RESEARCH REPORT
SWINE HEALTH

Title: Assessment of the infectivity of asymptomatic carriers, to understand the dynamics of *Mycoplasma hyopneumoniae* infection in reproductive herds- NPB #05-013

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Abstract

Swine Enzootic Pneumonia continues to be one of the most important respiratory diseases for the industry. Although many eradication efforts have been attempted, yielding a high rate of success, the science behind them remains unclear. This study was designed to clarify a central issue with eradication, namely, duration of infectivity. It involved 54 experimentally infected pigs which were placed in direct contact with susceptible pigs, vaccinated as well as unvaccinated, in three different transmission experiments, performed at 80, 200 and 240 days post inoculation. Transmission from infected to susceptible pigs occurred at 80 and 200 post inoculation; however, after 240 days of infection, experimentally infected pigs were not positive to *Mycoplasma hyopneumoniae* and did not transmit the bacteria to sentinel pigs. The transmission rate, as evaluated by the number of infected pigs, was significantly lower at 200 than at 80 days post infection. Transmission rate among vaccinated and unvaccinated animals was not significantly different. These results indicate that transmission of *Mycoplasma hyopneumoniae* occurs for at least 200 days after initial infection, even though infected animals did not show clinical signs associated to the disease and most of them resulted negative when being evaluated for antibodies against *Mycoplasma hyopneumoniae*. However, transmission did not occur at 240 days infection, since inoculated animals appeared to cleare the infection by that time.

Introduction

Enzootic Pneumonia, a high morbidity and low mortality condition, highly prevalent in swine herds is caused by *Mycoplasma hyopneumoniae* (Mare and Switzer, 1965; Goodwin *et al*., 1965). The disease is characterized by a chronic respiratory condition, which does not represent a threat by itself, but often appears combined with a number of bacterial and viral pathogens such as *Pasteurella multocida* or porcine respiratory reproductive virus, thus playing a central role in swine pneumonia.

Introduction of naïve replacement gilts to reproductive herds infected with *Mycoplasma hyopneumoniae* has always been a challenging problem. The goal is to minimize the detrimental effect to both the existing herd and the newly introduced animals, which would lead to a herd destabilization. Vaccination of incoming gilts prior to entry into infected gestation barns is a common practice, but it is not known if vaccination prevents infection of these gilts.

The objective of this study was to assess transmission of *Mycoplasma hyopneumoniae* from asymptomatic persistently infected carriers to unvaccinated as well as vaccinated sentinel pigs, and to compare the infected proportions between the two animal populations.

Objectives:

To determine whether asymptomatic persistently infected carriers can transmit *Mycoplasma hyopneumoniae* to susceptible pigs up to 240 days of infection.

Materials and Methods:

Animals and housing

This experiment was performed at the Swine Disease Eradication Centre Research Farm under the Institutional Animal Care and Committee protocol No. 0503A68487 (University of Minnesota).

A total of one hundred fifty nine animals were included in the study. All animals were obtained from a source known to be negative to *Mycoplasma hyopneumoniae* as well as porcine respiratory reproductive syndrome virus and were distributed as follows:

- Nine, three-week old piglets, which served as negative controls through study. Piglets were divided into three groups of three and were introduced at different dates; the first group at the beginning of the study, second at third at second and third transmission experiments, respectively.

- Fifty four, fifteen-week old female pigs were experimentally infected. The experimental infection consisted of an intratracheal inoculation of 20mL per pig of a standardized lung suspension inoculum, produced with a culture of *Mycoplasma hyopneumoniae* strain 232 resuspended to a final dose of $1x10^5$ colour-changing units (CCU) per mL.
- Forty five unvaccinated sentinel gilts.
- Forty five vaccinated sentinel gilts. Vaccination consisted in the application of two doses of a *Mycoplasma hyopneumoniae* commercial vaccine (RespiSure® Pfizer) 30 and 15 days prior to starting the transmission experiments.

Experimental Design

The study design consisted of three independent transmission experiments on days 80, 200 and 240 after experimental infection. The date of 80 days after infection was selected for the infectivity assessments in order to use *Mycoplasma hyopneumoniae* asymptomatic carriers.

