

# Establishing *Mycoplasma hyopneumoniae* herd status classification criteria for breeding herds

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## Summary

A standardized system for classifying the *Mycoplasma hyopneumoniae* status of swine breeding herds was developed by defining a set of diagnostic guidelines to determine the exposure and shedding status of herds. The classification is based on epidemiological and ecological features of *M hyopneumoniae* and reflects current field control and elimination practices. The classification was developed by a working group composed of representatives from academia, industry, swine practitioners, American Association of Swine Veterinarians (AASV), and the National Pork Board, and approved by the AASV Board of Directors on October 2, 2019. Clear and concise terminology will facilitate communication across all stakeholders.

**Keywords:** swine, *Mycoplasma hyopneumoniae*, herd classification, disease status, diagnostics

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## Resumen - Establecimiento de los criterios de clasificación del estatus de *Mycoplasma hyopneumoniae* para piaras de reproductoras

Se desarrolló un sistema estandarizado para clasificar el estado de *Mycoplasma hyopneumoniae* en las piaras reproductoras mediante la definición de un conjunto de pautas de diagnóstico para determinar su estado de exposición y eliminación. La clasificación se basa en las características epidemiológicas y ecológicas de *M hyopneumoniae* y refleja las prácticas actuales de control y eliminación en el campo. La clasificación fue desarrollada por un grupo de trabajo integrado por representantes de la academia, la industria, los profesionales especialistas en cerdos, la Asociación Americana de Veterinarios Especialistas en Cerdos (AASV), y el Consejo Nacional de Porcicultores, y aprobada por la Junta Directiva de la AASV el 2 de octubre de 2019. Esta terminología clara y concisa facilitará la comunicación entre todas las partes interesadas.

## Résumé - Établissement de critères de classification du statut des troupeaux envers *Mycoplasma hyopneumoniae* pour les troupeaux reproducteurs

Un système standardisé de classification du statut des troupeaux porcins reproducteurs envers *Mycoplasma hyopneumoniae* a été développé en définissant un ensemble de directives de diagnostic pour déterminer l'exposition et le statut d'excrétion des troupeaux. La classification est basée sur les caractéristiques épidémiologiques et écologiques de *M hyopneumoniae* et reflète les pratiques actuelles de contrôle et d'élimination sur le terrain. La classification a été élaborée par un groupe de travail composé de représentants du monde universitaire, de l'industrie, des praticiens du porc, de l'American Association of Swine Veterinarians (AASV) et du National Pork Board, et approuvée par le conseil d'administration de l'AASV le 2 octobre 2019. Une terminologie claire et concise facilitera la communication entre toutes les parties prenantes.

MJC, DJH, RM: Veterinary and Diagnostic Production Animal Medicine Department, College of Veterinary Medicine, Iowa State University, Ames, Iowa.

MJC: Pig Improvement Company, Hendersonville, Tennessee.

LGP, TP, DB: Zoetis, Parsippany, New Jersey.

PY: Swine Veterinary Center, St. Peter, Minnesota.

CJ: Carthage Veterinary Service, Ltd, Carthage, Illinois.

MS: Audubon Manning Veterinary Clinic, LLC, Audubon, Iowa.

EF: Boehringer Ingelheim Animal Health USA Inc, Duluth, Georgia.

EM: Pipestone Veterinary Services, Pipestone, Minnesota.

LB: National Pork Board, Clive, Iowa.

LG: TriOak Foods, Oakville, Iowa.

HS: American Association of Swine Veterinarians, Perry, Iowa.

AM: The Maschhoffs, Carlyle, Illinois.

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**M***ycoplasma hyopneumoniae* is the etiologic agent of porcine enzootic pneumonia, an infectious respiratory disease characterized by a nonproductive cough, reduced daily weight gain, and poor feed conversion.<sup>1</sup> *Mycoplasma hyopneumoniae* represents a significant burden for the swine industry, especially when combined with viral co-infections, causing losses of up to \$10/pig.<sup>2</sup> From a system-wide perspective, control of *M hyopneumoniae*-associated disease largely depends on minimizing transmission from sow to piglet. In fact, a high prevalence of *M hyopneumoniae* in weaned pigs has been associated with elevated disease in the growing phase.<sup>3</sup> In another study, this significant correlation between weaning status and clinical disease at slaughter was not observed.<sup>4</sup> Still, efforts that largely focus on controlling transmission within the breeding herd and minimizing the prevalence at weaning likely have the highest impact on disease reduction.<sup>5,6</sup> In production systems where elimination is not pursued, the main focus for control programs has been on the safe exposure of young, naive gilt populations with the resident *M hyopneumoniae* strain. Promoting early gilt exposure to *M hyopneumoniae*-positive cull sows in gilt development units or implementing controlled exposure programs (intratracheal or aerosol inoculation), followed by sufficient time for the development of a robust immunity (at least 240 days) and decreased bacterial shedding, has shown to be an effective way of reducing disease in downstream populations.<sup>5-10</sup>

