

Impact of a modified-live porcine reproductive and respiratory syndrome virus vaccine intervention on a population of pigs infected with a heterologous isolate

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Abstract

The objectives of this study were to evaluate the effects of a therapeutic vaccine intervention with a modified-live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine on the dynamics of a heterologous viral infection in a population of pigs, and to determine the clinical and virological response of previously exposed and vaccinated pigs against a second virulent heterologous challenge. A population of 320 pigs were infected with a field isolate, PRRSV MN-30100, alone or followed by Ingelvac[®] PRRS MLV vaccine administered one to three times at 30 days intervals beginning 1 week after infection. Vaccine intervention reduced the duration of viral shedding, but did not reduce the viral load in tissues or the proportion of persistently infected pigs. A different and highly virulent field isolate, MN-184, was then given as a heterologous viral challenge at 97 days after first exposure. Previously infected and vaccinated pigs showed a significant reduction in clinical signs and enhanced weight gain after the highly virulent challenge with PRRSV MN-184, but infection with and shedding of the challenge isolate were not prevented.

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1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an economically significant viral disease of swine, estimated to cost the US pork producing industry approximately 560 million dollars in direct losses per year. While the clinical presentation of PRRS includes both a reproductive and a respiratory component, 88% of the total cost of PRRS is due to the effect of the virus in post-weaning pigs [1]. Clinical signs of PRRS virus (PRRSV) infection in growing pigs include anorexia, lethargy, hyperemia of the skin, dyspnea, hyperther-

mia, increase in mortality rates and reduction in average daily gain (ADG) [2–4]. PRRSV replicates in pulmonary alveolar macrophages [5], facilitating the incidence of bacterial co-infections and resulting in cases of streptococcal meningitis, septicemic salmonellosis, Glasser's disease and bacterial bronchopneumonia [6]. Infection of susceptible pigs with highly virulent PRRSV isolates results in higher viral concentrations in blood and tissues [7,8]. The pathogenic effects and immune response of the pigs to PRRSV are directly related to these higher viral loads [7].

PRRSV is an arterivirus [9]. Viruses of this genus, including lactate dehydrogenase-elevating virus, equine arteritis virus and simian hemorrhagic fever virus, initially replicate in macrophages and establish non-clinical persistent infec-

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tions [10]. PRRSV has the ability to continuously undergo genetic change [11]. This rapid evolution is driven by mutations, genetic recombination and geographical redistribution of PRRSV genotypes [12], and results in emergence of new isolates with different levels of pathogenicity and virulence expressed as a wide variety of clinical presentations [13,14]. Genetic diversity also affects the efficacy of current vaccines [15].

To further complicate the situation, the immune response against PRRSV is ineffective in resolving viral infection, resulting in a prolonged viremia and persistent infection in lymphoid tissues [16]. PRRSV RNA has been detected in the lymphoid tissues of pigs up to 251 days post-inoculation (DPI) [17]. The virus continuously replicates at a low level [18] and can be transmitted to susceptible animals following direct contact with pigs inoculated up to 86 days after infection [19]. The prolonged viremia and persistent infection as well as the presence of subpopulations of PRRSV-naïve and positive swine co-existing within endemically infected herds perpetuate the infection in pig populations [20]. Techniques such as herd closure [21,22], gilt acclimation [23–25] and mass exposure [26–29] can eliminate subpopulations and reduce the risk of PRRSV shedding. However, results vary in success and outcomes have been inconsistent across farms.

The use of vaccination to immunize and protect naïve pigs against PRRSV infection has been widely evaluated at the individual animal level. The induction of both humoral and cell-mediated immune responses has been described following the application of PRRS modified-live virus (MLV) vaccines in pigs [30–32]. Multiple experiments have shown that preventive vaccination with MLV significantly reduces lesions and clinical signs following homologous or heterologous PRRSV challenge [30,31,33–35], although the protection against heterologous infection is incomplete [33]. Inactivated vaccines are also available, but in general they are considered less efficacious than MLV vaccines when used in naïve animals [6].

Little scientific information is available describing the effect of PRRSV vaccination in the face of an acute outbreak under commercial conditions of swine management. Under field conditions, the strategic combination of mass vaccination using PRRS MLV products and management of pig flow is a successful approach to control PRRSV transmission in acutely infected swine herds [36–39]. However, these studies were observational in nature and lacked controls. Recently, the therapeutic effect of a PRRS MLV product on the dynamics of homologous PRRSV infection in a population of pigs was evaluated. Results indicated that vaccination of the entire herd (mass vaccination) was beneficial for reducing persistence and duration of shedding, but not for elimination of the wild-type virus from the pigs. In the same study, a subset of pigs from each group was challenged with a heterologous and highly virulent PRRSV isolate (PRRSV MN-184) 3 months following the first inoculation. The previous exposure to PRRSV provided significant levels of protection against

