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Evaluation of a 20 year old porcine reproductive and respiratory syndrome (PRRS) modified live vaccine (Ingelvac® PRRS MLV) against two recent type 2 PRRS virus isolates in South Korea

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ABSTRACT

Type 2 porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) was first isolated in Korea in 1994. The commercial PRRS modified live vaccine (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica Inc., St. Joseph, Missouri, USA) based on type 2 PRRSV, was first licensed for use in 3- to 18-week-old pigs in Korea in 1996. The objective of the present study was to evaluate the efficacy of this 20 year old commercial PRRS modified live vaccine (MLV) against two recent PRRSV isolates. Two genetically distant type 2 PRRSV strains (SNUVR150004 for lineage 1 and SNUVR150324 for lineage 5), isolated in 2015, were used as challenge virus. Regardless of the challenge virus, vaccination of pigs effectively reduced the level of viremia, the lung lesions, and of the PRRSV antigen within the lung lesions. The induction of virus-specific interferon-γ secreting cells by the PRRS vaccine produced a protective immune response, leading to the reduction of PRRSV viremia. There were no significant differences in efficacy against the two recently isolated viruses by the PRRS MLV based on virological results, immunological responses, and pathological outcomes. This study demonstrates that the PRRS MLV used in this study is still effective against recently isolated heterologous type 2 PRRSV strains even after 20 years of use in over 35 million pigs

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) has been one of the most economically important global viral diseases for over two decades. PRRS is characterized by reproductive failures in sows and respiratory distress in growing pigs (Zimmerman et al., 2012). The etiologic agent of PRRS is the PRRS virus (PRRSV), which is a member of the *Arterivirus* genus, *Arteriviridae* family and *Nidovirales* order (Snijder and Meulenberg, 1998). The PRRSV genome is approximately 15 kb in length and contains at least ten open reading frames (ORFs) (Snijder et al., 2013). PRRSV can be divided into two genetically distinct genotypes: type 1 PRRSV, which is the major genotype circulating in Europe, and type 2 PRRSV, which is the major genotype found in North America and Asian countries (Allende et al., 1999; Murtaugh et al., 2010). Type 2 PRRSV is the most dominant and economically significant genotype in Korea. Type 2 PRRSV is further classified into 9 lineages based on global genotyping classification (Shi et al., 2010). Among those, type 2 PRRSV belonging to lineage 1 and 5 is commonly isolated in Korea (Shi et al., 2010).

PRRSV is considered one of the most rapidly evolving viruses on the planet (Normile, 2007). Mutation and recombination are two common evolutionary mechanisms for PRRSV, which can lead to enhanced fitness for survival or increased virulence (Gorbalenya et al., 2006; Domingo and Holland, 1997). Rapid evolution of PRRSV is an important driving force for the emergence of new strains capable of vaccine resistance (Chand et al., 2012). The commercial PRRS modified live vaccine (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica Inc., St. Joseph, Missouri, USA) has been used to control PRRSV for 20 years in Korean pig farms. After 20 years of use, some swine producers and practitioners have raised the concerns about the efficacy of this PRRS MLV due to genetic and antigenic change of field viruses. Therefore, the objective of this study was to evaluate this PRRS MLV against two recent PRRSV isolates.

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2. Materials and methods

2.1. PRRSV inoculum

Type 2 PRRSV (SNUVR150004 strain, lineage 1, GenBank no. KU301047) was isolated from lung samples of growing pigs at 84 days of age in a 500-sow herd in 2015. This herd showed severe respiratory problems in growing pigs aged 10–18 weeks old. Another type 2 PRRSV (SNUVR150324 strain, lineage 5, GenBank no. KU301048) was isolated from lung samples of weaned pigs at 42 days of age in a 1000-sow herd in 2015. This herd had suffered recent losses due to type 2 PRRSV infection and respiratory diseases in weaned pigs.

2.2. Experimental design

A total of 130 colostrum-fed, cross-bred, conventional piglets were purchased at 14 days of age from a commercial PRRSV-free farm. All piglets were negative for PRRSV, porcine circovirus type 2 (PCV2), and *Mycoplasma hyopneumoniae* according to routine serological testing. Serum samples were negative for PCV2 and PRRSV, and nasal swabs were negative for *M. hyopneumoniae* when tested by real-time polymerase chain reaction (PCR) (Dubosson et al., 2004; Wasilk et al., 2004; Gagnon et al., 2008).

The pigs were randomly divided into 5 groups: Vac/Ch2L1 ($n = 30$), Vac/Ch2L5 ($n = 30$), UnVac/Ch2L1 ($n = 30$), UnVac/Ch2L5 ($n = 30$), and UnVac/UnCh ($n = 10$) (Table 1). The pigs in Vac/Ch2L1 and Vac/Ch2L5 were vaccinated with Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica; Lot No. 2451017A) and challenged with type 2 PRRSV lineage 1 and 5, respectively. A dose of 2 mL of Ingelvac PRRS MLV (Boehringer Ingelheim Vetmedica) was delivered by intramuscular injection on the right side of the neck at 21 days of age, according to the manufacturer's instructions.

