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Protection provided by a commercial modified-live porcine reproductive and respiratory syndrome virus (PRRSV) 1 vaccine (PRRSV1-MLV) against a Japanese PRRSV2 field strain

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ABSTRACT

Background: Porcine reproductive and respiratory syndrome virus (PRRSV) vaccines do not provide full cross-protection, mainly due to the virus genetic variability. Despite this, vaccines based on modified-live PRRSV (PRRSV-MLV) reduce the disease impact.
Objectives: To assess the efficacy of two commercial vaccines—one based on PRRSV1 (PRRSV1-MLV) and another on PRRSV2 (PRRSV2-MLV)—against a Japanese PRRSV2 field strain.
Methods: Two groups of three-week-old piglets were vaccinated (G1: PRRSV1-MLV; G2: PRRSV2-MLV) and two were kept as non-vaccinated (INF and CTRL). One month later, G1, G2, and INF were challenged with a PRRSV2 field strain.
Results: After the challenge, clinical signs were only observed in INF. Moreover, the highest rectal temperatures and values for the area under the curve (AUC) were observed in INF. Regarding viral detection, both AUC and the proportion of positive samples in blood were higher in INF. In G1, viremic animals never reached 100%. At necropsy (21 d after the challenge), differences for titers among groups were only found in tonsils (G1 < G2 and INF). One animal (belonging to G1) was negative in all tissues. Regarding humoral responses, G1 and G2 seroconverted after vaccination, as detected in the corresponding enzyme-linked immunosorbent assay. Specific neutralizing antibodies (NA) against PRRSV1-MLV were already detected at 14 d after vaccination in G1, showing a significant booster after the challenge, while PRRSV2-MLV NA were detected in G2 at the end of the experiment.
Conclusions: Despite genetic differences, PRRSV1-MLV has been demonstrated to confer partial protection against a Japanese PRRSV2 strain, at least as good as PRRSV2-MLV.

Keywords: Swine; PRRSV; attenuated vaccine; immune response; cross protection

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Conflict of Interest

Joel Miranda, Salvador Romero, Lidia de Lucas, Fumitoshi Saito, and Mar Fenech are employees of Laboratorios Hipra.

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INTRODUCTION

Due to the enormous impact that causes on the swine industry worldwide, porcine reproductive and respiratory syndrome (PRRS) is considered one of the most important diseases in pigs [1]. For instance, during 2008–2009, it has been estimated that PRRS had a higher impact on the Vietnamese meat market than any other animal disease [2]; in Japan, the total losses associated with PRRS have been estimated to be around US $280 million/year [3].

One of the main characteristics of the PRRS causative agent, an RNA virus commonly known as PRRS virus (PRRSV), is its extremely high genetic variability. Because of this, it is classified into two different species, formally designed as *Betaarterivirus suid 1* (PRRSV1) and *Betaarterivirus suid 2* (PRRSV2) [4]. Several subtypes, clades, and lineages have been described within both species, demonstrating their ever-expanding genetic diversity [5]. Regarding their distribution, although PRRSV1 predominates in Europe and PRRSV2 in America and Asia, both species can be detected worldwide [1].

Although PRRSV vaccines are far from providing universal protection against the plethora of existing field strains, they are one of the main pillars of controlling the disease [6,7]. Until now, available vaccines provide partial cross-protection at most. This phenomenon may be basically due to the high PRRSV antigenic diversity, which, in turn, is the consequence of the huge genetic variability [8]. Despite the lack of absolute cross-protection, even among strains belonging to the same species, several authors have demonstrated that vaccines reduce the impact of the disease, both in terms of clinical and virological parameters [6,7,9].

The aim of the present study was to evaluate the efficacy of a commercial vaccine based on a modified-live PRRSV1 (PRRSV1-MLV) against a Japanese PRRSV2 strain and to compare it to the protection conferred by a commercial PRRSV2-MLV. Clinical protection was measured in terms of clinical signs, rectal temperatures, and body weight gains. Virological protection was measured in terms of PRRSV presence in blood and tissues. Also, antibodies by commercial enzyme-linked immunosorbent assays (ELISAs, PRRSV1 and PRRSV2-antibodies) and neutralizing antibodies (NA) by viral neutralization test were determined.

MATERIAL AND METHODS

Experimental design

Twenty piglets aged three-week-old were randomly selected from a Japanese PRRSV-negative farm. Animals were transported to the experimental facilities, ear tagged, randomly divided into four groups (G1, G2, INF, and CTRL), and left to acclimatize. Animals were re-confirmed to be free of PRRSV1 and PRRSV2-antibodies by commercial ELISAs (CIVTEST SUIS E/S for PRRSV1 and CIVTEST SUIS A/S for PRRSV2; HIPRA Laboratories, Spain). Before the acclimatization phase ended, one animal in G2 suffered a limb dislocation, being withdrawn from the study. At 4 wk of age, namely 0 days post-vaccination (dpv), the vaccination phase started; animals were intramuscularly vaccinated, G1 (n = 5) with a PRRSV1-MLV (2 mL), and G2 (n = 4) with a PRRSV2-MLV (2 mL). The remaining piglets were kept as unvaccinated animals: INF (n = 5) and CTRL (n = 5).