heterologous challenge but did not prevent re-infection [40]. Since it is unlikely that such a homologous relationship between vaccine virus and field virus would occur under commercial production conditions, an attempt was made to develop a more broadly relevant field-based challenge model. This model not only included the infection and later application of different protocols of mass vaccination to a large population of pigs, but it also simulated the introduction of a highly virulent heterologous PRRSV isolate to previously infected and/or vaccinated pig populations. Here, we hypothesized that a PRRS MLV vaccine would significantly reduce persistence and transmission of PRRSV in a population of pigs infected with a heterologous PRRSV isolate and that previous exposure to PRRSV would improve the clinical response of pigs against a highly virulent heterologous challenge. The specific aims of the study were to evaluate the effect of vaccination on persistence of PRRSV in the population, and on PRRSV transmission to susceptible pigs, and to determine the clinical and virological response of previously vaccinated pigs following challenge with a highly virulent heterologous PRRSV isolate.

2. Materials and methods

2.1. Animals and housing

Three hundred fifty-two 6–8 week-old pigs (principals) were obtained from a herd known to be free of PRRSV on the basis of 10 years of diagnostic testing. After arrival, pigs were confirmed to be PRRSV naïve using enzyme-linked immunosorbent assay (ELISA, Herd Chek PRRS Antibody 2XR Test Kit, IDEXX Laboratories, Westbrook, ME) and reverse transcriptase-polymerase chain reaction (RT-PCR, Taqman RT-PCR kit, Perkin-Elmer Applied Biosystems, Foster City, CA). Pigs were individually identified using ear tags and randomly assigned to six different groups (A–F) that were housed in separate rooms at the research farm at the University of Minnesota Swine Disease Eradication Center in west-central Minnesota. Groups were designated as follows: group A (wild-type PRRSV only, positive control), group B (PRRSV + 1 dose of MLV vaccine), group C (PRRSV + 2 doses of MLV vaccine), group D (PRRSV + 3 doses of MLV vaccine), group E (sham-inoculated negative control) and group F (MLV vaccine only) (Table 1). Groups A, B, C and D had 80 pigs each, group E had 12 pigs and group F had 20 pigs. All pigs were vaccinated on arrival against *Haemophilus parasuis* and *Erysipelothrix rhusiopathiae* (Ingelvac HPE-1, Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO) and *Lawsonia intracellularis* (Enterisol Ileitis, Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO). All protocols and procedures of pig management and care were approved by the University of Minnesota Institutional Animal Care and Use Committee. Personnel practiced PRRSV-specific biosecurity protocols [41] across all study groups throughout the experiment.

Table 1
Experimental design of the first phase of the study: animal flow and specific actions

Group	DPI						
	0	7	37	67	97	127	
A	MN-30100 inoculation		P	P	P	P	
				T-1	T-2	T-3	
B	MN-30100 inoculation	MLV	P	P	P	P	
				T-1	T-2	T-3	
C	MN-30100 inoculation	MLV	MLV	P	P	P	
				T-1	T-2		
D	MN-30100 inoculation	MLV	MLV	MLV	P	P	
					T-1		
E	Sham inoculation		P	P	P	P	
			T-1	T-2	T-3		
F		MLV			P	P	

DPI: Days post-inoculation; MLV: time of PRRS Ingelvac MLV application; P: assessment of persistence, involving sacrifice and collection of tissues from 10 originally inoculated pigs to determine the proportion of persistently infected pigs; T-1, T-2 or T-3: assessment of transmission, involving the introduction of groups of 10 sentinel pigs, placed in contact with originally inoculated pigs for 30 days and then removed, slaughtered and sampled. Group A: wild-type virus infection only (positive control) ($n=80$); Group B: wild-type virus plus one dose of MLV ($n=80$); Group C: wild type virus plus two doses of MLV ($n=80$); Group D: wild-type virus plus three doses of MLV ($n=80$); Group E: negative control ($n=12$); Group F: MLV vaccine only ($n=20$).

2.2. Experimental design

2.2.1. Phase 1: the effect of mass vaccination on PRRSV persistence and transmission

On day 0, all 320 pigs in groups A–D were intranasally (IN) inoculated with 2 mL of cell culture fluid containing 10^4 TCID₅₀ (50% tissue-culture infective dose)/mL of PRRSV MN-30100 at cell culture passage four. PRRSV MN-30100 was obtained from a persistently infected sow in a commercial production site and shown to persist and be shed for long periods of time [19,42,43]. It replicates at low levels in blood and tissues and induces only mild clinical signs (transient depression, lack of appetite for 24–48 h and mild fever of 40–41 °C) following inoculation of growing pigs [8,19,43,44]. MN-30100 is shed at significantly lower levels than highly pathogenic isolates after experimental inoculation [8].

