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NOTE

Virology

Comparative evaluation of the efficacy of commercial and prototype PRRS subunit vaccines against an HP-PRRSV challenge

Do Tien DUY[1)#], Hanjin KIM[2)#], Jiwoon JEONG[2)], Kee Hwan PARK[2)], Siyeon YANG[2)], Taehwan OH[2)], Seeun KIM[2)], Ikjae KANG[2)] and Chanhee CHAE[2)*]

[1)]Faculty of Animal Husbandry and Veterinary Medicine, Nonglam University, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam

[2)]Seoul National University, College of Veterinary Medicine, Department of Veterinary Pathology, 1 Gwanak-ro, Gwanak-gu, Seoul, 08826, Republic of Korea

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ABSTRACT. The objective of this study was to compare the efficacy of a commercial porcine reproductive and respiratory syndrome (PRRS) subunit vaccine and a prototype PRRS II subunit vaccine against a highly pathogenic PRRS virus (HP-PRRSV) in pigs. Both vaccines were administered intramuscularly in 2 doses at 21 and 42 days of age, and the pigs were challenged intranasally with HP-PRRSV at 63 days of age. Pigs vaccinated with the prototype PRRS II subunit vaccine had significantly higher anti-PRRSV antibody titers, a greater number of interferon-γ-secreting cells, and a greater reduction in lung lesion scores compared to pigs vaccinated with the commercial PRRS subunit vaccine. Therefore, the commercial PRRS subunit and prototype PRRS II subunit vaccines are efficacious against HP-PRRSV.

KEY WORDS: highly pathogenic porcine reproductive and respiratory syndrome virus, porcine reproductive and respiratory syndrome, subunit vaccine

Highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) was first reported in the literature after being isolated from large-scale outbreaks of atypical PRRSV epidemics in China in 2006 [17]. Since then, the virus has been isolated from several Southeast Asian countries [5, 6, 14, 20]. Infections are characterized by high fever (40–42°C), and high mortality (20–70%) in young and adult pigs, resulting in more than one million deaths in pig farms [17]. So far, all HP-PRRSV strains share a common molecular hallmark: an identical discontinuous deletion of 30 amino acids in nonstructural protein 2 [17].

HP-PRRSV infections have resulted in enormous economic losses, representing the most severe swine disease currently threatening the Asian swine industry. So far, vaccines have been the most effective tool in controlling HP-PRRSV infection. Commercial PRRS modified-live virus vaccines have been reported as effective against HP-PRRSV [4, 11]. However, no study has reported the efficacy of commercial PRRS subunit vaccines. The objective of this study was to evaluate the efficacy of a commercial PRRS subunit vaccine and a new prototype PRRS subunit vaccine against HP-PRRSV, based on clinical, virological, immunological, and pathological evaluations.

In this study, 48 colostrum-fed, cross-bred, conventional piglets were purchased from a commercial farm at 14 days of age. All piglets were tested using an enzyme-linked immunosorbent assay and real-time polymerase chain reaction (RT-PCR), the results of which were negative for PRRSV [18]. Pigs were randomized, using the Excel random number function (Microsoft Corporation, Redmond, WA, U.S.A.), into 4 groups (12 pigs/group) (Table 1). At −42 and −21 days post challenge (dpc, 21 and 42 days of age), the pigs in the VacReber/Ch group were administered a 2.0-m*l* dose of the commercial PRRS subunit vaccine (PRRSFREE™ PRRS subunit vaccine, Lot No. F5002, Reber Genetics Co., Ltd., Taipei, Taiwan, Republic of China) as an intramuscular injection on the right side of the neck. The pigs in the VacProto/Ch group were similarly administered a 2.0-m*l* dose of the prototype PRRS II subunit vaccine (manufacture date 11/15/2016, Reber Genetics Co., Ltd.). At 0 dpc (63 days of age), the pigs in the VacReber/Ch, VacProto/Ch, and UnVac/Ch groups were inoculated intranasally with 3 m*l* of tissue culture fluid containing a 50% tissue culture infective dose ($TCID_{50}$) of $10^{5.5}$/m*l* of Vietnamese HP-PRRSV (strain MB6, 4th passage in MARC-145 cells). Vietnamese HP-PRRSV (strain MB6, GenBank number KM244760) is a highly virulent strain that was isolated from a 30-sow herd in a northern region of Vietnam in 2009 [3]. The negative control pigs in the UnVac/UnCh group were neither vaccinated nor challenged.