At 28 dpv, the challenge phase started; G1, G2 and INF were intranasally challenged with the Japanese field strain Chiba NOSAI ($10^{3.5}$ $TCID_{50}$/mL; 2 mL, 1 mL each nostril), whilst CTRL

was kept as unvaccinated and unchallenged control. Pigs were followed up 3 wk, namely 21 days post-infection (dpi). Then, animals were euthanized, and tissue samples were collected.

Animals were kept in approved experimental facilities and subjected to veterinary inspection in terms of health and welfare. All experiments involving pigs were carried out following the guidelines of the Japanese ministerial ordinances. The experimental design was submitted and approved by the National Veterinary Assay Laboratory.

Vaccines and field virus strain

Animals in G1 were vaccinated with a commercial PRRSV1-MLV based on a European strain ($10^{5,2}$ $TCID_{50}$/mL; UNISTRAIN PRRS; HIPRA Laboratories). Animals in G2 were vaccinated with a commercial PRRSV2-MLV based on an American strain ($10^{5,9}$ $TCID_{50}$/mL; INGELVAC PRRS MLV; Boehringer-Ingelheim). Both vaccines were titrated in MA104 cells. Vaccines were diluted with the vehicles recommended by each of the manufacturers.

The virulent field strain Chiba NOSAI was isolated from the lungs during a PRRS outbreak in a Japanese farm in 2012 (accession number: OQ850312). It was isolated and propagated in porcine alveolar macrophages (PAM). The ORF5 sequence was obtained with Sanger sequencing and nucleotide identity (p-distance using MEGAv11) and compared with the vaccine strains PRRSV1-MLV and PRRSV2-MLV (accession numbers: MK134483 and AF066183, respectively). For comparison purposes, several Japanese PRRSV2 field strains were downloaded from GenBank: Hokkaido (AB175720), Gunma (AB175721), Ibaraki3 (AB175722), Tochigi (AB175708), Niigata (AB175698), Shizuoka08-1 (AB546118). The dataset was completed with PRRSV1 and PRRSV2 prototype strains—Lelystad virus (M96262) and VR-2332 (U87392), respectively—, and the highly pathogenic PRRSV2 (HP-PRRSV2) strain JXA1 (EF112445).

The evolutionary relationships among sequences were evaluated by means of a phylogenetic tree. The tree was constructed with MEGA v11 [10], applying a heuristic maximum likelihood method and the Tamura-Nei substitution rate with a uniform pattern throughout the fragment analyzed. The confidence of the internal branches was assessed with 100 bootstrap replicates.

Clinical follow-up and sampling scheme

Table 1 summarizes the clinical follow-up and sampling scheme applied during the vaccination (from 0 to 28 dpv) and challenge (from 0 to 21 dpi) phases. Individual clinical signs and rectal temperatures were recorded daily from 2 d before vaccination (−2 dpv) till the end of the study. Clinical signs, in terms of appetite and respiratory distress, as well as depression, were scored using a scale from 0 to 3 (**Table 2**). Individual body weight was measured before vaccination (0 dpv), before the challenge (28 dpv, namely 0 dpi), and at the end of the experiment. Serum samples were collected before vaccination, immediately before the challenge, and at 2, 4, 6, 8, 11, 13, 16, and 21 dpi. Samples were kept frozen at −80°C until needed. At the end of the study, piglets were euthanized and samples from submandibular and bronchial lymph nodes, tonsils and lungs were collected.

Virological analysis

The presence of the virus in sera and tissues was determined on PAM cultures by viral isolation [11]. Firstly, tissues were homogenized in a medium (1:4) and centrifugated at 12,000 g for 20 min. Then, supernatants were filtered (0.22 µm). Sera and supernatants were serially diluted, inoculated, and incubated on PAM cultures. After incubation, diluted samples were removed, and the medium was replaced with a new medium containing 2%

Table 1. Experimental design

Variables	Vaccination phase (dpv)				Challenge phase (dpi)								
	0	7	14	21	0 (28 dpv)	2	4	6	8	11	13	16	21
	G1				PRRSV2 challenge								
	G2												
	INF												
					CTRL								
Clinical observation and temperatures					Daily recorded from −2 dpv to 21 dpi								
Individual body weight	X				X								X
Serum samples	X	X	X	X	X	X	X	X	X	X	X	X	X
Euthanasia[a]													X

Clinical follow-up and sampling scheme. Pigs were vaccinated at 4 wk of age. G1: PRRSV1-MLV; G2: PRRSV2-MLV; INF: non-vaccinated; CTRL: non-vaccinated and non-challenged.
dpv, days post-vaccination; dpi, days post-infection; PRRSV, porcine reproductive and respiratory syndrome virus; MLV, modified-live vaccine.
[a]Samples from submandibular and bronchial lymph nodes, tonsils and lungs were collected.