The economic impact, coupled with recent diagnostic improvements, increased knowledge on the ecology of *M hyopneumoniae*, and availability of naive breeding stock has led to an increase in the frequency of successful *M hyopneumoniae* control, prevention, and elimination programs in North America.<sup>11</sup> The wide implementation and continued use of the porcine reproductive and respiratory syndrome virus (PRRSV) swine herd classification system since 2011, has been proven to be a valuable tool for disease management; facilitating communication between swine producers, veterinarians, diagnosticians, and breeding stock companies, monitoring the status of herds, evaluating and executing strategies for disease control and prevention, and supporting regional control and elimination efforts.<sup>12-15</sup>

Our objective was to provide an updated standardized system for classifying the *M hyopneumoniae* status of swine breeding herds by defining a set of diagnostic guidelines to determine the exposure and shedding status of herds.

## Methods

The classification system incorporated objective diagnostic criteria based on the relevant biological and ecological features of *M hyopneumoniae*. The previous breeding herd classification systems developed for *M hyopneumoniae* were used as the foundation, as well as standards and definitions developed for the PRRSV herd status classification for consistency between systems.<sup>8,12,16</sup> The working group held a workshop in Hendersonville, Tennessee on November 28-29, 2018. The terminology and classification criteria approved by the working group was presented to the American Association of Swine Veterinarians (AASV) Committee on Transboundary and Emerging Diseases (CTED) at the 50<sup>th</sup> AASV Annual Meeting in Orlando, Florida on March 9, 2019. This was followed up with the distribution of the working document to all CTED members. On August 7, 2019, an online meeting was held to further discuss the classification with all CTED members and the working group where additional input was obtained. The CTED approved the classification on September 7, 2019. The final document was approved by the AASV Board of Directors on October 2, 2019.

## Considerations

### Diagnostic criteria for category establishment

The two diagnostic criteria used to determine the *M hyopneumoniae* shedding and exposure status of a herd were 1) detection of the agent in the respiratory tract and 2) antibody detection. These criteria are used to frequently monitor a subpopulation of the breeding herd and determine its status. In addition to the diagnostic criteria, the use of *M hyopneumoniae* vaccine was used to determine the status of farms.

Detection of the agent in lung lesions or the respiratory tract can be achieved using a variety of tests.<sup>6</sup> Polymerase chain reaction (PCR) is the most common and preferred test for detection of *M hyopneumoniae* in tissue and samples from live pigs. While immunohistochemistry,

fluorescent antibody, *in situ* hybridization, and bacterial culture are used by diagnostic laboratories for detection of the agent within affected tissue, they are not frequently performed for monitoring populations.<sup>6</sup> To evaluate the infection and shedding status of live pigs, it is critical to sample *M hyopneumoniae* colonization sites characterized by respiratory type epithelium, such as the trachea and bronchi. Therefore, deep tracheal samples are the preferred antemortem samples for *M hyopneumoniae* detection, compared to nasal and laryngeal swabs.<sup>17-21</sup> While aggregate samples, such as oral fluids, are used for *M hyopneumoniae* surveillance, current knowledge suggests variable and inconsistent detection capabilities, questioning its diagnostic value for accurate determination of the shedding status of a herd.<sup>19,22,23</sup> Finally, the use of pooling strategies to reduce testing cost has proven to be of value and maintain diagnostic accuracy for detection of other agents, such as PRRSV and, more recently, *M hyopneumoniae*.<sup>24,25</sup>

