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Duration of immunity for an inactivated *Mycoplasma hyorhinis* vaccine in pigs

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ABSTRACT

Mycoplasma hyorhinis (Mhr) is a pathogen of pigs causing polyserositis and polyarthritis. The most susceptible population are nursery pigs of approximately 7 weeks of age, although we have shown that clinical signs can persist into finishing aged animals after a late-nursery infection. We have previously demonstrated the efficacy of a novel inactivated Mhr vaccine for the reduction of lameness and polyserositis in caesarian-derived colostrum-deprived (CDCD) pigs vaccinated at 3 weeks and challenged with Mhr at 6 weeks of age. Here we evaluated the duration of immunity (DOI) of the same vaccine. Vaccine or placebo was administered to CDCD pigs at 3 weeks of age. Pigs were challenged with Mhr at either 10 weeks of age (=7 week DOI) or 13 weeks of age (=10 week DOI). In the 7 week DOI, vaccination provided significant reductions in lameness ($p = 0.0018$), arthritis ($p = 0.0002$), and pericarditis ($p = 0.0312$) versus the placebo control. In the 10 week DOI, a significant reduction in arthritis ($p = 0.0320$) was observed in the vaccine group as compared to the placebo group. Both vaccine groups showed a significant increase ($p < 0.0001$) in the post-challenge average daily gain (ADG), gaining 0.2 kg/day more than their respective placebo groups.

1. Introduction

Infection of pigs by *Mycoplasma hyorhinis* (Mhr) can lead to polyserositis, lameness and arthritis (Ennis et al., 1971; Lin et al., 2006; Straw et al., 2006; Rovira et al., 2010; Martinson et al., 2017a, b). This in turn can result in reduced performance indicators, such as weight gain (Murray, 2012; Clavijo et al., 2017; Martinson et al., 2017b). We have previously established the efficacy of an inactivated Mhr vaccine which was cultivated in a eukaryotic cell system (Martinson et al., 2018). In that study, a single 2 mL dose of vaccine was administered to 3 week old caesarian-derived colostrum-deprived (CDCD) pigs which were then challenged with Mhr at 6 weeks of age. We have also demonstrated a progressive decline in the severity of disease in Mhr challenged pigs from nursery age (7 weeks) to finishing age (16 weeks) (Martinson et al., 2017b). While pericarditis was mostly absent in the older animals, it is important to note that disease was still present, in particular arthritis and associated lameness. It is therefore important to producers and veterinarians to define the duration of the protective immunity induced by this vaccine to help formulate Mhr disease mitigation plans.

The objective of the present study was to evaluate the efficacy of our inactivated Mhr vaccine at 7 or 10 weeks post-vaccination to determine the duration of the protective immune response generated.

2. Materials and methods

2.1. Vaccine

Boehringer Ingelheim Animal Health USA's (BI AH USA) inactivated Mhr vaccine and minimum immunizing dose have been described previously (Martinson et al., 2018). Briefly, murine fibroblasts in suspension were infected with Mhr and allowed to incubate at 37 °C until cytopathic effect (CPE) was observed. The culture was then inactivated with binary ethylenimine and adjuvanted with Seppic Montanide™ ISA207 VG at 50% (w/w). The dose was 7.41×10^7 CCU per 2.0 mL as determined by pre-inactivation color changing units (CCU). The adjuvanted placebo contained all components of the vaccine except Mhr. Vaccination was performed by an individual not involved with data collection.

2.2. Challenge

The Mhr challenge isolate, preparation of challenge material and administration have been described previously (Martinson et al., 2017a, b; Martinson et al., 2018). Briefly, Mhr was inoculated onto confluent monolayers of Madin-Darby canine kidney (MDCK) cells and allowed to incubate at 37 °C until CPE was observed. Cultures were harvested by

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freeze-thaw, combined with sterile glycerol, aliquoted into working volumes and stored at ≤ −60 °C until challenge. Challenge material was quickly thawed in a 37 °C water bath before each challenge and the dose was determined by CCU.