Table 2. Clinical scores

Variables	Score			
	0	1	2	3
Appetite	Normal	Slightly declined	Declined	Abolition
Respiratory	Normal	Mildly increased respiratory rate	Laboured breathing	Abdominal breathing
Depression	Alert, active	May appear lethargic, but upon stimulation appears normal	Apathy. May be recumbent but is stable to stand	May be recumbent and reluctant to rise. Head carried low with eyes dull and ears drooping

fetal bovine serum. Cultures were incubated for 7 d (37°C, 5% CO_2). Infection of inoculated cells was evaluated by determining the cytopathic effect (CPE). The titration of the virus was calculated using the Reed-Muench method.

Humoral responses

Commercially available ELISA kits were used to determine the presence of antibodies against PRRSV1 and PRRSV2 (CIVTEST SUIS E/S and A/S, respectively; HIPRA Laboratories). The relative index ×100 (IRPC) was calculated for each sample following the manufacturer's instructions; IRPC higher than 20 was considered positive.

PRRSV1-MLV and PRRSV2-MLV-specific NA were measured in cell line MA104 [12,13]. All sera were two-fold diluted and PRRSV1-MLV or PRRSV2-MLV viral suspensions were adjusted to 100 $TCID_{50}$. The culture plates containing MA104 were examined for CPE at 3-, 5- and 7-dpi. Titers were expressed as $\log_2$ of the reciprocal of the highest dilution without CPE.

Statistical analysis

Statistics were performed using StatsDirect v2.7.7 (StatsDirect Ltd, UK). The Kruskal-Wallis non-parametric test for multiple comparisons (Conover-Iman method) was used to compare averages among groups. The Friedman test was used to compare kinetics inside groups. Proportion comparisons were determined by χ^2 test (Fisher's exact test). F-test of equality of variances was used to compare variances among groups. The area under the curve (AUC) for temperatures and viremia was calculated using the trapezoidal approach [14]. The 5% level of significance ($p < 0.05$) was used to assess statistical differences.

RESULTS

Virus sequencing and phylogenetic analysis

According to the open reading frame (ORF) 5 sequence, Chiba NOSAI strain was classified as PRRSV2 and grouped in Cluster III, the most prevalent Cluster in Japan [15], with the rest of

the Japanese strains analyzed in the present paper (**Fig. 1**). The nucleotide identity between Chiba NOSAI and the rest of Japanese strains ranged from 89.3% to 91.1%, while it was 85% to the Chinese HP-PRRSV2. Nucleotide identity between ORF5 Chiba NOSAI and PRRSV1-MLV and PRRSV2-MLV was 63.3% and 85.9%, respectively.

Clinical observation and temperatures

During the vaccination phase, animals in G1 and G2 did not show clinical signs. The score for unvaccinated animals (INF and CTRL) was also null. Regarding rectal temperatures, some animals sporadically reached 40ºC—one animal during 1 d in each vaccinated group; two animals during 1 d in INF; and two animals on three different days in CTRL—. Nevertheless, daily average temperatures never reached 40.0ºC in any group (maximum values: 39.4ºC for G1; 39.2ºC for G2; 39.5ºC for INF; and 39.4ºC for CTRL). No significant differences among groups for average temperatures or for the AUC were found (AUC: 98.0 ± 0.3 for G1; 97.8 ± 0.5 for G2; 97.7 ± 0.3 for INF; and 97.8 ± 0.3 for CTRL; $p > 0.2$).

After the challenge, clinical scores were only recorded in INF. From a potential maximum of 315 points (3 points × 21 d × 5 animals), the total sum was 100 for appetite, 134 for respiratory, and 87 for depression scores. From 12 to 21 dpi, all animals in that group showed scores > 1 for all the clinical parameters.

After the challenge, five individual temperatures higher than 40ºC were documented in G1, four in G2, 23 in INF, and none in CTRL. Although averages in vaccinated groups (G1 and G2) never went over 40ºC, both groups showed significantly higher averages than CTRL for 4 d (from 5 to 8 dpi; $p < 0.05$) (**Fig. 2**). On the contrary, averages in INF were significantly higher than CTRL for 10 d (from 5 to 14 dpi; $p < 0.05$). When AUC for body temperatures

