRDC. If a farm is co-infected with PCV2 and PRRSV, vaccination of pigs with PCV2 is preferable because PCV2 infection decreases the efficacy of the PRRSV vaccine (109).

14. Correlation between efficacy and genetic similarity in the vaccine viruses

The genetic diversity of PRRSV has been expanding and could be the main issue; such a situation has created the coincidental expansion of antigenic diversity that has rendered vaccines ineffective in imparting relevant protection. It has been widely acknowledged that live, attenuated PRRSV vaccines do not continuously offer whole protection against challenges with various isolates of the type 1 or type 2 genotype (68), a dilemma made more complex by vaccine efficacy not being strictly related to the genetic correlation of the transmitting strains (152).

These outcomes infer that some major immunological targets essential for protection are preserved in spite of high levels of genetic and antigenic diversity. Thus, the replication process of the type 2 vaccine strains containing highly homogeneous sequences primarily share crucial sequences with all type 2 PRRSVs even if genetic gaps between highly categorized isolates and commercially available vaccines exhibit a pattern of broadening diversity over time (139).

Genotype involved in clinical disease help to predict that genetic sequence similarity and clinical similarity are highly interrelated. On the other hand, the correlation coefficients from the Mantel test are small at less than 0.1, specifying a weak-positive correlation between the similarity in PRRSV sequence and clinical similarity. These findings infer that a potential correlation between sequence similarity and clinical similarity might not be consistent across the whole range of sequence similarity in the dataset and encourage further investigation of the data with generalized additive models (122).

A previous study examined similarity in clinical signs and symptoms, such as abortion, sow mortality, pre-weaning mortality, and respiratory disease in nursery pigs, respiratory disease in growing/finishing pigs, and a longer time to market for growing/finishing pigs (45). The researchers identified significant correlation

between similarity in sow mortality and similarity in PRRSV ORF5 by the application of the Mantel test. However, the current study identified a nonlinear correlation, thus the Mantel test was not able to detect significant correlation. The outcomes for the similarities in pre-weaning mortality showed a general positive trend throughout the entire range of sequence similarity. However, the generalized additive models illuminated that the slope of the line became negative for isolates which had above 96% similarity; this is very weak evidence that genotype is associated with pre-weaning mortality in a herd (122).

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PART 1. Comparative efficacy of concurrent administration of a porcine circovirus type 2 (PCV2) vaccine plus a porcine reproductive and respiratory syndrome virus (PRRSV) vaccine from two commercial sources in pigs challenged with both viruses

ABSTRACT

The objective of first study was to compare clinical, virological, immunological and pathological indicators in pigs each treated simultaneously with a porcine circovirus type 2 (PCV2) and a porcine reproductive and respiratory syndrome virus (PRRSV) vaccine from one of two commercial products and challenged with field strains of PCV2 and PRRS virus. Pigs in one group administered with simultaneously Fostera PCV® and Fostera PRRS® (Zoetis, Florham Park, New Jersey) and pigs in another group administered simultaneously Ingelvac Circo FLEX® and Ingelvac PRRS MLV® (Boehringer Ingelheim Vetmedica Inc., St Joseph, Missouri) at study day -28 (21 days of age) were challenged with PCV2 and PRRS virus at study day 0 (49 days of age). No significant differences were seen between two immunized group with transmission in clinical, virological (except PCV2 viremia at day 14), immunological and pathological examinations. Under this study condition, there was no difference in protection whether PCV2 and PRRSV vaccines were treated simultaneously or not. Simultaneous immunization is efficient tool for controlling co-challenge with PCV2 and PRRSV.

Keywords:

Fostera®

Ingelvec®

Porcine circovirus type 2 (PCV2)

Porcine reproductive and respiratory syndrome virus (PRRSV)

Vaccine

1. INTRODUCTION

Porcine respiratory disease complex (PRDC) causes a severe health problem in growing and finishing pigs normally about 16 to 22 weeks of age, and is featured by slow growth, poor feed, lethargy, anorexia, fever, cough and dyspnea (1). Pathogens implicated in PRDC may be viral, bacterial or both. Dual-infection with porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) is generally known etiology of PRDC (2). Hence, regulating both PCV2 and PRRSV transmission is the most important issue for the international swine industry. Because immunization is one of the main tool to handle PCV2 and PRRSV transmission, this is absolutely necessary to handle PRDC effectively.

Recent study (30) reported outcome of co-infection with PCV2 and PRRSV. When PRRSV type 2 were infected in the pig with PCV2 virus, PCV2 antigens in the tissue, DNA loads in the sera and lesion in the lung and lymph nodes were

observed, In contrast, there was no effect seen with regard to the PRRSV induced replication or lesions in those organs. When the PRRSV vaccine was challenged into a pig, which was co-infected with PCV2 and PRRSV, the PCV2 replication and lymphocyte activation was increased. When PCV2 vaccine was challenged into a pig, there no effect observed in relation to the PRRSV infection. However, PCV2 viremia and lymphoid lesions were severe. It was predicted that either PRRSV vaccine or PCV2 vaccine could reduce viremia, DNA or RNA replication and lymphoid lesions induced by those viruses. The results were opposite to the expectation. Only the double vaccination with both PCV2 vaccine and PRRSV vaccine resulted in the decrease of viremia caused by those viruses.

Lately, a new commercially released live PRRSV vaccine (Fostera PRRS®, Zoetis, Florham Park, New Jersey) was launched into the international market to handle respiratory disorder in growing pigs. At the site, swine growers consistently treat both singe-dose PCV2 and PRRSV vaccines simultaneously to handle PRDC. Therefore, field condition may lead to reflect distinguished usage of single-dose PCV2 and PRRSV vaccines, which were treated simultaneously. On the other hand, no one has proposed differentiation so far based on clinical, virological, immunological and pathological parameters when commercially introduced single-dose PCV2 and PRRSV vaccines were treated simultaneously. Therefore, the objective of this study was to distinguish growth, virologic, immunologic and

pathologic parameters in wean-to-finishing pigs, which were simultaneously immunized with a PCV2 vaccine and a PRRSV vaccine from two commercial products, respectively.

2. MATERIALS AND METHODS

All animal protocols were authorized by the Seoul National University Institutional Animal Care and Use Committee.

2.1 Experimental Study

Sixty colostrum-fed, crossbred, standard piglets were acquired at 5 days of age from a commercially registered Korean farm. At a research facility, all piglets in this study were proven negative for PCV2 and PRRSV upon their arrival when test (PCV2 Ab Mono Blocking ELISA; Synbiotics, Lyon, France and PRRS X3 Ab test; Idexx Laboratories Inc., Westbrook, Maine) was conducted. Meanwhile, all piglets were also proven negative for PCV2 and PRRSV viremia by real-time PCR (polymerase chain reaction) was applied (3, 4).

60 total pigs were randomly separated into four groups employing Excel's random number generation function (Microsoft Corporation, Redmond, Washington) (Table 2). Sample size was determined presuming a 90% power ($1 - \beta = .90$) of identifying the difference at the 5% level of significance ($\alpha = .05$) based on the

expected outcomes of ELISA antibody titers (PCV2 and PRRSV), virus contents (PCV2 and PRRSV) evaluated by real-time PCR, and lung and lymphoid lesions indicated by scores (5). The timeline for the administration is presented in Table 2. According to the manufacturers' instructional label, pigs in Group 1 were injected intramuscularly with 2.0 mL dose of Fostera PCV® (Zoetis) and 2.0 mL dose of PRRS (Zoetis) into the right and left sides of the neck, respectively at the study day -28 (21 days of age). Likewise, pigs in Group 2 were injected intramuscularly with 1.0 mL dose of Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica Inc., St Joseph, Missouri) and 2.0 mL dose of Ingelvac PRRS MLV® (Boehringer Ingelheim Vetmedica Inc.) into the right and left sides of the neck, respectively at the study day -28 (21 days of age). At the study day 0 (49 days of age), individual pig in Group 1, 2 and 3 was administered intranasally with 2.0 mL of PCV2b (strain SNUVR000463; 5th passage; 1.2 x 10^5 median $TCID_{50}$ [Tissue Culture Infective Doses] per mL). In the same day afternoon, same pigs were administered intranasally with 2.0 mL of PRRSV (strain SNUVR090851; 5th passage; 1.2 x 10^5 $TCID_{50}$ per mL). Dual-infection with these PCV2b and PRRSV strains caused serious interstitial pneumonia and lymphoid depletion of lymph nodes in treated pigs (6). Pigs in Group 3 played a role as the positive control animal (challenged but unimmunized), and pigs in Group 4 played a role as the negative-control animal (unchallenged and unimmunized). Each group was gathered in separate room (five pigs per room) within the same facility. Blood samples were collected

at the study day -42, -28, 0 (49 days of age), 14, 28, 63, 91 and 126 (175 days of age). At the study day 126, each pig was anesthetized via intravenous injection of azaperon (Stresnil; Janssen Pharmaceutica, Beerse, Belgium) and then euthanized for necropsy. Lung and lymph nodes were collected for histopathological and immunohistochemical examinations.

2.2 Clinical Evaluation

From the starting day when Group 1, 2 and 3 were administered (day 0), all pigs were observed daily for physical condition and score recorded weekly to monitor severity of respiratory disease using scoring system. The scores were ranged from 0 (normal) to 6 (severe dyspnea, abdominal breathing, and death) (7). Immunization status was blinded to observers. Rectal body temperature was recorded daily from day 0 to day 21.

2.3 Assessment of Growth Performance

Body weight of each pig in Group 1, 2, 3 and 4 was measured at the study day -28, 0, 21, 63 and 126. ADG (Average Daily Gain) was measured over four time period between the day -28 and 0; between the day 0 and 21; between the day 21 and 63; between the day 63 and 126, respectively. The ADGs during these diverse growth stages was measured as the difference between the starting and final weights separated by the term of stage. Dead pigs were included for the data

calculation.