2.3. Animals and housing

Male and female CDCD piglets were purchased from Struve Labs International (SLI; Manning, IA). Animal identification was by unique ear tags. Pigs were confirmed to be Mhr negative by real-time PCR of nasal swabs (eSwab™, Copan; Murrieta, CA) collected seven days before vaccination. Testing for *Mycoplasma hyosynoviae* (Mhs) was not performed in this study as we have previously established the source herd to be negative for Mhs. Pigs were serologically negative for *Mycoplasma hyopneumoniae* (Mhp) and porcine reproductive and respiratory syndrome virus (PRRSV) by ELISA (M. hyo Ab Test and PRRS X3 Ab Test, IDEXX Laboratories, Inc; Westbrook, MA) from blood collected one day before vaccination. No serological assays were available for Mhr or Mhs at the time of this study. Prophylactic treatments with Excede® (ceftiofur, Zoetis; Florham Park, NJ) were administered to all pigs on day -5 (0.25 mL/pig) and day 15 (0.5 mL/pig), as per label instructions (5.0 mg ceftiofur equivalent per kg body weight). No other biologicals or pharmaceuticals were administered during the study. Animals were fed a diet of milk replacer until they were able to be weaned on unmedicated, dry food, at which time water was provided *ad libitum*. Daily observations were performed to ensure they had sufficient feed and water.

Pigs were kept in individual isolators at SLI until 2 weeks of age, when they were moved to brooders containing 2–3 pigs. Pigs were 22 days of age at vaccination (day 0). Pigs were transported to Veterinary Resources, Inc. (VRI; Cambridge, IA) on day 16 at which time the 7 week and 10 week duration of immunity (DOI) groups were housed separately for the remainder of the study. Animals in the 7 week DOI group were randomly assigned to two rooms with two pens per room and 15–17 pigs per pen. To accommodate larger animals, pigs in the 10 week DOI group were randomly assigned to two rooms with four pens each and 5–10 pigs per pen. Litters were penned together. Each treatment was represented within each litter and pen. Pens were on raised decks with metal slatted floors. To prevent exposure to Mhr, **NTX** pigs were segregated from the treatment groups at the time of challenge and housed in a separate room. Personnel performing data collection and laboratory assays were blinded to the treatment groups.

2.4. Experimental design

This study was a randomized complete block design. Piglets (n = 139) were first randomly assigned by litter to either a 7 week or 10 week DOI group. Within each of these DOI groups, piglets were blocked by litter and randomly assigned to the vaccine (**V**) or placebo (**P**) group (n = 32 animals each). Animal numbers were determined assuming an incidence rate of disease in the placebo group of at least 50%. Group size of twenty-eight animals per treatment was expected to provide approximately 80% power to detect a difference of 35% affected animals between the vaccine and placebo for a two-sided test using $\alpha = 0.05$. Four additional pigs were added per treatment group to allow for attrition.

The schedule of events is listed in Table 1. Weights were measured one day before vaccination. Pigs were administered the vaccine or placebo on day 0 at 3 weeks of age. Pigs in the vaccine groups (**V7** and **V10**) received 2.0 mL of the minimum immunizing dose (MID) of the inactivated Mhr vaccine (Martinson et al., 2018) intramuscularly in the right neck; pigs in the placebo groups (**P7** and **P10**) received a placebo control product in the same fashion. A set of non-vaccinated/non-challenged controls (**NTX**) were included with each DOI group (n = 5 for 7 week DOI, **NTX7**; n = 6 for 10 week DOI, **NTX10**). Observations for general health were made daily until six days before each challenge. Nasal swabs (eSwab™) and blood were collected before each challenge. Swabs were tested by real-time PCR as described previously (Martinson et al., 2017a). Sera were tested for antibodies against Mhp and PRRS by ELISA (IDEXX). Weights were measured again one day before challenge.

Animals were inoculated with Mhr on three consecutive days, by three separate routes of administration. Pigs in the 7 week DOI were inoculated at 20 mL/intraperitoneal (IP) on day 49, 10 mL/intravenous (IV) on day 50, and 10 mL/intranasal (IN) on day 51 for a total three-day dose of 4.55×10^9 CCU per pig. Pigs in the 10 week DOI were inoculated at 20 mL/IP on day 70, 10 mL/IV on day 71, and 10 mL/IN on day 72 for a total three-day dose of 4.61×10^9 CCU per pig. From six days before challenge through to euthanasia, clinical observations were made for signs of respiratory distress, coughing, and lameness. Clinical observations and scoring were modified from a previous study (Martinson et al., 2018) and are described in Table 2. Pigs were considered lame if they received lameness score of ≥1 on two or more consecutive days. Animals were euthanized for humane reasons if they received a lameness score of 4 (recumbent) at any time.