To measure *M hyopneumoniae* exposure, the most performed antibody test is the enzyme-linked immunosorbent assay (ELISA). Seroconversion within a population can take several weeks to be detected by ELISA, and therefore timing should be considered. It is also important to recognize that current commercially available serological assays are unable to differentiate natural infection from vaccination, and thus alternative diagnostic tests, such as PCR, should be used to determine status correctly.<sup>26</sup> Evaluation and comparison of the diagnostic performance of several commercially available ELISAs is available and can aid veterinarians in determining the most suitable test, or combination of tests, for their diagnostic objectives.<sup>27,28</sup> As noted in previous publications, false-positive results can occur with these assays, requiring an in-series testing approach or collection of additional samples from the population to troubleshoot unexpected results. A common process carried out by veterinary diagnostic laboratories involves testing the unexpected ELISA-positive samples using a different serological assay than the one used initially. Veterinarians can also decide to collect additional samples from the reacting animals or other animals within the population, such as tracheal swabs or lung samples, which are then tested by PCR.

Clinical signs associated with *M hyopneumoniae* infection are characterized by a dry, nonproductive cough, exacerbated by physical exertion, decreased appetite, and labored breathing. Microscopic lesions consist of lobular distribution of peribronchiolar and perivascular lymphocytic cuffing.<sup>6</sup> Alveoli and airways may contain serous fluid with a few macrophages and neutrophils. The airway epithelium is intact and sometimes slightly hyperplastic.<sup>6</sup> Clinical signs and lesions are not pathognomonic of *M hyopneumoniae* infection; thus, determining the shedding and exposure status is best achieved by detection of the agent in the respiratory tract and antibodies to the bacterium in serum.

### Gilt acclimation and *M hyopneumoniae* control

Control of *M hyopneumoniae* infection in pig populations is typically based on establishing sow herd immunity by means of effective gilt acclimation (ie, deliberate infection of gilts at an early age), strategic medication, and vaccination. The overarching goal of creating robust herd immunity is to minimize shedding of *M hyopneumoniae* by breeding females and vertical transmission to their piglets.<sup>5</sup> However, the duration of shedding in infected pigs is quite long (approximately 254 days).<sup>29</sup> Therefore, the goal of acclimating gilts to *M hyopneumoniae* is to allow them to become infected early in life so they can develop immunity and decrease shedding before being introduced into the sow farm.<sup>5-10</sup> This reduces the number of positive piglets at weaning, which can be a predictor for *M hyopneumoniae* clinical disease in grow-finish populations.<sup>3</sup>

Herds that have an acclimation program where replacement gilts are exposed to *M hyopneumoniae*, either naturally or through controlled exposure methods, by a maximum of 80 days of age are expected to have a low incidence of *M hyopneumoniae* disease in the breeding herd and are therefore considered *M hyopneumoniae* controlled herds.<sup>5,9</sup> However, the classification described herein does not require a specific gilt acclimation protocol and, thus, relies on the farm veterinarian and producer to define an acclimation program that suits their production system.

## *M hyopneumoniae* herd status classification

The classification system focuses on the breeding herd and is divided in 4 distinct categories: positive uncontrolled (I), positive controlled (II), provisionally negative (III), and negative (IV; Table 1). Category III is subdivided into two subcategories: unvaccinated (IIIA) and vaccinated (IIIB).

### Positive uncontrolled (I)

The following herds fall into category I: 1) breeding herds going through an *M hyopneumoniae* outbreak; 2) herds that have not performed the necessary testing described and the status is unknown; and 3) herds that have performed the necessary testing but do not qualify for status II, III, or IV.

### Positive controlled (II)

In these herds, the agent is not detected in parity 1 (P1) sows and the herd is serologically positive. For herd classification purposes, P1 sows are those that have weaned their first litter and have not farrowed their second. Herds in this category likely have an ongoing *M hyopneumoniae* gilt acclimation program where gilts are exposed at an early age; however, this is not a requirement. This status will be considered the goal for those herds that do not wish to pursue elimination and decide to only control *M hyopneumoniae* (Figures 1 and 2). Diagnostic evidence to promote a herd to this category includes 4 consecutive negative monthly samplings of a minimum of 30 tracheal swabs from P1 sows up to 30 days post weaning. This narrows the P1 age range that is tested and avoids testing P1 sows that are close to farrowing their second litter. A sample size of 30 is based on the number of samples required to detect at least 1 positive animal if the agent is present at an expected prevalence of 10% with 95% confidence for any population size greater than 1000 assuming a diagnostic test sensitivity greater than 95% and random sampling from a population with a homogenous distribution of positive animals.<sup>30-32</sup> While larger sample sizes and increased sampling events would have improved the confidence level, the chosen sample size of 30 collected 4 times was carefully selected to balance cost and inconvenience of testing and the confidence to detect a low prevalence. Such evidence would suggest that efforts to reduce

shedding in replacement animals by the end of the first parity (Figures 1, 2, and 3) are succeeding. However, evidence supporting the absence of detection of the agent in P1 sows does not rule out the possibility that there is continued *M hyopneumoniae* transmission in the herd. It is presumed that, over time, category II herds will have a low level of infection in piglets at weaning.