2.4 PCV2 Serology

Serum samples were analyzed with a commercial PCV2 ELISA (Synbiotics) and serum virus neutralization was tested with heterologous PCV2b (strain SNUVR000463) (8). According to manufacturer's instruction label, serum samples were indicated as positive for anti-PCV2 antibody if the exchangeable ELISA titer was higher than 350. Nab (Neutralizing Antibody) data were reshaped to base 2 logarithms for the evaluation.

2.5 PRRSV Serology

Serum samples were analyzed with a commercial PRRSV ELISA (Idexx Laboratories, Inc.) and serum virus neutralization was tested with heterologous PRRSV (strain SNUVR090851) (9). According to the manufacturer's instruction label, serum samples were indicated as positive for anti-PRRSV antibody if sample-to-positive (S:P) ratio was higher than 0.4. Nab data were reshaped to base 2 logarithms for the evaluation.

2.6 Quantification of PCV2 DNA

QLAamp DNA Mini Kit (Qiagen, Inc. Valencia, California) was applied to isolate DNA from serum samples. The DNA isolation was applied to count the numbers

of PCV2 genomic DNA copies by real-time PCR (3). Number of genomic copies of PCV2 DNA per mL in the serum was reshaped to base 10 logarithms for the evaluation.

2.7 Quantification of PRRSV RNA

QLAamp RNA Mini Kit (Qiagen, Inc. Valencia, California) was applied to isolate RNA from serum samples. The RNA isolation was applied to count the numbers of PRRSV genomic RNA copies by real-time PCR (4). Number of genomic copies of PRRSV RNA per mL in the serum was reshaped to base 10 logarithms for the evaluation.

2.8 Enzyme-linked Immunospot Assay

The numbers of PCV2- and PRRSV- specific IFN-γ-SC (Interferon-γ Secreting Cells) were detected in the PBMC (Peripheral Blood Monoclonal Cells) by ELISPOT (Enzyme-linked Immunospot) method (6, 10). Whole PCV2b and PRRSV (the strains applied for the transmission), each one at a multiplicity of infection of 0.01, were adopted to catalyze PBMC. Phytohemaglutinin (10 μg per mL; Roche Diagnostics GmbH, Mannheim, Germany) and phosphate buffered saline were adopted as positive and negative controls, respectively. The outcomes were shown as the numbers of IFN-γ-SC per million PBMC.

2.9 Interleukin-10

The protein concentration of IL-10 (Interleukin-10) were evaluated in the supernatants of PBMC cultures (2 x 10^6 cells per well; 250 μL) in vitro for 20 hours with transmittable PRRSV (multiplicity of infection of 0.01) or phytohemaglutinin (10 μg per mL). According to manufacturer's instruction label, commercial ELISA kits (pig Interlukin-10 ELISA kit; Cusabio Biotech, Wuhan, China) was used for the evaluation. The detection limit for IL-10 was 1.5 pg per mL.

2.10 Histopathologic Examination

To score histopathological lesion in lymph nodes via morphometric analysis, the superficial inguinal lymph node was gathered from each pig, and three segments of lymph node tissue were observed histologically with disclosed animal IDs (11, 12). Lymphoid lesions were graded as a score scale from 0 to 3; 0 = no lymphoid depletion or granulomatous replacement; 1 = mild lymphoid depletion; 2 = moderate lymphoid depletion; and 3 = severe lymphoid depletion and histiocytic replacement (11).

To score histopathological lesion in lung via morphometric analysis, eight samples of lung tissue (one from the ventromedial part of the right caudal lobe, two from the right middle lobe, two from the right cranial lobe, one from the dorsomedial

part of the right caudal lobe, one from the mild-lateral part of the right caudal lobe, and one from the accessory lobe) were gathered from each pig, and three segments of lung tissue were observed histologically by one of the authors (JJ) with disclosed animal IDs. Lung lesions were graded on a score 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 interstitial pneumonia.

Immunochemical analysis for PCV2 antigen was conducted using PCV2 polyclonal antibody (Iowa State University, Ames Iowa) (13), and Immunochemical analysis for PRRSV antigen was conducted using SR30 monoclonal antibody (Rural Technologies Inc., Brookings South Dakoda) (14). Number of lymphoid cells showing positive for PCV2 antigen in lymph node (12), and number of pulmonary cells showing positive for PRRSV and PCV2 antigen in lung per unit area (0.25 mm^2) (15) were measured using an NIH Image J 1.45s program (http://imagej.nih.gov/ij/download.html).

2.11 Statistical Analysis

Continuous data such as rectal body temperature, body weight and PCV2 DNA (log_{10} PCV2 genomic copies per mL) evaluated by real-time PCR; PRRSV RNA (log_{10} PRRSV genomic copies per mL) evaluated by real-time PCR; PCV2 and

PRRSV serum titer and number of IFN-γ-SC per 10^6 PBMC evaluated by ELISPOT assay; numbers of lung segments showing positive for PRRSV antigen and PCV2 antigen per unit area (0.25 mm^2) evaluated by immunohistochemistry were all examined using repeated estimates of ANOVA for each time point. If the ANOVA exhibited significant outcome, Tukey's test for multiple comparison was conducted at each time point. Fisher's exact test was applied for various data (respiratory lesion scores, lung lesion scores and lymphoid lesion scores). A chi-square test was applied for mortality rate. A vale of $P < .05$ was deemed to be significant.

3. RESULTS

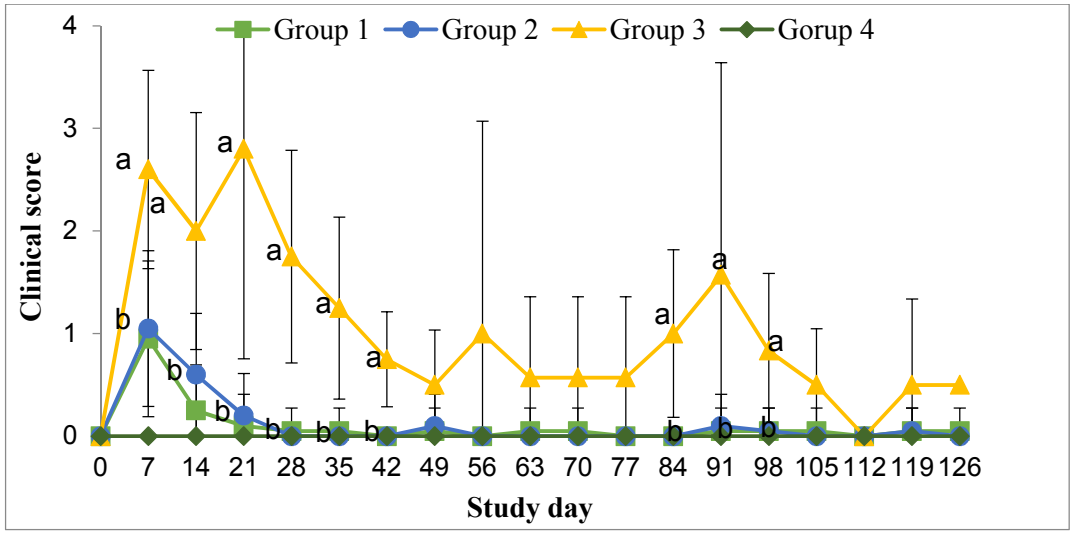
3.1 Clinical Evaluation

Significantly higher ($P > .05$) mean respiratory lesion scores were present in challenged pigs with no immunization (Group 3) than in challenged pigs with immunization (Group 1 and Group 2) from the day 7 to 42 and from the day 84 to 98 (Figure 1A). Significantly higher ($P > .05$) mean rectal temperature ranging from 39.7°C to 40.2°C was present in challenged pigs with un-immunization (Group 3) than in challenged pigs with immunization (Group 1 and Group 2) from the day 4 to 7 (Figure 1B). Mortality rate in each group was 5% (one out of 20 pigs) in Group 1, 10% (two out of 20 pigs) in Group 2, 30% (three out of 10 pigs) in Group 3 and 0% (0 out of 10 pigs) in Group 4. No significant difference in

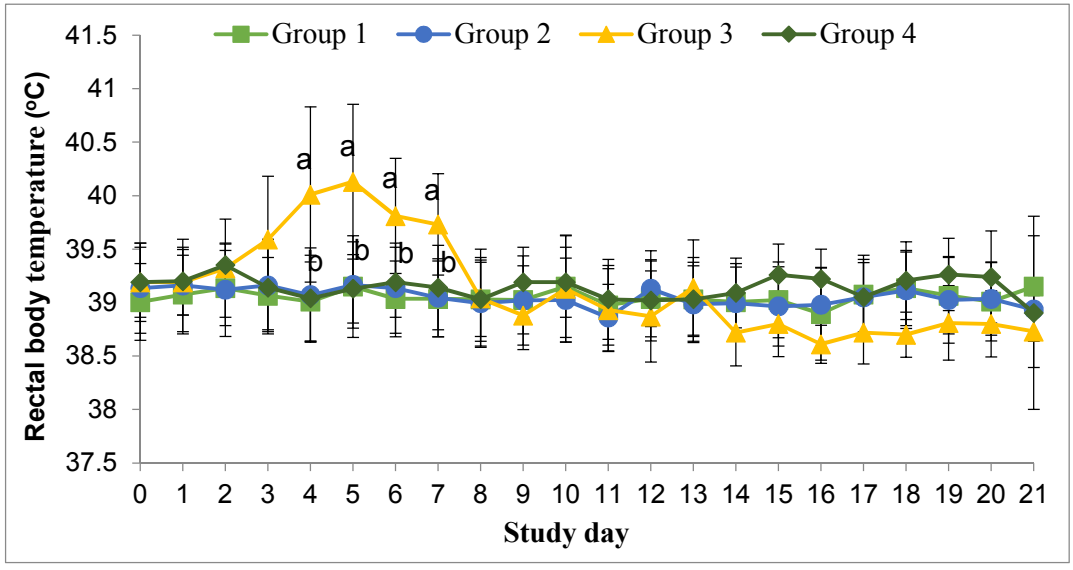
mortality rate between challenged pigs with immunization (Group 1 and Group 2) and challenged pigs with no immunization (Group 3) was observed. Diagnostic outcomes demonstrated that pig deaths were mostly associated with severe pneumonia.