Weights were measured one day before necropsy. Pigs were anesthetized, euthanized and necropsied 21 days after challenge. The elbows, carpi, stifles, and tarsi were exposed and examined for signs of arthritis (e.g. excess or abnormal synovial fluid, abnormal synovial membranes, abnormal articular surfaces). A single swab (eSwab™) was used to sample the articular surfaces of the elbows and carpi; a second swab was used to sample the stifles and tarsi. The thoracic cavity was examined for pericarditis and a swab (eSwab™) of the visceral pericardium was collected. Joint swabs and pericardial swabs were tested by Mhr-specific real-time PCR as described previously (Martinson et al., 2017a).

The study was performed following the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010) in ABSL-2, USDA inspected facilities. Before study initiation, the protocol was reviewed and approved by the United States Department of Agriculture Center for Veterinary Biologics (USDA-CVB).

2.5. Statistical analysis

Analyses were conducted using SAS version 9.4 (SAS Institute, Inc.; Cary, North Carolina, USA). The 7 week and 10 week DOI groups were analyzed separately. Lameness (0 = no, 1 = yes), pericarditis (0 = absent, 1 = present), and arthritis (0 = absent, 1 = signs of arthritis in any of the eight joints) were coded as binomial outcomes and analyzed using the GLIMMIX procedure of SAS. The model included the fixed effect of treatment group and random effect of litter within room and utilized a binomial distribution and logit link function. Abnormal respiration, coughing, the number of affected joints per pig, and the number of PCR positive swabs were each summarized as frequency distributions by treatment group. Body weight at vaccination was summarized by treatment group. Weight gain during the challenge phase was analyzed using a repeated measures mixed model using the MIXED procedure of SAS. The model included the fixed effect of treatment, day, and treatment by day interaction. Litter was included as a random effect. An unstructured covariance structure was utilized. Comparisons to the placebo group are reported within day using $\alpha = 0.05$. ADG for treatment groups was estimated using the ESTIMATE statement with least squares means (LSM) and 95% CIs reported.

3. Results

3.1. Animal removals

One **P7** pig, one **P10** pig, and two **V10** pigs died between day 6 and day 12. Each of these animals had a fluid-filled jejunum and enlarged mesenteric lymph nodes and was diagnosed with enterotoxemic colibacillosis. A second **P10** pig was euthanized for humane reasons on day

Table 1
Schedule of events.

Study Day	Event	Group*	Samples
Day -7	Sample collection	All	Nasal swabs for PCR
Day -5	Antibiotic treatment	All	None
Day -1	Sample collection	All	Blood for ELISA; weight measurements
Day 0	**Vaccination**	**P7, V7, P10, V10**	None
Day 15	Antibiotic treatment	All	None
Day 16	Transport	All	None
Day 43	Sample collection	P7, V7, NTX7	Nasal swabs for PCR; blood for ELISA
	Observations	P7, V7, NTX7	Clinical observations and lameness scoring
Day 44 to Day 47	Observations	P7, V7, NTX7	Clinical observations and lameness scoring
Day 48	Sample collection	P7, V7, NTX7	Weight measurements
	Observations	P7, V7, NTX7	Clinical observations and lameness scoring
Day 49	**Challenge by IP****	**P7, V7**	None
	Observations	P7, V7, NTX7	Clinical observations and lameness scoring
Day 50	**Challenge by IV****	**P7, V7**	None
	Observations	P7, V7, NTX7	Clinical observations and lameness scoring
Day 51	**Challenge by IN****	**P7, V7**	None
	Observations	P7, V7, NTX7	Clinical observations and lameness scoring
Day 52 to Day 63	Observations	P7, V7, NTX7	Clinical observations and lameness scoring
Day 64	Sample collection	P10, V10, NTX10	Nasal swabs for PCR; blood for ELISA
	Observations	P7, V7, NTX7; P10, V10, NTX10	Clinical observations and lameness scoring
Day 65 to Day 68	Observations	P7, V7, NTX7; P10, V10, NTX10	Clinical observations and lameness scoring
Day 69	Sample collection	P7, V7, NTX7	Weight measurements
	Observations	P7, V7, NTX7; P10, V10, NTX10	Clinical observations and lameness scoring
Day 70	Observations	P7, V7, NTX7; P10, V10, NTX10	Clinical observations and lameness scoring
	Euthanasia	**P7, V7, NTX7**	Gross pathology; pericardial and joint swabs for PCR
	Challenge by IP	**P10, V10**	None
Day 71	**Challenge by IV**	**P10, V10**	None
	Observations	P10, V10, NTX10	Clinical observations and lameness scoring
Day 72	**Challenge by IN**	**P10, V10**	None
	Observations	P10, V10, NTX10	Clinical observations and lameness scoring
Day 73 to Day 89	Observations	P10, V10, NTX10	Clinical observations and lameness scoring
Day 90	Sample collection	P10, V10, NTX10	Weight measurements
	Observations	P10, V10, NTX10	Clinical observations and lameness scoring
Day 91	Observations	P10, V10, NTX10	Clinical observations and lameness scoring
	Euthanasia	**P10, V10, NTX10**	Gross pathology; pericardial and joint swabs for PCR