### Provisionally negative (III)

In these herds, the agent is not detected in the breeding herd population, however, the population may be serologically positive. Category III is divided into two subcategories. The first is provisional negative unvaccinated (IIIA). Herds in this subcategory have completed a whole herd elimination program, which refers to any set of procedures implemented at the sow herd level that succeeds in the complete removal of the targeted infectious agent from the population. It is not the intent of this classification system to define the procedures required to achieve whole herd elimination, but rather rely on each production system to determine the ideal program for their herds. A recent review on *M hyopneumoniae* elimination provides comprehensive information on the different approaches to disease elimination.<sup>11</sup>

To be classified as IIIA, herds need to meet one of two diagnostic requirements: 1) prior to introduction of negative replacement gilts, perform two consecutive negative samplings of a minimum of 60 tracheal swabs from breeding females in the last subpopulation exposed before the elimination program started or 2) two consecutive monthly negative samplings of a minimum of 30 serum samples or 30 tracheal swabs from negative replacement gilts after a minimum of 120 days post entry (Figures 1 and 3). The working group proposed the latter testing scheme to allow production systems, particularly commercial ones, to avoid delaying introduction of naive gilt replacements and, thus, achieve breeding targets. A sample size of 60 was based on the number of samples required to detect at least 1 positive animal at an expected prevalence of 5% with 95% confidence for any population size greater than 1000 assuming a diagnostic test sensitivity greater than 95% and random sampling from a population with a homogenous distribution of infection.<sup>30-32</sup> For the second testing scheme, a sample size of 30 might be considered small, but delaying testing to

**Table 1:** *Mycoplasma hyopneumoniae* breeding herd status classification criteria and summary of required supporting evidence

Breeding herd category and mapping symbol	Diagnostic criteria		Description and supporting diagnostic evidence to promote a herd into category
	Agent detection in respiratory tract	Serology	
Positive uncontrolled (I) 	Positive	Positive	<i>M hyopneumoniae</i> is detected within lesions, in the respiratory tract. Most herds will be serologically positive, while farms experiencing recent outbreaks might still be seronegative. Untested herds are category I by default.
Positive controlled (II) 	Negative in P1 sows	Positive	Herds implementing gilt acclimation programs where early exposure of incoming replacement gilts is achieved. Evidence to promote a herd to category II is monthly sampling of 30 tracheal swabs of P1 sows, tested individually for <i>M hyopneumoniae</i> . All samples are negative for 4 consecutive months.
Provisionally negative (III) 	Unvaccinated (IIIA)	Negative	Herds that have completed a whole herd elimination program. Evidence to promote a herd to category IIIA is either: 1. Monthly sampling of 60 tracheal swabs from animals in last exposed population before herd reopening, tested individually for <i>M hyopneumoniae</i> . All samples are negative for 2 consecutive months. 2. Monthly sampling of 30 serum samples or 30 tracheal swabs from negative replacement gilts after a minimum of 120 days post entry, tested individually for <i>M hyopneumoniae</i> . All samples are negative for 2 consecutive months.
	Vaccinated (IIIB) 	Negative	Positive
Negative (IV) 	Negative	Negative	Herds undergoing elimination efforts should have been category IIIA and completely rolled over the breeding herd to fall into category IV. Newly established herds and herds that underwent complete depopulation and repopulation are considered Category IV. To maintain negative status, a minimum of 30 monthly negative serology or 30 tracheal swabs results from various parity sows should be obtained.

120 days post naive replacement introduction would likely ensure a detectable prevalence if *M hyopneumoniae* persisted in the herd post elimination.