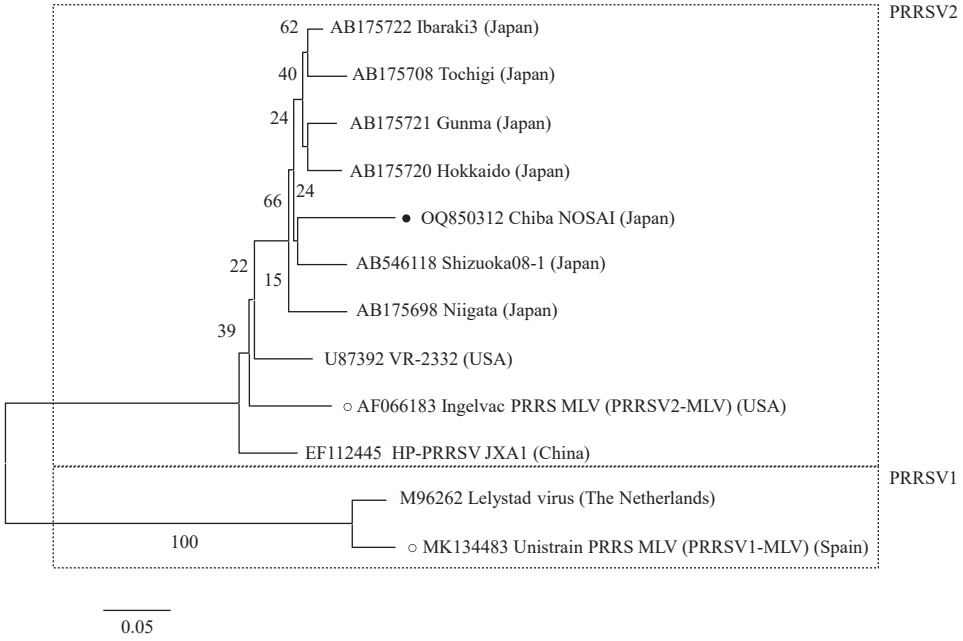

Fig. 1. Phylogenetic tree depicting the evolutionary relationships among the strains analyzed (Material and Methods for details). Numbers along the branches represent the confidence probability estimated using 100 bootstrap replicates. In the present study, the efficacy of two commercial vaccines—PRRSV1-MLV and PRRSV2-MLV— (empty circles) was assessed against a Japanese PRRSV2 field strain (black circle).
PRRSV, porcine reproductive and respiratory syndrome virus; MLV, modified-live vaccine.

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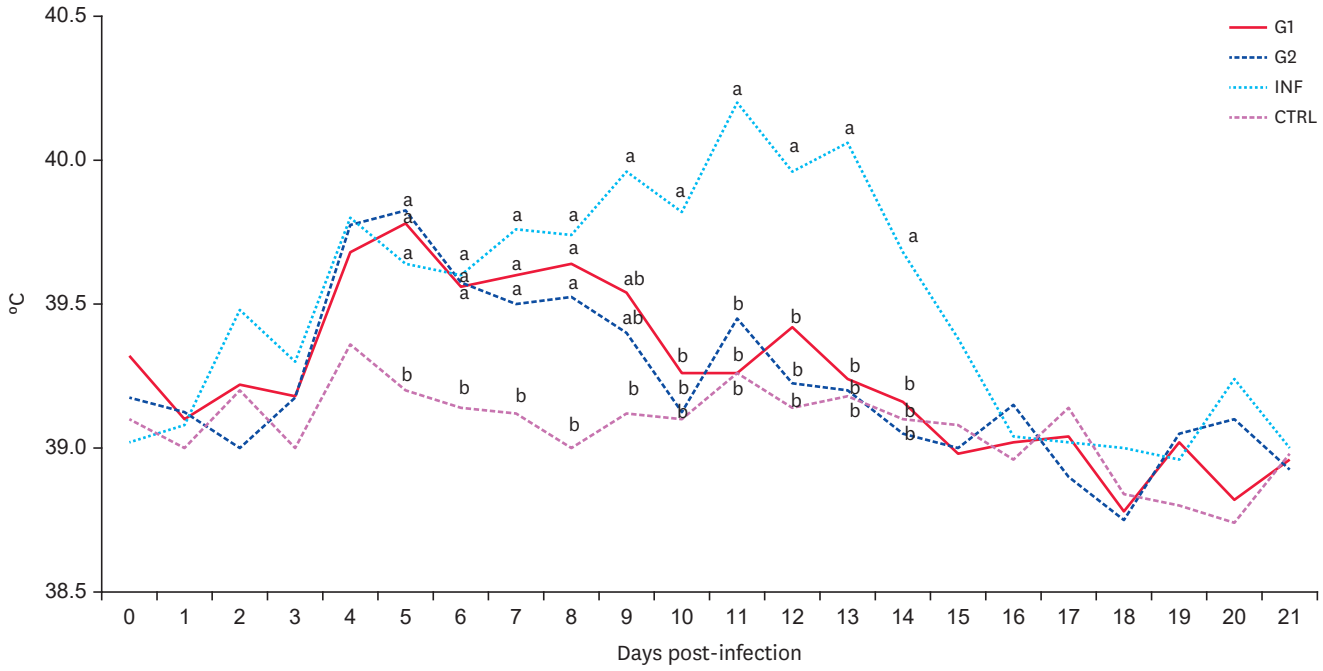

Fig. 2. Rectal temperatures. Average rectal temperatures during the challenge phase (0–21 dpi). The Kruskal-Wallis non-parametric test for multiple comparisons (Conover-Iman method) was used to compare groups in a given day. G1: PRRSV1-MLV; G2: PRRSV2-MLV; INF: non-vaccinated; CTRL: non-vaccinated and non-challenged.
PRRSV, porcine reproductive and respiratory syndrome virus; MLV, modified-live vaccine.
Superscript letters show significant differences (a > b; $p < 0.05$).

was calculated, values for both vaccinated groups were significantly lower than INF and significantly higher than CTRL (INF: 849.2 ± 5.9; G1: 843.9 ± 2.8; G2: 843.4 ± 2.4; CTRL: 840.0 ± 0.7) (INF > G1 and G2, $p = 0.04$; INF > CTRL, $p < 0.001$; G1 = G2, $p > 0.99$; and G1 and G2 > CTRL, $p = 0.01$).