* P7 = placebo, 7 week DOI; V7 = vaccine, 7 week DOI; P10 = placebo, 10 week DOI; V10 = vaccine, 10 week DOI; NTX7 = non-vaccinated/non-challenged, 7 week DOI; NTX10 = non-vaccinated/non-challenged, 10 week DOI.

** IP = intraperitoneal, IV = intravenous, IN = intranasal.

Table 2
Description of clinical observation scoring.

Score	Abnormal Respirations	Cough	Lameness
0	**Normal**—no respiratory discomfort	**Normal**—no cough	**Normal**—no visible lameness at a walk
1	**Mild—**mild increase in respiratory rate	**Mild**—slight cough that does not seem to disturb normal activities	**Mild**—difficult to observe lameness as the animal walks around the pen; not constantly lame when walking; walks at a normal speed; is weight bearing while walking and standing. Lameness is indicated by intermittent reduced weight bearing on one limb or shortening of the stride.
2	**Moderate—**notable increase in respiratory rate	**Moderate**—loud, pronounced cough that disrupts normal activities	**Moderate**—constant and observed throughout every step at a walking pace; bearing some weight on the leg at a walk and standing but short-striding one or more legs while walking; walks at a normal speed. Animals may appear to stand hunched with limbs extended farther cranially than expected in an attempt to shift weight to the pelvic limbs.
3	**Severe—**thumping	**Severe**—dry, hacking cough that appears painful	**Severe**—puts no weight on the leg(s) the first few steps after standing; constant obvious lameness while at a walking pace; putting very little to no weight on the leg(s) at a walk or while standing. Lameness requires the pig to slow its speed of walking.
4	n/a	n/a	**Recumbent—**will not stand even with assistance.

70 due to severe lameness. Necropsy revealed a thickened and fibrosed right tarsal joint with several abscessed areas on the joint. Three pigs in group **P10** were found dead during the challenge phase, one on day 83, one on day 89, and one on day 91. Death was attributed to gastric ulceration, which was present in each pig.

3.2. Clinical observations

A significant ($p = 0.0018$) reduction in lameness (Fig. 1) was achieved in the 7 week DOI; lameness was observed in 61.3% (95% CI [43.0, 76.9]) of the pigs in group **P7** and 18.7% (95% CI [8.5, 36.4]) of the pigs in group **V7**. There was no significant difference in lameness between the vaccine and placebo groups in the 10 week DOI; lameness was observed in 33.0% (95% CI [16.6, 54.9]) of the pigs in group **P10** and 16.1% (95% CI [6.0, 36.4]) of the pigs in group **V10**.

