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Efficacy of an inactivated *Mycoplasma hyorhinis* vaccine in pigs

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ABSTRACT

Lameness and polyserositis in pigs caused by *Mycoplasma hyorhinis* are generally treated with antibiotics and may require multiple doses. The costs of these antibiotics combined with economic losses from culling and reduced feed conversion due to lameness are hardships to the swine producer. In this study we have demonstrated efficacy of an inactivated *M. hyorhinis* vaccine administered to three-week old caesarian-derived colostrum-deprived piglets. Three doses of vaccine (high, medium, and low) were evaluated and compared to a placebo control. *Mycoplasma hyorhinis* challenge occurred three weeks after vaccination. Pigs were observed for lameness and respiratory distress for three weeks following challenge. Pigs were then euthanized and a gross pathological evaluation for polyserositis and arthritis was performed. A minimum immunizing dose of vaccine was defined as containing at least 7.41×10^7 CCU of *M. hyorhinis* per 2.0 mL dose as represented by the medium dose vaccine. This vaccine provided significant reductions in lameness and pericarditis with preventive fractions of 0.76 (95% CI [0.26, 0.92]) and 0.58 (95% CI [0.31, 0.74]), respectively, compared to the placebo control group. A significant increase in post-challenge weight gain ($P < .0001$) was also achieved with this vaccine, with an average daily gain (ADG) of 0.92 lbs/day compared to 0.57 lbs/day in the placebo group.

1. Introduction

Among the species of mycoplasma which are pathogenic in swine, *Mycoplasma hyorhinis* (MHR) has the most diverse range of clinical effects. Similar to *Mycoplasma hyopneumoniae* (MHP) and *Mycoplasma hyosynoviae* (MHS), MHR colonizes the upper respiratory tract and can easily be recovered from the nasal cavity and tonsils of infected pigs [1,2]. MHR-associated disease occurs in the lungs and joints where the bacteria can cause pneumonia and polyarthritis, respectively [3–6]. It is also a common cause of polyserositis [3,5,6]. In particular, we have noted severe pericarditis in experimentally infected animals [7]. MHR has also been implicated in cases of eustachitis and otitis [3,8].

The most severe MHR infections, in particular polyserositis and lameness, lead to reductions in weight gain and feed conversion as well as culling of lame animals which result in economic losses to producers [9,10]. As no licensed commercial vaccine is currently available, treatment of MHR has typically been with prophylactic and therapeutic antibiotics.

The objective of this study was to determine the minimum immunizing dose of an inactivated MHR vaccine in caesarian-derived colostrum-deprived (CDCD) piglets at three weeks of age for protection against MHR.

2. Materials and methods

2.1. Experimental design

In this randomized complete block design, 120 piglets were blocked by litter and enrolled into one of four treatment groups, 28 animals per group. Assuming an incidence rate in the placebo group of at least 50%, 28 animals per treatment group was expected to provide approximately 80% power to detect a difference of 35 percentage points between the treatment and control for a two-sided test using $\alpha = 0.05$. Animals received one of three doses of an experimental MHR vaccine (HIGH, MED, and LOW) or a placebo control product (PLAC). A fifth group of eight animals received no treatment (NTX) throughout the study, acting as non-vaccinated/non-challenged controls. Twenty-two days after vaccination, all animals except NTX were challenged with MHR. Animals were observed daily post-challenge for signs of respiratory distress, coughing, lameness and well-being. Twenty-one days

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after challenge, all animals were euthanized and a gross pathological examination was performed. The primary parameters to determine the effect of vaccination were lameness and pericarditis. The minimum immunizing dose was defined as the lowest dose of vaccine with a positive preventive fraction (PF) with a lower confidence interval (CI) > 0. The study was performed following the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* [11] in BSL-2, USDA inspected facilities. The study protocol was approved by the United States Department of Agriculture Center for Veterinary Biologics (USDA-CVB) prior to study initiation.