Figure 1. Means scores for clinical signs (Panel A) and rectal body temperature (Panel B) in pigs.

A



B



工: Standard deviation

Different alphabets (a, b) represent significant differences among four groups.

3.2 Growth Performance

Significantly higher ($P < .05$) mean ADGs were shown in challenged pigs with immunization (Group 1 and Group 2) and unchallenged pigs with no immunization (Group 4) than challenged pigs with no immunization (Group 3) during the experiment. However, no difference in mean ADG was observed two groups with immunized, challenged pigs (Group 1 and Group 2) (Table 1).

Table 1. Mean number for ADG (g/day) in pigs.

Period between study days*	Age (days)	Group 1	Group 2	Group 3	Group 4
-28 to 0	21-49	329 (31)	330 (28)	326 (25)	340 (27)
0 t0 21	49-70	629 (33)[a]	612 (35)[a]	519 (24)[b]	626 (43)[a]
21 to 63	70-112	792 (44)[a]	785 (47)[a]	672 (45)[b]	804 (39)[a]
63 to 126	112-175	734 (43)[a]	718 (39)[a]	650 (33)[b]	728 (42)[a]
-28 to 126	21-175	662 (33)[a]	651 (34)[a]	579 (39)[b]	664 (43)[a]

ADG: Average daily gain.

* The body weight of each pig in each group was measured and ADGs were compared among four groups.

a, b Within a row, two different alphabets show significantly different numbers ($P < .05$).

3.3 Quantification of PCV2 DNA in Serum Samples

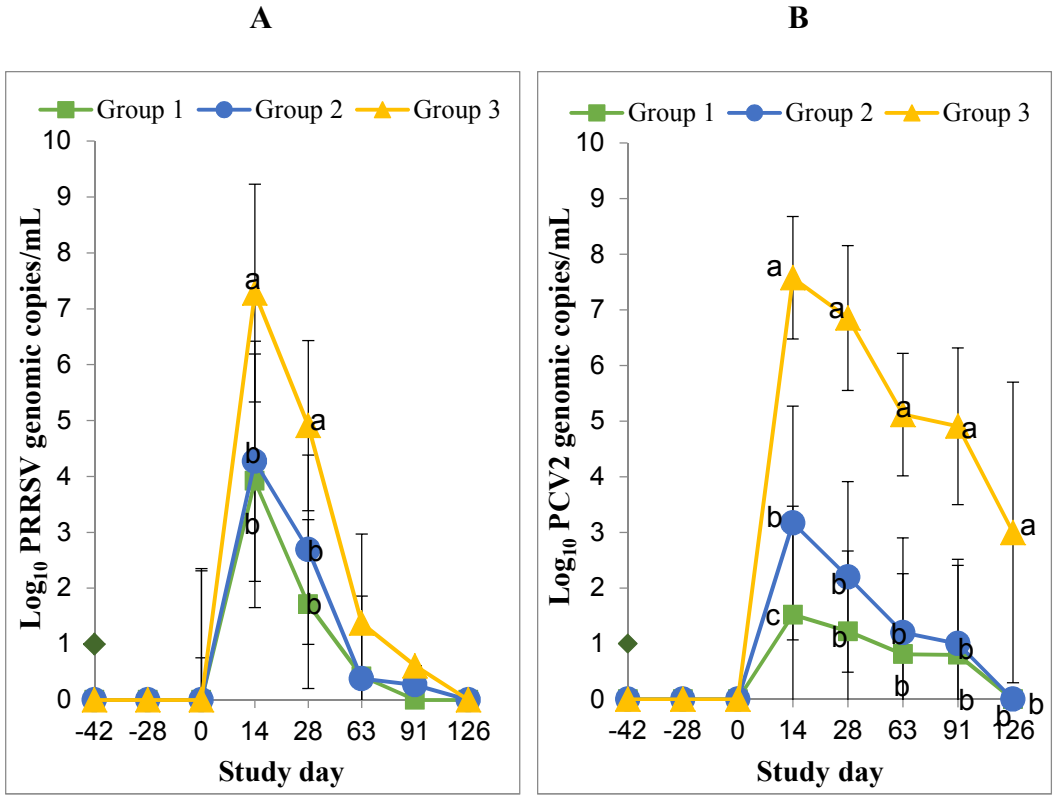
The serum samples did not contain PCV2 DNA examined at days -42, -28 and 0. Significantly lower ($P < .05$> numbers of genomic copies of PCV2 in the serum were present in Group 1 and Group 2 (challenged pigs with immunization) than in

Group 3 (challenged pigs with no immunization) from the day 14 through 126. Numbers of genomic copies of PCV2 in the serum were different between two groups challenged pigs with immunization (Group 1 and Group 2) at the day 14 (Figure 2A). There was no PCV2 DNA found in the serum samples of unchallenged pigs with no immunization (Group 4) during the experiment.

3.4 Quantification of PRRSV RNA in Sera

There was no PRRSV RNA found in the serum samples of pigs examined at days -42, -28, and 0. Significantly lower ($P < .05$) numbers of genomic copies of PRRSV in the serum were present in Group 1 and Group 2 (challenged with immunization) than in group 3 (challenged pigs with no immunization) from the day 14 through 28. Numbers of genomic copies of PRRSV in the serum were not different between two groups of challenged pigs with immunization (Group 1 and Group 2) (Figure 2B). There was no PRRSV RNA in the serum samples of unchallenged pigs with no immunization (Group 4) during the experiment.

Figure 2. Mean number of genomic copies of PCV2 DNA (Panel A) and PRRSV RNA (Panel B) in serum samples from pigs.



PRRSV: Porcine reproductive and respiratory virus.

PCV2: Porcine circovirus type 2.

工: Standard deviation

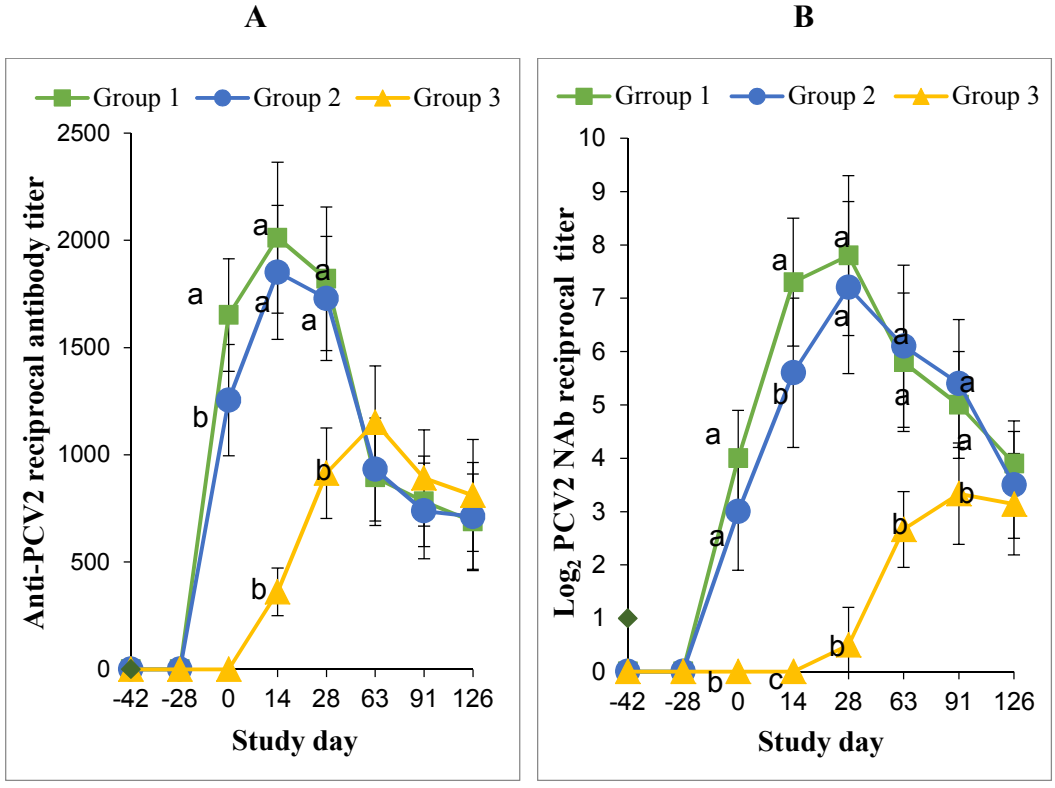
Different alphabets (a, b) represent significant differences among groups.

3.5 Immunological Responses to PCV2

Significant higher (P > .05) anti-PCV2 antibody titers were seen in challenged

pigs with immunization (Group 1 and Group 2) than in challenged pigs with no immunization (Group 3) from the day 0 through 28. Anti-PCV2 antibody titers were different between two groups of challenged pigs with immunization (Group 1 and Group 2) at the day 0 (Figure 3A). Significantly higher ($P > .05$) mean NAb titers were seen in challenged pigs with immunization (Group 1 and Group 2) than in challenged pigs with no immunization (Group 3) from the day 0 through 91. Mean NAb titers were different between two groups of challenged pigs with immunization (Group 1 and Group 2) at the day 14. (Figure 3B). Significantly higher ($P > .05$) numbers of PCV2-specific IFN-γ-SC in challenged pigs with immunization (Group 1 and Group 2) than in challenged pigs with no immunization (Group 3) from the day 0 through 28. Number of PCV2-specific IFN-γ-SC were different between two groups of challenged pigs with immunization (Group 1 and Group 2) at the day 0 and 14 (Figure 3C). There were no anti-PCV2 antibodies or PCV2-specific NAb or IFN-γ-SC found in unchallenged pigs with no immunization (Group 4).

Figure 3. Mean value for anti-PCV2 reciprocal ELISA antibody titers (Panel A); group means for NAb reciprocal titers (Panel B); and mean value for PCV 2-specific IFN-γ-SC in PMBC (Panel C).



PCV2: Porcine circovirus type 2.