Each exposure period was set at two weeks during which animals (experimentally infected + sentinels) were housed in direct contact in a three-room barn. Rooms included 3 pens from which the two pens located in the extremes were used for the study, the central pen was left empty. Three experimentally infected pigs were housed together with five unvaccinated sentinels in one pen, while three experimentally infected pigs were housed with five vaccinated sentinels in the other pen.

Subsets of experimentally infected pigs to be included in the infectivity assessments were randomly selected out of the fifty four inoculated pigs.

Unvaccinated and vaccinated sentinels arrived to the farm on the exposure period starting date. All pigs included in the transmission experiment were sent to slaughter as a group, thus animals used in all three exposure periods were different pigs.

The rest of the experimentally infected pigs were always kept together in a separate barn within the same farm.

Sample collection dates

Blood and nasal swabs, from negative controls and the soon-to-be experimentally infected pigs were collected on day 0.

Nasal swabs were collected from all animals on days 7 and 34, while blood samples were drawn from negative controls and experimentally infected pigs on days 34 and 60. Experimental infection was also assessed by the onset of clinical signs. The last day when pigs presented cough was therefore recorded.

The transmission experiment sampling protocol was similar for the three assessments. All animals – experimentally infected, unvaccinated and vaccinated sentinels- were sampled at the beginning and the end of

the exposure period. Animals were then sent to slaughter, where a bronchial swab and lung tissue of each individual was taken and lung lesion scores were measured.

Diagnostic tests and measurements

Blood samples were separated in order to obtain a serum sample to be evaluated for the presence of antibodies against *Mycoplasma hyopneumoniae* using a commercial kit for antibody detection (DAKO® ELISA) (Feld *et al*., 1998).

Nasal and bronchial swabs were frozen and taken to the laboratory were DNA was extracted and a nested polymerase chain reaction test (Calsamiglia et al., 1999) was run for the identification of a fragment of DNA specific to *Mycoplasma hyopneumoniae*.

The lung lesion score was measured (Pointon *et al*., 1999) and tissue samples were collected. Tissue selection as well as the conservation method was chosen depending on sample destination. Samples to be processed for histopathology were collected from macroscopic lesions and from a consistent location in pigs without visible lesions, and then suspended in a 10% formaldehyde solution. Samples from day 254, to be processed for scanning electron microscopy were included in a 4% glutaraldehyde solution, these samples were taken from the upper region from where a bronchial swab was previously collected.

Results:

All animals were negative to *Mycoplasma hyopneumoniae* on Day 0. Negative control animals remained negative to *M. hyopneumoniae* until euthanasia. Experimental infection of inoculated animals was confirmed by development of clinical signs on Day 13 and by means of antibody detection on Days 34 and 60, and at the beginning and the end of each infectivity assessment. Clinical signs were last recorded on Day 68 of the study.

Infectivity Assessment 1 – Days 80 to 94:

All experimentally infected pigs were positive to *M. hyopneumoniae* by n-PCR from bronchial swabs. 7/18 had lung lesions at slaughter.

Unvaccinated sentinels were negative to *M. hyopneumoniae* antibodies at the beginning and end of the transmission experiment and were also negative by n-PCR from nasal swabs when the exposure period started.

Numerical differences between proportion of animals (unvaccinated vs. vaccinated) positive to *M. hyopneumoniae* by n-PCR and presenting lung lesions at slaughter were not significant; p-values 0.30 and 0.40, respectively.

Antibodies against *M. hyopneumoniae* were detected in all but one vaccinated sentinel on days 80 and 94, while all were negative by n-PCR from nasal swabs at the beginning of the transmission experiment.

On day 94, positive n-PCR results were found in nasal swabs from unvaccinated (5/15), vaccinated (8/15) and experimentally infected animals (11/18).

Infectivity Assessment 2 – Days 200 to 214:

Eleven out of eighteen experimentally infected pigs were positive to *M. hyopneumoniae* by n-PCR from bronchial swabs, while 3/18 had lung lesions suggestive of *M. hyopneumoniae* at slaughter.