Body weight

At the beginning of the study (0 dpv) and before the challenge (28 dpv), average body weights did not show significant differences. During the infection phase, the average body weight gain in uninfected control animals was significantly higher than those from the infected groups (CTRL > G1, $p < 0.005$; CTRL > G2, $p < 0.003$; CTRL > INF, $p < 0.001$; G1 = G2, $p = 0.7$; G1 = INF, $p = 0.12$; G2 = INF, $p > 0.24$). No significant differences among groups for coefficients of variation were found (**Fig. 3**).

Presence of PRRSV in blood and tissues

All sera from G1, G2, and INF groups at 0dpv and 0dpi were negative for PRRSV isolation. All animals in CTRL group were negative throughout the experiment. After the challenge, all piglets were viremic regardless of the infected group (G1, G2, or INF), although the percentage of positive animals never reached 100% in G1 (**Table 3**). Total positive piglets in vaccinated groups were significantly lower than those in INF (G1 and G2 yielded 19 and INF 33; INF > G1 and G2, $p = 0.01$).

All pigs in INF were viremic from 4 to 13 dpi (average titers from 3.2 to 4.1 $\log_{10}$/mL). The peak of viral load ($\log_{10}$/mL) was observed at 8 dpi in INF, while it was detected at 6 dpi in both vaccinated groups. Afterward, both the proportion of positive samples and the viral load in

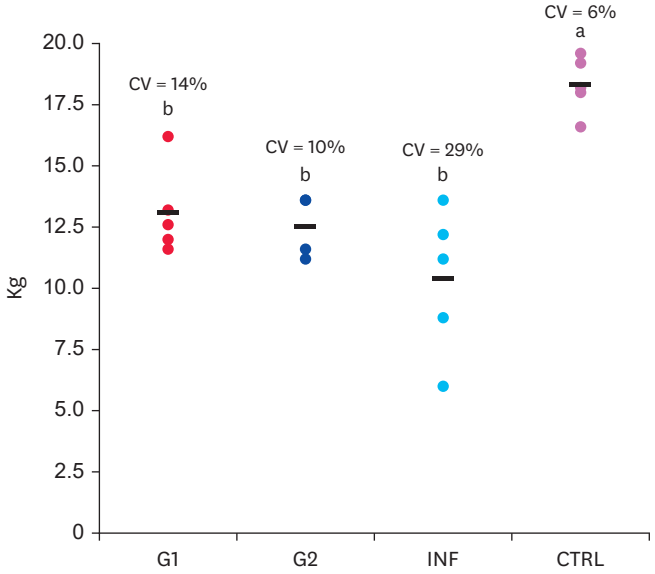

Fig. 3. Body weight gains. Individual body weight gains from 0 to 21 dpi. Black lines show the averages for each group. G1: PRRSV1-MLV; G2: PRRSV2-MLV; INF: non-vaccinated; CTRL: non-vaccinated and non-challenged.
CV, coefficient of variation (ratio of the SD to the average); PRRSV, porcine reproductive and respiratory syndrome virus; MLV, modified-live vaccine.
Superscript letters show significant differences among groups (a > b; $p < 0.01$). The Kruskal-Wallis non-parametric test for multiple comparisons (Conover-Iman method) was used to compare groups in a given day. F-test of equality of variances was used to compare variances among groups.

vaccinated groups slowly declined until 16 dpi, when no positive samples were found. At that time, four animals were still positive in INF. When the total viral load was calculated, AUC for both vaccinated groups were significantly lower than INF ($p < 0.03$). Among vaccinated groups, the lowest AUC was found in G1, although no statistical differences were found ($p = 0.4$).

In all animals, at least one tissue was positive for PRRSV, except one from G1 (**Table 4**). Average titers and proportions of positives in each tissue were similar among G1, G2, and INF, except for proportions in submandibular lymph nodes, where no positive animals were found in G1; plus, average titers in tonsils, where G1 showed the lowest value ($p < 0.02$). The lowest number of positive samples among infected groups was observed for G1, although significant differences were not found (G1 = G2 = INF; $p = 0.1$).

