**FOOD/FARMED ANIMALS**

**PCR detection of *Mycoplasma hyopneumoniae* in piglet processing fluids in the event of a clinical respiratory disease outbreak**

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**SUMMARY**

Diagnosis of early infection with *Mycoplasma hyopneumoniae* in breeding herds remains challenging. *M hyopneumoniae* has been recently detected in processing fluids (PFs), an emerging sample suitable for porcine reproductive and respiratory syndrome virus monitoring in pig breeding farms. This clinical report describes the unusual detection of *M hyopneumoniae* in PF at the same time that a clinical respiratory disease outbreak occurred in a previously *M hyopneumoniae*-negative sow farm. These results provide new insights into the value that testing PF to detect *M hyopneumoniae* may have in breeding herds.

**BACKGROUND**

*Mycoplasma hyopneumoniae* is the aetiological agent of porcine enzootic pneumonia and one of the major pathogens involved in the porcine respiratory disease complex (PRDC). Piglets are thought to be free from *M hyopneumoniae* at birth, but colonisation may occur as early as the first week of life. Notwithstanding, colonisation prevalence at weaning is highly variable, ranging from 0 to over 50 per cent in farms endemic for *M hyopneumoniae*.

Sampling procedures that better approximate to the lower respiratory airways are considered the most sensitive for detection of this bacterium in live pigs. However, these sample types (eg, tracheobronchial swabs) are invasive and, therefore, less conveniently obtained. This, together with the high piglet colonisation variability, makes it extremely challenging to monitor early stages of infection with *M hyopneumoniae* in breeding farms. Processing fluids (PF), which consist of serosanguineous exudates from tissues obtained during newborn piglet processing activities, have arisen as an appropriate sample for monitoring porcine reproductive and respiratory syndrome virus (PRRSV) presence in pig breeding herds. Moreover, a recently published study highlighted the feasibility of detecting *M hyopneumoniae* in PF.

The present case report describes the detection of *M hyopneumoniae* in PF by means of real-time PCR in the event of a clinical respiratory disease outbreak that took place in a previously *M hyopneumoniae*-negative sow farm. Results provide new insights for the potential use that PF may have to detect *M hyopneumoniae* in breeding farms.

**CASE PRESENTATION**

**Case herd**

The sow farm was located in the US Midwest and housed approximately 5450 sows and gilts divided in six barns: four for breeding and gestation, and two for farrowing. The farm had a 50 to 55 per cent average annual replacement rate and received replacement gilts from an on-site gilt development unit (GDU) of approximately 1600–1800 gilts managed by continuous flow. Gilts were obtained at weaning age from a single high health status multiplier. A PRRSV and *M hyopneumoniae* eradication herd closure was conducted at the sow farm early in 2015. However, the herd experienced a porcine reproductive and respiratory syndrome outbreak in October 2017. A modified live PRRSV vaccine was then applied to the breeding herd population. For the purpose of monitoring PRRSV, PF were collected weekly starting in March 2018. By then, the farm was deemed as clinically stable for PRRSV and negative for *M hyopneumoniae*. The farm was regarded as seronegative for *M hyopneumoniae* since no vaccination was applied. In addition, periodical laboratory tests from the wean-to-finish flow sourced from the sow farm were negative to *M hyopneumoniae* during the period 2016–2018.

**Outbreak description**

An outbreak of respiratory disease in the breeding herd was detected during the first week of August 2018. The outbreak was initially characterised by sudden coughing in the farrowing and gestation units. No cough was reported in the on-site GDU. To diagnose the ongoing respiratory disease, blood and nasal swab samples were immediately collected across clinically affected sows in the farrowing units. As the outbreak progressed, sow mortality and abortion rate increased in gestation barns. By mid-September, lungs from sow mortalities were submitted for further analysis and additional blood samples were obtained from gestating sows.

