

Infection of a naïve sow herd with *Mycoplasma hyopneumoniae*

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Summary

A 2500-sow herd previously free of, and unvaccinated for, *Mycoplasma hyopneumoniae* (MHP) became infected. Both MHP and influenza A H1N1pdm09 virus were identified in sows showing clinical signs. Coughing lasting 2 to 4 days was observed in approximately 10% of sows and 26 sows died over the course of the outbreak. There was no apparent impact on performance indicators. Polymerase chain reaction and serological results showed that MHP progression within the herd was fast and that infection may have occurred within a few weeks. An elimination program was quickly implemented so that sale of negative animals could resume.

Keywords: swine, *Mycoplasma hyopneumoniae*, epidemiology, elimination

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Resumen - Infección de una piara de cerdas libre con *Mycoplasma hyopneumoniae*

Una piara de 2500 cerdas previamente libre y no vacunada contra *Mycoplasma hyopneumoniae* (MHP) se infectó. Tanto el MHP como el virus de la influenza A H1N1pdm09 fueron identificados en cerdas que mostraban signos clínicos. Se observó tos que duró de 2 a 4 días en aproximadamente el 10% de las cerdas, 26 cerdas murieron durante el transcurso del brote. No hubo un impacto aparente en los parámetros de producción. La reacción en cadena de la polimerasa y los resultados serológicos mostraron que el movimiento del MHP dentro de la piara fue rápido y que la infección pudo haber ocurrido en unas pocas semanas. Rápidamente se implementó un programa de eliminación para que pudiera reanudarse la venta de animales negativos.

Résumé - Infection d'un troupeau de truies naïves par *Mycoplasma hyopneumoniae*

Un troupeau de 2500 truies précédemment exemptes et non vaccinées contre *Mycoplasma hyopneumoniae* (MHP) a été infecté. *Mycoplasma hyopneumoniae* et le virus de la grippe A H1N1pdm09 ont été identifiés chez des truies présentant des signes cliniques. Une toux d'une durée de 2 à 4 jours a été observée chez environ 10% des truies et 26 truies sont mortes au cours de l'écllosion. Il n'y a pas eu d'impact apparent sur les indicateurs de performance. La réaction d'amplification en chaîne par la polymérase et les résultats sérologiques ont montré que la progression de MHP au sein du troupeau était rapide et que l'infection pouvait s'être produite en quelques semaines. Un programme d'élimination a été rapidement mis en place afin que la vente des animaux négatifs puisse reprendre.

Infection of naïve herds with *Mycoplasma hyopneumoniae* (MHP) can be associated with significant clinical signs and losses.¹ Transmission of this organism is often slow compared to other pathogens like porcine reproductive and respiratory syndrome virus and influenza A virus in swine (IAV-S).²⁻⁴ This case report describes a naïve sow herd infected with MHP where clinical signs in most animals were mild, a low percentage of sows were affected, and within farm transmission may have occurred at a faster rate than what is commonly observed.³ The elimination program and diagnostic results are also discussed.

Animal care and use

The animals in the case herd were adequately housed, and humanely cared for.

Case description

Clinical signs, interventions, and timing of infection

The 2500-sow, high-health herd had remained negative to MHP since the farm was populated in 2007. The herd had not been vaccinated for this organism. The negative status was based on absence of clinical signs and lesions consistent with MHP infection in the sow herd and their progeny, no identification of the

organism in diagnostic material submitted to the laboratory, and on monthly negative serological testing of the sow herd for 13 years. Table 1 summarizes observations and testing completed before and after the first clinical signs were noticed. An H1N1 IAV-S strain had been identified in the herd in the past, but not the 2009 novel influenza A virus (H1N1pdm09). Coughing was first observed among the sows on February 22, 2020, and 3 to 4 new sows per day began coughing thereafter. Clinical signs gradually decreased after the herd was treated with medication on April 12 and completely stopped by May 10. Overall, between 250 and 300 sows were

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Table 1: Observations and diagnostic test results made before and after appearance of clinical signs for *Mycoplasma hyopneumoniae* (MHP)