Provisional negative vaccinated (IIIB) herds have completed a whole herd *M hyopneumoniae* elimination program and have fulfilled the diagnostic requirements for subcategory IIIA, but vaccination of breeding females for *M hyopneumoniae* continues. Herds that have been stocked with negative gilts but implement *M hyopneumoniae* vaccination of any type, regardless of vaccine type or brand also fall under this category. Herds may decide to continue vaccinating and remain in category IIIB indefinitely (Figures 1 and 3). Clinical signs and lesions suggestive of *M hyopneumoniae* in the breeding herd would trigger a diagnostic investigation.

### Negative (IV)

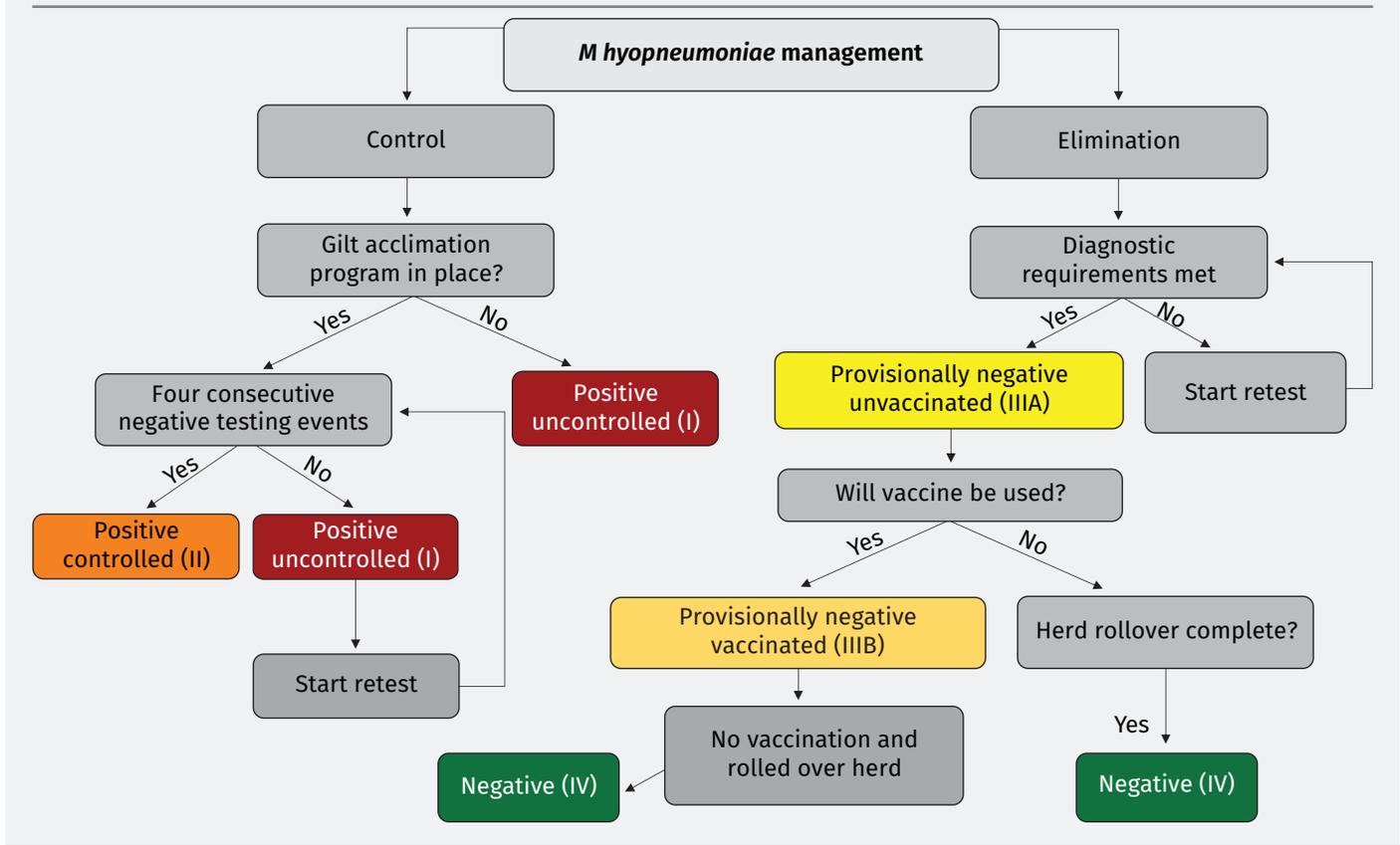
In these herds, the agent is not detected in any type of sample from any subpopulation in the breeding herd and the herd is serologically negative. Herds undergoing elimination efforts will be promoted from category IIIA to category IV when all previously infected animals in the herd are removed (Figures 1 and 3). Newly established negative herds and those that went through complete depopulation and repopulation efforts fall within category IV. To maintain a negative status, a minimum of 30 monthly negative serology or tracheal PCR results from various parity sows should be obtained. A sample size of 30 is based on the number of samples required to detect at least 1 positive animal if the agent is present at an expected prevalence of 10% with 95.76% confidence for any population