**Diagnostic test results and control measures**

Table 1 summarises test results obtained from samples collected from sows during the outbreak. *M hyopneumoniae*-like pneumonia lesions consisting of acute bronchopneumonia with lymphoplasmacytic alveolitis and perivasculitis were observed in lungs submitted for histopathology. Bacteriological examination of lung samples also revealed...
implication of *Pasteurella multocida* and *Streptococcus suis*. Altogether, findings pointed out that the respiratory problems were associated with an outbreak of PRDC as other bacterial agents and porcine circovirus type 2 (PCV2) were coinfections, along with *M. hyopneumoniae*. Lymphoid tissues were not examined. Thus, the full extent of PCV2 infection was uncertain. At the first suspicion of *M. hyopneumoniae* infection, feed containing 200 parts per million tilmicosin phosphate (Pulmotil 200 Premix, Elanco Animal Health) was pulsed to the breeding herd for a period of 3 weeks starting on August 24 2018.

**PF sampling and testing procedures**

PF collection consisted in gathering all tails and testicles from 15 farrowing gilts, 15 second parity sows, and 15 third parity sows and above, on a weekly basis. Tails and testicles were kept frozen for 1 or 2 weeks at the farm prior to submission to the University of Minnesota. At arrival, processing tissues were thawed at room temperature and PFs were aggregated by sow parity in a sterile tube. As a result, three PF samples were obtained on a given processing week: PF1 from primiparous sows’ litters, PF2 from second parity sows’ litters and PF3+ from third or more parity sows’ litters. In addition, individual litter samples from one of the processing weeks per month were also stored. All collected PF were frozen (−80°C) until tested. Once the PRDC outbreak was confirmed, a retrospective investigation was performed. PF samples were associated with an outbreak of PRDC as other bacterial agents and porcine circovirus type 2 (PCV2) were coinfections, along with *M. hyopneumoniae*. Lymphoid tissues were not examined. Thus, the full extent of PCV2 infection was uncertain. At the first suspicion of *M. hyopneumoniae* infection, feed containing 200 parts per million tilmicosin phosphate (Pulmotil 200 Premix, Elanco Animal Health) was pulsed to the breeding herd for a period of 3 weeks starting on August 24 2018.

**Laboratory findings**

A total of 90 PF were tested by real-time PCR for *M. hyopneumoniae*. All PF tested negative, except for three samples collected on August 13, 20 and 27, which showed cycle threshold (Ct) values of >37 (figure 1). The first two suspect PF were obtained from parity 3+ sows, whereas the last one was obtained from parity 2 sows. Remarkably, all three suspect PF were collected while the clinical respiratory disease outbreak was taking place, starting from the first week of August and ending by the second week of September. Individual litter PF gathered from the positive PF2 (week of August 27 2018) were available for further testing. PF2 was composed of PF from 12 litters that were individually tested by real-time PCR. All litter PF were detected negative, except for one litter with a Ct value of 32.21. The above-mentioned PF were retested by real-time PCR and their DNA were relatively quantified by a standard curve method. The number of DNA copies per millilitre of PF were 2.2, 1.4 and 3.5 × 10³ for the samples from August 13, 20 and 27 August, respectively. The individual litter PF had 350 × 10³ copies/ml. Results are depicted in figure 2.

**DISCUSSION**

PF have been postulated as an appropriate sample for monitoring PRRSV in pig herds, and recently published data showed detection of *M. hyopneumoniae* in this sample type. This latter finding is difficult to explain as *M. hyopneumoniae* is regarded as an extracellular pathogen that resides uniquely in the respiratory tract of pigs by attaching to the cilia that lines its...
epithelium. Nonetheless, *M. hyopneumoniae* has been cultured from tissues outside the respiratory tract, such as the liver, spleen or kidneys of experimentally infected pigs. Additionally, recent evidence suggested that this bacterium could persist intracellularly and traffic to extrapulmonary sites. Although the potential mechanisms or routes by which *M. hyopneumoniae* is detected in PF remain largely unknown, the role that this sample could play in detection of early stages of *M. hyopneumoniae* infection deserves to be further evaluated.