*Correspondence to: Chae, C.: swine@snu.ac.kr
#These authors contributed equally to this work.

Table 1. Clinical signs and pathology in pigs among 4 groups

	dpc	Groups			
		VacReber/Ch	VacProto/Ch	UnVac/Ch	UnVac/UnCh
Vaccine (age in days)		PRRSFREE (21, 42)	Prototype PRRS II (21, 42)	None (−)	None (−)
Challenge (age in days)		HP-PRRSV (63)	HP-PRRSV (63)	HP-PRRSV (63)	None (−)
Loss of appetite	3	0/12	0/12	0/12	0/12
	5	0/12	2/12	3/12	0/12
	7	1/12	4/12	5/12	0/12
	10	0/8	3/8	5/8	0/8
	14	0/4	0/4	2/4	0/4
Reluctant to move	3	0/12	0/12	0/12	0/12
	5	0/12	0/12	6/12	0/12
	7	1/12	0/12	10/12	0/12
	10	1/8	2/8	6/8	0/8
	14	0/4	0/4	3/4	0/4
Eye discharge/swollen	3	0/12	0/12	7/12	0/12
	5	2/12	0/12	8/12	0/12
	7	0/12	0/12	8/12	0/12
	10	0/8	1/8	5/8	0/8
	14	0/4	0/4	3/4	0/4
Erythema/cyanosis	3	0/12	0/12	0/12	0/12
	5	0/12	0/12	3/12	0/12
	7	1/12	4/12	5/12	0/12
	10	0/8	2/8	4/8	0/8
	14	0/4	0/4	2/4	0/4
Shivering	3	0/12	0/12	0/12	0/12
	5	0/12	0/12	0/12	0/12
	7	0/12	0/12	5/12	0/12
	10	1/8	0/8	4/8	0/8
	14	0/4	0/4	1/4	0/4
Macroscopic lung lesion score	7	$75.93 \pm 1.11^{a)}$	$80.00 \pm 4.08^{a)}$	$82.00 \pm 4.76^{a)}$	$0 \pm 0^{b)}$
	10	$66.50 \pm 4.51^{a)}$	$70.00 \pm 4.08^{a)}$	$74.50 \pm 8.43^{a)}$	$1.50 \pm 3.00^{b)}$
	14	$62.50 \pm 2.04^{b)}$	$67.25 \pm 2.06^{a, b)}$	$72.00 \pm 6.75^{a)}$	$1.00 \pm 2.00^{c)}$
Microscopic lung lesion score	7	$2.00 \pm 0.36^{b)}$	$2.00 \pm 0.41^{b)}$	$2.75 \pm 0.21^{a)}$	$0 \pm 0^{c)}$
	10	$2.33 \pm 0.21^{a,b)}$	$2.28 \pm 0.21^{b)}$	$2.80 \pm 2.24^{a)}$	$0 \pm 0^{c)}$
	14	$2.13 \pm 0.48^{a,b)}$	$2.00 \pm 0.41^{b)}$	$2.68 \pm 0.13^{a)}$	$0 \pm 0^{c)}$
Lung antigen score	7	$1.50 \pm 0.58^{b)}$	$1.63 \pm 0.25^{b)}$	$3.50 \pm 0.58^{a)}$	$0 \pm 0^{c)}$
	10	$1.25 \pm 0.50^{b)}$	$1.75 \pm 0.29^{a, b)}$	$3.00 \pm 0.82^{a)}$	$0 \pm 0^{c)}$
	14	$1.25 \pm 0.50^{a)}$	$1.38 \pm 0.48^{a)}$	$2.00 \pm 0.82^{a)}$	$0 \pm 0^{b)}$

Different letters (a, b, and c) indicate significant differences ($P<0.05$) among the groups at the same number of days post challenge (dpc).

Each of the groups was housed separately within the facility. Blood samples were collected at −42, −21, 0, 7, 10, and 14 dpc. Pigs were sedated using an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 7, 10, and 14 dpc, as previously described [1]. Tissues were collected from each pig at necropsy. All of the methods used were previously approved by the Nonglam University Institutional Animal Care and Use, and Ethics Committee.

Clinical respiratory disease severity was scored daily using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) [7]. Rectal temperatures were also recorded daily at the same time by the same member of personnel. Serum samples were collected from individual pigs, RNA was extracted, and RT-PCR was performed to quantify PRRSV genomic cDNA copy numbers, as previously described [3, 18].

