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Journal of Vaccines & Vaccination

Research Article

Efficacy of Ingelvac PRRS® Modified Live Virus Vaccine against Heterologous Porcine Reproductive and Respiratory Syndrome Virus Challenges

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is an infectious disease caused by the PRRS virus (PRRSV) and characterized by reproductive failure, respiratory disease and weight loss in swine.

Two randomized, blinded vaccination-challenge studies evaluated the efficacy of Ingelvac PRRS® modified live virus (MLV) vaccine in protecting pigs from the virulent heterologous PRRSV isolates, restriction fragment length polymorphism (RFLP) 1-3-4 and 1-7-4. In separate challenge studies, pigs were vaccinated on Day 0 with Ingelvac PRRS MLV or placebo 'challenge control' and challenged on Day 28 with PRRSV 1-3-4 or 1-7-4.

In the 1-3-4 challenge study, pigs vaccinated with Ingelvac PRRS MLV demonstrated significantly lower median viraemia (area-under-the-curve for Day 28–42 [AUC28–42]; P<0.0001) compared with unvaccinated controls. Vaccinated pigs also had significantly higher average daily weight gain (ADWG) than unvaccinated controls (P<0.0001). At Day 42, vaccinated pigs had significantly lower least square mean lung lesion scores than unvaccinated controls (P<0.001). Mortality was significantly higher with challenge control (61%) than with Ingelvac PRRS MLV (15%; P<0.01).

In the 1-7-4 challenge study, significantly lower AUC28–42 viraemia levels were observed with Ingelvac PRRS MLV compared with challenge control (P=0.031). Median rectal temperatures were significantly lower with Ingelvac PRRS MLV than with challenge controls at Days 29 and 42 (P<0.01 for both). Pigs vaccinated with Ingelvac PRRS MLV had significantly higher ADWG during the challenge phase (P<0.05) and significantly lower least square mean lung lesion scores at Day 42 compared with unvaccinated controls (P<0.05).

These data indicate that Ingelvac PRRS MLV provides heterologous protection against two relatively new and particularly virulent PRRSV field strains responsible for a growing number of infections in the US.

Keywords: Swine; ADWG; Heterologous protection; Modified live virus vaccines; PRRSV

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is a virally transmitted disease among swine that was first recognised in 1987 in the US, and later in Japan and Europe [1]. It now has a significant global presence, occurring in most swine-producing countries, having a profound impact on the swine industry.

Infection with the causative PRRS virus (PRRSV) affects pigs of all ages leading to viraemia, pyrexia, pneumonia with abnormal respiratory behaviour, reduced average daily weight gain (ADWG) and increased mortality rates [2-4]. The most devastating effects are observed in young piglets and pregnant sows. In pregnant sows, PRRSV causes late-term abortions and mummification of foetuses in utero. Live-born piglets from PRRSV infected sows are often weak and display severe

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Received: August 27, 2019; **Accepted**: September 09, 2019; **Published**: September 16, 2019

Citation: Patterson A, Haiwick G, Hermann J, Fergen B, Wakeland K, Chittick W, et al. (2019) Efficacy of Ingelvac PRRS® Modified Live Virus Vaccine against Heterologous Porcine Reproductive and Respiratory Syndrome Virus Challenges. J Vaccines Vaccin 10:407. Doi: 10.35248/2157-7560.19.10.407

respiratory symptoms [1,5]. The severe respiratory distress observed is due to active PRRSV replication in alveolar macrophages and subsequent damage in lung tissues [6], which in turn increases susceptibility to bacterial co-infections, resulting in cases of streptococcal meningitis, septicaemia salmonellosis, Glasser's disease and bacterial bronchopneumonia [3].

To further complicate the situation; the immune response against PRRSV is only partially effective, resulting in a prolonged viraemia and persistent infection in lymphoid tissues [7], which can be detected up to 251 days post-inoculation [8]. High level of viraemia leads to viral shedding and elevated levels of airborne virus, which can remain infectious at over nine kilometres from the infected herd [9]. Furthermore, swine can be infected by very low doses of virus, especially in naïve animals [10-13].

Due to the reduction or loss of pregnancies, death in young piglets, and decreased growth rates in all infected pigs, PRRSV is responsible for significant economic losses within the swine industry. Holtkamp et al. [14] estimated that the total cost of productivity losses due to PRRSV in the US was more than $650 million annually – approximately $104 million higher than the $560 million annual cost estimated in 2005 [15], despite PRRSV control and elimination strategies. In Europe however, the cost of PRRSV to the industry is considerably more, estimated as a €1.5 billion annual loss in 2013 [16].