The present work reports the detection of *M. hyopneumoniae* on a breeding farm that collected PF on a weekly basis for PRRSV monitoring. In the event of a PRDC outbreak, PF were retrospectively tested for *M. hyopneumoniae* by real-time PCR. Remarkably, the unique three samples that turned to be suspect were collected in the period in which clinical signs of respiratory disease took place. In a previous study, *M. hyopneumoniae* was consistently detected by real-time PCR in daily PF over a 2-month period. Report indicated the sow farm to be subclinically infected, as no clinical signs suggestive of *M. hyopneumoniae* were observed, but were occasionally detected in the progeny, in the finishing pig population. A different clinical picture was seen in the present farm, where *M. hyopneumoniae* was detected only in PF during a clinical respiratory disease outbreak in a previously *M. hyopneumoniae*-negative farm. Most probably, the antibiotic feed grade pulsed to the breeding herd in response to the outbreak mitigated shedding, preventing further detections of *M. hyopneumoniae*. In any case, PFs have been shown to be useful for detecting *M. hyopneumoniae* in two different clinical scenarios.

The origin of the genetic material detected in PF remains uncertain and environmental contamination cannot be ruled out, especially since *M. hyopneumoniae* has been shown to survive outside the host for up to 8 days. In this investigation, *M. hyopneumoniae* was detected with Ct values of >37. PF with Ct values between 37.01 and 40.0 have been regarded as doubtful. Fortunately, PF from each of the litters gathered in one of the suspect samples could be individually tested. One out of 12 of the gathered PF tested positive with a Ct value of 32.21. Additionally, all suspects could be relatively quantified using a standard curve method by real-time PCR, which confirmed the presence of *M. hyopneumoniae* genetic material in PF. Since PFs are an aggregated sample, the sensitivity to detect a positive pig or litter, if present in the sample, may decrease as more negative pigs or litters are collected. For PRRSV, however, it has already been estimated that aggregation of up to 40 litters does not hinder detection by real-time PCR when a pig with a Ct value of −33 is present in the sample. Here, ~15 litters were aggregated. Thus, even Ct values above 33 should have been detected. Besides possible environmental contamination, the high Ct values observed in the present work might be due to a very low proportion of *M. hyopneumoniae*-positive litters and the dilution factor inherent to aggregation and pooling in PF.

The fact that *M. hyopneumoniae* has been consistently detected in PF from two different epidemiological contexts has raised questions about the current understanding of the pathogenesis and epidemiology of this pathogen. While with the current data it is not possible to draw definite conclusions, more efforts are needed to elucidate the origin of the genetic material from *M. hyopneumoniae* detected in PF. Even in the case of environmental contamination, the value of using this accessible sample type to detect *M. hyopneumoniae* in breeding herds deserves further investigation.

**Learning points**

- Genetic material of *Mycoplasma hyopneumoniae* from processing fluid (PF) was quantified by real-time PCR.
- The origin of *Mycoplasma hyopneumoniae* genetic material detected in PF needs to be clarified.
- PF are a potential sample to detect *M. hyopneumoniae* in breeding herds.

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**REFERENCES**

1. Thacker EL, Minion CF. Mycoplasmosis. In: Zimmerman JJ, Karriker LA, Ramirez A, eds. *Diseases of swine*. 10th ed. Ames, IA: Wiley-Blackwell, 2012: 779–98.
2. Sibilia M, Nofrarias M, López-Soria S, et al. Chronological study of *Mycoplasma hyopneumoniae* infection, seroconversion and associated lung lesions in vaccinated and non-vaccinated pigs. *Vet Microbiol* 2007;122:97–107.
3. Sibilia M, Nofrarias M, López-Soria S, et al. Exploratory field study on *Mycoplasma hyopneumoniae* infection in suckling pigs. *Vet Microbiol* 2007;121:352–6.
4. Moorikamp L, Hewicker-Trautwein M, Grosse Beilage E. Occurrence of *Mycoplasma hyopneumoniae* in coughing piglets (3-6 weeks of age) from 50 herds with a history of endemic respiratory disease. *Transbound Emerg Dis* 2009;56:54–6.
5. Fano E, Piipaan C, Dee S, et al. Effect of *Mycoplasma hyopneumoniae* colonization at weaning on disease severity in growing pigs. *Can J Vet Res* 2007;71:195–200.
6. Villarreal JL, Vranckx K, Duchateau L, et al. Early *Mycoplasma hyopneumoniae* infections in European suckling pigs in herds with respiratory problems: detection rate and risk factors. *Vet Med* 2010;55:318–24.
7. Nathues H, Woeske H, Doehring S, et al. Herd specific risk factors for *Mycoplasma hyopneumoniae* infections in suckling pigs at the age of weaning. *Acta Vet Scand* 2013;55:30.
Vilalta C, et al. Vet Rec Case Rep 2020;8:e001045. doi:10.1136/vetreccr-2019-001045