At 7 DPI, pigs in groups B, C, D and F were vaccinated with 2 mL of the modified-live PRRSV vaccine Ingelvac® PRRS MLV (Boehringer Ingelheim Vetmedica, Inc. St. Joseph, MO) via the intramuscular route. Groups C and D were then re-vaccinated 30 days later (37 DPI), and a final dose was administered to group D on day 67 PI. Pigs in group E were sham-inoculated with 2 mL of sterile saline solution via the IN route (Table 1). To monitor the protocols of infection and vaccination 10 pigs from groups A–D and F and 6 pigs in group E were randomly selected and serum samples were collected in sterile vacuum tubes (Becton-Dickinson Vacutainer, Franklin Lakes, NJ) via jugular venipuncture at 0, 7, 14, 37, 67, 97 and 127 DPI. In conjunction with the 352 principal animals, 108 PRRSV-naïve age-matched sentinel pigs were introduced at designated periods following the initiation of the study (Table 1).

To evaluate whether different protocols of vaccination affected the proportion of persistently infected pigs or the

load in tissues through time, 10 pigs per group were randomly selected, slaughtered and sampled 30 days following the last vaccination (Table 1). The study was terminated and all remaining pigs slaughtered and sampled at 127 DPI. Tonsil, sternal and superficial inguinal lymph nodes were collected in the slaughterhouse, stored in separate plastic bags and transported on ice to the laboratory for testing. Tissue samples were tested by quantitative RT-PCR to determine the number of PRRSV RNA copies per gram of tissue (RNAc/g).

To determine whether different protocols of vaccination affected virus shedding, groups of 10 PRRSV-naïve sentinel pigs were introduced 30 days after the last vaccination in each treatment group (Table 1). All sentinel pigs were removed, slaughtered and sampled 30 days after introduction. Viral transmission to at least one sentinel pig per group was determined by the detection of PRRSV nucleic acid in serum or tissues by RT-PCR or by the detection of PRRSV antibodies in an ELISA followed by an indirect fluorescent antibody (IFA) test [45].

2.2.2. Phase 2: the effect of PRRSV infection status on clinical and virological responses following a highly virulent-heterologous challenge

At 97 DPI, 10 randomly selected pigs from every group and two additional negative control pigs were transported to the isolation facilities of the College of Veterinary Medicine at the University of Minnesota. Each group was divided in two rooms (5 pigs per room) at a density of 1.2 m² per pig. Each isolation room had an independent ventilation system and slurry pit to prevent cross-contamination of pathogens between rooms. All pigs in groups A–D and F and 10 pigs from E were inoculated (IN) with 2 mL of cell culture fluid containing 10^4 TCID₅₀/mL of PRRSV MN-184 isolate at cell culture passage five. Challenge control PRRSV-naïve pigs

were labeled as E+ and negative control pigs were sham-inoculated IN with 2 mL of sterile saline solution and labeled as E-. The challenge virus, PRRSV MN-184, was a highly virulent isolate recovered in 2001 from a farm in southern Minnesota that demonstrated clinical signs of severe reproductive disease with a high incidence of sow mortality [8]. Since its initial recovery from the field, MN-184 has been well-documented to replicate to high levels in blood and tissues of infected pigs and induce high mortality in experimentally infected animals [7,8].

All 62 pigs were bled at 0, 1, 3, 7, 18 and 24 DPI to evaluate viremia and antibody response. At 24 DPI all pigs were slaughtered and samples of tonsil, sternal and superficial inguinal lymph node were collected in separate plastic bags. To determine whether wild-type PRRSV inoculation only or with one to three vaccine exposures affected shedding, 1 PRRSV naïve pig of the same age as the inoculated pigs was introduced per room (2 sentinels per group, 12 total) 3 days after MN-184 inoculation. Sentinels were kept in the rooms until 24 DPI when the pigs were slaughtered and samples of serum and tissue were collected to perform ELISA and RT-PCR.

To evaluate the clinical response of each group following heterologous challenge rectal temperature, appetite and mortality were measured at 0, 1, 3, 7, 18 and 24 DPI. Pigs were weighed at 0 and 24 DPI to calculate ADG per group. Rectal temperature was measured by the same person every sampling day between 8 and 9 am. Every morning the same operator measured the volume of feed still remaining from the previous day in each room to estimate the percent reduction in feed intake. Results were consolidated into three different periods (1–8, 9–16 and 17–24 DPI). Study personnel were blinded across treatments.