Type 2 PRRSV inoculum consisted of either SNUVR150004 strain or SNUVR150324 strain, which was propagated on MARC-145 cells to a titer of 10^5 50% tissue culture infective doses ($TCID_{50}$)/mL. At 56 days of age (0 day post challenge, dpc), the pigs in Vac/Ch2L1 and UnVac/Ch2L1 were inoculated intranasally with 3 mL of type 2 PRRSV (SNUVR150004 strain, lineage 1) inoculums by setting them on their buttocks perpendicular to the floor and extending the neck fully back. The inoculum was slowly dripped into both nostrils of the pigs taking approximately 3–5 min/pig as previously described (Halbur et al., 1995). The pigs in Vac/Ch2L5 and UnVac/Ch2L5 were inoculated intranasally with 3 mL of type 2 PRRSV (SNUVR150324 strain, lineage 5) inoculums by the same manner. The pigs in UnVac/UnCh served as negative controls and were neither vaccinated nor challenged. Fifteen and five pigs from each treatment (Vac/Ch2L1, Vac/Ch2L5, UnVac/Ch2L1, and UnVac/Ch2L5) and negative control (UnVac/UnCh) group, respectively, were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 7 and 14 dpc as previously described (Beaver et al., 2001)

The pigs in each group were housed in separate experimental rooms equipped with air conditioning and high-efficiency particulate air filtration to avoid possible transmission of the pathogen between groups throughout the experiment in the research facility. Following PRRSV inoculation, the physical condition of the pigs was monitored daily including rectal temperatures. Blood samples were collected at −35, −28, −21, −14, 0, 3, 7, 10, and 14 dpc. All of the methods were previously approved by the Seoul National University Institutional Animal Care and Use, and Ethics Committee.

2.3. Clinical observation

Following vaccination and PRRSV challenge, the pigs were monitored weekly for physical conditions and scored daily for clinical respiratory disease severity using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (Halbur et al., 1995). Observers were blinded to vaccination status. Stress was induced daily by pig handler by holding the pig under his arm and taking the rectal temperature (Halbur et al., 1996b). Rectal thermometer (Digital Fever Thermometer, Becton-Dickinson, Franklin Lakes, New Jersey, USA) was lubricated and inserted approximately 6–7 cm into the rectum and readings were taken when the thermometer beeped (Thoresen et al., 2001). Rectal temperatures were recorded daily at the same time by same personnel.

2.4. Quantification of PRRSV RNA

RNA was extracted from serum samples to quantify PRRSV genomic cDNA copy numbers, as previously described (Wasilk et al., 2004). For the challenge type 2 PRRSV, the forward and reverse primers were 5′-TGGCCAGTCAGTCAATCAAC-3′ and 5′-AATCGATTGCAAGCAGAGGGAA-3′, respectively (Park et al., 2014). For the vaccine virus, the forward and reverse primers were 5′-CTAACAAATTTGATTGGGCAG-3′ and 5′-AGGACATGCAATTCTTTG-CAA-3′, respectively (Han et al., 2011). Real-time PCR for the

Table 1
Experimental design and results of lesion score and porcine reproductive and respiratory syndrome virus (PRRSV) RNA within lung lesion at 7 and 14 days post challenge (dpc).

Groups	PRRSV		*dpc* (*n*)	Lung lesion score		PRRSV-positive cells within lung lesion
	Vaccination (21 days)	Challenge (56 days)		Macroscopic	Microscopic	
Vac/Ch2L1	Yes	Lineage 1	7 (15)	31.06 ± 6.40^a	1.93 ± 0.25^a	30.33 ± 5.66^a
			14 (15)	20.46 ± 4.41^a	1.27 ± 0.44^a	21.27 ± 4.33^a
Vac/Ch2L5	Yes	Lineage 5	7 (15)	32.09 ± 6.80^a	1.8 ± 0.4^a	28.4 ± 5.21^a
			14 (15)	21.73 ± 5.66^a	1.2 ± 0.4^a	21.07 ± 3.60^a
UnVac/Ch2L1	No	Lineage 1	7 (15)	56.13 ± 8.17^b	3.67 ± 0.47^b	42.6 ± 4.64^b
			14 (15)	38.25 ± 5.79^b	3.27 ± 0.57^b	34.13 ± 4.36^b
UnVac/Ch2L5	No	Lineage 5	7 (15)	61.33 ± 8.16^b	3.67 ± 0.60^b	42.36 ± 5.84^b
			14 (15)	38.48 ± 6.24^b	3 ± 0.37^b	34.81 ± 3.96^b
UnVac/UnCh	No	No	7 (5)	4.44 ± 2.64^c	0.2 ± 0.4^c	0^c
			14 (5)	4.76 ± 3.56^c	0.4 ± 0.49^c	0^c

*n = Numbers of pigs were necropsied at 7 and 14 dpc. Different letters (a, b, and c) indicate significant ($P < 0.05$) difference among groups.