Very few pigs in either DOI group were observed with abnormal respiration or cough. In the 7 week DOI, abnormal respiration was observed in 4/31 pigs in group **P7** and 0/32 pigs in **V7**. Coughing was not observed in any pig (0/31) in group **P7** and was observed in 2/32 pigs in group **V7**. For the 10 week DOI groups, abnormal respiration was noted in 1/31 pigs in group **P10** and was not observed in any pig (0/30) in group **V10**. Coughing was observed in 1/31 pigs in both

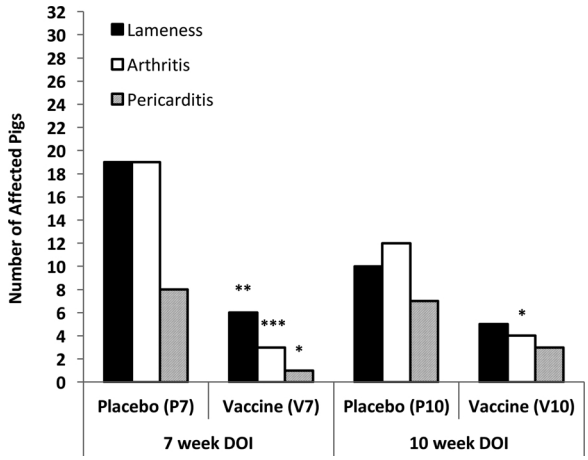

Fig. 1. Clinical findings for lameness, arthritis, and pericarditis. A pig was deemed lame (black bars) if it received a lameness score on two or more consecutive days during the clinical observation period. A pig was deemed positive for arthritis (white bars) if any of the eight joints evaluated was observed with signs of arthritis at necropsy. Pigs were observed for pericarditis (shaded bars) at necropsy. Data represents the number of affected animals in each group. 7 week DOI: P7 = placebo control (n = 31), V7 = MHR vaccine (n = 32); 10 week DOI: P10 = placebo control (n = 30[1]), V10 = MHR vaccine (n = 30). The non-vaccination/non-challenge groups (NTX) were not observed with lameness, arthritis or pericarditis and are not represented. [1]One P10 pig was removed before necropsy due to lameness but was included in results for this observation; therefore, n = 31 for P10 lameness category. Asterisks above bars represent significant reductions compared to respective placebo group: *p < 0.05, **p < 0.01, ***p < 0.001.

groups **P10** and **V10**.

All **NTX** pigs remained free from lameness and abnormal respiration. One **NTX7** pig was observed coughing on a single day (day 44).

3.3. Gross pathology

Pericarditis (Fig. 1) was observed in at least one animal in all treatment groups. Vaccination provided a significant (p = 0.0312) reduction in pericarditis in the 7 week DOI group; pericarditis was present in 26.0% (95% CI [12.4, 46.5]) of the pigs in group **P7** and 3.0% (95% CI [0.4, 19.7]) of the pigs in group **V7**. There was no significant difference in pericarditis between the vaccine and placebo groups in the 10 week DOI; pericarditis was observed in 22.0% (95% CI [8.4, 46.4]) of the pigs in group **P10** and 8.3% (95% CI [2.1, 27.2]) of the pigs in group **V10**.

A pig was considered positive for arthritis if lesions were seen in any of the eight joints examined. A significant (p = 0.0002) reduction in arthritis was seen in both the 7 week DOI (p = 0.0002) and the 10 week DOI (p = 0.0320): **P7** = 63.9% (95% CI [37.9, 83.7]), **V7** = 7.6% (95% CI [1.9, 26.0]); **P10** = 39.8% (95% CI [22.0, 60.7]), **V10** = 13.3% (95% CI [4.6, 32.7]). A summary of the total number of affected joints for individual animals in each DOI can be seen in Fig. 2A.

None of the **NTX** pigs had pericarditis or arthritis.

3.4. Body weight

Comparisons for body weight are summarized in Table 3. No significant difference in weight was seen at the time of challenge between the placebo and vaccine groups in either DOI. At the conclusion of the challenge period for the 7 week DOI, the vaccine group **V7** weighed significantly more (p = 0.0004) than the placebo group **P7**. There was no significant difference in body weight between group **P10** and **V10** at the conclusion of the challenge period for the 10 week DOI.

Average daily gain (ADG) was calculated for each treatment group. The difference in ADG between vaccinates and placebo's was significant for both the 7 week DOI (p < 0.0001) and the 10 week DOI (p < 0.0001): **P7** = 0.49 kg/d (95% CI [0.44, 0.54]), **V7** = 0.68 kg/d (95% CI [0.63, 0.73]); **P10** = 0.57 kg/d (95% CI [0.51, 0.63]), **V10** = 0.76 kg/d (95% CI [0.70, 0.81]).

