Practice Tip

Development of a herd-specific lung homogenate for exposure to *Mycoplasma hyopneumoniae* under field conditions

Rebecca C. Robbins, DVM, PhD; Alyssa M. Betlach, DVM; Maria R. Mondragon-Evans, MVZ; Maria Pieters, DVM, PhD

Summary

The swine industry is known for holding high standards of disease control and elimination. However, partial disease control for *Mycoplasma hyopneumoniae* at the farm level has been evident and has driven initiatives for unconventional health management strategies. Several approaches focused on gilt exposure for *M. hyopneumoniae* using a herd-specific lung homogenate have been performed in the field. Nevertheless, variations in efficacy are apparent and a publicly available protocol for producing *M. hyopneumoniae* lung homogenate under field conditions is not available. In this practice tip, a protocol is described for developing a herd-specific lung homogenate for *M. hyopneumoniae* exposure intended for use in veterinary-supervised elimination or control programs. A herd-specific lung homogenate inoculum, free of secondary respiratory pathogens for the herd of intended use and with an adequate *M. hyopneumoniae* concentration, was obtained through extensive diagnostic testing and evaluation of *M. hyopneumoniae* localization within the lung. Molecular methods were applied to characterize the *M. hyopneumoniae* present in the lung and to evaluate the genomic stability of the bacterium during the exposure process. In doing so, a herd-specific *M. hyopneumoniae* lung homogenate for gilt acclimation was obtained under field conditions.

Keywords: swine, *Mycoplasma hyopneumoniae*, gilt acclimation, lung homogenate, disease control and elimination

Accepted: March 5, 2019

Résumé – Développement d’un homogénate de poumon spécifique de troupeau pour exposition à *Mycoplasma hyopneumoniae* dans des conditions de terrain

L’industrie porcine est reconnue pour le maintien de standards élevés en ce qui a trait à la maîtrise et à l’élimination des maladies. Toutefois, à la ferme la maitrise partielle de l’infection par *Mycoplasma hyopneumoniae* est évidente et a entraîné des initiatives pour des stratégies non-conventionnelles de gestion de la santé. Plusieurs approches ont mis sur l’exposition de cochettes à *M. hyopneumoniae* en utilisant un homogénat de poumon spécifique au troupeau ont été réalisées sur le terrain. Cependant, des variations dans l’efficacité sont apparentes et un protocole disponible à tous pour produire en condition de terrain un homogénat pulmonaire contenant *M. hyopneumoniae* n’est pas disponible. Dans la présente astuce de pratique, un protocole est décrit pour développer et utiliser, sous supervision vétérinaire, un homogénat pulmonaire spécifique de troupeau contenant *M. hyopneumoniae* dans le cadre de programmes de maîtrise ou d’élimination. Un inoculum d’homogénat de poumon spécifique de troupeau, exempt d’agents pathogènes respiratoires secondaires pour le troupeau sélectionné et avec une concentration adéquate de *M. hyopneumoniae*, fut

RCR, MRM-E: Seaboard Foods, Guymon, Oklahoma.

AMB, MP: College of Veterinary Medicine, University of Minnesota, St Paul, Minnesota.

AMB: Swine Vet Center, St Peter, Minnesota.

Corresponding author: Dr Maria Pieters, Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, 1365 Gortner Ave, 225 VMC, St Paul, MN 55108; Tel: 612-624-7947; Fax: 612-625-6241; Email: pieters0094@umn.edu.

This article is available online at [http://www.aasv.org/shap.html](http://www.aasv.org/shap.html).


Journal of Swine Health and Production — Volume 27, Number 4
obtenu à la suite d’épreuves diagnostiques nombreuses et à l’évaluation de la localisation de *M. hyopneumoniae* dans le tissu pulmonaire. Des méthodes moléculaires furent utilisées afin de caractériser les *M. hyopneumoniae* présents dans le poumon et pour évaluer la stabilité génomique de la bactérie durant le processus d’exposition. Ainsi, un homogénat de poumon spécifique de troupeau contenant *M. hyopneumoniae* pour l’acclimatation des cochettes fut obtenu dans des condition de terrain.