8 Calsamiglia M, Pijoan C, Trigo A. Application of a nested polymerase chain reaction assay to detect Mycoplasma hyopneumoniae from nasal swabs. J Vet Diagn Invest 1999;11:246–51.
9 Otagiri Y, Asai T, Okada M, et al. Detection of Mycoplasma hyopneumoniae in lung and nasal swab samples from pigs by nested PCR and culture methods. J Vet Med Sci 2005;57:801–5.
10 Fablet C, Marois C, Kobisch M, et al. Estimation of the sensitivity of four sampling methods for Mycoplasma hyopneumoniae detection in live pigs using a Bayesian approach. Vet Microbiol 2010;143:238–45.
11 Pieters M, Daniels J, Rovira A. Comparison of sample types and diagnostic methods for in vivo detection of Mycoplasma hyopneumoniae during early stages of infection. Vet Microbiol 2017;203:103–9.
12 Lopez WA, Angulo J, Zimmerman JJ, et al. Porcine reproductive and respiratory syndrome monitoring in breeding herds using processing fluids. J Swine Health Prod 2018;26:146–50.
13 Vilalta C, Sanhueza J, Alvarez J, et al. Use of processing fluids and serum samples to characterize porcine reproductive and respiratory syndrome virus dynamics in 3 day-old pigs. Vet Microbiol 2018;225:149–56.
14 Vilalta C, Sanhueza JM, Murray D, et al. Detection of Mycoplasma hyopneumoniae in piglet processing fluids. Vet Rec 2019;185:510.
15 Vangroenweghe F, Karriker L, Main R, et al. Assessment of litter prevalence of Mycoplasma hyopneumoniae in preweaned piglets utilizing an antemortem tracheobronchial mucus collection technique and a real-time polymerase chain reaction assay. J Vet Diagn Invest 2015;27:606–10.
16 Strait EL, Madsen ML, Minion FC, et al. Real-Time PCR assays to address genetic diversity among strains of Mycoplasma hyopneumoniae. J Clin Microbiol 2008;46:2491–8.
17 DeBey MC, Ross RF. Ciliostasis and loss of cilia induced by Mycoplasma hyopneumoniae in porcine tracheal organ cultures. Infect Immun 1994;62:5312–8.
18 Le Carrou J, Laurentie M, Kobisch M, et al. Persistence of Mycoplasma hyopneumoniae in experimentally infected pigs after marbofloxacin treatment and detection of mutations in the parC gene. Antimicrob Agents Chemother 2006;50:1959–66.
19 Marois C, Le Carrou J, Kobisch M, et al. Isolation of Mycoplasma hyopneumoniae from different sampling sites in experimentally infected and contact SPF piglets. Vet Microbiol 2007;120:96–104.
20 Woolley LK, Fell S, Gonsalves JR, et al. Evaluation of clinical, histological and immunological changes and qPCR detection of Mycoplasma hyopneumoniae in tissues during the early stages of mycoplasmal pneumonia in pigs after experimental challenge with two field isolates. Vet Microbiol 2012;161:186–95.
21 Raymond BBA, Turnbull L, Jenkins C, et al. Mycoplasma hyopneumoniae resides intracellularly within porcine epithelial cells. Sci Rep 2018;8:17697.
22 Browne C, Loeffler A, Holt HR, et al. Low temperature and dust favour in vitro survival of Mycoplasma hyopneumoniae; time to revisit indirect transmission in pig housing. Lett Appl Microbiol 2017;64:2–7.
23 Vilalta C, Baker J, Sanhueza J, et al. Effect of litter aggregation and pooling on detection of porcine reproductive and respiratory virus in piglet processing fluids. J Vet Diagn Invest 2019;31:625–8.
24 Spackman E, Suarez DL. Type A influenza virus detection and quantitation by real-time RT-PCR. Methods Mol Biol 2008;436:19–26.