Comparative sequencing analysis of the PRRSV strains native to Europe and those found in North America has revealed there are two major genotypes of PRRSV: Type 1 (European) and Type 2 (North American; Nelsen et al. 1999) [17]. There is growing diversity among PRRSV strains due to mutation and recombination [18], which leads to variation in the severity of outbreaks [19]. Historically, increases in genetic diversity have coincided with increased numbers of virulent outbreaks [20]. These waves of outbreaks have typically been referred to by the restriction fragment length polymorphism (RFLP); for example, the Minnesota, US 1-8-4 outbreaks in 2001–2004 [21]. Since 2014, 1-7-4 and 1-3-4 RFLP type isolates have been frequently identified and considered virulent [22-24].

One method to protect pigs against PRRSV infection is vaccination. PRRS modified live virus (MLV) vaccines have demonstrated a significant reduction of clinical signs of PRRS, viraemia and lung lesions, and improved health and performance compared with non-vaccinated swine in challenge studies [25-29]. PRRS Type 2 MLV vaccines have been shown to improve ADWG and survival in PRRSV-challenged swine [26,30]. Furthermore, vaccines reduce airborne transmission of PRRSV, allowing control of PRRSV among and between herds, and in PRRS-prevalent areas [26-29]. However, with the ever-expanding diversity of PRRSV field strains, successful vaccines need to provide heterologous protection [18]. Ingelvac PRRS® MLV vaccine (Boehringer Ingelheim Animal Health, Duluth, Georgia, US) has been shown to provide heterologous protection against both Type 1 and Type 2 PRRSV strains [25,30]. The Ingelvac PRRS MLV vaccine cross-protects against genetically diverse PRRSV isolates, including strains currently circulating in the US (Patterson et al. 2013) [28], with vaccinated pigs demonstrating a significant reduction in aerosol shedding of wild-type PRRSV compared with non-vaccinated pigs (Dee et al. 2014) [29].

This study evaluated the efficacy of Ingelvac PRRS MLV vaccine in protecting against the currently circulating and particularly virulent heterologous PRRSV field strains RFLP 1-3-4 and 1-7-4 using a 3/4-week-old pig respiratory challenge model. The efficacy of Ingelvac PRRS MLV vaccine in this model was compared with the efficacy of Fostera® PRRS MLV vaccine (Zoetis Inc, Kalamazoo, Michigan, US), a more recently developed, commercially available Type 2 PRRS MLV vaccine created from a US field isolate [1].

MATERIALS AND METHODS

Study design and objectives

This was a randomised, blinded vaccination-challenge study to evaluate the efficacy of Ingelvac PRRS MLV vaccination in protecting swine against a heterologous challenge with either PRRSV 1-3-4 or 1-7-4 field strains. A secondary objective was to compare the efficacy of Ingelvac PRRS MLV and Fostera PRRS MLV vaccinations in protecting swine from the two challenges. The challenge studies for PRRSV 1-3-4 and 1-7-4 were conducted separately.

Animal information

Pigs, owned by Boehringer Animal Health, were sourced from Wilson Prairie View Farms, Inc. Burlington, Wisconsin, US. The standard of care of the pigs involved in the study was reviewed and approved by the Institutional Animal Care and Use Committee. For inclusion, pigs had to be in good health as assessed by the study investigators, and be negative for PRRSV based on enzyme-linked immunosorbent assay (ELISA) testing for anti-PRRSV antibody serology and quantitative reverse transcription polymerase chain reaction (qRT-PCR) assessment of viral RNA load. In total, 146 pigs aged 21 ± 6 days were used in the 1-3-4 RFLP challenge study, including pigs from 18 different litters, and 159 pigs aged 28 ± 3 days were used in the 1-7-4 challenge study, including pigs from 27 different litters.

Study procedures

For each of the challenge studies, pigs were randomly assigned to one of four groups (Table 1).

Table 1: Study design for randomised, blinded, vaccination-challenge studies.

Group	Number of pigs	Vaccination	PRRSV challenge
Study 1			
1	35	Ingelvac PRRS MLV	RFLP 1-3-4
2	35	Fostera PRRS MLV	RFLP 1-3-4
3	36	Challenge control	RFLP 1-3-4
4	5	No treatment (environmental control)	None*
Study 2			
5	45	Ingelvac PRRS MLV	RFLP 1-7-4
6	45	Fostera PRRS MLV	RFLP 1-7-4
7	64	Challenge control	RFLP 1-7-4
8	5	No treatment (environmental control)	None*

*Necropsied on Day 28 (day of challenge)

MLV: Modified Live Virus; PRRS: Porcine Reproductive and Respiratory Syndrome PRRSV: PRRS Virus; RFLP: Restriction Fragment Length Polymorphism.