Date	Observations	Results
12/30/2019 & 02/04/2020	No clinical signs; 20 blood samples from sows for each date	All negative for MHP*
01/01/2020 to 02/12/2020	3 groups of 400 gilts sold at weaning; tested extensively after delivery on remote locations	All negative for MHP*†
02/22/2020	A few lactating sows coughing	No tests conducted
02/27/2020	Nasal swabs from 5 coughing sows, 2 pools	Both pools positive for MHP [†] and Influenza A H1N1pdm09 virus [‡]
03/04/2020	20 blood samples on sows	1 of 20 positive for MHP*
03/09/2020	Tracheobronchial swabs from a coughing sow and the sow that was seropositive on March 4, and lungs of 2 suckling piglets from one litter with dyspnea	Sow swabs positive for MHP [†] ; lungs of piglets only positive for Influenza A H1N1pdm09 virus [‡]
03/24/2020	31 blood samples from females which had not shown clinical signs	22 positive for MHP*
03/24/2020	170 gilts that were in isolation are introduced in the herd; 20 had been serologically tested before introduction	All tested negative for MHP*
04/07/2020	30 gilts introduced on March 24 tested with tracheobronchial swabs, 12 pools of 2 or 3	11 of 12 pools positive for MHP [†]
03/18/2020 to 04/11/2020	26 sows reported to have died of the respiratory condition	No autopsy performed

* Enzyme-linked immunosorbent assay (*M. hyo* Ab test; IDEXX).

† Real-time polymerase chain reaction assay (Swinecheck *M. hyopneumoniae* PCR; Biovet).

‡ Real-time polymerase chain reaction assay (Swinecheck Influenza A virus PCR; Biovet).

coughing that, for most animals, lasted 3 or 4 days with or without treatment. Approximately 20% of the coughing animals also had reduced appetite and were treated with tulathromycin (Draxxin; Zoetis). The manager reported that a total of 26 sows died of their respiratory condition between March 18 and April 11. All these sows reportedly died rapidly, within two days of starting to show a deep cough and anorexia, and most were in late gestation between 2 to 3 weeks and 2 to 3 days of their farrowing date. All the females that died had farrowed at least one previous litter. No apparent impact was observed on performance indicators such as wean-to-estrus interval, farrowing rate, born alive, preweaning mortality, and number weaned per litter in the months during or after clinical signs were observed (data not shown).

Considering the time needed to produce a detectable serological response, approximately 3 weeks or more, it seems reasonable to assume that a large proportion of the sows present in the herd at the time of infection had been infected

with MHP by early March.^{1,5} Similarly, most of the gilts introduced on March 24 were MHP positive by April 7 as shown by polymerase chain reaction (PCR). These gilts had not shown clinical signs while in isolation and tested serologically negative before their introduction into the sow herd.

Elimination program

As soon as the MHP infection was confirmed, the decision was made to eliminate it from the sow herd so that sale of MHP-negative animals could resume. The herd was closed after transferring the 170 gilts from the quarantine barn to the sow herd on March 24. Clinically affected (coughing) sows were placed strategically within the gilt area to encourage rapid transmission of MHP. No medication other than individual treatments was used at the time so as not to reduce organism transmission within the herd. The PCR testing conducted on April 7 suggested that a large percentage of gilts had already come in contact with MHP. Coupled with the March 24 results,

it appeared that most, and perhaps all, females present in the herd at the time of infection had apparently come in contact with the organism. Therefore, it was decided to start medication treatment on April 12. Tylvalosin (Aivlosin, Pharmgate) was added to the feed for 9 weeks at a dose of 2.125 mg/kg of live weight. Tulathromycin (Draxxin, Zoetis) was used on piglets at the time of processing and at 12 days of age for a period of 6 weeks starting one month after sows were medicated. The whole breeding herd was vaccinated with an MHP vaccine on April 13 and again on August 3 and September 1.

On June 22, 7 days after sow medication had concluded, 220 tracheobronchial samples were collected to determine if the organism could still be detected by PCR. Animals positive or suspicious on June 22 were retested until all were found to be negative on October 26. Table 2 shows the results that were obtained over time. Because two gilts were suspicious or positive on August 17, a 1-month feed medication period

(September 26 to October 26) was added with the same product and dosage as previously used. The practitioner elected to add a third feed medication period (November 7 to December 7) as an extra precaution, again using the same product and dosage.

Because of cost and labor concerns, only a subset of females found negative on June 22 were retested (data not shown in Table 2). Fifteen gilts found negative on June 22 were retested on August 17 and all were negative. Twenty-four animals found negative on July 12 were retested on August 17 and found negative. Finally, 33 and 41 gilts introduced on March 24 but had not yet been tested were sampled on October 26 and January 18, respectively, and none were positive. Given these results it seems reasonable to suggest that most if not all animals found negative on June 22 would likely have remained negative on subsequent testing dates.