2.3. Diagnostic testing

The PRRSV antibody response was evaluated by a commercial ELISA test (Herd Chek PRRS Antibody 2XR Test Kit, IDEXX Laboratories, Westbrook, ME). The presence of PRRSV nucleic acid in serum and tissues was determined by RT-PCR (Taqman RT-PCR kit, Perkin-Elmer Applied Biosystems, Foster City, CA). Tissue samples collected at

slaughter were pooled by individual animal and 0.5 g was placed in 7.5 mL of lysis buffer (Nucleospin II kit, BD Biosciences, Palo Alto, CA) into a sterile plastic tube (Falcon tube, Becton-Dickinson, Franklin Park, NJ). After homogenization (Polytron PT 3100, Kinematica AG, Lucerne, Switzerland), samples were clarified by centrifugation at 3000 rpm for 15 min. Total RNA was extracted and purified from 200 µL of serum or 50 µL of the middle layer of the homogenized tissue supernatant using the Nucleospin II kit (BD Biosciences, Palo Alto, CA), according to the manufacturer's protocol. RNA was eluted in 50 µL of water, dried in a vacuum centrifuge (Savant Speedvac, GMI Inc, Ramsey, MN) and rehydrated in 5 µL of water. Every sample was assayed in duplicate using 2 µL of the rehydrated sample in a 20-µL RT-PCR reaction with primers and probe directed to the open reading frame (ORF) 7 region of the North American PRRSV [46]. All reactions were conducted in a real-time PCR instrument (ABI 7700, Perkin-Elmer Applied Biosystems, Foster City, CA).

Samples of cell culture fluid of both PRRSV wild-type isolates (MN-30100 and MN-184) and the MLV vaccine were submitted to the University of Minnesota Veterinary Diagnostic Laboratory to sequence the PRRSV ORF 5 region. The PRRSV challenge strains (MN-30100 and MN-184) had a 16.1% difference in the ORF 5 region. Ingelvac PRRS MLV was 11.6% different from PRRSV MN-30100 and 15% different from PRRSV MN-184. Quantitative strain specific RT-PCR was also performed using primers unique to the ORF5 region of each strain (Table 2). The PCR reaction included the RT-PCR kit (Taqman RT-PCR kit, Perkin-Elmer Applied Biosystems, Foster City, CA), the isolate-specific forward and reverse primers and the isolate-specific probe (Table 2). A standard curve was developed for the quantitative RT-PCR procedure by preparing 10-fold dilutions of the specific isolate stock. Results were reported as the number of RNAC/g of tissue.

2.4. Statistical analysis

The proportion of PRRSV persistently infected pigs was compared between groups within the same DPI with Fisher's Exact Test. The number of PRRSV RNA copies per g of tissue

Table 2

Primers and TaqMan[®] fluorescent probes used in isolate-specific real time RT-PCR for absolute quantification of the PRRSV RNAC/g of tissue

PRRSV Isolate	Sequence
MN-184	Forward primer: 5' TAACTTAACGATATGTGAGCTGAATGGCAC 3' Reverse primer: 5' ACACAGTGATCAGGCCGACC 3' Probe: 5' 6FAM-CTGGCTGAACAATCATTTTAGTTGGGCAGTGGAGACTTTCGTTATC-TAMRA 3'
MN-30100	Forward primer: 5' TAACTTGACGCTATGCGAGCTGAAT 3' Reverse primer: 5' AAACCCGGCGGTGGATACA 3' Probe: 5' VIC-TTGGCTGGCTGGAAAGTTTGATTGGGCAGTGG-TAMRA 3'
Ingelvac MLV	Forward primer: 5' GCA GCT CCC ATC TAC AGC TGA TT 3' Reverse primer: 5' AGACAATGTGAGTCAAAACGGGAAAGAT 3' Probe: 5' NED-TTGGCTAGCTAACAAATTTGATTGGGCAGTGGAGAGTTT-TAMRA 3'

Note: 6FAM, VIC and NED = the fluorescent reporter dye; TAMRA = fluorescent quencher dye.

Table 3
Virological and serological response of pigs inoculated with PRRSV MN-30100

Group	DPI											
	7		14		37		67		97		127	
	Pro Vir	ELISA s/p ratio	Pro Vir	ELISA s/p ratio	Pro Vir	ELISA s/p ratio	Pro Vir	ELISA s/p ratio	Pro Vir	ELISA s/p ratio	Pro Vir	ELISA s/p ratio
A	10/10 ^b	0.25 ± 0.1 ^b	10/10 ^b	1.19 ± 0.2 ^b	2/10 ^a	1.57 ± 0.2 ^a	1/10 ^a	1.16 ± 0.2 ^a	0/10 ^a	1.43 ± 0.2 ^a	0/10 ^a	1.16 ± 0.3 ^a
B	10/10 ^b	0.47 ± 0.1 ^b	10/10 ^b	1.58 ± 0.2 ^b	5/10 ^a	1.83 ± 0.2 ^a	0/10 ^a	1.47 ± 0.2 ^a	0/10 ^a	1.37 ± 0.3 ^a	0/10 ^a	0.71 ± 0.4 ^a
C	10/10 ^b	0.31 ± 0.1 ^b	10/10 ^b	1.03 ± 0.3 ^b	5/10 ^a	1.88 ± 0.2 ^a	0/10 ^a	1.37 ± 0.2 ^a	0/10 ^a	1.46 ± 0.2 ^a	0/10 ^a	1.77 ± 0.3 ^a
D	10/10 ^b	0.21 ± 0.1 ^b	10/10 ^b	0.85 ± 0.1 ^b	5/9 ^a	1.67 ± 0.2 ^a	0/10 ^a	1.38 ± 0.2 ^a	0/10 ^a	1.12 ± 0.2 ^a	0/10 ^a	1.09 ± 0.4 ^a
F	0/10 ^a	0.01 ± 0.01 ^a	5/9 ^a	0.14 ± 0.1 ^a	3/9 ^a	1.54 ± 0.2 ^a	2/9 ^a	1.26 ± 0.3 ^a	0/9 ^a	0.82 ± 0.3 ^a	NA	NA