NTX animals were segregated from the other groups at the time of challenge and could not be directly compared to the treatment groups. For reference, the mean weight and standard deviations for **NTX** groups were calculated for the 7 week DOI (**NTX7**; day -1 = 3.0 ± 0.6 kg; day 48 = 26.4 ± 5.1 kg; and day 69 = 44.4 ± 6.8 kg) and 10 week DOI (**NTX10**; day -1 = 2.8 ± 0.5 kg; day 69 = 45.5 ± 8.3 kg; and day 90 = 59.6 ± 9.7 kg). The ADG for **NTX** groups was calculated to be 0.85 kg/d for **NTX7** and 0.67 kg/d for **NTX10** during their respective post-challenge periods.

3.5. Detection of Mhr in sample swabs

Pairwise comparisons were not performed for PCR results. Nasal swabs collected on day -7 (pre-vaccination), day 43 (pre-challenge, 7 week DOI), and day 64 (pre-challenge, 10 week DOI) were all PCR negative for Mhr. Fewer than 20% of the pericardial swabs collected at necropsy were PCR positive for Mhr, similar to findings in our previous studies (Martinson et al., 2017a, b; Martinson et al., 2018): **P7** = 19.4% (6/31), **V7** = 9.4% (3/32); **P10** = 6.9% (2/29), and **V10** = 0% (0/30).

A pig was considered joint swab positive for Mhr if either the elbow-carpal swab or the stifle-tarsal swab was PCR positive. Results were similar in each DOI: **P7** = 61.3% (19/31), **V7** = 15.6% (5/32); **P10** = 63.3% (19/30), **V10** = 13.3% (4/30). A summary of the total number of PCR positive joint swabs for individual animals in each DOI can be seen in Fig. 2B. In all treatment groups, there were more PCR positive samples detected from the hind limb joints than from the

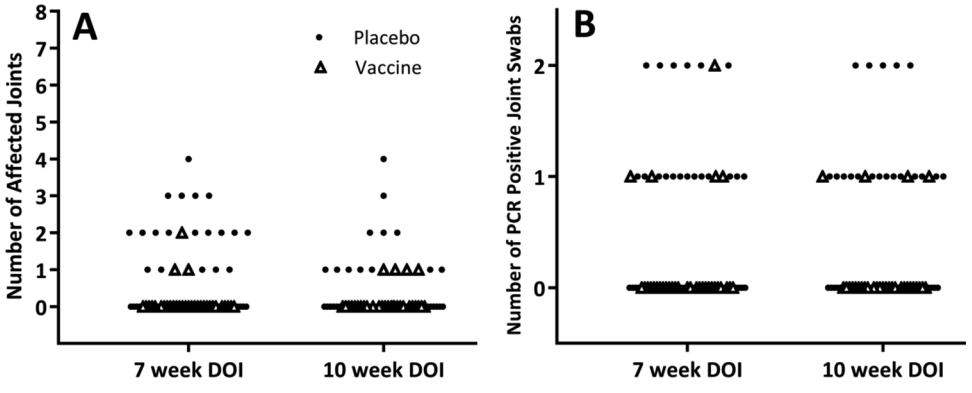

Fig. 2. Distribution of the number of affected joints and PCR positive joint swabs. **A.** At necropsy, eight joints (carpi, elbows, tarsi, and stifles) were evaluated for signs of arthritis. Data points represent individual animals with the respective total number of joints observed with arthritis. **B.** After gross examination, a single swab was used to sample the articular surfaces of both elbows and both carpi for each pig; a second swab was used to sample both stifles and both tarsi. Swabs were tested by Mhr-specific PCR. Data points represent individual animals with the respective total number of swabs PCR positive for Mhr. Dots = placebo (P7, 7 week DOI (n = 31); P10, 10 week DOI (n = 30)). Triangles = vaccine (V7, 7 week DOI (n = 32); V10, 10 week DOI (n = 30)). The non-vaccination/non-challenge groups (NTX7, NTX10) showed no signs of arthritis, had no PCR positive joint swabs, and are not represented here.

Table 3
Weight gain (kg) post-challenge.