NAb: Neutralizing antibody.

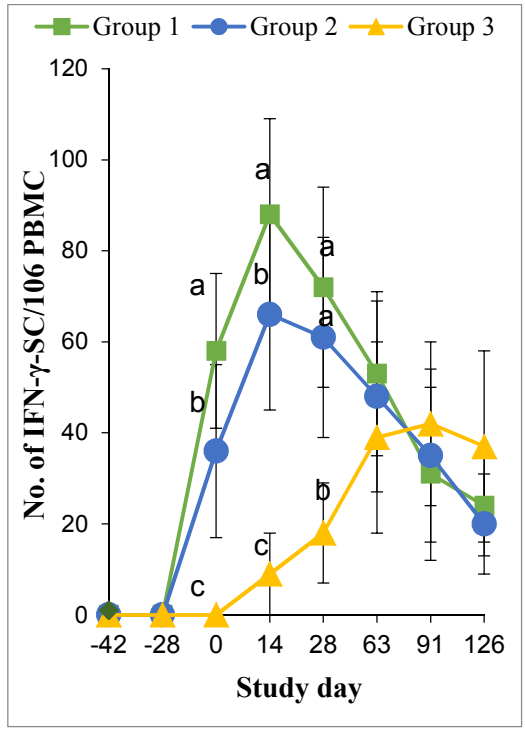
IFN-γ-SC: Interferon-γ-secreting cells.

PBMC: Peripheral blood mononuclear cells.

工: Standard deviation

Different alphabets (a, b) represent significant differences among three groups.

C



PCV2: Porcine circovirus type 2.

NAb: Neutralizing antibody.

IFN-γ-SC: Interferon-γ-secreting cells.

PBMC: Peripheral blood mononuclear cells.

工: Standard deviation

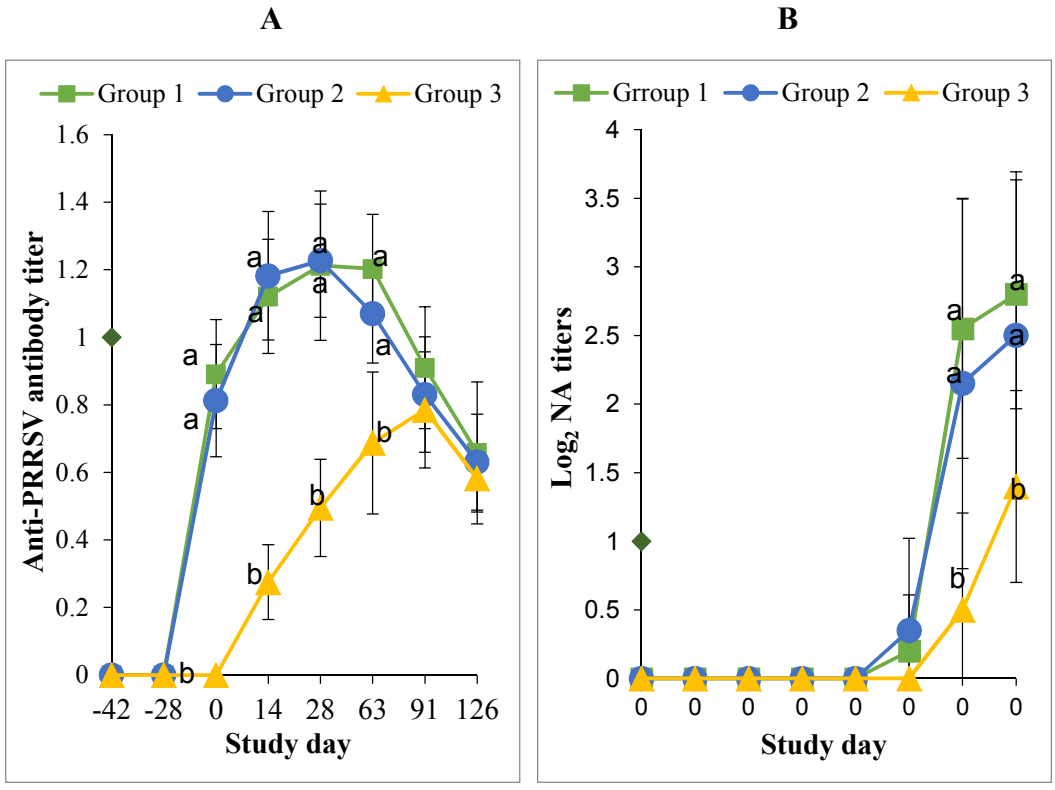
Different alphabets (a, b) represent significant differences among three groups.

3.6 Immunologic Responses to PRRSV

Significantly higher (P > .05) anti-PRRSV antibody titers were present in

challenged pigs with immunization (Group 1 and Group 2) than in challenged pigs with no immunization (Group 3) from the day 0 through 63 (Figure 4A). Significantly higher ($P > .05$) man NAb titers were present challenged pigs with immunization (Group 1 and Group 2) than in challenged pigs with no immunization (Group 3) from the day 91 to 126 (Figure 4B). Significantly higher ($P > .05$) numbers of PRRSV-specific IFN-γ-SC were present in challenged pigs with immunization (Group 1 and Group 2) than in challenged pigs with no immunization (Group 3) from the day 0 through 28 (Figure 4C). There were no anti-PRRSV antibodies or PRRSV-specific NAb or IFN-γ-SC found in unchallenged pigs with no immunization (Group 4).

Figure 4. Mean value for commercial PRRSV ELISA S:P ratio (Panel A); group means for NAb reciprocal titers (Panel B); and mean value for PRRSV-specific IFN-γ-SC in PBMC (Panel C).



S:P: Sample-to-positive.

NAb: Neutralizing antibody.

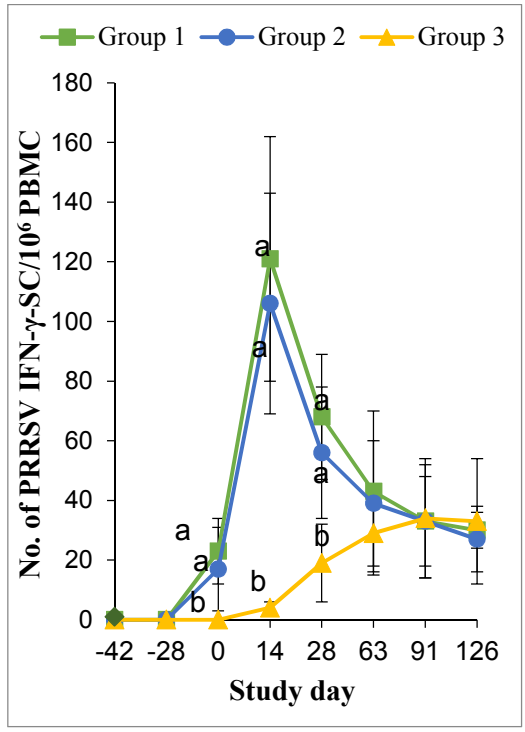
IFN-γ-SC: Interferon-γ-secreting cells.

PBMC: Peripheral blood mononuclear cells.

⊥: Standard deviation

Different alphabets (a, b) represent significant differences among three groups.

C



S:P: Sample-to-positive.

NAb: Neutralizing antibody.

IFN-γ-SC: Interferon-γ-secreting cells.

PBMC: Peripheral blood mononuclear cells.

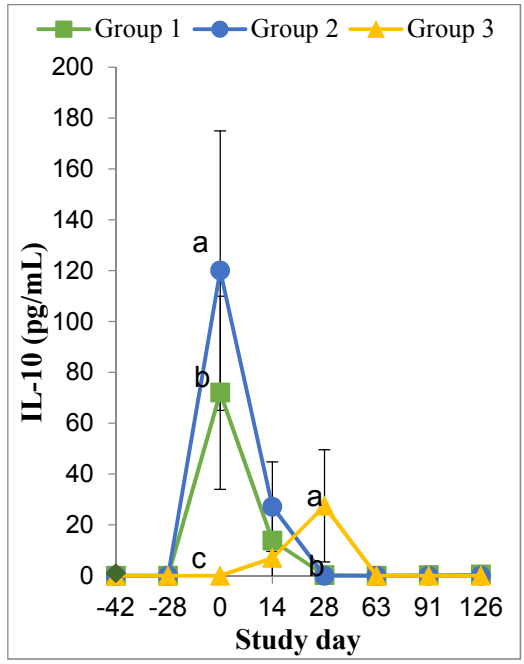
工: Standard deviation

Different alphabets (a, b) represent significant differences among three groups.

Significantly higher (P > .05) IL-10 levels were seen in challenged pigs with immunization (Group 1 and Group 2) than in challenged pigs with no

immunization (Group 3) on the day 0. Two groups of challenged pigs with immunization (Group 1 and Group 2) showed different concentration of IL-10 on the day 0. Significantly higher (P > .05) IL-10 concentration was present in challenged pigs with no immunization (Group 3) than in challenged pigs with immunization (Group 1 and Group 2) on the day 28 (Figure 5). There was IL-10 found in unchallenged pigs with no immunization (Group 4).

Figure 5. Mean value for PRRSV-specific IL-10 concentrations in serum samples from pigs.



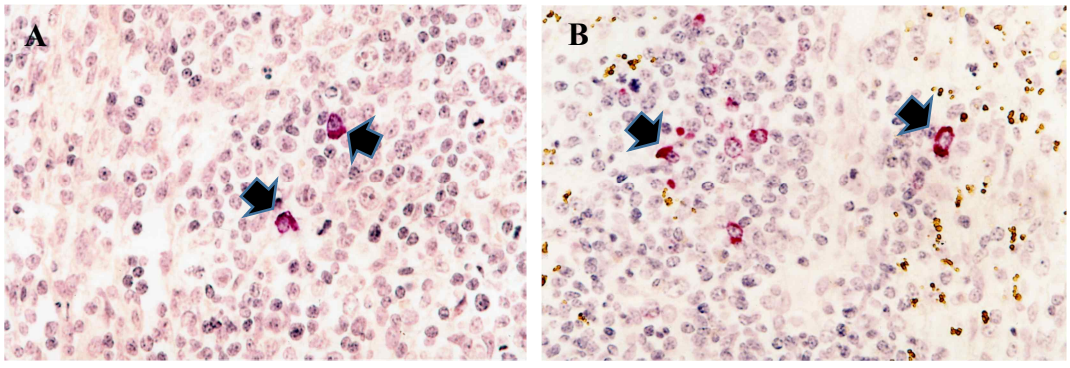
工: Standard deviation

Different alphabets (a, b) represent significant differences among three groups.