Notes: Values of ELISA s/p ratio are group means ± SE. Different superscripts within same DPI were statistically different ($P < 0.05$). All serum samples collected before inoculation (0 DPI) were PRRSV PCR and ELISA negative (results not shown). Negative control pigs (group E) remained ELISA and PCR negative throughout the study (results not shown). Pigs in group F were not inoculated at 0 DPI but vaccinated 7 days later. Pro Vir: the number of serum PCR positive individuals/total number of monitors in the group.

was log-transformed to stabilize the variance prior to analysis. Rectal temperature, ELISA sample/positive (s/p) ratio mean, ADG and log₁₀ RNAC/g of tissue were compared among groups by one-way ANOVA. All analyses were performed with standard software (Statistix 8, Analytical Software, Tallahassee, FL).

3. Results

3.1. Effect of vaccine intervention on viremia and antibody response

Mild fever, depression and lack of appetite were detected for 2 days after PRRSV MN-30100 inoculation. PRRSV nucleic acid was detected in serum at 7 DPI in 100% of monitor pigs from all inoculated groups (Table 3). The proportion of viremic individuals declined over time to 67 DPI when nearly all serum samples in all groups were PCR negative except for one pig in group A (positive control) and one pig in group F (vaccine only). The higher proportion of viremic monitor pigs in group F (56%) was detected 7 days after the vaccination (Table 3).

At 14 DPI 85% of monitor pigs in all inoculated groups (A–D) were ELISA positive (s/p ratio greater than 0.4). No statistically significant differences in mean s/p ratio were observed among inoculated groups (A–D) through the 127 days of the study independently of the vaccination protocol ($P \geq 0.05$). At 37 DPI, 30 days after the vaccination, 90% of the monitor pigs in group F were ELISA positive. The ELISA s/p ratio mean of group F was statistically undistinguishable from all inoculated groups from 37 to 127 DPI ($P \geq 0.05$). ELISA results for all groups are summarized (Table 3). Negative control pigs (group E) remained ELISA and PCR negative throughout the study.

3.2. Effect of vaccine intervention on viral persistence and viral load in lymphoid tissues

The proportions of persistently infected pigs per group every sampling day are summarized (Table 4). PRRSV MLV vaccine did not reduce the proportion of persistently infected pigs at 37, 67, 97 or 127 DPI ($P \geq 0.05$). The group averages of PRRSV RNAC/g of tissue in the PCR positive samples were not statistically different across groups ($P \geq 0.05$); the viral load in lymphoid tissues was not affected by the vaccination protocol (Table 4).

All PCR positive tissue samples from persistently infected pigs in groups B, C and D from 37 to 127 DPI (48 total samples) were tested by two isolate-specific PCR tests in order to differentiate the presence of the wild-type PRRSV (MN-30100) from the MLV. Eight samples were found to be negative by both tests, 39/48 tissue samples were carrying the wild-type PRRSV isolate MN-30100 only and one pig had the MLV. No tissue sample tested positive for both viruses.

Table 4
Effect of vaccine intervention on PRRSV infection, persistence and shedding

Group	DPI											
	37		67			97			127			
	P	VL	P	VL	T	P	VL	T	P	VL	T	
A	9/10 ^a	6.4 ± 0.2 ^a	9/10 ^a	3.2 ± 0.3 ^a	Yes	7/9 ^a	2.7 ± 0.3 ^a	Yes	3/27 ^a	3.8 ± 0.7 ^a	Yes	
B	9/10 ^a	6.5 ± 0.2 ^a	6/8 ^a	3.8 ± 0.4 ^a	Yes	6/9 ^a	3.1 ± 0.2 ^a	Yes	2/27 ^a	2.6 ± 0.7 ^a	No	
C	n.t.	n.t.	6/9 ^a	3.6 ± 0.3 ^a	n.t.	5/9 ^a	3.1 ± 0.2 ^a	Yes	6/37 ^a	3.9 ± 0.3 ^a	No	
D	n.t.	n.t.	n.t.	n.t.	n.t.	3/7 ^a	3.8 ± 0.2 ^a	n.t.	4/43 ^a	4.6 ± 0.5 ^a	No	
E	n.t.	n.t.	0/6 ^b	0 ^b	No	0/6 ^b	0 ^b	No	0/6 ^b	0 ^b	No	
F	n.t.	n.t.	n.t.	n.t.	n.t.	6/9 ^a	3.7 ± 0.3 ^a	n.t.	n.t.	n.t.	n.t.	