Treatment[1]	Pre-challenge[2] LSM (95% CI)	Difference (P-V)	p-value	Pre-necropsy[3] LSM (95% CI)	Difference (P-V)	p-value
P7	26.2 (25.3, 27.2)	0.2530	0.666	36.6 (35.0, 38.1)	−3.7646	0.0004
V7	26.0 (25.0, 27.0)			40.3 (38.8, 41.9)		
P10	45.3 (43.2, 47.3)	1.2581	0.2224	57.1 (54.7, 59.6)	−2.7245	0.0526
V10	44.0 (41.9, 46.1)			59.9 (57.4, 62.3)		

LSM = least squares mean weight (kg); CI = confidence interval.
[1] P7 = placebo, 7 week DOI; V7 = vaccine, 7 week DOI; P10 = placebo, 10 week DOI; V10 = vaccine, 10 week DOI.
[2] Pre-challenge: 7week DOI = Day 48, 10 week DOI = Day 69.
[3] Pre-necropsy: 7 week DOI = Day 69, 10 week DOI = Day 90.

forelimb joints: elbow-carpal swabs—**P7** = 29.0% (9/31), **V7** = 6.3% (2/32), **P10** = 33.3% (10/30), **V10** = 0% (0/30); stifle-tarsal swabs—**P7** = 51.6% (16/31), **V7** = 12.5% (4/32), **P10** = 46.7% (14/30), **V10** = 13.3% (4/30).

All pericardial and joint swabs collected from the **NTX** groups were PCR negative for Mhr.

3.6. Serology

All sera collected on day -1, day 43, and day 64 were negative for Mhp and PRRSV by ELISA.

4. Discussion

The objective of this study was to evaluate the efficacy of an inactivated Mhr vaccine in pigs challenged at 7 weeks or 10 weeks after vaccination. In a previous study, we demonstrated a decrease in the severity of Mhr disease in progressively older animals, challenged at 7, 10, 13 or 16 weeks of age (Martinson et al., 2017b). It was anticipated that the lower numbers of animals expected to be affected at 16 weeks of age would require considerable increases in the number of animals per group to detect a protective effect of the vaccine. Therefore, we included challenges for both 10-week old (= 7 week DOI) and 13-week old (= 10 week DOI) pigs in this trial.

The natural route of infection for Mhr has yet to be identified (Straw et al., 2006; Rovira et al., 2010). Our Mhr challenge is severe; however, the model has consistently resulted in sufficient numbers of affected animals to effectively evaluate vaccine efficacy for multiple parameters (Martinson et al., 2017a,b). Our vaccine has been shown to be effective in the face of this severe challenge in nursery-aged animals challenged three weeks after vaccination (Martinson et al., 2018). In this study, the inactivated Mhr vaccine had significant reductions in lameness, arthritis, and pericarditis, as well as a significant increase in post-challenge ADG in pigs challenged seven weeks after vaccination. A significant reduction in arthritis and a significant increase in post-challenge ADG was seen in pigs challenged ten weeks after vaccination.

The reduced severity of Mhr associated disease in older pigs makes it difficult to evaluate vaccine efficacy in these animals. Whether this is due to the limitations of our Mhr challenge model or represents a true age limit to Mhr infection is unknown. Previous research suggests nursery-aged pigs are the most susceptible population (Friis and Feenstra, 1994; Straw et al., 2006; Rovira et al., 2010; Clavijo et al., 2017). The results generated in this study demonstrate that this vaccine can provide partial protection to pigs from Mhr-associated disease through late nursery ages and into early finishing. Vaccination of production animals in the face of natural exposure and infection will ultimately determine the utility or our vaccine under field conditions. Development of serological assays and monitoring of additional immunological criteria in future studies will also help define Mhr infection beyond production parameters and improve our understanding of the pathogenesis of disease caused by Mhr.

In conclusion, we have confirmed the efficacy of an inactivated Mhr vaccine in CDCD pigs vaccinated at 3 weeks of age and challenged with Mhr at either 10 weeks or 13 weeks of age, resulting in a DOI of at least 7 weeks following vaccination.

Conflict of interest

This study was funded entirely by Boehringer Ingelheim Animal Health USA (BI AH USA). All authors are employees of BIAH.

Contributions

B.M., W.Z., and K.B. were involved with study design and management. J.K. was involved with study design and approvals. L.B. performed statistical analyses. B.M. managed laboratory testing and wrote the manuscript which has been read and approved by all authors.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.02.021.

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