3.7 Pathology Testing

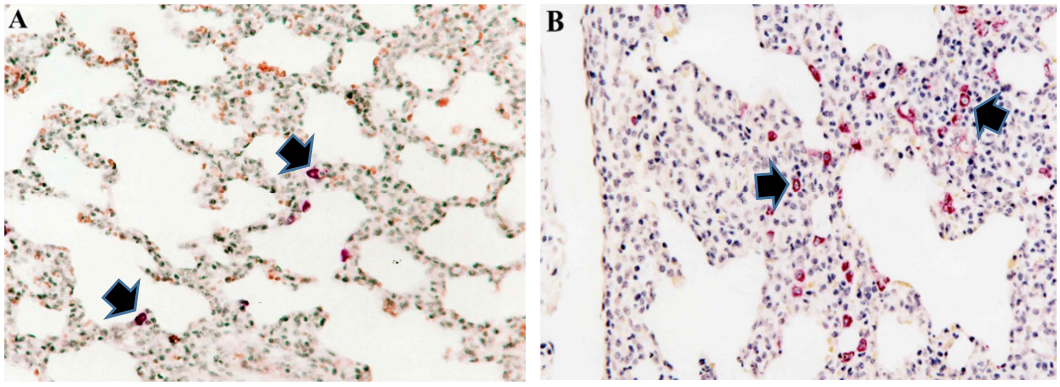
Significantly lower ($P < .05$) lymphoid and pulmonary lesion scores were detected in challenged pigs with immunization (Group 1 and Group 2) than in challenged pigs with no immunization (Group 3). Significantly lower ($P < .05$) numbers of lymphoid cells showing positive for PCV2 antigen (Figure 6), and pulmonary cells showing positive for PRRSV antigen (Figure 7) and PCV 2 antigen (Figure 8) were seen in challenged pigs with immunization (Group 1 and Group 2) than in challenged pigs with no immunization (Group 3) (Table 2).

Figure 6. Small number of PCV2 antigen-positive cells were identified in macrophages in Group 1 pigs (Panel A). Several PCV2 antigen-positive cells were identified in macrophages in Group 3 pigs (Panel B).



Immunohistochemical examination to identify porcine circovirus type 2 (PCV2) antigen in lymph nodes of pigs adopting PCV2 polyclonal antibody (Iowa State University, Ames Iowa). 400 x magnification.

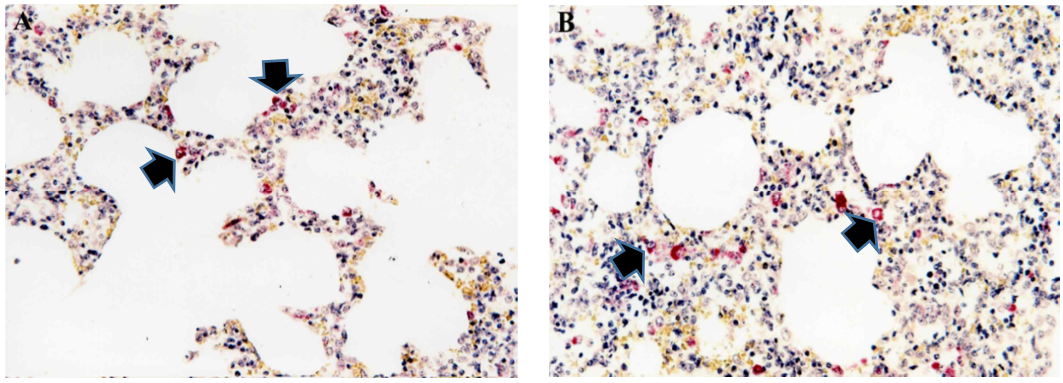
Figure 7. Small number of PRRSV antigen-positive cells were identified in macrophages in pigs from Group 1 (Panel A). Several PRRSV antigen-positive cells were identified in macrophages in pigs from Group 3 (Panel B).



Immunohistochemical examination to identify porcine reproductive and respiratory syndrome virus (PRRSV antigen in lungs of pigs adopting SR30 monoclonal antibody (Rural Technologies Inc., Brookings, South Dakoda).

200 x magnification.

Figure 8. Small number of PCV2 antigen positive cells were identified in macrophage in pigs from Group 1 (Panel A). Several PCV2 antigen-positive cells were identified in macrophages in pigs from Group 3 (Panel B).



Immunohistochemical examination to identify porcine circovirus type 2 (PCV2) antigen in lungs of pigs adopting PCV2 polyclonal antibody (Iowa State University, Ames Iowa).

200 x magnification.

Table 2. Mean scores for lymphoid and pulmonary lesion and mean numbers of positive cells for lymphoid PCV2 antigen and PRRSV antigen in pigs immunized simultaneously with PCV2 and PRRSV vaccines and challenged with PCV2 and PRRSV.

			Lymph Node		Lung		
Group	Vaccination (21 days of age)	Challenge (49 days of age)	Lesion Score†	No. of PCV2+ cells¢	Lesion Score†	No. of PRRSV+ cells¢	No. of PCV2+ cells¢
1	Fostera PCV® and Fostera PRRSV®	PCV2 and PRRSV	0.43 (0.53)[a]	3.15 (3.52)[a]	1.15 (0.23)[a]	1.76 (3.87)[a]	1.65 (2.19)[a]
2	Ingelvac CircoFLEX® and Ingelvac PRRS MLV®	PCV2 and PRRSV	0.71 (0.59)[a]	7.05 (5.45)[a]	1.23 (0.38)[a]	1.54 (3.43)[a]	2.09 (2.60)[a]
3	None	PCV2 and PRRSV	2.11 (0.73)[b]	20.70 (8.17)[b]	2.23 (0.44)[b]	2.95 (3.31)[b]	6.78 (5.21)[b]
4	None	None	0.28 (0.41)[a]	0	0.11 (0.54)[c]	0	0

PRRSV: Porcine reproductive and respiratory syndrome virus.

PCV2: Porcine circovirus type 2.

* Pigs in Group 1 were simultaneously admnistered with Fostera PCV® and Fostera PRRS® vaccines (Zoetis, Florham Park, New Jersey) and pigs in Group 2 were simultaneously administered with Ingelvac CircoFLEX® and Ingelvac MLV® vaccines (Boehringer Ingelheim Vetmedica Inc., St Joseph Missouri) and both groups were challenged with both viruses. The body weight of each pig was recorded. Blood samples were gathered from pigs for Interferon-γ-secreting cells and serologic testing without anticoagulant. Nasal swabs were also gathered on

the same study days.

† Pigs in all groups were euthanized at 175 days of age. Puperficial inguinal lymph node and lung were gathered for histopathological examination and immunohistochemical analysis. Scores for lymphoid lesion and lung lesion were recorded on a scale from 0 to 4. Mean scores were compared among four groups adopting Fisher's exact test.

₰ Number of positive lymphoid and pulmonary cells for PCV2 antigen and number of positive pulmonary cells for PRRSV antigen, per unit area (0.25 mm^2) of lung were counted adopting an NIH Image J 1.45s program (http://image.nih.gov/ij/download.html). Numbers of positive cells were contrasted among groups adopting Tukey's test.

abc Within a column, three different alphabet letters show significantly different numbers ($P < .05$).

4. DISCUSSION

This study indicated that single-dose immunization treatment using PCV2 and PRRSV vaccine is effective for handling dual-infection with PCV2 and PRRSV. Without considering types of vaccines, higher ADG and lower mortality rate were seen in challenged animals with immunization than in challenged animals with no immunization.

PCV2 caused lymphoid lesions are associated with PCV2 (porcine circovirus type 2) viremia (16, 17). Hence, PCV2 viremia is a relevant element to assess PCV2 vaccine. The generation of PCV2-specific NAb and IFN-γ-SC is related to a lower number of genomic copies of PCV2 DNA (16-20). In this study, only vaccinated animals displayed PCV2-specific NAb and IFN-γ-SC. Vaccinated pigs with Fostera PCV® and Fostera PRRSV® vaccine (Group 1) contained higher titers of PCV2-specific NAb and higher numbers of IFN-γ-SC than pigs vaccinated with Ingelvac CircoFLEX® and Ingelvac PRRS MLV® vaccines (Group 2). These differences probably affected the lower numbers genomic copies of PCV2 DNA in Group 2. Thus, these outcomes might coincide with earlier discoveries that the Fostera PCV® vaccines led to significantly lower numbers of genomic copies of PCV2 DNA and outstanding vaccinated immunity (elevated titers of PCV2-specific NAb and elevated number of IFN-γ-SC) when contrasted to the Ingelvac CircoFLEX® vaccine.

An important parameter to assess the efficacy of vaccines in regulation of PRRSV treatment is dependent upon the number of genomic PRRSV RNA copies in serum samples (22). In this study, it is shown that PRRSV viremia can be treated before NAbs are formed. Hence, the lower number of genomic PRRSV RNA copies is not critical for the development of NAbs as described in earlier studies (23, 24). Furthermore, there is no proof that PRRSV antibodies spotted by ELISA

perform a significant function in defense action against PRRSV treatment (25). On the other hand, a lower number of PRRSV genomic RNA copies is observed concurrently with the presence of PRRSV-specific IFN-γ-SC in challenged animals with vaccination. Hence, PPRSV-specific IFN-γ-SC can clear out PRRSV, even if the purpose of IFN-γ-SC in a lower number of PRRSV RNA copies is still controversial (23, 26). In this study, no significant differences were identified in two PRRSV vaccines to provoke PRRS-specific IFN-γ-SC and to lower PRRSV viremia as earlier study indicated (10).