On Day 0, pigs in Groups 1–3 and 5–7 were vaccinated intramuscularly into the right neck with 2 mL of either Ingelvac PRRS MLV (Groups 1 and 5), Fostera PRRS MLV (Groups 2 and 6) or placebo 'challenge control' (phosphate buffered saline; Groups 3 and 7). Pigs in Groups 4 and 8 were not vaccinated and acted as environmental controls. Pigs were housed separately according to the vaccination received, and then commingled at the time of challenge.

On Day 28, all pigs in Groups 1 – 3 were challenged intramuscularly (2 mL) and intranasally (1 mL in each nostril; 2 mL in total) with serum PCR-positive for a virulent heterologous PRRSV 1-3-4 isolate (Boehringer Ingelheim Animal Health, Inc. St. Joseph, Missouri, US). The amount of virus present in the serum was quantified by qRT-PCR and found to be 7.97 log genomic copies/mL. Ingelvac PRRS MLV and Fostera PRRS MLV have 85.5% and 86.5% similarity, respectively, to the 1-3-4 RFLP virus isolate present in the serum based on open reading frame (ORF) 5 sequence.

Pigs in Groups 5–7 were challenged intramuscularly (2 mL) and intranasally (1 mL per nostril; 2 mL in total) with virulent heterologous PRRSV 1-7-4 isolate (Boehringer Ingelheim Animal Health, Inc. St. Joseph, Missouri, US) on Day 28. The mean concentration of PRRSV 1-7-4 challenge was 4.6 TCID50/mL. Ingelvac PRRS MLV and Fostera PRRS MLV have 88% and 87% similarity, respectively, to the 1-7-4 RFLP virus challenge based on ORF 5 sequence.

Environmental control pigs (Groups 4 and 8; n=5 per group) were necropsied on Day 28 without a challenge. All pigs in Groups 1–3 and 5–7 were necropsied on Day 42 (14 days post-challenge).

Clinical observations

Pigs were examined each day for signs of abnormal respiration, abnormal behaviour or the presence of coughing, with each category rated as absent (0) or present (1). Pigs in the PRRSV 1-3-4 challenge groups were weighed on Days 0, 27 and 42 (study termination), or when removed from the study, to assess ADWG. Pigs in the PRRSV 1-7-4 challenge groups were weighed on Days 0, 28 and 42. To assess for the presence of pyrexia (defined as a rectal temperature of >40°C), rectal temperatures were taken on Day 0 (before vaccination) and regularly throughout the study.

Viraemia and presence of PRRSV antibodies

Viraemia and PRRSV antibody levels were assessed from serum samples. Venous whole blood (6–15 mL) was collected on specified days between Day 0 and Day 42. Serum samples were tested by qRT-PCR (Life Technologies) to assess for the presence of PRRSV RNA and results were recorded as genomic copies/mL. IDEXX PRRSV X3 ELISA (IDEXX Laboratories Inc, Westbrook, Maine, US) was used to test for PRRSV antibodies. Results were recorded as sample-to-positive (S:P) ratios, with an S:P ratio of ≥0.4 (cut-off value) considered as positive.

Evaluation of lung lesions

Lung lesions were described in general terms and scored for the presence of macroscopic lesions. Lung total score was calculated based on the following formula: lung total score=(% of lesions in the right cranial lobe *0.11)+(% of lesions in the right middle lobe *0.1)+(% of lesions in the right caudal lobe *0.34)+(% of lesions in the left cranial lobe *0.05)+(% of lesions in the left middle lobe *0.06)+(% of lesions in the left caudal lobe *0.29)+(% of lesions in the accessory lobe *0.05).

Statistical methods

Pigs were randomised to treatment groups, blocking on litter using SAS® version 9.4 (SAS Institute Inc.; 2015). The number of pigs per treatment across litters was evenly distributed, except for the environmental control groups which included five pigs per group.

Statistical analysis was conducted using SAS version 9.4. Data were analysed using a linear mixed model. Fixed effects were group, time (where applicable) and group by time interaction (where appropriate), random effects were study housing and animal (where appropriate). A covariance structure was used to incorporate the covariance among outcomes measured repeatedly. Pairwise comparisons between groups were conducted, with adjustment for multiplicity using the multivariate-T method. A level of significance of 0.05 was used to indicate statistical significance and all tests conducted were two-sided. Transformations used in the analysis of study data included the arcsin square-root for lung lesion scores and log10 for viraemia (genomic copies/mL).