Table 3. PRRSV isolation in blood

Variables	Days post-infection								
	0	2	4	6	8	11	13	16	Prop pos
									AUC
G1	0/5	3/5	3/5	4/5	3/5	2/5	4/5	0/5	$19/35^{B}$
	-	2.2 ± 2.1^{b}	$2.1 \pm 1.9^{a,b}$	2.5 ± 1.4^{a}	2.3 ± 2.1^{a}	$1.7 \pm 2.3^{a,b}$	$2.0 \pm 1.1^{a,b}$	0 ± 0^{b}	12.8 ± 7.7^{b}
G2	0/4	3/4	3/4	4/4	4/4	3/4	2/4	0/4	$19/28^{B}$
	-	3.1 ± 2.1^{a}	2.8 ± 1.9^{a}	3.4 ± 0.3^{a}	3.4 ± 0.8^{a}	$2.3 \pm 1.6^{a,b}$	1.2 ± 1.4^{b}	0 ± 0^{b}	16.2 ± 4.3^{b}
INF	0/5	4/5	5/5	5/5	5/5	5/5	5/5	4/5	$33/35^{A}$
	-	2.2 ± 1.3^{b}	3.6 ± 0.8^{a}	3.3 ± 0.9^{a}	4.1 ± 0.3^{a}	3.5 ± 0.5^{a}	3.2 ± 0.5^{a}	2.2 ± 1.3^{a}	22.1 ± 3.5^{a}
CTRL	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	$0/35^{C}$
	-	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{c}

Proportion of positive samples and average ± SD of titer (log_{10}/mL). The last column shows the Prop pos and AUC (average ± SD). G1: PRRSV1-MLV; G2: PRRSV2-MLV; INF: non-vaccinated; CTRL: non-vaccinated and non-challenged.
Prop pos, proportion of total positive samples; AUC, area under the curve; PRRSV, porcine reproductive and respiratory syndrome virus; MLV, modified-live vaccine.
Superscript letters show significant differences among groups in titers for a given day sampling (a > b > c; $p < 0.05$), in the Prop pos (A > B > C; $p = 0.01$) and comparing AUC (a > b > c; $p < 0.03$). The Kruskal-Wallis non-parametric test for multiple comparisons (Conover-Iman method) was used to compare groups in terms of titers and AUC. Prop pos were compared using χ^2 test (Fisher's exact test).

Table 4. Distribution of PRRSV challenge strain in tissues

Variables	Submandibular lymph nodes	Bronchial lymph nodes	Tonsils	Lungs	Proportion of total positive samples
G1	0/5	1/5	4/5	1/5	6/20[A]
	0 ± 0	0.6 ± 1.3	2.4 ± 1.3[b]	0.6 ± 1.3	
G2	2/4	1/4	4/4	2/4	9/16[A]
	1.5 ± 2.1	0.7 ± 1.5	3.6 ± 0.0[a]	1.6 ± 2.0	
INF	3/5	2/5	5/5	1/5	11/20[A]
	1.9 ± 1.3	1.2 ± 1.6	3.7 ± 0.3[a]	0.6 ± 1.3	
CTRL	0/5	0/5	0/5	0/5	0/20[B]
	0 ± 0	0 ± 0	0 ± 0[c]	0 ± 0	

Proportion of positive pigs and average ± SD of titer (log_{10}/mL) for each tissue. The last column shows the proportion of total positive samples. G1: PRRSV1-MLV; G2: PRRSV2-MLV; INF: non-vaccinated; CTRL: non-vaccinated and non-challenged.
PRRSV, porcine reproductive and respiratory syndrome virus; MLV, modified-live vaccine.
Superscript letters show significant differences in titers for a given tissue (a > b > c; $p < 0.02$), and in the proportion of total positive samples among groups (A > B; $p < 0.01$). The Kruskal-Wallis non-parametric test for multiple comparisons (Conover-Iman method) was used to compare titers. Proportion of total positive samples was compared using χ^2 test (Fisher's exact test).

Humoral responses

All animals were seronegative for both PRRSV1 and PRRSV2-antibodies at 0 dpv. Animals in the CTRL group remained negative for both ELISAs throughout the study.

Regarding PRRSV2-antibodies (**Fig. 4A**), animals in G1 and INF were negative at 0 dpi; but all of them seroconverted after the challenge. In G2, all animals were already positive before the challenge, later showing a significant booster ($p < 0.001$). For PRRSV1-antibodies (**Fig. 4B**), all animals in G2 and INF were negative throughout the study, while in G1 all animals seroconverted after vaccination. Later, a significant booster was observed in this group ($p < 0.001$).

All animals in CTRL and INF were negative for both PRRSV1 and PRRSV2-MLV specific-NA throughout the experiment (**Table 5**). For PRRSV2-MLV specific-NA, animals in G2 they yielded positive results only at the end of the study. For PRRSV1-MLV specific-NA, positive samples were