Notes: P: number of persistently infected pigs/number of necropsied pigs; VL: viral load (log₁₀ RNAc/g group) mean ± SE of positive animals. Different superscripts within same DPI are statistically different (*P* < 0.05). T: transmission to the sentinel group (yes or no). n.t.: Not tested.

3.3. Duration of viral transmission to sentinel pigs

Between 97 and 127 DPI, sentinel pigs introduced to vaccinated groups (B–D) remained uninfected whereas sentinels in group A (positive control) became infected as determined by PCR or ELISA (Table 4). PRRSV transmission was detected at 37–67 DPI and 67–97 DPI into all groups in which sentinel pigs were introduced (Table 4).

All PCR positive tissue samples from sentinel pigs introduced to groups B, C and D from 37 to 127 DPI (25 total samples) were tested by the isolate-specific PCR tests. One sample was found negative for MN-30100 and Ingelvac MLV, 19/25 tissue samples harbored the wild-type PRRSV isolate MN-30100 only and 5 sentinel pigs had both PRRSV strains in lymphoid tissues.

3.4. Effect of vaccination on the clinical and virological response to MN-184 challenge

On day of challenge and 1 day post-challenge with PRRSV MN-184, viremia was not detected in any pig. At 3 and 7 DPI, PRRSV RNA was identified in serum of 9 of 10 newly infected pigs (group E+) and 10 of 10 pigs that received vaccine alone 120 days previously (group F), but in only 2–4 of 10 previously infected and vaccinated pigs (Table 5, groups A–D). Viremia was still present at 18 DPI in groups C and D,

but was absent in all pigs from the other groups (Table 5). The mean ELISA s/p ratio was not statistically different among previously infected and/or vaccinated groups (A–D, F) before MN-184 inoculation (*P* = 0.567), 1 DPI (*P* = 0.787) or 3 DPI (*P* = 0.457). Seven days after MN-184 challenge, all pigs in group E+ were ELISA negative. In contrast, previously exposed pigs demonstrated an increase in their ELISA s/p ratios by day 7, and pigs in the vaccine control group (F) had a significantly higher s/p ratio average than all other previously exposed groups (*P* = 0.029). At 18 DPI, every pig in group E+ was ELISA positive and the s/p ratio averages were statistically indistinguishable (*P* = 0.213) among all inoculated groups (Table 5). Similar results were obtained 24 days after MN-184 inoculation (*P* = 0.123). Negative control pigs (E–) remained ELISA and PCR negative during this phase of the study, but given the small sample size this group was not included in the statistical analysis.

PRRSV MN-184 was detected in tissue samples of 47% of the pigs from all challenged groups at 24 DPI. Six of 9 challenge controls (group E+) and 7 of 10 vaccine-only controls (group F) contained PRRSV RNA in lymphoid tissues. A lower incidence of PRRSV RNA-positive tissues ranging from 10 to 55% was observed in groups A–D (Table 5). PRRS MLV was not identified in tissue samples of any pig and only one individual was carrying both MN-184 and MN-30100 isolates. No statistically significant difference among groups

Table 5
Virological and serological response of pigs challenged with PRRSV MN-184

Group	DPI						Proportion persistently infected	log ₁₀ RNAc g
	3		7		18			
	Proportion viremic	ELISA s/p ratio	Proportion viremic	ELISA s/p ratio	Proportion viremic	ELISA s/p ratio		
A	2/10 ^b	1.2 ± 0.2 ^a	3/10 ^b	1.1 ± 0.2 ^b	0/10 ^a	1.7 ± 0.3 ^a	3/9 ^{ab}	3.4 ± 0.5 ^a
B	3/10 ^b	0.8 ± 0.2 ^a	4/10 ^b	1.2 ± 0.3 ^b	0/10 ^a	1.8 ± 0.3 ^a	1/10 ^b	3.5 ± 0.8 ^a
C	4/10 ^b	0.7 ± 0.1 ^a	3/10 ^b	0.7 ± 0.1 ^b	4/10 ^a	1.3 ± 0.2 ^a	5/10 ^{ab}	3.6 ± 0.4 ^a
D	4/10 ^b	1.2 ± 0.3 ^a	2/10 ^b	1.2 ± 0.3 ^b	1/10 ^a	1.9 ± 0.3 ^a	5/9 ^a	3.5 ± 0.4 ^a
E+	9/10 ^a	0.03 ± 0.01 ^b	10/10 ^a	0.1 ± 0.01 ^c	0/10 ^a	2.2 ± 0.2 ^a	6/9 ^a	3.1 ± 0.3 ^a
F	10/10 ^a	1.1 ± 0.3 ^a	2/10 ^b	1.9 ± 0.3 ^a	0/10 ^a	2 ± 0.3 ^a	7/10 ^a	3.2 ± 0.3 ^a

Notes: Values of ELISA s/p ratio and log₁₀ RNAc/g of tissue are group means ± S.E. Different superscripts within same DPI were statistically different (*P* < 0.05). All serum samples of 0, 1 and 24 DPI were PRRSV PCR negative (results not shown). Proportion viremic: the number of serum PCR positive individuals/total number of animals in the group. Proportion persistently infected: the number of tissue PCR positive pigs/total number of pigs in the group at day 24.