RESULTS

Viraemia

Viraemia after PRRSV 1-3-4 challenge, as measured by median RNA genomic copies per mL, peaked between Days 29 and 35 in all groups (Figure 1a). Viraemia levels after challenge were significantly lower in the Ingelvac PRRS MLV and Fostera PRRS MLV vaccinated groups compared with the challenge control groups based on area-under-the-curve (AUC) between Day 28 and Day 42 (P<0.0001 for both PRRS MLV vaccinated groups).

Viraemia after PRRSV 1-7-4 challenge peaked between Days 29 and 35 in all groups (Figure 1b). Viraemia levels after challenge were significantly lower with Ingelvac PRRS MLV compared with challenge control (P=0.031) and Fostera PRRS MLV (P=0.011) based on AUC between Day 28 and Day 42, but there was no significant difference between challenge control and Fostera PRRS MLV in this regard (P=0.73). Mean viraemia on Day 42 was significantly lower in pigs vaccinated with Ingelvac PRRS MLV than in pigs vaccinated with Fostera PRRS MLV (P=0.0017) or challenge control (P<0.001).

Figure 1. Viremia by vaccination group

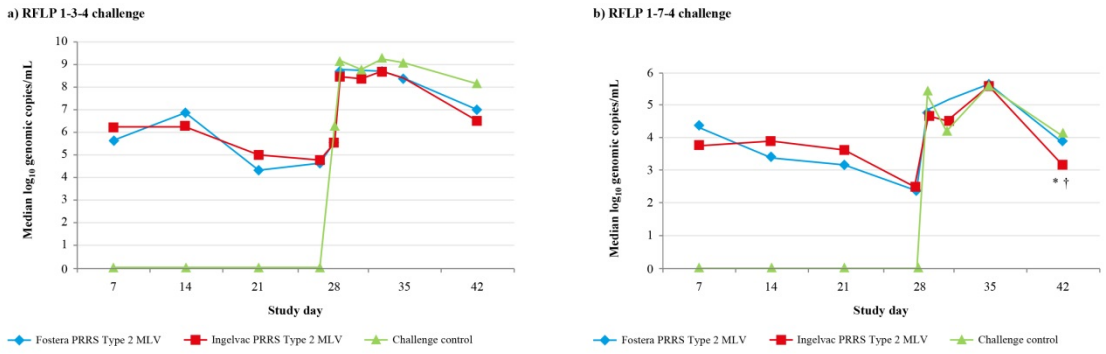

Figure 1: Viraemia by vaccination group.

a) RFLP 1-3-4 challenge

For AUC between Day 28 and Day 42, P<0.0001* for both vaccinated groups vs. the challenge control group

*Multivariate T-method

AUC: Area-Under-The-Curve; MLV: Modified Live Virus; PRRS: Porcine Reproductive and Respiratory Syndrome; RFLP: Restriction Fragment Length Polymorphism.

b) RFLP 1-7-4 challenge

*P<0.001 for Ingelvac PRRS MLV vs. challenge control

†P=0.0017 for Ingelvac PRRS MLV vs. Fostera PRRS MLV

For AUC between Day 28 and Day 42, P=0.031 for Ingelvac PRRS MLV vs. challenge control and P=0.011 for Ingelvac PRRS MLV vs. Fostera PRRS MLV

p-values calculated using the multivariate T-method

AUC: Area-Under-The-Curve; MLV: Modified Live Virus; PRRS: Porcine Reproductive and Respiratory Syndrome; RFLP: Restriction Fragment Length Polymorphism.

Anti-PRRSV antibody response

In the 1-3-4 challenge study, all pigs vaccinated with Ingelvac PRRS MLV or Fostera PRRS MLV seroconverted by Day 14. Pigs

in the challenge control groups remained seronegative throughout the vaccination period, indicating validity of the study. There was no significant difference between the group mean anti-PRRSV antibody responses in pigs vaccinated with Ingelvac PRRS MLV and pigs vaccinated with Fostera PRRS MLV; however, S:P ratios were numerically higher in the Ingelvac PRRS MLV group compared with the Fostera PRRS MLV group at all time points after Day 7 (Figure 2a).

In the 1-7-4 challenge study, 44 out of 45 pigs in both vaccinated groups seroconverted prior to challenge on Day 28. Pigs in the challenge control groups remained seronegative throughout the vaccination period. During the vaccination period, S:P ratios were numerically higher in pigs vaccinated with Ingelvac PRRS MLV compared with pigs vaccinated with Fostera PRRS MLV at all timepoints after Day 14. Following challenge with PRRSV 1-7-4, all pigs in the challenge control groups seroconverted by Day 42. Pigs vaccinated with Ingelvac PRRS MLV maintained significantly higher mean antibody titres than pigs vaccinated with Fostera PRRS at Days 35 and 42 ($P<0.01$; Figure 2b).