Antibodies for *M. hyopneumoniae* were not detected in unvaccinated sentinel pigs at the beginning or the end of the exposure period. All vaccinated sentinels pigs were positive to *M. hyopneumoniae* antibodies on day 200 and 14/15 were positive on day 214.

A significant difference was found when comparing proportions of pigs with positive bronchial n-PCR swabs between 80 and 200 days post infection, for both the unvaccinated and vaccinated populations (p-values 0.0003 and 0.005). Statistically significant results were also obtained when comparing lung lesion score of unvaccinated and vaccinated pigs (p-values of 0.006 and 0.005).

Infectivity Assessment 3 – Days 240 to 254:

Regarding the detection of antibodies against *M hyopneumoniae*, on day 240, five out of eighteen experimentally infected animals and fourteen out of fifteen vaccinated sentinels resulted positive; while on day 254, 12/18 experimentally infected and 13/15 vaccinated sentinels were still positive to the test. Conversely, all unvaccinated sentinels were negative at the beginning of the exposure period and remained negative until the end of the infectivity assessment.

Results from the n-PCR run on bronchial swabs were negative for all experimentally infected pigs. Therefore, all sentinels (vaccinated as well as vaccinated) resulted negative to this test.

Results from scanning electron micrographs of experimentally infected pigs showed a varied degree of tissue damage, from healthy-looking respiratory tissue to damaged tissue with severe loss of respiratory cilia and epithelial cells. However, *M. hyopneumoniae*-like structures were not identified in any of the evaluated samples.

Discussion:

Under the conditions of this study, animals infected 80 and 200 days previously with *M. hyopneumoniae* were able to transmit the agent to both susceptible unvaccinated and vaccinated pigs after a 14-day exposure period, showing that vaccination against *M. hyopneumoniae* did not prevent infection and that infected incoming gilts, unvaccinated or vaccinated are likely to shed the organism.

These results are in accordance with those of Meyns *et al.*, (2006) in which the transmission ratio for *M. hyopneumoniae* among vaccinated and unvaccinated populations was not significantly different. This result

suggests that vaccination of incoming gilts prior to their entry into the reproductive herd may be a less valuable management tool than previously though and that different strategies have to be used in order to protect the reproductive herd after the introduction of new replacement gilts groups.

The infectious potential of chronically infected asymptomatic pigs for periods up to 150 days post infection had been previously reported (Fano *et al*., 2005). This is the first time that transmission of *M. hyopneumoniae* from experimentally infected carrier pigs (200 days post infection) to sentinels pigs has been demonstrated; thus confirming the infectious potential of the disease-recovered animals to transmit the microorganism to susceptible pigs after long periods following disappearance of clinical signs.

Transmission of *M. hyopneumoniae* in terms of proportion of infected pigs was lower at 200 than at 80 days post infection. However, since only 11 out of the 18 experimentally infected pigs were confirmed positive to *M. hyopneumoniae* by the end of the second infectivity assessment (214 days), it could be hypothesized that a decrease in the number of infected pigs was responsible for a lower transmission rate.

The results from this study show the elimination of *M. hyopneumoniae* from the experimentally infected pigs 8 months after initial infection. This information validates the 10 months cut-off age used in the European programs for eradication of *M. hyopneumoniae*, by partial depopulation, assuming that infection of those animals occurs before they are 2 months old.

Lay Interpretation:

Designing disease control and/or eradication protocols depends mostly in how much we know about the pathogen we want to eliminate. Regarding *Mycoplasma hyopneumoniae*, the causative agent of Enzootic Pneumonia, many questions remain unanswered. A central question is the duration in time that infected pigs can infect incoming naïve animals. This is critical when designing herd closure schemes for eradication. The results of this study described the longest period of persistence of the bacteria in adult animals, which was approximately 7 months after infection when the bacteria was still able to be transmitted to naïve animals. It was also shown that elimination of *Mycoplasma hyopneumoniae* from the adult pigs occurred some time before 8 months after infection. The information from this study also showed that vaccination of naïve animals was not enough to protect them against infection, even in the chronic stages of the disease.

The knowledge gained from this experiment results crucial for the definition of strategies to contain or eliminate *Mycoplasma hyopneumoniae* in swine farms.