Table 6
Clinical responses to PRRSV MN-184 challenge in pigs with various histories of PRRSV exposure

Group	Temperature (°C)			Mortality	Reduction in appetite (%) [*]	ADG (g)
	1 DPI	3 DPI	7 DPI			
A	39.2 ± 0.1 ^b	39.5 ± 0.2 ^a	39.8 ± 0.2 ^{ab}	0	1% 1 st period	792 ± 55 ^a
B	39.2 ± 0.1 ^b	39.9 ± 0.4 ^a	39.3 ± 0.1 ^b	0	6% 1 st period and 1% 2 nd period	767 ± 80 ^a
C	39.2 ± 0.1 ^b	39.6 ± 0.2 ^a	39.5 ± 0.2 ^b	0	1% 1 st period	837 ± 104 ^a
D	39.5 ± 0.1 ^{ab}	40 ± 0.3 ^a	39.6 ± 0.2 ^b	0	3% 1 st period	803 ± 75 ^a
E+	40 ± 0.1 ^a	40.1 ± 0.2 ^a	40.4 ± 0.2 ^a	1 pig at 12 DPI	16% 1 st period, 8% 2 nd period and 3% 3 rd period	288 ± 36 ^b
F	39 ± 0.1 ^b	40.2 ± 0.3 ^a	39.8 ± 0.2 ^{ab}	0	6% during 1 st period and 1% 2 nd period	672 ± 57 ^a
E- ^{**}	39.6 ± 0.3	39.2 ± 0.1	39.5 ± 0.1	1 pig at 20 DPI	None	N/A

Notes: Values of rectal temperature (°C) and ADG (g) are group means ± S.E. Different superscripts within same DPI are statistically different ($P < 0.05$). N/A: not available.

^{*} Reduction in appetite is presented as the % of reduction in feed intake compared to the provided ration in 3 different periods: 1–8 DPI (1st period), 9–16 DPI (2nd period) and 17–24 DPI (3rd period).

^{**} Not included in statistical analysis due to small sample size, $n = 2$.

was detected in the average number of PRRSV RNA copies per g of tissue ($P = 0.366$).

Every one of the 12 sentinel pigs introduced to challenged groups (A–D, E+ and F) between 3 and 24 days after MN-184 inoculation seroconverted and harbored PRRSV MN-184 RNA in lymphoid tissues. PRRSV transmission was detected in all challenged groups irrespective of the previous PRRSV infection status.

Overall, pigs from groups that were exposed previously to PRRSV showed reduced clinical signs compared to challenge controls. One challenge group animal was humanely sacrificed 12 days post-challenge, after presenting with hyperthermia (41.5 °C), hyperemia of the extremities, dyspnea, lethargy and anorexia. Interstitial pneumonia was observed histopathologically, and PRRSV RNA was detected in lymph nodes and serum. One pig in the negative control group (E–) died suddenly at 20 DPI due to intestinal torsion. No PRRS-related clinical signs were observed in group E–. Average rectal temperature in all groups was statistically indistinguishable before MN-184 inoculation ($P = 0.090$). No statistically significant difference in average rectal temperature was found between challenged groups (A–D, E+ and F) at 3 ($P = 0.321$), 18 ($P = 0.079$) and 24 DPI ($P = 0.121$). However, at 1 and 7 DPI, pigs with primary infection (group E+) had significantly higher rectal temperature than pigs in other challenged groups ($P < 0.05$, Table 6). A 3–16% average reduction in feed intake was recorded in group E+ and ADG was significantly lower than in pigs of groups A–D and F ($P = 0.001$, Table 6).

4. Discussion

The aim of this study was to evaluate the effects of a therapeutic vaccine intervention with a modified-live PRRSV vaccine on the dynamics of a heterologous viral infection in a population of pigs, and to determine the clinical and virological response of previously exposed and vaccinated pigs against a second virulent heterologous challenge. The goal was to determine whether therapeutic vaccination could be a

potential tool in PRRS control and/or eradication programs. The experimental design attempted to recreate commercial swine rearing conditions to evaluate outcomes of transmission to susceptible pigs, persistence, and the response against a challenge with a second heterologous and highly virulent isolate. Overall, we observed that the study not only reinforced a number of previously reported observations, it brought forth new information on the dynamics of PRRSV infection at the population level and the impact of vaccination on several defined outcomes.