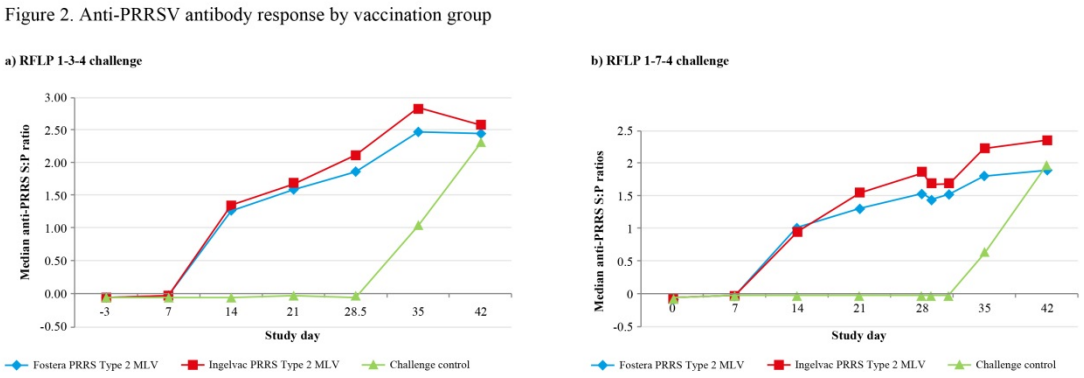

Figure 2: Anti-PRRSV antibody response by vaccination group.

a) RFLP 1-3-4 challenge

MLV: Modified Live Virus; PRRS: Porcine Reproductive and Respiratory Syndrome; RFLP: Restriction Fragment Length Polymorphism; S:P: Sample-to-Positive.

b) RFLP 1-7-4 challenge

MLV: Modified Live Virus; PRRS: Porcine Reproductive and Respiratory Syndrome; RFLP: Restriction Fragment Length Polymorphism; S:P: Sample-to-Positive.

CLINICAL OBSERVATIONS

PRRSV 1-3-4 study

Abnormal behaviours (e.g. depression, lethargy, anorexia, piling) were observed in over 85% of pigs in all groups within 1 day of challenge with PRRSV 1-3-4 (Day 29). There was a trend towards lower mean behaviour scores in pigs vaccinated with Ingelvac PRRS MLV compared with pigs vaccinated with Fostera PRRS MLV or challenge control (data not shown).

Abnormal respiratory signs (dyspnoea, open-mouthed breathing, and rough hair) were observed in over 68% of pigs by Day 32 (four days post-challenge with PRRSV 1-3-4). There was a trend towards lower respiratory scores in pigs vaccinated with Ingelvac PRRS MLV vs. Fostera PRRS MLV-vaccinated pigs or challenge control pigs from Day 38 to Day 42 (Figure 3a). Mean percentage coughing scores after 1-3-4 challenge increased in all groups throughout the study, but were numerically lower in PPRS MLV vaccinated groups at Days 36–42 compared with the challenge control groups.

Median rectal temperature increased in all groups after challenge with PRRSV 1-3-4. By Day 42, median rectal temperature was numerically lower in both PRRS MLV vaccinated groups than in the challenge control groups (Figure 4a).

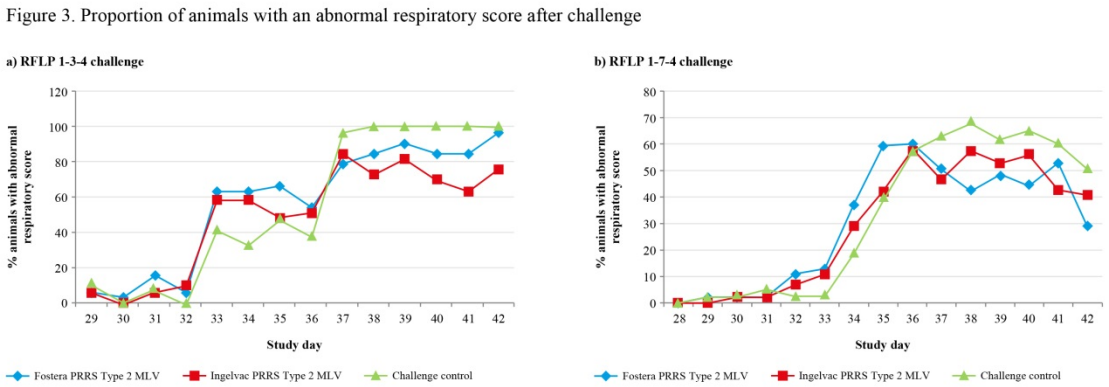

Figure 3: Proportion of animals with an abnormal respiratory score after challenge.

a) RFLP 1-3-4 challenge

MLV: Modified Live Virus; PRRS: Porcine Reproductive and Respiratory Syndrome; RFLP: Restriction Fragment Length Polymorphism.

b) LP 1-7-4 challenge

MLV: Modified Live Virus; PRRS: Porcine Reproductive and Respiratory Syndrome; RFLP: Restriction Fragment Length Polymorphism.