Serum samples were tested using a commercially available PRRSV enzyme-linked immunosorbent assay (HerdCheck PRRS X3 Ab, IDEXX Laboratories Inc., Westbrook, ME, U.S.A.). Serum samples were considered positive for PRRSV antibody when the sample-to-positive ratio was ≥0.4, according to the manufacturer's instructions. The numbers of PRRSV-specific interferon (IFN)-γ-secreting cells (IFN-γ-SC) stimulated withVietnamese HP-PRRSV were determined from peripheral blood mononuclear cells (PBMC), as previously described [2, 12, 15].

Macroscopic and microscopic lung lesions were scored and analyzed morphometrically, as previously described [7].

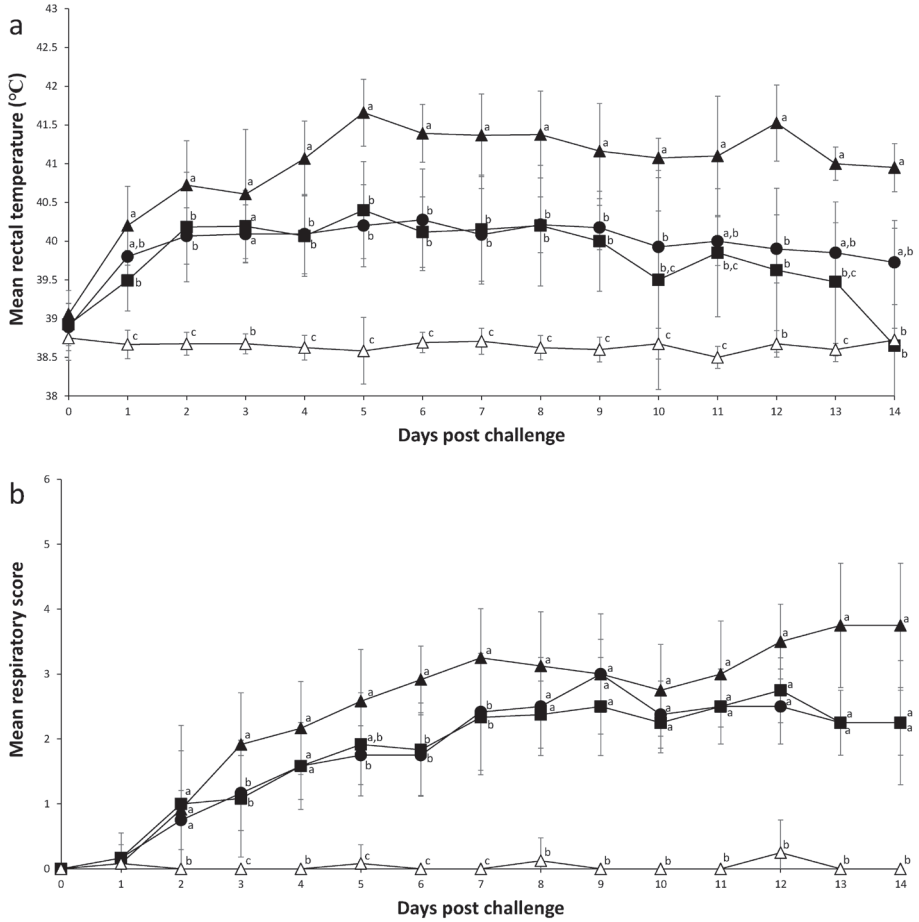

Fig. 1. Mean rectal temperature (a) and mean respiratory score (b) in the pigs in the VacReber/Ch (●), VacProto/Ch (■), UnVac/Ch (▲), and UnVac/UnCh (△) groups. Variation is expressed as the standard deviation. Different letters (a, b, and c) indicate significant differences (P<0.05) among the groups at same number of days post challenge.

Immunohistochemistry for PRRSV was performed using SR30 monoclonal antibody (Rural Technologies Inc., Brookings, SD, U.S.A.) and analyzed morphometrically, as previously described [8, 9].

RT-PCR data were transformed to $\log_{10}$ values prior to statistical analysis. Continuous data (rectal temperature, PRRSV RNA, serology, IFN-γ-SC, and macroscopic lung lesion score) were analyzed using a one-way analysis of variance followed by Tukey's multiple comparison test at each time point (−42, −21, 0, 7, 10, and 14 dpc) Discrete data (respiratory clinical score, PRRSV antigen score, and microscopic lung lesion score) were analyzed using Mann–Whitney tests. A value of P<0.05 was considered significant.