Veterinarians are responsible for applying their knowledge to improve animal health and welfare. The swine industry aims for high herd health to rear healthy pigs and safe pork. To do so, veterinarians, producers, industry professionals, and scientists attempt to implement practical and science-driven solutions that can be applied in the field. The herd veterinarian is tasked with recommending solutions based on professional judgement, scientific literature, experience, field research, and consultation with colleagues and experts. Historically, herd management practices have evolved in response to issues faced in the field and are adopted as ethically and scientifically substantiated solutions. In the case of disease control, the swine industry has been keen to develop and apply strategies towards disease management and elimination, including the use of biosecurity and the modification of production practices to decrease the detrimental effect of disease transmission (eg, early weaning and all-in/all-out production). In cases where ideal disease control cannot be achieved with the available tools, novel solutions are generated.

The administration of a herd-specific infectious product for disease control has been used in veterinary medicine to confer complete and strain-specific protection when other measures have proven inadequate to contain the disease process. In some instances, administration of a herd-specific tissue homogenate is the best option for a controlled exposure to indigenous pathogens when the exposure is intended to protect the larger population. Use of herd-specific tissue homogenate for controlled exposure requires veterinary oversight and must adhere to any applicable regulations ensuring that it does not adversely affect the health and performance of the individual animal exposed. For example, the control of viruses (ie, porcine parvovirus and porcine enterovirus) known to cause stillbirths, mummification, embryonic deaths, and infertility has been achieved by exposing dams to infectious feedback material composed of feces or tissues from contaminated litters. This exposure serves to homogenize herd immunity and acclimate incoming gilts to prevent herd disequilibrium. Immunity to porcine epidemic diarrhea virus (PEDV) and porcine rotavirus has been accomplished by using pre-farrow oral controlled exposure of dams with infectious feedback material, resulting in protection of piglets through the development of humoral and cell-mediated immunity.

*Mycoplasma hyopneumoniae* causes a chronic respiratory condition in pigs known as enzootic pneumonia (EP), which affects herds worldwide. Control measures for EP include the use of immunization, antimicrobial medication, increased biosecurity practices, parity segregation, all-in/all-out movement, and elimination strategies. However, in certain situations such as gilt acclimation, partial control can be obtained with the use of these measures, even if they are employed in combination. Thus, veterinary professionals have proposed the use of alternative measures to control *M. hyopneumoniae* infections in the field, which are tailored to be herd-specific and include pathogen exposure using lung homogenate.

**Statement of the problem**

Replacement gilts play an important role in the dynamics of a sow farm, as approximately half of the herd is replaced with young females every year for genetic improvement and maintenance of parity structure. However, every new batch of replacement females needs to be evaluated for their potential to cause disturbance of the sow farm dynamics, especially as it pertains to infectious agents. Incoming gilts may introduce new pathogens not currently prevalent in the herd or be naïve to existing pathogens on the recipient sow farm. Gilt health status is closely surveilled before and after transportation and during introduction to the recipient herd. Assurance from suppliers regarding freedom from economically important swine pathogens (ie, porcine reproductive and respiratory syndrome virus [PRRSV], PEDV, and *M. hyopneumoniae*) may or may not be required by the buyer. Although freedom of infectious agents and disease is a desirable attribute in replacement animals, it is hypothesized that in certain circumstances the health conditions of the recipient farm may be more severely affected by the introduction of naïve pigs. This is the case for *M. hyopneumoniae* infections, which are considered endemically prevalent in a significant proportion of swine farms. Introduction of naïve gilts into *M. hyopneumoniae*-positive farms is hypothesized to be a risk factor for sow herd disequilibrium and results in difficulty to control disease presentation in downstream flows.

Various options can be pursued to address the issue of naïve gilt introductions into *M. hyopneumoniae* endemically infected farms. Disease elimination is most favorable for any swine production unit, and recently efforts for *M. hyopneumoniae* eradication have increased in the United States. One of the most commonly utilized strategies for *M. hyopneumoniae* elimination, which is herd closure and medication, implies uniform exposure of the entire herd at the same time prior to the start of closure. A protocol directed at exposure with *M. hyopneumoniae* is needed when pursuing disease elimination. To achieve and maintain the elimination of *M. hyopneumoniae*, farm geographical location, area prevalence, facility design, production system flow, and constant and continuous supply of negative gilts should be accounted for. However, these factors often cannot be modified to achieve successful elimination. Therefore, disease control is viewed as one of the oldest and most cost-effective strategy to deal with *M. hyopneumoniae* eradication on endemically infected farms, keeping in mind the necessity to maintain the health of incoming and resident dam populations.