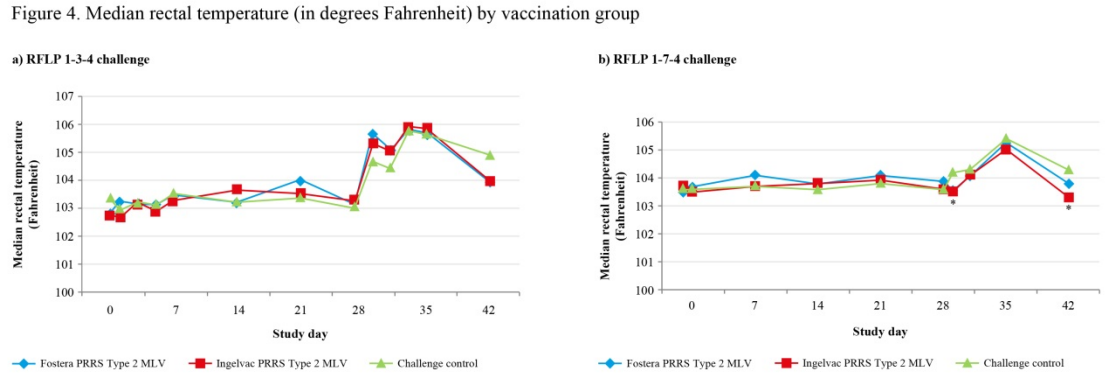

Figure 4: Median rectal temperature (in degrees Fahrenheit) by vaccination group.

a) RFLP 1-3-4 challenge

MLV: Modified Live Virus; PRRS: Porcine Reproductive and Respiratory Syndrome; RFLP: Restriction Fragment Length Polymorphism.

b) RFLP 1-7-4 challenge

*P<0.01 for both vaccination groups vs. challenge control group.

p-values calculated using the multivariate T-method
MLV: Modified Live Virus; PRRS: Porcine Reproductive and Respiratory Syndrome; RFLP: Restriction Fragment Length Polymorphism.

PRRSV 1-7-4 study

In the PRRSV 1-7-4 challenge study, clinical signs of PRRSV infection were observed in all pigs approximately five days post-challenge (Day 33). However, due to the lack of large numerical differences between PRRS MLV vaccinated pigs and challenge control pigs, no statistical comparison by group was completed on respiratory (Figure 3b), behaviour, or cough scores.

An increase in median rectal temperatures was observed in all groups challenged with PRRSV 1-7-4 between Day 28 (day of challenge) and Day 35 (Figure 4b). Median rectal temperatures were significantly lower in both of the PRRS MLV vaccinated

groups compared with the challenge control groups at Days 29 and 42 (P<0.01 for both).

Average daily weight gain

PRRS MLV vaccinated pigs had significantly higher ADWG than the challenge control groups after PRRSV 1-3-4 challenge (P<0.0001). Pigs vaccinated with Ingelvac PRRS MLV had a significantly higher ADWG than Fostera PRRS MLV-vaccinated pigs during the challenge phase (Day 28–42) of the study P=0.035; Table 2). For pigs that were removed from the study before Day 42, ADWG was calculated using weight of pig on day of necropsy.

Table 2: Average Daily Weight Gain in the challenge phase (Days 27–42).

Vaccination	PRRSV challenge	ADWG, lbs (95% CI)	p-value* vs. challenge control	p-value* vs. Fostera PRRS MLV
Ingelvac PRRS MLV	RFLP 1-3-4	0.317 (0.19–0.45)	<0.0001	0.04
Fostera PRRS MLV	RFLP 1-3-4	0.052 (-0.09–0.19)	0.03	N/A
Challenge control	RFLP 1-3-4	-0.25 (-0.42–0.085)	N/A	N/A
Ingelvac PRRS MLV	RFLP 1-7-4	0.61 (0.53–0.70)	<0.0001	0.124
Fostera PRRS MLV	RFLP 1-7-4	0.49 (0.41–0.58)	<0.0001	N/A
Challenge control	RFLP 1-7-4	0.24 (0.17–0.31)	N/A	N/A