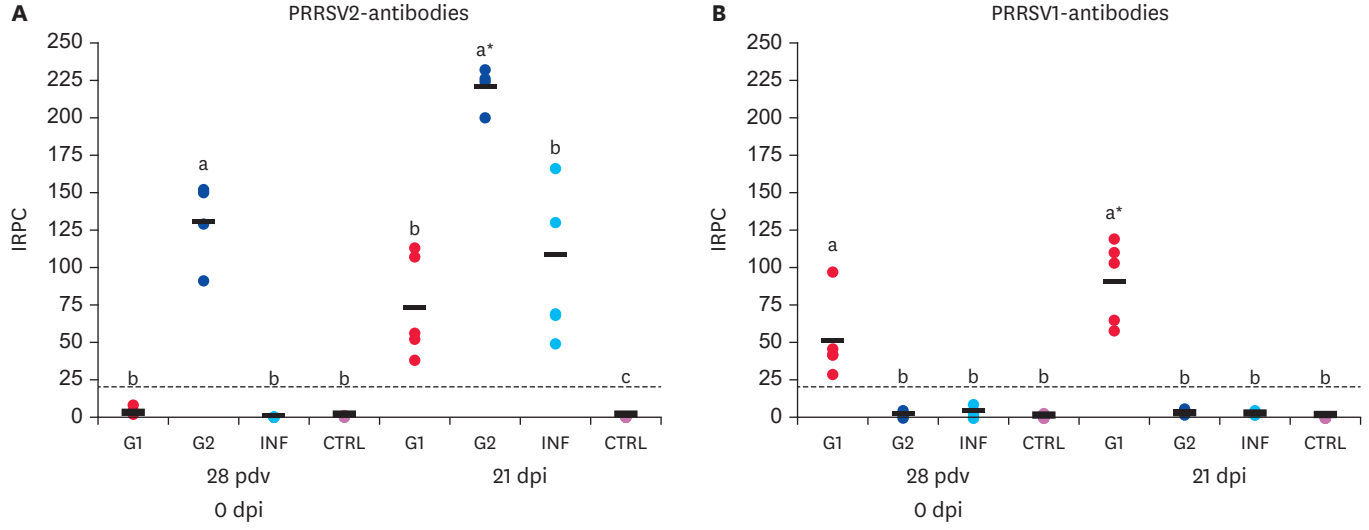

Fig. 4. (A) PRRSV2 and (B) PRRSV1-specific antibodies as determined by commercial ELISAs. Black lines show the averages (IRPC) for each group at each time point. The dotted line shows the cut-off value of the test (> 20). G1: PRRSV1-MLV; G2: PRRSV2-MLV; INF: non-vaccinated; CTRL: non-vaccinated and non-challenged. IRPC, relative index ×100; PRRSV, porcine reproductive and respiratory syndrome virus; MLV, modified-live vaccine; dpv, days post-vaccination; dpi, days post-infection.
Superscript letters show significant differences among groups on each sampling day (a > b > c; $p < 0.001$).
*Significant increase in IRPC compared to 0 dpi in each group ($p < 0.001$). The Kruskal-Wallis non-parametric test for multiple comparisons (Conover-Iman method) was used to compare groups on each sampling day. The Friedman test was used to compare kinetics inside groups (booster).

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Table 5. Viral neutralization test

Variables	PRRSV2-MLV specific-NA (dpv)						PRRSV1-MLV specific-NA (dpv)					
	0	7	14	21	28 (0 dpi)	21 dpi	0	7	14	21	28 (0 dpi)	21 dpi
G1	-	-	-	-	-	-	-	-	2/5 1.0 ± 1.7	3/5 1.4 ± 1.7	4/5 2.0 ± 1.4	5/5 4.4 ± 2.1[a]
G2	-	-	-	-	-	4/4 2.2 ± 0.5	-	-	-	-	-	-
INF	-	-	-	-	-	-	-	-	-	-	-	-
CTRL	-	-	-	-	-	-	-	-	-	-	-	-

Proportion of positive samples and average ± SD of titers (log_2). The Friedman test was used to compare kinetics inside group G1. G1: PRRSV1-MLV; G2: PRRSV2-MLV; INF: non-vaccinated; CTRL: non-vaccinated and non-challenged.
dpv, days post-vaccination; dpi, days post-infection; PRRSV, porcine reproductive and respiratory syndrome virus; MLV, modified-live vaccine; NA, neutralizing antibodies.
[a]Significant increase compared to titer detected at 0 dpi ($p = 0.01$).

already detected in G1 at 14 dpv (2/5); 4 out of 5 were positive immediately before the challenge, and all reached positive results at 21 dpi, showing a significant booster ($p = 0.01$).

DISCUSSION

The economic impact of PRRS in the swine industry is huge, particularly in those countries where PRRSV2 is predominant [2,3,16]. Firstly, HP-PRRSV2 emerged in China in 2006 and rapidly spread all over Asia, causing a devastating scenario with high mortality in pigs of all ages [17]. Secondly, it seems that PRRSV2 isolates, even non-highly pathogenic ones, induce more severe respiratory disease than PRRSV1 [18]. Besides, to reduce the impact of the disease on farms, PRRSV control programs must be based on four cornerstones: monitoring and diagnostics, management, biosecurity, and immunization, which in piglets this last one is achieved by vaccination.