One common question in the industry is whether control can be achieved with commercial products directed at treating or controlling *M. hyopneumoniae* infections. The species-specific vaccines and antimicrobial drugs with activity towards mycoplasmas play an important role in decreasing the negative outcomes of EP. However, it is widely known that partial protection is conferred by *M. hyopneumoniae* bacterins and vaccinated pigs can become colonized after contact with shedding pigs. In addition, elimination of the bacterium from the respiratory tract of pigs has not been achieved with antimicrobial treatment alone, even during the chronic phase of infection. Therefore, a need exists for a practical protocol for herd exposure to *M. hyopneumoniae*. In this practice tip, we describe a procedure to develop a herd-specific lung homogenate for *M. hyopneumoniae* exposure under field conditions to potentially stimulate immunity and decrease the proportion of susceptible
animals in the population. This practice tip is intended to be used as a resource for swine veterinarians who are designing gilt acclimation strategies that involve the procurement of a herd-specific lung homogenate.

Definitions
For the purpose of providing clarity to this practice tip, the following definitions are proposed:

- **Gilt acclimation**: The process of adapting gilts to a new environment or exposure to an infectious agent prior to introduction into a recipient breeding herd.13,21
- **Lung homogenate**: Lung tissue made uniform through a blending process that is used for exposure.

Animal care
All animals were under veterinary oversight and care with a veterinarian-client-patient relationship and Pork Quality Assurance Plus certification in place. Feed and water were available ad libitum in stainless steel feeders and through water nipples, respectively. Pigs and their environment were monitored daily by caretakers. All feed rations were formulated to meet or exceed nutritional recommendations for swine.22 Gilts were raised in standard indoor production facilities with fully slatted floors, fed a diet to meet or exceed their nutritional needs, and received immunizations against porcine circovirus type 2 (PCV2), PRRSV, and Mycoplasma species positive farm and was selected at 31 weeks of age when she exhibited clinical signs (ie, dyspnea and loss of body condition) suggestive of M. hyopneumoniae infection.26 Alternatively, an initial donor may be chosen through testing of ante-mortem samples (eg, laryngeal swabs)27 using sterile swabs (BBL CultureSwab, Sparks, Maryland) and tested for M. hyopneumoniae by species-specific real-time polymerase chain reaction (real-time PCR) to confirm infection.28 The donor was humanely euthanized and lung tissue harvested if macroscopic lesions (ie, consolidation of apical and cardiac lung lobes) consistent with M. hyopneumoniae infection were observed26 and no lesions of secondary bacterial infection (eg, polyserositis) were evident. A bronchial swab was obtained by inserting a sterile swab into bilateral bronchioles of affected lung tissue and submitted to the University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL), along with a portion of the affected lung lobe for diagnostic testing. Remaining lung tissue was stored at −20°C for a minimum of 48 hours and until diagnostic testing was completed to ensure a high recovery of M. hyopneumoniae.

Procuring a herd-specific M. hyopneumoniae lung homogenate
Under experimental conditions, viable culture and tissue homogenates have been administered to stimulate M. hyopneumoniae exposure.23-25 However due to the fastidious growth of this microorganism, the procurement of a herd-specific lung homogenate was proposed. To obtain a herd-specific lung homogenate, a procedure focusing on lung homogenate preparation from tissue donor gilts was developed for use in field scenarios (Figure 1). Several factors including farm history and health status, clinical observations, and diagnostic testing were taken into consideration by the herd veterinarian during the selection of donor gilts and lung tissue. With diagnostic aid, the concentration of M. hyopneumoniae and presence of secondary agents were evaluated to ensure adequate lung homogenate quality. It was up to the herd veterinarian to consider the herd’s indigenous organisms when developing parameters for homogenate quality. In addition, the infectivity and genomic stability of the M. hyopneumoniae lung homogenate were assessed under field conditions.

Donor gilt selection
**Initial tissue donor gilt**
The initial tissue donor gilt was from a PRRSV, influenza A virus (IAV), PCV2, and Mycoplasma species positive farm and was selected by the veterinarian. In addition, the infectivity of the lung homogenate was confirmed by testing lung tissue homogenate against all species of the farm. In the event of a positive test, the gilt was euthanized and lung tissue was removed for diagnostic testing. The initial gilt was selected by fitting a standard curve with the diagnostic criteria for M. hyopneumoniae infection and to confirm the presence of M. hyopneumoniae in the lung homogenate. The diagnostic parameters were designed to prevent the introduction, amplification, or spread of secondary respiratory pathogens, including but not limited to PRRSV, IAV, PCV2 and H. parasuis, which could cause unintended infection and compromise gilt health. Mycoplasma hyorhinis is a commensal microorganism in swine; however, clinical disease associated with polyserositis is often evident at high bacterial concentrations.29 Therefore, an M. hyorhinis Ct value ≥ 33 was chosen as the cut-off parameter while considering the ubiquitous nature of this microorganism in swine herds and the clinical history of the herd. A PCV2 Ct value ≥ 30 was chosen as the cut-off parameter due to the endemic nature of this microorganism in swine herds.30 If additional respiratory pathogens were detected, continuation of lung homogenate development protocol was at the discretion of the veterinarian.