Pathologic examination is another important parameter to evaluate the efficacy of the PCV2 and PRRSV vaccines under experimental circumstances. Typical microscopic lesion induced by dual-infection with PCV2 and PRRSV were serious interstitial pneumonia and lymphoid depletion in challenged animals with no immunization as present and earlier studies indicated (2, 27). Single dose vaccination with PCV2 and PRRSV at a 21 days of age was efficacious in reducing scores for lung and lymphoid lesions in challenged animals with vaccination contrasted to in challenged animals with no immunization, without considerable differences between Fostera PCV®-PRRS® and Ingelvac CircoFLEX®-PRRS MLV®.

There may be potential interference with the efficacy of one vaccine to another

when animals immunized with both PCV2 and PRRSV vaccines at the same time. Especially, induction of IL-10 by PRRSV vaccine raised concerns that PRRSV immunization may disturb the efficacy of PCV2 immunization (28). IL-10 is a widely known cytokine synthesis interfering factor and blocks cell-mediated immune responses (29). Both PRRSV vaccines applied in this study produced maximal level of IL-10 at a study day 0 (28 days of post immunization) that thereafter reduced quickly. Although PCV2- and PRRSV-specific IFN-γ-SC elevated steadily, initiating at the study day 0 and reaching a peak at the study day 14, even in the appearance of IL-10. These outcomes imply that the initiation of IL-10 by PRRSV vaccines may not disturb the cell-mediated immunity initiated by PCV2 vaccines. This information is clinically valuable for swine growers, who prefer to treat both vaccines simultaneously, saving work efforts and leading to less stress to the animals.

Even if dual-infection can be handled by other means, such as upgraded management, pig flow, biosafety tools and housing conditions, immunization is still the most efficient method to handle the PRDC induced by dual-infection with PCV2 and PRRSV. This study outcome may accommodate swine physicians and growers with another alternative in handling PRDC via simultaneous treatment of single-dose PRRSV and PCV2 vaccines together.

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PART 2. Comparison of growth performance under field conditions in growing pigs each vaccinated with one of two commercial modified-live porcine reproductive and respiratory syndrome vaccines

ABSTRACT

The objective of second study was to distinguish the efficacy of two MLV PRRS vaccines at the field site condition, in healthy pigs from a herd treated with PRRSV-2. Nowadays, two commercial PRRS MLVs (modified live vaccines) are accessible in Korea. The clinical trial at the site was performed on a 1,000-sow herd with two-site generation: farrowing nursery and growing-finishing system. The farm have had difficult times of losing animals due to respiratory disease occurred by type 2 PRRSV in post-weaning and late-growing pigs at the same time of study. All pigs were regularly immunized with a commercial porcine circovirus type 2 (PCV2) vaccine at 3 weeks of age. Type 2 PRRSV (SNUVR 150324 strain, lineage 5, GenBank No. KU301048) was extracted from lung samples of weaned pigs at 42 days of age, before the initiation of this study. Fostera PRRS® vaccine and Ingelvac PRRS MLV® vaccine virus both show 91.3% nucleotide similarity for ORF5.

Pigs in Group 1 were administered intramuscularly with 2.0 mL of the Fostera PRRS® vaccine (Zoetis, Lot No. A405013B). Pigs in Group 2 were administered

intramuscularly with 2.0 mL of the Ingelvac PRRS MLV® (Boehringer Ingelheim Vetmedica Inc., Lot No. 245-659A). Pigs in Group 3 were administered into the same spot with 2.0 mL of phosphate buffered saline (0.01M, pH 7.4).

This study indicated that pigs immunized with MLV vaccines for PPRS, which are Fostera PRRS® (Zoetis, Florham New Jersey) and Ingelvac PRRS MLV® (Boehringer Ingelheim Vetmedica Inc., St Joseph Missouri), showed better growth performance and fewer lung injuries than unimmunized controls under wild condition. Moreover, no significant discrepancies were detected between two commercial MLV PRRSV vaccines in this study based upon four types of results: clinical signs (ex. ADG), immunologic (antibodies), virologic (PCR testing) and pathologic (lesions and viral antigen). No difference from the number of genomic copies of type 2 PRRS wild-type virus RNA was not detected between immunized and unimmunized pigs under wild conditions. Without considering the commercial MLV PRRS vaccine, better growth performance and fewer lung lesions were observed in immunized pigs than in unimmunized pigs.

Keywords:

Fostera®

Ingelvec®

Modified live vaccine (MLV)

Porcine reproductive and respiratory syndrome (PRRS)

1. INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) virus is characterized by enveloped positive-stranded RNA virus classified under the order *Nidovirales*, family *Arteriviridae* and genus *Arterivirus* (1). PRRS virus (PRRSV) is separated into PRRSV-1 (European) and PRRSV-2 (North American) genotypes based upon the 3'-terminal structural genes of the whole genomes (2, 3). PRRS is one of the most disastrous disease of swine, enabling huge economic disturbances in the international swine industry because of reproductive breakdown in sows and respiratory disease in raising pigs (4). Immunization is yet a main weapon for the handling of PRRSV exposure. Nowadays, two commercial PRRS MLVs (modified live vaccines) are accessible in Korea. Fostera PRRS® (Zoetis, Florham New Jersey) and Ingelvac PRRS MLV® (Boehringer Ingelheim Vetmedica Inc., St Joseph Missouri). Therefore, the goal of this study was to distinguish the efficacy of two MLV PRRS vaccines at the site condition, in healthy pigs from a herd treated with PRRSV-2.

2. MATERIALS AND METHODS

All animal protocols were authorized by the Seoul National University Institutional Animal Care and Use Committee.

2.1 *Clinical Trial*

The clinical trial at the site was performed on a 1,000-sow herd with two-site generation: farrowing nursery and growing-finishing system. The farm have had difficult times of losing animals due to respiratory disease occurred by PRRSV-2 in post-weaning and late-growing pigs at the same time of study. In contrast, reproductive breakdown had been addressed in breeding females from the farm 4 months before the study. All pigs were regularly immunized with a commercial porcine circovirus type 2 (PCV2) vaccine 3 weeks of age, on the contrary clinical signs, which were features of PCV2 had not been shown.

2.2 *Nucleotide similarity*

PRRSV-2 (SNUVR 150324 strain, lineage 5, GenBank No. KU301048) was extracted from lung samples of weaned pigs at 42 days of age, before the initiation of this study. The SNUVR 150324 strain and Fostera PRRS® vaccine (GenBank No. AF494042) both show 91.5% nucleotide similarity for ORF5 (open reading frame 5). The SNUVR 150324 strain and Ingelvac PRRS MLV® vaccine virus (GenBank No. AF066183) both show 99.1% nucleotide similarity for ORF5. Fostera PRRS® vaccine and Ingelvac PRRS MLV® vaccine virus both show 91.3% nucleotide similarity for ORF5.

2.3 *Experimental Design*

This study developed a randomized, blinded, weight-matched, controlled clinical design (Table 2). Sample size was computed expecting a 90% power ($1 - \beta = .90$) of identifying a discrepancy at the 5% level of significance ($\alpha = .05$), which depended on anticipated outcomes of ADG (average daily gain) (5). To reduce the sow variation, six piglets at 7 days of age were chosen form each sow adopting random number generation function in Excel (Microsoft Corporation, Redmond Washington). Pigs were allocated equally to three groups (30 pigs per group) using Excel's random number generator. According to the manufacturer's instruction label, pigs in Group 1 were administered intramuscularly with 2.0 mL of the Fostera PRRS® vaccine (Zoetis, Lot No. A405013B) into the right side of the neck at 21 days of age. Pigs in Group 2 were administered intramuscularly with 2.0 mL of the Ingelvac PRRS MLV® (Boehringer Ingelheim Vetmedica Inc., Lot No. 245-659A) into the right side of the neck at 21 days of age. Pigs in Group 3 were administered into the same spot with 2.0 mL of phosphate buffered saline (0.01M, pH 7.4).

2.4 *Clinical evaluation*

Pigs in each group were randomly assigned to three pens (10 pigs per pen) adopting the Excel random number generator and were placing in the barn. Pigs were observed daily for physical condition and mean scores for respiratory track

were counted once weekly. Scores ranged from 0 (normal) to 6 (severe dyspnea, abdominal breathing and death) at the study day 0 to the study day 91 (Figure 1) (6). Immunization status were confidential to observers. Mortality rate was estimated as the number of dead pigs divided by the number of pigs initially allocated to that group within batch.

The body weight of each pig in group 1, 2 and 3 was recorded at the study day 0 (21 days of age), 49, 91 and 147 (168 days of age). The ADG (grams per pig per day) was measured over three time periods; between study day 0 and 49; between 49 and 91; between 91 and 147, respectively (Table 1). The ADG during such a diverse development stages was estimated as the difference between the starting and final weight divided by the term of the stage. Data from dead pigs were included in the computation.

2.5 *Serology*

Blood samples from pigs were gathered at the study day 0, 21, 49, 70, 91 and 147. Blood samples were also gathered from sows at the study day 0, 21, 49, 70 and 91. Serum samples from sows at the study day 0, 21, 49, 70 and 91 were examined using commercial PRRSV ELISA (Idexx Laboratories Inc., Westbrook Maine). According to manufacturer's instruction label, serum samples were deemed positive for anti-PRRSV antibody if the sample-to-positive ratio (S : P) was ≥ 0.4

2.6 *Quantification of PRRSV RNA*

QIAmp RNA Mini Kit (Qiagen Inc., Valencia California) was applied to isolate RNA from the serum samples of pigs at study day 0, 21, 49, 70, 91 and 147. The RNA extracts were used to assess the number of PRRSV genomic RNA copies by real-time PCR (7, 8). Real-time PCR for the vaccine strain as also conducted to assess the number of PRRSV genomic RNA copies (8, 9). Number of copies of PRRSV genomic RNA per mL of serum were changed to base 10 logarithms for analysis.