*Multivariate T-method

ADWG: Average Daily Weight Gain; CI: Confidence Interval; MLV: Modified Live Virus; N/A: Not Applicable; PRRS: Porcine Reproductive and Respiratory Syndrome; PRRSV: PRRS Virus; RFLP: Restriction Fragment Length Polymorphism

In the PRRSV 1-7-4 challenge study, vaccinated pigs had significantly higher ADWG than the challenge control groups during the challenge phase (P<0.05; Table 2). Pigs vaccinated with Ingelvac PRRS MLV had a numerically higher ADWG than pigs vaccinated with Fostera PRRS MLV, although this difference was not statistically significant.

Lung lesions

Lung lesions were observed in all pigs challenged with PRRSV 1-3-4 at time of necropsy (Table 3). Pigs in both Ingelvac PRRS MLV and Fostera PRRS MLV vaccinated groups had significantly lower least square mean lung lesion scores than the challenge controls (P<0.001). The group vaccinated with Ingelvac PRRS MLV had a numerically lower least square mean lung lesion score (22.7%) than the group vaccinated with Fostera PRRS MLV (30.1%), but this difference was not statistically significant.

In the PRRSV 1-7-4 challenge study, PRRS MLV vaccinated pigs had significantly lower least square mean lung lesion scores than challenge controls (P<0.05; Table 3). The group vaccinated with Ingelvac PRRS MLV had numerically lower least square mean lung lesion score (11.4%) than the group vaccinated with Fostera PRRS MLV (12.7%), but again this difference was not statistically significant.

Table 3: Least square mean lung lesion scores after necropsy by vaccination group.

Group	Number of pigs	Vaccination	Least square mean lung lesion score	95% CI	p-value (vs. challenge control)
Study 1: RFLP 1-3-4 challenge					

1	35	Ingelvac PRRS MLV	22.7	14.0–32.8	<0.001
2	35	Fostera PRRS MLV	30.1	20.5–40.6	<0.001
3	36	Challenge control	58.4	47.6–68.9	N/A
Study 2: RFLP 1-7-4 challenge					
5	45	Ingelvac PRRS MLV	11.4	6.6–17.2	<0.05
6	45	Fostera PRRS MLV	12.7	7.6–18.7	<0.05
7	64	Challenge control	28.3	21.7–35.5	N/A

Mortality

In the PRRSV 1-3-4 challenge study, 40 pigs died or were euthanised as a result of the challenge. Mortality was significantly higher in the challenge control group (61%) than in the group vaccinated with Ingelvac PRRS MLV (15%) or the group vaccinated with Fostera PRRS MLV (21%; P<0.01 for each vs. challenge controls; Table 4).

Table 4: Post-challenge mortality by vaccination group.

Group	Number of pigs	Vaccination	Deaths, n	Mortality rate, %	p-value* (vs challenge control)
Study 1: RFLP 1-3-4 challenge					
1	33	Ingelvac PRRS MLV	5	15	0.002
2	33	Fostera PRRS MLV	7	21	0.009
3	36	Challenge control	22	61	N/A
Study 2: RFLP 1-7-4 challenge					
5	45	Ingelvac PRRS MLV	1	2	ND
6	45	Fostera PRRS MLV	0	0	ND
7	64	Challenge control	0	0	N/A

*Multivariate T-method

MLV: Modified Live Virus; N/A: Not Applicable; ND: Not Determined; PRRS: Porcine Reproductive and Respiratory Syndrome; RFLP: Restriction Fragment Length Polymorphism

In the PRRSV 1-7-4 challenge study, only one animal died during the challenge period; this animal was in the Ingelvac PRRS MLV group and was confirmed to have a secondary bacterial infection.

DISCUSSION

The growing diversity of PRRSV strains and their potential to cause severe disease outbreaks has focused attention on the need for PRRS vaccines to provide heterologous protection across strains [18,19].

Ingelvac PRRS MLV vaccine has been shown to cross-protect against eight genetically diverse PRRSV isolates, including a number of field strains currently circulating in the US that have been associated with high mortality in growing swine [28]. In the present study, we have demonstrated the ability of Ingelvac PRRS MLV vaccine to protect against two particularly virulent heterologous PRRSV isolates, RFLP 1-3-4 and 1-7-4, which are known to be responsible for severe PRRSV outbreaks in the US (Dr. P. Gauger, personal communication).