The M. hyopneumoniae Ct value of ≤ 26 was selected by fitting a standard curve with known concentrations of bacterial infectivity (color changing units/mL [CCU/mL]) to the real-time PCR assay and obtaining a Ct value equivalent to 1 × 10^3 CCU/mL. A concentration of 1 × 10^5 CCU/mL of M. hyopneumoniae has been suggested as the minimum required infectious dose for successful colonization of a pig’s lung in experimental conditions.31 Differences in virulence across M. hyopneumoniae strains have been observed,32 therefore, a potentially lower infectious dose equivalent of 1 × 10^3 CCU/mL was chosen by the veterinarian. In addition, within-sample variation was assumed based on the nature of the sample, therefore, the infectious dose may potentially vary. Lungs fulfilling the diagnostic criteria, with the intent to inoculate M. hyopneumoniae-negative gilts, were used to make enough homogenate for the herd-specific gilt acclimation program recommended by the veterinarian.

Donor gilts for amplification and lung homogenate procurement
To amplify and procure lung homogenate for M. hyopneumoniae exposure for replacement gilts to a 65,000-sow herd, 3- to 5-week old PRRSV, IAV, and M. hyopneumoniae-negative
**Figure 1:** Procedure to obtain a *Mycoplasma hyopneumoniae* lung homogenate. *Mhp* = *Mycoplasma hyopneumoniae*; PCR = polymerase chain reaction; Ct = cycle threshold; CCU = color changing units; *Mhr* = *Mycoplasma hyorhinis*; PRRSV = porcine reproductive and respiratory syndrome virus; IAV = influenza A virus; PCV2 = porcine circovirus type 2; *H* parasuis = *Haemophilus parasuis.*

Gilt expressing clinical signs suggestive of *Mhp* infection

- Laryngeal swab tested by real-time *Mhp* PCR
  - *Mhp* negative or high Ct value (> 30)
  - *Mhp* positive (Ct value < 30)
  - Humanely euthanize and evaluate lung for macroscopic lesions suggestive of *Mhp* infection
  - Bilateral bronchial swab and accessory lung lobe tested to meet criteria
    - *Mhp* PCR Ct value ≤ 26 and obtain a concentration of 1 x 10^5 CCU/mL or higher
    - *Mhr* PCR Ct value ≥ 33
    - PRRSV and IAV PCR negative
    - PCV2 Ct value ≥ 30
    - No *H* parasuis growth on culture
    - < 1 + aerobic bacteria
    - Freeze remaining lung at -20° C for 48 hours or until diagnostic testing is complete.
  - Gilt is withdrawn for donor selection
- Samples don’t meet diagnostic criteria

Gilt donor selection

- Diluted lung homogenate
  - Thaw lung tissue at 4° C
  - Homogenize lung tissue with modified-medicated Friis broth (70:30 ratio)
  - Aliquot 2mL of homogenate sample and submit for *Mhp* real-time PCR. Perform PCR in triplicate
    - *Mhp* Ct value > 30
      - Lung homogenate is withdrawn for further processing
    - *Mhp* Ct value ≤ 30 and obtain a concentration of 1 x 10^5 CCU/mL or higher

Initial lung homogenate

- Dilute lung homogenate with Friis media to 1:9 ratio. Once prepared, the sample should be used within 4 hours
  - Administer 10mL dose of the diluted lung homogenate to an individual pig for *Mhp* exposure (amount is based on pig age)
gilts (n = 38) were intra-tracheally inoculated with 10 mL of the diluted lung homogenate.