Pigs in both of the vaccinated groups (VacReber/Ch and VacProto/Ch) exhibited a delay in the onset of disease by several days and no mortality occurred throughout the experiment. Four pigs in the UnVac/Ch group died at 7 (n=2) and 10 (n=2) dpc. Pigs in the UnVac/UnCh group did not show any clinical signs throughout this study (Table 1).

Mean rectal temperatures at 2, 4–10, and 12 dpc were significantly lower (P<0.05) in the pigs in the VacReber/Ch and VacProto/Ch groups compared to the pigs in the UnVac/Ch group (Fig. 1a). Mean respiratory scores at 3, 6, and 7 dpc in the pigs in the VacReber/Ch and VacProto/Ch groups were significantly lower (P<0.05) compared to the pigs in the UnVac/Ch group. The pigs in the UnVac/UnCh group did not exhibit any respiratory symptoms throughout this study (Fig. 1b).

The number of genomic copies of HP-PRRSV detected in the serum samples collected at 7 dpc was significantly lower (P<0.05) in the pigs in the VacReber/Ch and VacProto/Ch groups compared to the pigs in the UnVac/Ch group at 7 dpc. No genomic copies of HP-PRRSV were detected in the blood of the pigs in the UnVac/UnCh group (Fig. 2a).

The pigs in the VacProto/Ch group had significantly higher (P<0.05) anti-PRRSV antibody titers compared to the pigs in the VacReber/Ch group at 7, 10, and 14 dpc. At 7 dpc, the pigs in the VacReber/Ch group also had significantly higher (P<0.05) anti-PRRSV antibody titers compared to pigs in the UnVac/Ch group, however the anti-PRRSV antibody titers were significantly lower than in the pigs in the UnVac/Ch group at 14 dpc. No anti-PRRSV antibody titers were detected in the pigs in the UnVac/UnCh group at any time.

The number of HP-PRRSV-specific IFN-γ-SC induced at 0 and 7 dpc was significantly higher (P<0.05) in the pigs in the VacReber/Ch and VacProto/Ch groups compared to the pigs in the UnVac/Ch group. Of the 2 vaccinated groups, the pigs in

the VacProto/Ch group produced significantly higher (P<0.05) numbers of HP-PRRSV-specific IFN-γ-SC at 7 dpc compared to the pigs in the VacReber/Ch group. The mean frequencies of HP-PRRSV-specific IFN-γ-SC in the pigs in the UnVac/UnCh group remained at basal levels (<20 cells/10^6 PBMC) throughout this study (Fig. 2b).

At 14 dpc, the pigs in the VacReber/Ch group had significantly lower macroscopic lung scores compared to the pigs in the UnVac/Ch group. Both vaccinated groups had significantly lower mean microscopic lung lesion scores at 7 dpc compared to the UnVac/Ch group, whereas only the VacProto/Ch group had significantly lower mean microscopic lung lesion scores at 10 and 14 dpc compared to the UnVac/Ch group. No lung lesions were observed in the pigs in the UnVac/UnCh group throughout this study (Table 1). PRRSV antigens were detected exclusively within the cytoplasm of macrophages in the lung tissues of pigs in the challenged groups. Sections collected from the pigs in the vaccinated groups (VacReber/Ch and VacProto/Ch) had a significantly lower mean number of PRRSV-positive cells per lung area unit compared to the pigs in the UnVac/Ch group at 7 dpc. The pigs in the VacReber/Ch group also exhibited significantly lower PRRSV-positive cells per lung area unit compared to the pigs in the UnVac/Ch group at 10 dpc. No PRRSV-positive cells were detected in the lung tissues collected from the pigs in the UnVac/UnCh group (Table 1).

Infection with HP-PRRSV typically results in high mortality (20–70%) and hyperpyrexia (fever over 41°C) in young and adult pigs, which more than likely contributes to the high mortality [19]. Therefore, clinical evaluation (i.e., body temperature and mortality) are crucial parameters in assessing the efficacy of a vaccine. Immunization of pigs with either the commercial or the prototype PRRS II subunit vaccine prevented hyperpyrexia and mortality after challenge with a HP-PRRSV. Another symptom typically associated with infection is the consistent formation of lung lesions in the form of severe interstitial pneumonia [3]. Both vaccines were able to completely prevent the formation of macro and microscopic lung lesions.