Discussion

Some findings associated with this case were considered unexpected or original. First, the low number of animals that showed clinical signs was unexpected given that the herd was totally naïve to the organism at the time of infection. A second observation from the case was the very short period during which animals showed clinical signs. Coughing lasted only 2 to 4 days and most affected animals recovered without significant losses. When pigs are experimentally infected with MHP at the same time, pigs

begin to cough about 2 weeks post infection, peak about 2 weeks later, and then coughing gradually declines.^{1,5} Because both MHP and IAV-S were identified in coughing sows and no necropsy was performed, it is difficult to determine the respective role that each organism played or if something else could have contributed to the problem. Typically IAV-S will cause coughing for only a few days to a week in an individual animal, while MHP can cause coughing that often lasts weeks.^{1,5-7} In this respect, the short period of coughing in affected animals in this case would suggest IAV-S rather than MHP, but the long period where coughing was present in the herd (February 22 to May 10) seems more likely to be associated with MHP than with IAV-S. Also, no sows died of the condition after medication was administered to the sow herd on April 12, which again may suggest the role MHP played. It is also possible that both pathogens contributed to the outbreak. It has been reported that animals infected with both organisms may have more severe lesions and losses than those infected with only one of them.^{8,9} Studies have also shown that, as for most swine pathogens, strains of MHP can vary in virulence.^{10,11} The low mortality and number of clinically affected animals, lack of impact on performance indicators, and that two known respiratory pathogens were identified in sick animals emphasize the apparent low virulence of the MHP strain involved in the current case.

Another finding that differed from what is often seen in MHP cases is the rapid speed of the organism transmission

within the herd. Other authors have reported how slow the transmission of this organism within a population of naïve animals can be.²⁻⁴ In a recent study, only 27% of the naïve animals placed in contact with an infected gilt had become infected 8 weeks post exposure.³ In the case herd most animals present had become infected within a few weeks. Following experimental infection, it is estimated to take approximately 2 weeks or more for animals to begin coughing and 3 weeks or more to seroconvert.^{1,5} As a working hypothesis, this suggests that most females present in the herd at the time of infection came in contact with MHP between early February and early to mid-March. The last batch of quarantined gilts was introduced on March 24, and by April 7, 11 of 12 pools of tracheobronchial samples obtained from 30 of the 170 introduced gilts were positive. This last batch of gilts would have been exposed to the organism between late March and early to mid-April, about 3 to 4 weeks after the rest of the herd.

The difference between the percentage of positive or suspicious recently introduced gilts and that of the rest of the herd after the elimination program was implemented was of interest. According to the samples taken on June 22, a 3- to 4-week delay in the time of infection resulted in a percentage of MHP-positive and MHP-suspicious gilts that was 4.8 times higher than for the other females in the herd. This percentage was 31.3 times higher for samples taken on July 12. Pieters et al⁶ reported that 18 of 18 gilts (100%) were MHP-positive 94

Table 2: Number and percent of tested females already present in the herd at the time of infection and gilts introduced on March 24, 2020 that were found positive/suspicious over time by PCR on tracheobronchial samples

	Date tested			
	06/22/2020	07/12/2020	08/17/2020	10/26/2020
Tested females in the herd, No.	147	13*	1*	0*
Females positive/suspicious, No. [†]	13	1	0	0
Females positive/suspicious, % [‡]	8.8	0.7	0	0
Tested gilts introduced on March 24, No.	73	29*	16*	2*
Gilts positive/suspicious, No. [†]	31	16	2	0
Gilts positive/suspicious, % [‡]	42.5	21.9	2.7	0
Ratio of positive/suspicious gilts:females, %	4.8	31.3	-	-

* Only animals testing positive/suspicious on the previous test were retested on this date.

[†] Cycle threshold (Ct) values < 35 were considered positive and Ct = 35-38 were considered suspicious.

