

# Assessment of transmission of *Mycoplasma hyopneumoniae* by personnel

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## Summary

Respiratory disease in swine is a major economic concern for producers around the world. Enzootic pneumonia, one of the most important chronic diseases in swine, is caused by *Mycoplasma hyopneumoniae*. Direct contact with infected pigs has been established as the chief route of transmission, constituting the main point of entry of the agent into the herd. Latently infected animals, aerosol spread, and fomites are alternative routes of infection of naive

swine herds. Although the role of people acting as mechanical vectors in the transmission of pathogens between farms or groups of pigs has not been clearly defined, there are reports of isolation of foot-and-mouth disease virus, swine influenza, *Pasteurella multocida*, and porcine reproductive and respiratory syndrome virus from humans exposed to infected swine. In this case, *M hyopneumoniae* was not transmitted during a 20-week period when personnel

weekly contacted susceptible pigs in a naive herd immediately after close contact with pigs in an infected herd. Personnel used a standard hygiene protocol before entering the uninfected farm.

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*Mycoplasma hyopneumoniae*, the causative agent of enzootic pneumonia, is one of the most important chronic diseases in swine.<sup>1</sup> It has been reported that over 50% of pigs marketed in major swine-producing countries have pneumonic lesions typical of *M hyopneumoniae*.<sup>2</sup> Recently, a new respiratory syndrome, porcine respiratory disease complex (PRDC) has been described as an important cause of decreased productivity in the late phases of swine production. *Mycoplasma hyopneumoniae* is one of the most important pathogens associated with PRDC.<sup>3</sup> Vertical, horizontal, and airborne transmission of *M hyopneumoniae* have been documented,<sup>4</sup> as well as indirect transmission through fomites.<sup>4</sup>

For many years, pigs have been raised in continuous flow systems with all animals housed together, facilitating horizontal transmission of *M hyopneumoniae*.<sup>5</sup> Recent

changes in production systems, especially rearing of nursery and finisher pigs in separate buildings, either on the same or different sites, have produced changes in the epidemiology of mycoplasmosis. In one-site, all in-all out systems, clinical manifestations of mycoplasma infection are usually observed at the beginning of the finishing period. With the adoption of three-site production systems, clinical presentation of mycoplasmosis has moved to the late finishing period.<sup>6</sup> Pijoan<sup>7</sup> proposed that this change in the epidemiology of mycoplasmosis is due to a reduction in the prevalence of infected pigs at weaning, ie, if the prevalence of infected pigs is low, occurrence of clinical disease is delayed. Transmission of *M hyopneumoniae* appears to be slow and occurs primarily by nose-to-nose contact. Morris et al<sup>8</sup> reported that direct contact was the only significant variable associated with seroconversion, and naive pigs in direct contact with other

infected pigs were seven times more likely to seroconvert than those having indirect contact. Direct contact with infected pigs has therefore been established as the primary route of transmission and the main source of entry of the organism into a swine herd.<sup>4</sup> Latently infected animals,<sup>4</sup> aerosol-spread,<sup>8,9</sup> and fomites<sup>4</sup> are potential routes of infection for naive swine herds.

The role of personnel as mechanical vectors for transmission of pathogens between farms or groups of pigs is not clear.<sup>9-12</sup> There are reports of isolation of foot-and-mouth-disease virus,<sup>13</sup> swine influenza virus,<sup>14</sup> *Pasteurella multocida*,<sup>15</sup> and porcine reproductive and respiratory syndrome virus<sup>16,17</sup> from humans exposed to infected swine. In today's modern production systems, swine producers frequently practice strict measures of biosecurity to reduce the risk of pathogen introduction to the farms. These measures, referred to as "biosecurity protocols," include refraining from contact with swine for 24 to 72 hours ("down time"), changing clothing and footwear before entering the premises, and showering into and out of the facility. Scientific validation for the need of such protocols is lacking, despite their widespread acceptance in the swine industry.<sup>16</sup>

In this case, personnel repeatedly collected blood samples and nasal swabs from pigs in a commercial herd infected with *M hyopneumoniae*, then contacted pigs in an

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uninfected research herd, using a standard hygiene protocol before entry into the research facility.

## Case description

During 20 consecutive weeks in the summer and fall of 2001, three veterinarians visited two farms 60 km apart. Farm A was an 800-sow, three-site, farrow-to-finish commercial farm naturally infected with *M hyopneumoniae*. This herd (Herd A) had been positive for *M hyopneumoniae* for more than 10 years. In June of 2001, *M hyopneumoniae*-positive status was confirmed when 160 sows (20% of the herd) were tested by Tween-20 ELISA, with positive results in 149 sows (93%). Farm B was the University of Minnesota Swine Disease Eradication Center experimental farm. This herd (Herd B) was known to be negative for *M hyopneumoniae* on the basis of 5 years of diagnostic data, absence of clinical signs in all phases of production, and absence of lesions suggestive of *M hyopneumoniae* at the slaughterhouse.

During the first visit to Farm A, 200 of the total of 350 pigs weaned per week into a nursery on Site 2 of the farm were randomly selected and individually identified. During the next 18 weeks, the three investigators had close contact with these pigs for 3 to 4 hours weekly, either in the nursery (7 weeks) or in the finisher (11 weeks), collecting nasal swabs, blood samples, or both from the identified pigs on each occasion. During the contact period, investigators wore disposable coveralls and rubber boots, but not gloves, facemasks, or hairnets.