Four weeks post inoculation, laryngeal swabs were collected and tested for *M. hyopneumoniae* by species-specific real-time PCR to confirm infection. If swabs were positive, lungs were harvested at 5 weeks post inoculation and diagnostic testing was performed as previously described for the initial donor (Figure 1). The accessory lung lobe was submitted for diagnostic testing to evaluate the presence of viruses and secondary bacteria while preserving the remaining lung sections for subsequent lung homogenate development. Sample collection and tissue harvest took place 5 weeks post inoculation because peak *M. hyopneumoniae* shedding has been shown to occur at 4 weeks post inoculation under experimental conditions and to account for the lower *M. hyopneumoniae* infectious dose (1 × 10^3 CCU/mL). Lungs that fulfilled the diagnostic criteria were processed into lung homogenate and used to expose larger gilt populations as part of the herd-specific acclimation program.

**Lung homogenate preparation**

**Selection of lung tissue**

To identify the maximum amount of lung tissue meeting the diagnostic criteria for homogenate production, a pilot study was performed. Localization of *M. hyopneumoniae* was evaluated by determining the relative bacterial load within different anatomical lung sections using lung homogenate samples of two gilts, which were evaluated individually (Figure 2). The lung homogenates were obtained 5 weeks post inoculation and tested for *M. hyopneumoniae* using real-time PCR. Each lung homogenate was run in triplicate, in which the genetic material from 3 sample subsets was extracted and tested individually to account for possible diagnostic variation due to sample consistency. Of the 2 gilts sampled, *M. hyopneumoniae* bacterial loads were numerically higher in the proximal lung sections (median Ct values = 21.4 and 19.8) compared to the distal lung sections (median Ct values = 36.3 and 23.3) and the caudal diaphragmatic lobe (median Ct values = 21.7 and 30.4). However, the amount of viable *M. hyopneumoniae* based on anatomical lung sections was not assessed because of the difficulty to obtain an *M. hyopneumoniae* culture, especially under field conditions. In addition, the proportion of affected lung within each anatomical lung section was not evaluated.

For the detection of this microorganism, within-homogenate variance was observed for each anatomical lung region, but to a greater extent in distal and proximal lung sections compared to the caudal diaphragmatic lobe (Figure 2). The degree of within-homogenate variance could have resulted from the anatomic nature of the tissue as the homogenate includes cartilaginous airways, pleura, and lung tissue with the specific localization of the microorganism. In this investigation, a small sample size was evaluated, however, insight regarding the relative bacterial load based on anatomical lung section was gained at the individual pig level. Further research involving a larger sample size and evaluating the impact of different *M. hyopneumoniae* infection lengths and lung lesion scores on the relative bacterial load within each anatomical lung section is needed.
Since this microorganism was localized across the three different anatomical lung sections, the relative bacterial load of \textit{M. hyopneumoniae} within different lung homogenate preparations was evaluated in 38 gilts at 5 weeks post inoculation. \textit{Mycoplasma hyopneumoniae} Ct values were compared in bronchial swabs and 2 types of lung homogenate samples prepared from either whole lung tissue or from lesioned apical, cardiac, and diaphragmatic lobes that contained adjacent apparently non-affected tissue (Table 1). All bronchial swabs were collected from affected apical and cardiac lung lobes and the lung tissue was homogenized using 70% lung tissue and 30% modified, medicated Friis broth.\textsuperscript{34} Samples were submitted for \textit{M. hyopneumoniae} testing using real-time PCR, in which the homogenate samples were run in triplicate and the median Ct value was used for data analysis. For statistical analysis, a two-sample \textit{t}-test assuming equal variances was performed using R (v3.5.1; R Core Team, 2018) to compare lung homogenate Ct values based on preparation type. Differences were considered significant at \( P < .05 \). Based on the conditions of this study, the \textit{M. hyopneumoniae} lung homogenate derived from lesioned apical, cardiac, and diaphragmatic lung lobes showed significantly lower Ct values compared to whole lung tissue Ct values (\( P = .003 \); Table 1). In both lung homogenates, the mean \textit{M. hyopneumoniae} Ct values were 20.9 and 24.9, suggesting a high bacterial presence of the microorganism regardless of tissue preparation method (Table 1). In addition, tissue preparation using whole lung provided a larger volume of lung homogenate, resulting in the use of fewer donor gilts. Since the whole lung homogenate preparation met or exceeded the veterinarian’s homogenate quality criteria, the lung homogenate was prepared by incorporating the whole lung tissue.