2.7 *PCR material Reading*

Five serum samples from pigs with PCR-positive for field or vaccine virus, which were freely picked using the Excel random number generator at the study day 21, 49, 70, 91 and 147, were applied to examine the sequence of ORF5 by PCR (10). According to the manufacturer's instructional label, the PCR materials were refined adopting a commercial kit (Wizard PCR Preps DNA Purification and PCR Clean-Up System Promega Madison, Wisconsin), duplicated with the TOPcloner Blunt Kit (Enzynomics, Daejeon Korea) and disseminated in DH5α competent cells (Enzynomics). Plasmid DNA was refined with a plasmid purification kit (iNtRON Biotechnology, Sungnam Kyeonggi-do Korea) and sequenced by a commercial service (Sol Gent Co., Ltd, Daejeon Korea). Three duplicates of each PCR material were sequenced individually at least three times.

2.8 *Morphometric analysis*

Lung samples were gathered from all pigs in each group at the study day 147 (the time of euthanasia). For the scores in lungs from morphometric examination of histopathological wounds, eight portions of lung tissues (two pieces 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 mid-lateral part of the right caudal lobe, and one from the accessory lobe) were gathered from each pig. Three tissue segments from the eight lung portions were ready and two veterinary pathologists (authors JJ and CC) confidentially observed at the Seoul National University (Seoul, Republic of Korea) (6) Scores for lung damage 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 interstitial pneumonia (6). In situ hybridization to identify and separate PRRSV-1 and PRRSV-2 nucleic acids in lung tissues was conducted and examined morphometrically (9, 11).

2.9 *Statistical Analysis*

SPSS software (version 21; IBM, Armonk New York) was applied for the statistical analysis. Continuous data including ADG, which is the discrepancy between the starting and final weight divided by the term of the stage; PRRSV RNA (numbers of $\log_{10}$ PRRSV genomic copies per mL) by real-time PCR;

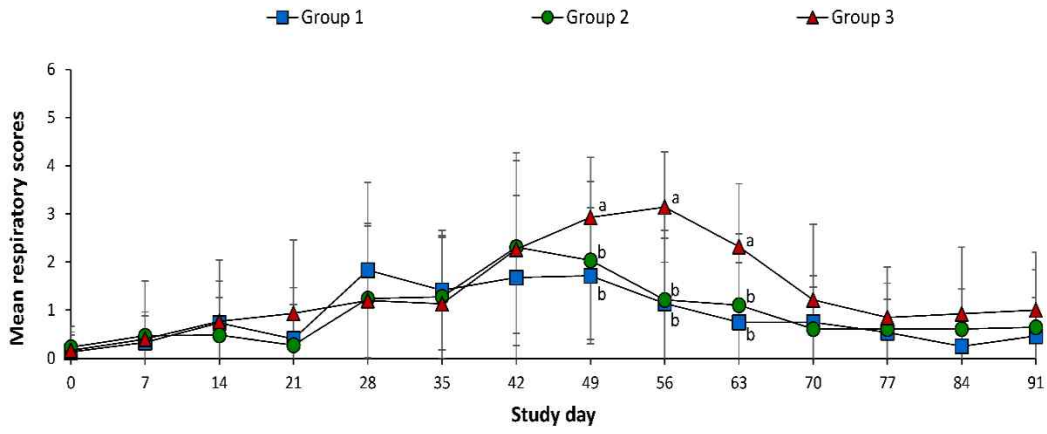
PRRSV antibody titer; and numbers of lung portions positive for PRRSV nucleic acid per unit area (0.25 mm^2) by in situ hybridization. Continuous data were estimated using Tukey's multiple comparisons test to compare the discrepancy among groups at each time point. Discrete data (clinical signs and lung injury scores) were evaluated with the Kruskal-Wallis test. When the outcome from Kruskal-Wallis test indicated significance, the Mann-Whitney test was conducted to evaluate the significant differences among groups. Fisher's exact test was adopted to determine mortality rate. A value of $P < .05$ was deemed significant.

3. RESULTS

3.1 *Clinical evaluation*

Significant lower ($P < .05$) mean scores for the respiratory lesion were detected in immunized pigs (Group 1 and Group 2) than in unimmunized pigs (Group 3) between the study day 49 and 63 (Figure 1). The entire morality rates were 6.6% (2 out of 30 pigs) in Group 1 and in Group 2, and 13.3% (4 out of 30 pigs) in Group 3, respectively. The results from diagnostic test showed the cause of death was mainly related to *streptococcal meningitis* in Group 1 and *pneumonic pasteurellosis* in Group 2 and Glasser's disease (*hemophilus parasuis*) in Group 3, respectively.

Figure 1. Mean respiratory scores of pig.



工: Standard deviation

Different alphabets (a, b) at a study day showed significant differences among three groups

Significant higher (P > .05) ADGs were found in immunized pigs (Group 1 and Group 2) than in unimmunized pigs (Group 3) between the study day 91 and 147 and between the study day 0 and 147 (Table 1).

Table 1. Mean numbers from ADG (g/day) in pigs immunized for PRRS vaccine (Group 1 and Group 2) or administrated with PBS (Group 3).

Period between study days	Age (days)	Group 1	Group 2	Group 3
0 to 49	21 to 70	400.51 (56.81)	405.77 (41.02)	396.57 (36.86)
49 to 91	70 to 112	631.57 (86.12)	639.13 (80.26)	607.14 (78.75)
91 to 147	112 to 168	786.80 (59.16)[a]	783.07 (71.85)[a]	738.61 (41.41)[b]
0 to 147	21 to 168	615.82 (34.43)[a]	615.85 (29.15)[a]	586.59 (30.38)[b]

ADG: Average daily gain.

PRRS: Porcine reproductive and respiratory syndrome.

PBS: Phosphate buffered saline.

* Group 1 pigs were immunized with a one-dose PRRS vaccine (Fostera PRRS®; Zoetis, Florham Park New Jersey); Group 2 pigs were immunized with a one-dose PRRS vaccine (Ingelvac PRRS MLV®; Boehringer Ingelheim Vetmedica Inc., St. Joseph Missouri); and Group 3 pigs were administered with phosphate buffered saline. The body weight of each pig in each group was monitored. ADG was compared among three groups using Tukey's multiple comparison test.

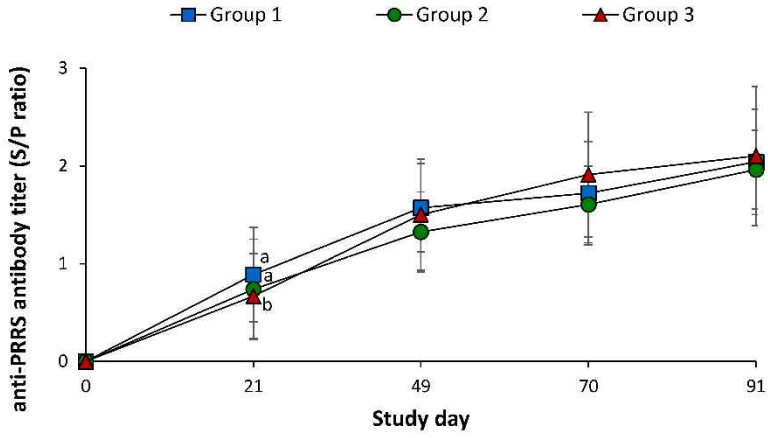
ab Whithin a row, numbers with different alphabet letters showed significant difference ($P < .05$).

3.2 *Quantification of PRRSV RNA in the blood*

On a study day 21, significantly higher ($P > .05$) anti-PRRSV antibody titers were

found in immunized pigs (Group 1 and Group 2) than in unimmunized pigs (Group 3) (Figure 2). 15 sows showed anti-PRRSV antibody titers, with S:P ratios ranging from 0.4 to 0.7.

Figure 2. Mean anti-PRRSV antibody serum titers of pigs. Blood samples were gathered from pigs for serological test (S:P ratio reported).



S:P: Sample-to-positive.

工: Standard deviation

Different alphabets (a, b) at a study day show significant differences among three groups.

3.3 *ORF5 sequence reading*

No difference in numbers of genomic copies of PRRS-2 wild virus in serum was

detected between immunized pigs (Group 1 and Group 2) and unimmunized pigs (Group 3) during the experiment. Five freely chosen serum samples in three groups expressed ORF5 sequences, which were highly homologous (99.1% to 100%) with PRRS wild virus (SNUVR150324 strain). Vaccine virus was identified in the blood of immunized pigs (Group 1) at a study day 21 (four pigs) and day 49 (one pig) ORF5 sequences from immunized pigs (Group 1) at the study day 21 and day 49 characterized the Fostera PRRS® vaccine virus. Vaccine virus was also identified in the blood of immunized Pigs (Group 2) at a study day 21 (five pigs) and day 49 (two pigs). ORF5 sequences from immunized pigs (Group 2) at a study day 21 and day 49 characterized the Ingelvac PRRS® vaccine virus. Analyzed by PRRSV ORF5 sequencing after immunization, cross-contamination of vaccine virus was not witnessed between Group 1 and Group 2 immunized pigs. Vaccine virus was not observed in the blood of unimmunized pigs (Group 3). PRRSV-1 was not observed in any of the three groups during the experiment.

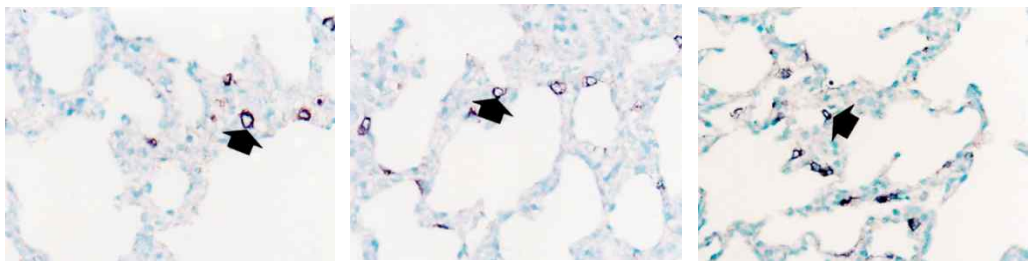
3.4 *Pulmonary lesion scores*

Significant lower ($P < .05$) pulmonary lesion scores were detected in immunized pigs (Group 1 and Group 2) than unimmunized pigs (Group 3) (Table 2). The number of lung cells showing positive signal for PRRSV-2 nucleic acid was significantly dissimilar between immunized pigs (Group 1 and Group 2) (Figure 3)

and unimmunized pigs (Group 3) (Table 2).