Immediately after collecting the samples from the *M hyopneumoniae*-infected herd, the investigators showered, changed clothing, and drove to Farm B. Farm B housed 120 *M hyopneumoniae*-naive animals obtained from a source known to be negative for *M hyopneumoniae*. The pigs were housed in a mechanically ventilated finishing building consisting of 10 pens with partially slatted floors. Animals were placed 12 per pen, and provided at least 1 m<sup>2</sup> space per pig. When the naive pigs entered the research facility at 4 months of age (Day 0), an index group of 30 animals was established by randomly selecting and eartagging three animals from each pen. Investigators donned cloth coveralls and rubber boots before entering the finishing building, but did not take a shower. They then spent at least 1 hour in close contact with the naive

pigs, collecting blood samples and nasal swabs from the identified animals. *Mycoplasma hyopneumoniae*-status of the naive herd was determined by testing of blood samples collected on Day 0 and Day 154 and nasal swabs collected on Day 154.

## Diagnostic testing

### Herd A (infected herd)

To assess the dynamics of *M hyopneumoniae* in Herd A, the 200 identified pigs were tested during the 18-week period after weaning. Blood samples collected when pigs were weaned at 19 days of age (Day 0) and at 145 days of age (Day 126) were tested for *M hyopneumoniae* antibodies by Tween-20 ELISA.<sup>18</sup> Titers were expressed as sample-to-positive (S:P) ratios, with values  $\geq 0.4$  considered positive. Nasal swabs collected on the same days were tested for *M hyopneumoniae* by a nested-polymerase chain reaction (N-PCR) technique.<sup>19</sup>

Of the 200 pigs sampled, 17 (8.4%) were seropositive for *M hyopneumoniae* antibodies by the Tween-20 ELISA on Day 0, and 70 (34.8%) were seropositive on Day 126. On Day 0, 20 of the 200 nasal swabs tested (10%) were positive for *M hyopneumoniae* by N-PCR, and on Day 126, 84 (42%) were positive.

### Herd B (naive herd)

Blood samples and nasal swabs were tested as for Herd A. All 30 index animals were seronegative for *M hyopneumoniae* on Days 0 and 154, and all nasal swab samples were negative for *M hyopneumoniae* by N-PCR on Day 154.

## Discussion

In this case, multiple diagnostic tests were used to increase the sensitivity of detecting *M hyopneumoniae* both in the infected and in the naive population. *Mycoplasma hyopneumoniae* is well recognized for inducing a delayed and variable immune response.<sup>20</sup> The *M hyopneumoniae*-positive status of the commercial herd (Herd A) was confirmed both by antibody detection and identification of the organism by N-PCR. In addition, N-PCR identified the spread of the organism in the population over time. On the other hand, *M hyopneumoniae* was not detected in any of the naive index animals in the research herd (Herd B) either by serological testing or by N-PCR. Calsamiglia et al<sup>21</sup> showed that N-PCR

was able to detect *M hyopneumoniae* before the animals seroconverted, and was therefore a better diagnostic test for *M hyopneumoniae* than the ELISA. In this case, nasal swabs from approximately 10% of the animals in the commercial herd were positive for *M hyopneumoniae* by N-PCR at weaning, and prevalence increased with time. Positive ELISA results for 19-day-old pigs in this herd probably represented passive immunity acquired via colostrum, as the sows in this herd were *M hyopneumoniae*-positive.

Pigs in Herd B were negative for *M hyopneumoniae* both by the Tween-20 ELISA and by N-PCR on Day 154. The *M hyopneumoniae*-negative status of these pigs was determined only by serological testing on Day 0; however, their *M hyopneumoniae*-negative status both by N-PCR and ELISA at the end of the study strongly suggests that these animals were indeed uninfected.

In this case, investigators made 20 consecutive weekly visits to the naive herd immediately after visiting the infected herd, without evidence of transmission of *M hyopneumoniae*. Furthermore, this was a "real-world" setting that involved commercial conditions and large populations. Transmission of *M hyopneumoniae* to the naive animals would likely have resulted in clinical signs and a high seroprevalence.<sup>1</sup> Animal age would not be a factor in the development of disease, as pigs of all ages in a naive population appear to be equally susceptible.<sup>1</sup> The probability of detecting at least one infected animal in Herd B with our sample size of 30 was  $> 99\%$ .<sup>22</sup> Since clinical signs were not observed in the naive animals, and swabs and serum samples were negative for *M hyopneumoniae* by N-PCR and Tween-20 ELISA, respectively, it is most likely that these pigs were truly uninfected 154 days after their first exposure to the personnel who had contacted the infected herd.

In this case, transmission of *M hyopneumoniae* from infected to susceptible pigs did not occur when personnel followed specific sanitation protocols, including a change of clothing and showering on the infected farm. However, there was no control group involving personnel who moved directly from *M hyopneumoniae*-infected animals to a group of naive animals without implementing any sanitation protocols. Also, we

did not confirm that the investigators were actually contaminated with *M hyopneumoniae* before or after they showered and changed clothing. The case included only one set of farms, and the infected herd was not always experiencing severe clinical signs of mycoplasmosis. Furthermore, farm visits were made only during periods of hot weather and were not repeated during cool weather. Finally, we did not conduct a statistically significant number of replicates to determine the frequency of the observed events. Since negative results cannot be interpreted, the efficacy of the sanitation protocol employed cannot be calculated. However, our results concur with those obtained by Goodwin,<sup>4</sup> who reported that *M hyopneumoniae* was not isolated from breath and hair samples from personnel exposed to pigs experimentally infected with *M hyopneumoniae*.

The observations made in this case suggest that the risk of *M hyopneumoniae* transmission may be reduced through the implementation of the basic sanitary measures proposed. This information may be of value to swine producers and practitioners as they begin to evaluate the validity of commercially applied biosecurity protocols for the prevention of *M hyopneumoniae* transmission by personnel. Hopefully, this case may serve as a pilot project to stimulate further evaluation of the effect of commercial biosecurity protocols on *M hyopneumoniae* transmission.

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