[‡] All percentages based on the number of sows (147) and gilts (73) initially tested on June 22; it was assumed that animals negative on June 22 would remain negative afterwards.

days post experimental infection and it took 254 days for gilts to test negative for the organism. The reason for such a large difference between the last introduced gilts and the rest of the females in the case herd is unknown. One possible hypothesis could be that animals with immune systems that had been more completely stimulated by earlier exposure to the organism would better respond to the medication and vaccination program and more rapidly clear the pathogen from their respiratory system. This could mean that in herds where elimination of the organism is the goal, ensuring that all females come in contact with the organism as soon as possible would be important. If, as is often the case, MHP-negative gilts are introduced in the sow herd, this hypothesis would suggest that efforts may have to be made to ensure that gilts are infected before or soon after introduction.

In North America, the strategies used to eliminate MHP in sow herds have usually involved a herd closure of several months coupled with a medication period of 3 to 4 weeks or more in sows and piglets.^{12,13} An 8-month herd closure is frequently recommended and different antimicrobials have been used in sows and piglets.^{12,13} The rationale for such a long herd closure is the study where it took 240 days for experimentally infected animals to stop infecting negative sentinels placed in contact.⁶ In elimination programs, the medication period is usually applied towards the end of the closure period.^{12,13} In the case herd described here, the initial medication of the sow herd lasted 9 weeks and began only 20 days after the last gilts were introduced into the herd, at a time when some animals were still showing clinical signs. It was hypothesized that the longer medication period and the product and dose used could allow reproductive animals to eliminate the infectious organism, without including a closure period in the elimination program.

The results obtained showed that on August 17, none of the PCR-tested females (147) that were already present in the herd when infection occurred were MHP positive or suspicious. Furthermore, 5 weeks earlier on July 12, only 1 (0.7%) of these 147 females was weakly positive (cycle threshold = 34.2) and it is not known if this represented infectious MHP. This could mean that 4 or 5 months (mid-March to mid-July or August) following infection of the last female already present in the herd, the organism may have been

eliminated from this group of animals. Other laboratory results suggest that the farm may have started to produce uninfected pigs at that time. Twenty-five pigs from a batch of about 3000 piglets born around July 27 were tested serologically at about 9 weeks of age (September 28) and found to be negative. Twenty of the same pigs were tested again when they were 23 weeks of age (January 6) and found to be negative. This is of particular interest because 21.9% of the gilts introduced on March 24 and tested on July 12 were MHP positive. Many of the recently introduced gilts had farrowed before the end of July since insemination had started while they were in isolation.

The diagnostic results and case interpretation suggest that the elimination program did succeed at least to an undetectable or low prevalence. For gilts introduced in March 2020 and had not yet been tested, 33 and 41 gilts were sampled using tracheobronchial catheters in October 2020 and January 2021, respectively. Between October 2020 and April 2021, tracheobronchial samples (average of 29 samples) were obtained 12 times either at weaning or at the end of the nursery period. In March and April 2021, 30 pigs from 4 different finishing units were tested at the end of the finishing period by both tracheobronchial samples and serology. Thirty negative sentinel gilts introduced in the sow herd in January 2021 were tested in April by tracheobronchial samples and serology, and 20 were retested by serology in May and June. In February, March, and April 2021, between 1200 and 1400 gilts were sold at weaning each month. Thirty of these gilts were tested by PCR (tracheobronchial samples) at 4- and 7-weeks post delivery. All these test results were negative. No evidence of MHP infection has been detected in the nursery and finishing units that received pigs from the case herd since July 2020.

If these elimination results were repeatable in other situations and with different strains, it could suggest that herd closure periods shorter than the 8 months often proposed in North America may be sufficient to eliminate MHP from sow herds. This would be consistent with other reports where elimination was achieved with very short or no herd closure.¹³⁻¹⁵ Given the increasing interest in MHP elimination programs, more work is needed to identify the programs that are more likely to succeed at the lowest cost and with the least impact on production results.

The PCR test used in this study also detected *Mycoplasma hyorhinis*. While the strategy used in the case herd was successful at eliminating MHP, *M. hyorhinis* could still be identified in the weeks and months following termination of the program. Most nursery pigs tested between October 2020 and February 2021 were found to be *M. hyorhinis*-positive by PCR (data not shown). Finally, an epidemiological investigation was undertaken to determine the source of MHP infection for the herd described in this case report, but none could be identified with certainty.

Implications

Under the conditions of this study:

- An infected MHP-naïve herd had mild clinical signs and rapid transmission.
- Clearance of the organism by medication can depend on timing of infection.
- Elimination of MHP may require a shorter herd closure period than commonly used.

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Conflict of interest

None reported.

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* Non-refereed references.