The patterns of viremia and seroconversion observed after the inoculation of pigs with PRRSV MN-30100 were consistent with previous observations involving other isolates [8,47]. The proportion of PRRSV persistently infected pigs and the viral loads in positive tissues were not reduced by any of the three vaccination protocols, in contrast to a positive effect of vaccine intervention on persistence of a homologous virus [40]. Given the similarity in experimental design between the present and the previous study [40], where the only difference is the use of a wild-type PRRSV heterologous to the MLV vaccine, it is reasonable to conclude that the genetic difference between the infecting strain and the vaccine virus affected the clearance process of the wild-type virus from the lymphoid tissues. The mechanism of vaccine-induced viral clearance is not well understood at this time. Interestingly, after single or repeated vaccination with a MLV product, the wild-type virus was predominantly detected in pigs at 127 DPI. These results support previous data indicating that PRRSV strains may differ in their ability to replicate in the pig and that some strains predominate over others [48]. However, in contrast with previous reports our results indicated that heterologous strains do not always predominate in the pig after challenge [49]. A potential explanation for this observation is that previous studies tested preventive vaccination in contrast to our therapeutic vaccination approach.

Therapeutic vaccine intervention prevented viral shedding to naïve sentinels introduced to the heterologous infected population during the last period (97–127 DPI). Prior to 97 DPI, wild-type virus was actively circulating in the infected populations since the PRRSV strain predominantly isolated

from sentinel pig tissue samples during the first two periods (37–67 and 67–97 DPI) was MN-30100. These results are consistent with those from a previous study in which vaccination decreased viral shedding but did not reduce persistence of the virus [40]. Limitations of this phase of the study include the use of a single wild-type PRRSV isolate, the absence of quantification of viremia, the inability to calculate the rate of shedding to sentinels and the termination of the study at 127 DPI. Selection of the MN-30100 PRRSV isolate for the experiment was based on its ability to persist and to shed within pig populations [35]. Given the duration of PRRSV shedding reported in the literature [14], we hypothesized that an effect of the vaccine on transmission or persistence would be observed within this period. The uniform effect of vaccine intervention treatments to reduce viral shedding to less than 100 days suggests that one vaccine treatment during acute infection, i.e. 7 DPI, is important. The mechanism of vaccine-induced reduction of shedding is not well understood at this time and further evaluation is necessary. At present, these conclusions are limited to infection of pigs with PRRSV similar to the MN-30100 isolate. However, if repeatable under both experimental and field conditions with other viral genotypes, the ability of an MLV vaccine to reduce the transmission of wild-type PRRSV may prove to be advantageous for use in regional control and eradication programs.

The final phase of the study replicated the frequently encountered situation of “area spread of PRRSV” or the re-introduction of an unrelated isolate to an endemically infected swine herd. Infection of pigs with PRRSV MN-184 occurred across groups irrespective of previous exposure to PRRSV MN-30100, MLV vaccine, or both. But animals that were previously exposed to PRRSV by infection or vaccination or both were partially protected against infection with the MN-184 strain. A significant reduction in clinical signs and significantly enhanced growth performance were observed in all groups that were previously exposed to PRRSV even with only one exposure to wild-type virus or one dose of vaccine. These results are consistent with previous experiments which demonstrated that vaccination with MLV significantly reduces lesions and clinical signs following PRRSV heterologous challenge [33–35]. However, under the conditions of this study, previous exposure to PRRSV clinically protected pigs following challenge. Viremia, serological response and shedding occurred in all groups regardless of the previous PRRSV infection status, but were greater in naïve animals (challenge control group). While similar results were observed in a previous experiment [50], to the author’s knowledge, this is the first study assessing the ability of a population of pigs with varying PRRSV status to actively shed the virus to naïve sentinels. Notwithstanding the limited ability of the experimental design of this phase of the study to fully replicate the diversity of field conditions, the findings indicate a significant potential benefit in disease reduction for swine herds located in high pig dense areas where the risk of area spread of PRRS is high. The beneficial effect of

a previous exposure to PRRSV on growth performance of PRRSV-infected pigs is notable since 88% of the economic losses due to PRRS occur in growing pigs [1].

In conclusion, under the conditions of this study, the use of a modified-live PRRSV vaccine reduced the duration of shedding of wild-type virus in a population of pigs infected with a heterologous PRRSV isolate and provided partial protection against a highly virulent and heterologous challenge. Therapeutic vaccination once or repeatedly at monthly intervals did not eliminate the wild-type virus RNA from lymphoid tissues or prevent heterologous infection. These results offer a detailed and controlled evaluation of the benefits and risks of the use of PRRSV mass vaccination under controlled field conditions involving a large population of pigs. Although anecdotal field experiences and studies have been reported [29–32], this study is to our knowledge, the first large scale controlled test of MLV vaccine intervention in a heterologous acutely infected swine herd. However, while the results are significant, further experiments testing different heterologous isolates, as well as testing of these observations in commercial farms are needed to better predict the effects of therapeutic vaccination on area-based PRRS control and eradication programs.

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