The virulence of the Japanese field strain used in the present study was already evaluated in piglets in a previous experiment. In that experiment, the wild strain caused fever, loss of appetite, leukopenia, cyanosis in ears, and lesions in the spleen and lymph nodes, as well as interstitial pneumonia (data not shown). In our study, a clear clinical impact was recorded only in INF animals. Interestingly, partial protection in terms of rectal temperatures and clinical scores was also conferred by both PRRSV1 and PRRSV2-MLVs ($p < 0.05$). Conversely, similar gains were observed among all infected groups, which were in turn, significantly lower than CTRL ($p < 0.05$). The low number of animals per group, along with the high individual variability observed in the infection outcome, could explain the lack of differences in weight gains between vaccinated groups and INF. For instance, two animals showed weight gains minor than 10 kg, both in the INF group: one of them corresponded to the animal with the highest clinical score and the highest temperature records. Accordingly, the range of body weight gains was lower in vaccinated groups (coefficient of variation = 14% and 10% for G1 and G2, respectively) compared to INF (29%), demonstrating that body weight gains were more homogenous within vaccinated than non-vaccinated groups. Again, probably due to the low number of animals studied, no significant differences were found. Indeed, maintaining homogenous weight groups in the farm is desirable to avoid the existence of delayed pigs, which could act as carriers of pathogens in a scenario where contact with younger animals will be facilitated [19]. In summary, both PRRSV1 and PRRSV2-MLV conferred a similar degree of partial clinical protection. Interestingly, differences between vaccinated groups could be observed when virological parameters were assessed.

In general, a PRRSV infection under experimental conditions causes lower respiratory clinical signs than the same strain in the field [20]. As clinical disorders may generally be not obvious during experimental infections, the importance of virological protection is even greater when evaluating a PRRSV vaccine. In this sense, although providing only partial protection, vaccines can significantly reduce the reproductive rate (R0), through the decrease of the proportion of viremic pigs, the extent of the viremia, and the viral shedding [7]. Thus, vaccines could eventually decrease the virus transmission and, therefore the infection pressure in the population. In the present study, the presence of PRRSV in blood was significantly lower in vaccinated groups than in INF in terms of proportion of total positive samples, titers, extent of viremia, and AUC. Interestingly, among vaccinated groups, the PRRSV1-MLV, which had the lowest ORF5 nucleotide identity compared to the PRRSV2 challenge strain, showed at least equal results to PRRSV2-MLV in terms of proportion of positive samples, titers at the peak and AUC. Differences among vaccinated animals were also evident when tissue samples were analyzed. The average titer detected in tonsils, where PRRSV can be typically found at high levels for long periods [21], was significantly lower in G1 compared to G2 and INF. Moreover, one animal in G1 was negative for all tissue samples, including tonsils. Since the virus was detected in the blood, we cannot conclude that sterilizing immunity was reached. Nevertheless, it is important to note that it showed the lowest total viral load in blood, being viremic only during two sampling days. Finally, we cannot rule out that some tissues were positive after the vaccine strain since positive samples were not sequenced.

Regarding humoral responses, all animals in vaccinated groups seroconverted during the vaccination phase, as expected. After the challenge, G1 pigs also seroconverted for PRRSV2-antibodies, while a booster was observed in G2 for those specific antibodies. Curiously, a booster was also observed in G1 for PRRSV1-antibodies after the PRRSV2 challenge. Although the reason behind this observation is unknown, some degree of similarity between ELISA antigens may not be discarded. Nevertheless, as it has been established, antibodies detected by ELISA are not related to protection. On the contrary, although their roles are not completely elucidated, both NA and cell-mediated immunity (CMI) have been linked to protection [22]. It has been demonstrated, by passive transfer studies, that NA can confer protection in terms of clinical and virological parameters [23]. Also, a pre-formed enough NA due to immunization can provide protection [24]. Interestingly, NA were already detected in G1 before the challenge, as soon as 14 dpv. The presence of pre-formed NA might explain the results observed in G1; however, they were found at low levels and never in 100% of the animals. Later, NA booster was observed in G1, showing the highest values than G2 at 21 dpi. This finding could play a role in the virus clearance and might explain why the infection outcome found in G1 was equal to or even better than in G2. Unfortunately, cross-neutralization against the challenge strain, which could shed more light on this issue, was not evaluated. Since the level of NA immediately before the challenge was low in G1 and null in G2, as expected in vaccinated piglets [25,26], CMI was likely to be responsible for the partial protection. As suggested by others, partial protection against a heterologous challenge within a given species or among PRRSV species could be attributed to the role of CMI, particularly when NA are not found [24,27]. Even CMI was not measured in our study, three other studies that evaluated the same PRRSV1-MLV vaccine demonstrated that partial protection could be achieved against PRRSV2 and HP-PRRSV2 field strains and that protection would be related to CMI [28-30].

Cross-protection between two heterologous strains is somewhat more complex than a matter of genetic similarity. It has been demonstrated that cross-protection cannot be simply predicted by a mere overview of the genetic identity, neither using ORF5 nor even

considering the whole genome [25,31-33]. In this sense, the immunological properties of the strains could be much more important than genetic similarities [24,34]. In our case, the vaccine with the lowest degree of similarity to the challenge strain (63.3%) provided equal, or in some cases even better, partial protection than that provided by the vaccine belonging to the same species (ORF5 nucleotide identity to the challenge strain = 85.9%). As discussed above, this phenomenon might be due to the ability of vaccines to induce interferon-γ release or NA production.

In summary, immunization with a commercial PRRSV1-MLV granted partial protection, both in terms of clinical and virological parameters, against a Japanese PRRSV2 strain. The protection conferred by this PRRSV1-MLV was at least as good as that provided by the PRRSV2-MLV.

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