2.2. Animals

CDCD piglets were purchased from Struve Labs International (SLI; Manning, IA) and were 18–21 days of age at the time of vaccination (Day 0). Both females and intact males were used. Animals were identified by uniquely numbered ear tags. Piglets were determined to be free from colonization by MHR by MHR-specific real-time PCR of nasal swabs (eSwab™, Copan; Murrieta, CA) collected on Day −6 (D −6) and tested as previously described [7]. Piglets were also determined to be free from antibodies to MHP (M. hyo. Ab Test, IDEXX Laboratories, Inc.; Westbrook, MA) and porcine reproductive and respiratory syndrome virus (PRRSV) by ELISA (PRRS X3 Ab Test, IDEXX Laboratories, Inc.; Westbrook, MA) from blood collected on D - 1. Pigs were given a prophylactic treatment of Excede® (Zoetis; Florham Park, NJ) at D - 6, per label instructions. No other biologicals or pharmaceuticals were administered during the study. All animals were deemed to be healthy before vaccination. A diet of milk replacer was given until pigs were able to wean to non-medicated, dry food at which time water was provided *ad libitum*. Pigs were observed daily to ensure sufficient feed, water, and well-being.

The vaccination phase of the study was conducted at SLI. Pigs were initially housed in individual isolators until approximately two weeks of age at which time they were moved into brooders of two to three litter-mates each. Five days before challenge animals were transported to Veterinary Resources, Inc. (VRI; Cambridge, IA) for the challenge phase of the study. Pigs were again blocked by litter and housed for equal representation of treatment groups within pens. Pens were a raised deck with metal slatted flooring. During the challenge phase, NTX animals were housed separately from the challenged animals to prevent exposure to MHR. Personnel involved in collecting data or performing laboratory assays were blinded to the allocation of pigs to treatment group.

2.3. Vaccine

Boehringer Ingelheim Animal Health's (BIAH) MHR vaccine isolate originated from a pig with clinical signs of *Mycoplasma hyorhinis* infection. Initial isolation and speciation was performed in modified Friis medium using standard techniques [12]. For experimental vaccine production, McCoy cells (murine fibroblasts) in suspension were infected with MHR and then subsequently inactivated with binary ethylenimine per BIAH's proprietary outline of production.

A high, medium, and low dose vaccine were generated. The high dose vaccine contained the complete culture described above blended with Seppic Montanide™ ISA207 VG at 50% (w/w). For the medium and low dose vaccines, the MHR culture was first diluted 1:2 or 1:10, respectively, in PBS before blending with adjuvant at 50% (w/w). The respective dose of MHR in the HIGH, MED, and LOW vaccine preparations was calculated to be 1.48×10^8, 7.41×10^7, and 1.48×10^7 CCU per 2.0 mL dose as determined by color changing units (CCU) from the pre-inactivation MHR harvest material.

An adjuvanted placebo was generated and contained the exact composition as the high dose experimental vaccine, without MHR.

Pigs were administered a single 2.0 mL dose of vaccine or placebo intramuscularly (IM) in the right side of the neck on D 0. Treatments were administered by an individual not involved with data collection.

2.4. Challenge

The MHR challenge isolate and preparation of material for challenge have been previously described [7]. Animals were administered MHR challenge as follows: 20 mL/intraperitoneal (IP) on D 22, 10 mL/intravenous (IV) on D 23, and 10 mL/intranasal (IN) on D 24 for a total dose of 5.39×10^8 CCU per animal.

2.5. Observations

Animals were observed daily for general health before challenge. From four days before challenge (D18) through study termination (Day 43), all pigs were observed daily for clinical signs of MHR infection including respiratory distress, coughing, and lameness as described in Table 1. A pig was considered lame if it received a lameness score of ≥1 on any two (or more) consecutive days. Animals receiving a lameness score of 4 for two consecutive days, or any animal with a lameness score of 5 was euthanized for humane reasons.

Table 1
Clinical observation scoring description.

Score	Respiration	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
3	**Severe**—thumping	**Severe**—dry, hacking cough that appears painful	**Moderately Severe**—puts no weight on the leg the first few steps after standing, constant, obvious lameness while at a walking pace, putting very little weight on the leg at a walk or while standing; lameness requires the pig to slow its speed of walking
4	N/A	N/A	**Severe**—will stand, may require assistance, for at least 3 min, non-weight bearing on one or more legs at walk or standing, still able to three-legged walk
5	N/A	N/A	**Recumbent**—will not stand even with assistance