**Initial lung homogenate**

Frozen whole lung tissue was homogenized using a ratio of 70% tissue and 30% modified medicated Friis broth\textsuperscript{34} using a Ninja Professional blender. This ratio was chosen based on the sampling procedure used for viral isolation by the UMN VDL and the feasibility to handle and process the material considering its viscosity. The blending process was repeated until lung tissue reached a slurry consistency. Friis medium was used to support \textit{M. hyopneumoniae} viability during the preparation and inoculation of the lung homogenate because this medium is commonly used for the culture and isolation of this microorganism.\textsuperscript{34} Lung tissue was processed, aliquoted, and stored at -80°C. Currently, there is minimal information regarding the freeze-thaw effect on \textit{M. hyopneumoniae} viability. It is hypothesized that thawing frozen lung tissue aids in the detachment of this microorganism from the targeted tissue leading to a higher bacterial recovery. However, further information on this topic is necessary to assess the viability and storage of frozen \textit{M. hyopneumoniae} clinical samples. Previous literature suggests that freezing a \textit{Mycoplasma} organism culture at -70°C and -30°C for up to 2 years may result in up to 1 and 2 log\textsubscript{10} reduction in bacterial titers, respectively.\textsuperscript{35} Prior to freezing, 2 mL of the lung homogenate was submitted for \textit{M. hyopneumoniae} real-time PCR and tested in triplicate, resulting in an average 25.5 Ct value.

**Evaluating lung homogenate infectivity and genomic stability**

**Lung homogenate infectivity**

Diagnostic monitoring post inoculation was performed to evaluate the diluted lung homogenate infectivity. The veterinarian considered the lung homogenate to be infectious if an \textit{M. hyopneumoniae} infection was observed or detected post inoculation. Laryngeal swabs were collected 4 weeks post inoculation for \textit{M. hyopneumoniae} detection using real-time PCR. All the pigs sampled (\( n = 38 \)) were \textit{M. hyopneumoniae} positive, evidencing sample infectivity. Post inoculation, clinical signs and mortality were closely monitored. If clinical signs suggestive of secondary bacterial infections (eg, unthriftiness, cough, thumping, or increased respiratory effort) were observed, antimicrobials without activity towards mycoplasmas (eg, Ceftiofur) were administered according to label directions.

**Genomic stability**

Multiple locus variable number tandem repeat analysis (MLVA)\textsuperscript{37} was employed to identify \textit{M. hyopneumoniae} types in the lung homogenate and to evaluate for potential genomic mutations that could have occurred during the tissue processing and inoculation. The molecular characterization method was performed from \textit{M. hyopneumoniae}-positive bronchial swabs that were collected from the initial and subsequent donor gilts’ lung tissue. All samples showed an MLVA type 11-15. This suggests a lack of detectable genomic change in the targeted amplicon during the initial lung homogenate preparation and throughout the subsequent exposure-harvest processes. This finding is supportive of other research that describes \textit{M. hyopneumoniae} \textit{in vitro} and \textit{in vivo} genomic stability.\textsuperscript{37,38}

---

**Table 1:** Detection of \textit{Mycoplasma hyopneumoniae} (Ct values) in bronchial swabs and lung homogenate samples based on tissue preparation. Different superscript letters represent significant difference (\( P < .05 \)) based on a two-sample \textit{t}-test. Ct = cycle threshold.

<table>
<thead>
<tr>
<th>Lung section</th>
<th>No. of samples</th>
<th>Bronchial swabs, Ct value (SD)</th>
<th>Lung homogenate, Ct value (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesioned apical, cardiac, and diaphragmatic lobes</td>
<td>14</td>
<td>22.6 (4.5)</td>
<td>20.9 (3.6)\textsuperscript{a}</td>
</tr>
<tr>
<td>Whole lung</td>
<td>24</td>
<td>22.9 (2.7)</td>
<td>24.9 (3.9)\textsuperscript{b}</td>
</tr>
</tbody>
</table>
Conclusion
In this practice tip, a procedure for the development of a herd-specific lung homogenate for *M. hyopneumoniae* exposure under field conditions is described. This practice tip details a step-by-step process focusing on lung homogenate preparation. In doing so, gilt acclimatization practices that encompass herd-specific pathogen exposure methods may be achieved to provide adequate *M. hyopneumoniae* exposure and immunization.

Acknowledgments
The authors would like to thank Dr Albert Rovira and Casey Thomlinson for their valuable contributions to the diagnostics phase and support in the application of this protocol.

Conflict of interest
None reported.

Disclaimer
Scientific manuscripts published in the *Journal of Swine Health and Production* are peer reviewed. However, information on medications, feed, and management techniques may be specific to the research or commercial situation presented in the manuscript. It is the responsibility of the reader to use information responsibly and in accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or region.

References

* Non-referenced references.