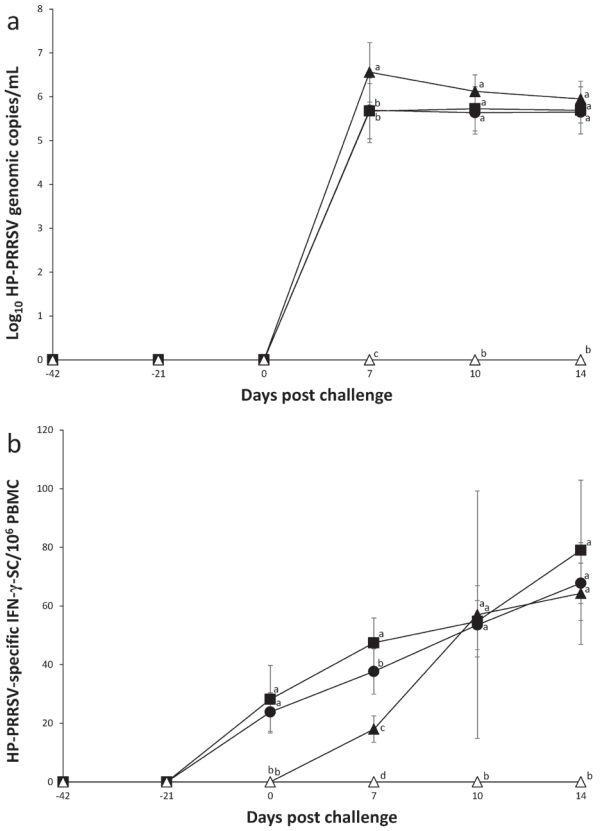

Fig. 2. Mean values of the genomic copy numbers of highly pathogenic PRRS virus (HP-PRRSV) from the serum (a) and mean numbers of HP-PRRSV-specific interferon-γ secreting cells from peripheral blood mononuclear cells (b) in the pigs in the VacReber/Ch (●), VacProto/Ch (■), UnVac/Ch (▲), and UnVac/UnCh (△) groups. Variation is expressed as the standard deviation. Different letters (a, b, c, and d) indicate significant differences (P<0.05) among the groups at same number of days post challenge.

In a previous study, HP-PRRSV viremia was resolved even before neutralizing antibodies were detected in the same HP-PRRSV-infected pigs [4]. In addition, the induction of neutralizing antibodies in vaccinated challenged and vaccinated unchallenged pigs was also limited (titer <1:2) using the same HP-PRRSV used in a previous study [4]. Hence, neutralizing antibodies are not essential for HP-PRRSV protection. By contrast, virus-specific IFN-γ responses are believed to play a role in viral clearance similar to inactivated PRRS vaccines [16]. Both commercial PRRS and prototype PRRS II subunit vaccines are able to enhance anamnestic virus-specific IFN-γ responses following HP-PRRSV challenge [16, 21]. The same commercial PRRS subunit vaccine also induces anamnestic IFN-γ responses following classical PRRSV challenge [10]. A previous study also showed that oral administration of a recombinant *Kluyveromyces lactis*-expressing GP5 of HP-PRRSV induced PRRSV-specific cellular immune responses in mice [20]. Vaccination of pigs with either of the PRRS subunit vaccines can reduce viremia at 7 dpc, coinciding with an increase in virus-specific IFN-γ-SC. However, at 10 and 14 dpc, despite higher numbers of IFN-γ-SC compared to 7 dpc, no further reduction in viremia occurs. This suggests that virus-specific IFN-γ responses are not the sole factor responsible for viral clearance.

To the best of our knowledge, this is the first comprehensive study to evaluate the efficacy of a commercial PRRS subunit vaccine and a new prototype PRRS II subunit vaccine against HP-PRRSV challenge based on clinical, virological, immunological, and pathological analyses under experimental conditions. Interest in inactivated PRRS vaccines has increased recently because of some of the safety issues resulting from modified-live virus (MLV) vaccines reverting to virulence in the field. A commercial MLV vaccine based on HP-PRRSV has already been used in China and southeastern Asian countries [6]. However, MLV vaccines based on HP-PRRSV carry the risk of reverting to virulence under field conditions, similar to an MLV vaccine based on classical PRRSV [13]. This could result in higher economic losses compared to those caused by a virulent MLV vaccine virus based on classical PRRSV. The efficacy data on these commercial PRRS subunit vaccines could provide swine practitioners and producers with valuable clinical information to help control HP-PRRSV infection while avoiding safety issues.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

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