Figure 3. Small number of PRRSV-2 nucleic acid-positive cells were identified in macrophages in pigs from Group 1 (Panel A), Group 2 (Panel B), or Group 3 (Panel C).

A **B** **C**



In situ hybridization test was conducted using a PRRSV-2 specific probe to identify PRRSV-2 nucleic acid in lungs for pigs.

PRRSV-2: Type 2 porcine reproductive and respiratory syndrome virus.

200 x magnification.

Table 2. Mean scores from pulmonary lesion and Mean numbers of pulmonary cells showing positive signal for PRRSV-2 nucleic acid.

Group (n)	Vaccination (21 days)	Lesion score‡	No. of type 2 PRRSV-positive cells€
1 (30)	Fostera PRRS®	0.69 (0.51)[a]	3.33 (1.35)
2 (30)	Ingelvac PRRS MLV®	0.81 (0.53)[a]	3.94 (1.85)
3 (30)	None	1.64 (0.44)[b]	4.17 (2.16)

PRRSV-2: Type 2 porcine reproductive and respiratory syndrome virus

* Study described in Table 1.

† Vaccines: Fostera PRRS®, Zoetis Florham New Jersey and Ingelvac PRRS MLV®; Boehringer Ingelheim Inc., St. Joseph Missouri

‡ Lung samples were gathered from pigs in each group, and three tissue portions from eight lung segments were observed blindly. Lung injuries were recorded. Lung injuries were recorded on a scale from 0 to 4.

€ Numbers of lung cells showing positive signal for PRRSV-2 nucleic acid per unit area (0.25 mm^2 of lung were counted using an NIH Image J 1.45s program (http://imagej.nih.gov/ij/download.html). Numbers of positive cells were compared among groups.

ab Within a column, numbers with different alphabetical letters showed significant difference ($P < .05$)

4. DISCUSSION

This study indicated that pigs immunized with MLV vaccines for PPRS showed

better growth performance and fewer lung injuries than unimmunized controls under wild condition. Moreover, no significant differences were detected between two commercial MLV PRRSV vaccines in this study based upon four types of results: clinical signs (ex. ADG), immunologic (antibodies), virologic (PCR testing) and pathologic (lesions and viral antigen). Evaluation of PRRSV viremia was one of the parameters in determining the efficacy of PRRS vaccines under an experimentally challenge study (12-14). On the other hand, in comparison to earlier studies (15-17), the present study demonstrated that no difference from the number of genomic copies of wild type PRRSV-2 RNA was not detected between immunized and unimmunized pigs under wild conditions. This difference may be detected from various conditions, such as ventilation and feeding systems in experimental and field sites. In this study, immunized and unimmunized pig were placed in a separate cages within the same animal house. Hence, immunized pigs might be infected with the prevalent PRRS wild-type virus. This situation may elucidate the reason why the number of genomic copies of wild-type PRRSV-2 RNA was not unlike significantly between immunized and unimmunized pigs.

Even though reproductive breakdown had happened within 4 months of this study in the sow farms, maternally inherited anti-PRRSV antibodies were not identified in any pigs from three groups. Low PRRSV ELISA S : P ratios of 15 sows applied in this study proposed that the most of newborn piglets could have acquired small

quantities of foremilk anti-PRRSV antibodies from their dams. These passively obtained antibodies might disintegrate in pigs by 21 days of age, which elucidated the reason why the 21-day-old pigs in this study did not possess observable maternally acquired anti-PRRSV antibodies at the time of immunization.

Discrimination between two commercial MLV PRRS vaccines accommodates swine physicians and growers with clinical information regarding the control of PRRSV infection. Without considering the commercial MLV PRRS vaccine, better growth performance and fewer lung lesions were observed in immunized pigs than in unimmunized pigs. However, no significant differences in growth performance lung lesions between pigs immunized with either commercial MLV PRRS vaccine.

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국문 논문 초록

두 가지 PRRSV와 PCV2 백신의 효능 비교 평가

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Porcine reproductive and respiratory syndrome virus (PRRSV)에 의해 발생되는 돼지 생식기 호흡기 증후군 (PRRS)은 세계적으로 돼지 산업에서 가장 커다란 경제적 손실을 일으켰다. 성장한 돼지를 PRRSV로 감염 시키면 심각한 호흡기 질환이 유발되어 이유자돈 및 성장돈의 성장이 억제된다. 또한 바이러스는 암퇘지에서는 생식 질병을 유발한다. 본 논문은 돼지에 두 가지 상용화된 PCV2 백신과 PRRSV 백신을 동시에 투여한 후 동일한 두 가지 바이러스에 감염시켰을 때 두 백신간의 효능을 비교 평가한 첫 번째 실험결과와 양돈장 현장에서 PRRSV Type 2에 감염된 육성돈을 대상으로 두 가지 상용화 백신 중

한가지씩 각각 투여한 후 그들의 효능을 비교 평가한 두 번째 실험결과로 구성되어 있다. 첫 번째 연구의 목적은 Zoetis사와 Boehringer Ingelheim사가 출시한 상용화 제품들 중 돼지 써코바이러스 2 형 (PCV2) 백신과 돼지 생식기 및 호흡기 증후군 바이러스 (PRRSV) 백신 2가지를 동시에 접종시킨 이유자돈들이 동일한 두 가지 바이러스에 복합 감염되었을 때 임상적, 바이러스학적, 면역학적 및 병리학적 지표를 비교하여 치료에 영향을 미치는지를 관찰해 보았다. -28일째(21일령)에 1군에는 Fostera PCV®와 Fostera PRRS® (Zoetis, Parsippany, New Jersey) 백신 바이러스를 동시에 투여하였고, 2군에는 Ingelvac Circo FLEX®와 Ingelvac PRRS MLV® (Boehringer Ingelheim Vetmedica Inc., St Joseph, Missouri) 백신 바이러스를 동시에 투여하였다. 시험 0 일째 (49 일령)에 PCV2 및 PRRS 바이러스에 감염시켰다. 임상적, 바이러스학적 (14 일째 PCV2 바이러스 혈증 제외), 면역학적 및 병리학적 지표에 의하면 1군 & 2군과 양성 대조군인 3군간에는 유의적인 차이가 관찰되었으나, 백신투여 Group 1과 Group2 군간에 유의한 차이는 관찰되지 않았다. 본 연구조건 하에서, 두 개발사가 출시했던 PCV2와 PRRSV 상용화 백신 각각의 복합 투여에 의한 효능을 비교해 보았을 때 유의적인 차이는 관찰되지 않았다. 그러나 두 가지 상용화 백신의 복합 투여는 PCV2와 PRRSV 바이러스의 활동을 제어하는 효율적인 방법이었다. 두 번째 연구의 목적은 양돈장 현장에서 PRRSV-2 바이러스에 감염된 돼지를 대상으로 위 두 개발사가 출시했던 2 개의 MLV PRRS 백신 각각의 효능을 비교 평가하는

것이었다. 현장은 시험개시 4개월 전부터 PRRSV-2에 의한 번식장애와 호흡기질환 등으로 인해 문제를 겪어왔다. PRRSV-2에 대한 실험에 집중하기 위해 PCV2 바이러스 감염을 방어할 목적으로 3주령 이상의 돼지를 대상으로 써코바이러스 제2형 (PCV2) 백신 바이러스를 정기적으로 접종하였다. 야생 바이러스와 PRRSV-2 백신간의 뉴클레오타이드 ID의 상동성을 파악하기 위해 42일령 돼지의 폐로부터 2형 PRRSV (SNUVR 150324 주, 계통 5, GenBank No. KU301048)를 추출하였다. Fostera PRRS® 백신 바이러스와 Ingelvac PRRS MLV® 백신 바이러스는 모두 ORF5에 대해 각각 91.5% 및 99.1%의 뉴클레오타이드 ID의 상동성을 나타내었다. Group 1은 Zoetis사 Fostera PRRS® 백신 바이러스를 투여 하였고, Group 2는 Boehringer Ingelheim사 Ingelvac PRRS MLV® 백신 바이러스를 투여하였다. Group 3은 대조군으로 PBS(0.01M, pH 7.4)를 투여하였다. 야생 상태에서 임상적, 면역학적, 바이러스학적 및 병리학적 지표들의 결과를 바탕으로 볼 때 1군 및 2군을 3군과 비교했을 때 유의적인 차이가 관찰되었으나 1군과 2군 간에는 유의적인 차이가 관찰되지 않았다. 아울러 1군, 2군 및 3군간에 2 형 PRRS 바이러스 양의 유의적인 차이는 관찰되지 않았음을 볼 때 3개 그룹을 동일한 곳간 안에 무작위 분산 배치했음에도 불구하고 면역화한 돼지들 조차 순화하는 PRRS Field 바이러스에 감염되었을 가능성이 높았다고 할 수 있겠다. 효능에 대해서는 야생 바이러스인 PRRSV-2 (SNUVR 15034 strain)와 Fostera PRRS® 백신 바이러스 그리고 Ingelvac PRRS® 백신 바이러스간에 높은 (> 90%)

뉴클레이타이드 ID를 공유하지만 PRRS MLV 백신 바이러스와 야생 바이러스간에 유전적 상동성과는 관계가 없다는 결론이 본 실험을 통해 밝혀진 내용이다.

주요어: 돼지 써코바이러스 2형 (PCV2), 돼지 PRRS 바이러스 제2형 (PRRSV-2), 돼지 생식기 및 호흡기 증후군 (PRRSV), 백신

학번: 2015-31176