Vaccination with Ingelvac PRRS MLV mitigates the biological consequences of infection with PRRSV 1-3-4 and 1-7-4. After challenge with both PRRSV strains, we observed significant reductions in group mean viraemia levels in pigs vaccinated with Ingelvac PRRS MLV compared with challenge control pigs. Vaccinated pigs had significantly higher ADWG and

significantly lower least square mean lung lesion scores than challenge control pigs. With the 1-3-4 challenge, mortality was significantly higher in challenge control pigs than in pigs vaccinated with Ingelvac PRRS MLV. The ability of Ingelvac PRRS MLV vaccine to cross-protect against both of these PRRSV strains can be described as heterologous protection since Ingelvac PRRS MLV has 85.5% similarity to the 1-3-4 RFLP virus and 88% similarity to the 1-7-4 RFLP virus based on ORF 5 sequence. To our knowledge, this is the first study to investigate the efficacy of a PRRSV MLV vaccine against the virulent field strain, PRRSV 1-3-4. A previous study [31] has reported the efficacy of Fostera PRRS MLV against PRRSV 1-7-4 challenge. Angulo et al. [31] concluded similar results of Fostera PRRS MLV vaccine performance as we have shown in our study; however, our study used different post-challenge sampling points, so a direct comparison cannot be made. This makes comparing the efficacy between Fostera PRRS MLV to the results obtained from Ingelvac PRRS MLV vaccine in our study a challenge; for example, by comparing data collected on the last day of each study, compared with challenge controls, Inglevac PRRS MLV vaccine reduced viraemia levels significantly more than Fostera PRRS MLV, ($P<0.001$ vs. $P\leq0.05$), but the study end days differed, Day 42 in our study vs. Day 37 in the Angulo et al. [31] study.

While the study demonstrated that both Ingelvac PRRS MLV and Fostera PRRS MLV vaccines show efficacy in protecting against PRRSV 1-3-4 and 1-7-4 challenges in young pigs, Ingelvac PRRS MLV showed consistent numerical advantages over Fostera PRRS MLV in this regard. Pigs vaccinated with Ingelvac PRRS MLV had a significantly higher ADWG than Fostera PRRS MLV-vaccinated pigs following challenge with PRRSV 1-3-4. Consistent with this finding, pigs vaccinated with Ingelvac PRRS MLV and challenged with PRRSV 1-3-4 showed a trend towards lower respiratory scores, mean behaviour scores, least square mean lung lesion scores, and mortality compared with pigs vaccinated with Fostera PRRS MLV. For pigs challenged with PRRSV 1-7-4, mean viraemia levels following challenge were significantly lower by Day 42 in the group vaccinated with Ingelvac PRRS MLV compared with the group vaccinated with Fostera PRRS MLV. Similarly, pigs vaccinated with Ingelvac PRRS MLV and challenged with PRRSV 1-7-4 had numerically lower least square mean lung lesion scores and numerically higher ADWG than pigs vaccinated with Fostera PRRS MLV.

Although these studies were not specifically designed to assess superiority of one vaccine over the other, as we were evaluating vaccine efficacy, we have also compared the results between the two vaccines. In addition, the study used young pigs that were negative for PRRSV antibodies and PRRSV RNA; further studies are therefore required to determine whether these results apply to pigs of different ages and immune status. While this study was appropriately sized to assess the efficacy of PRRSV 1-3-4 and 1-7-4 in a laboratory setting, a larger number of animals would be required to evaluate the efficacy of the vaccines in a field study.

CONCLUSION

The studies reported add to the accumulating data that demonstrate the ability of Ingelvac PRRSV MLV to protect swine against relevant PRRSV infection. In these latest studies, Inglevac PRRS MLV provided heterogenous protection against two new and particular virulent isolates responsible for a growing number of infections in the US. Ingelvac PRRS MLV vaccination significantly improved health and performance of infected swine, and displayed distinct advantages over Fostera PRRSV MLV vaccine.

HIGHLIGHTS

Ingelvac PRRS MLV provides heterologous protection against two new, virulent PRRSV field strains. Vaccination reduced clinical signs of PRRSV infection, improved health and ADWG performance. Ingelvac PRRS MLV outperformed Fostera against two virulent PRRSV challenges. Ingelvac PRRS MLV vaccine could directly improve swine production.

FUNDING

Both studies were funded by Boehringer Ingelheim Animal Health.

ACKNOWLEDGEMENTS

The authors thank Ian Marshall and Zoe Kelly, PhD on behalf of InterComm International Ltd, Cambridge, UK for medical writing and editorial support, with funding provided by Boehringer Ingelheim Animal Health.

DECLARATIONS OF INTEREST

All authors are employees of Boehringer Ingelheim Animal Health who funded the study.

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