size greater than 1000 assuming a diagnostic test sensitivity greater than 95% and random sampling from a population with a homogenous distribution of positive animals.<sup>30-32</sup>

### Discussion

Development of a disease status classification system must rely on the input from the end users for whom it is being developed. Thus, building on the successful and widely adopted AASV PRRSV classification efforts, an *M hyopneumoniae* working group was assembled in 2018 and composed of practitioners from private practice, industry representatives, academicians, and representatives from AASV and National Pork Board. The objective was to bring in the collective experience of the working group

**Figure 1:** Decision tree for *Mycoplasma hyopneumoniae* management and breeding herd status classification.



and develop an *M hyopneumoniae* classification that was practical, feasible, reliable, and easy to adopt. A standardized system for classifying the *M hyopneumoniae* status of swine breeding herds was developed by defining a set of objective diagnostic guidelines to determine the exposure and shedding status of herds. The classification is based on epidemiological and ecological features of *M hyopneumoniae* and current control and elimination programs.

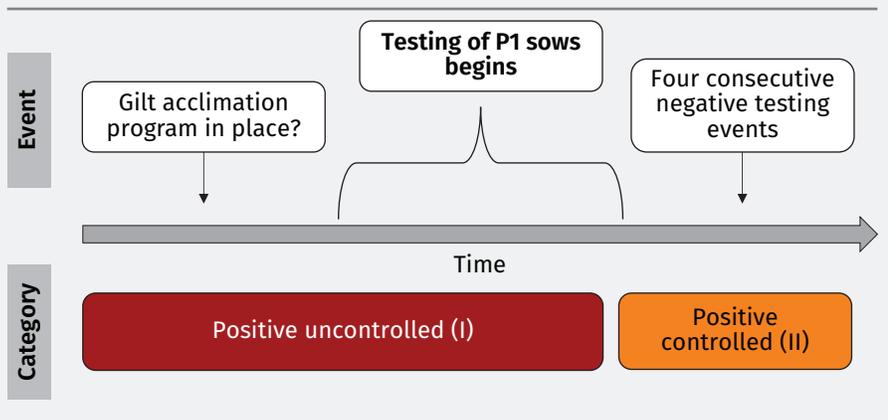
The working group used two previously proposed *M hyopneumoniae* classifications as a foundation for the one presented herein. In 2016, Galina and Clavijo developed an *M hyopneumoniae* breeding herd status classification, which was part of a manual titled *A Contemporary Review of Mycoplasma hyopneumoniae Control Strategies*.<sup>33-35</sup> The working group identified several limitations of the 2016 Galina and Clavijo *M hyopneumoniae* classification that needed revision. For example, the classification focused on the due-to-be weaned piglet population to measure *M hyopneumoniae* transmission between sows and piglets. It classified farms as stable or unstable depending on the disease prevalence in the due-to-be weaned piglet population

based on the Fano et al<sup>3</sup> paper on associations between prevalence at weaning and disease downstream. However, because of recent published information about *M hyopneumoniae* epidemiology and field experience shared by members of the working group, it was determined that the due-to-be weaned piglet population was not ideal to accurately measure breeding herd pathogen shedding.<sup>5,20,36,37</sup> Furthermore, the use of the term stability could lead to confusion within the swine industry, since it is utilized by the PRRSV classification with a different meaning and applied to a virus with a significantly different pathogenesis and epidemiology than *M hyopneumoniae*.<sup>12</sup> It was decided instead to use the term controlled to better describe herds that were implementing control efforts that would reduce sow-to-piglet transmission, such as gilt acclimation practices. Thus, the P1 sow population was chosen as the more appropriate population to measure the effectiveness of those efforts. However, the suitability of this population to measure the effectiveness of gilt acclimation protocols needs further validation. Finally, the Galina and Clavijo<sup>33</sup> classification used clinical signs and lesions as diagnostic criteria to

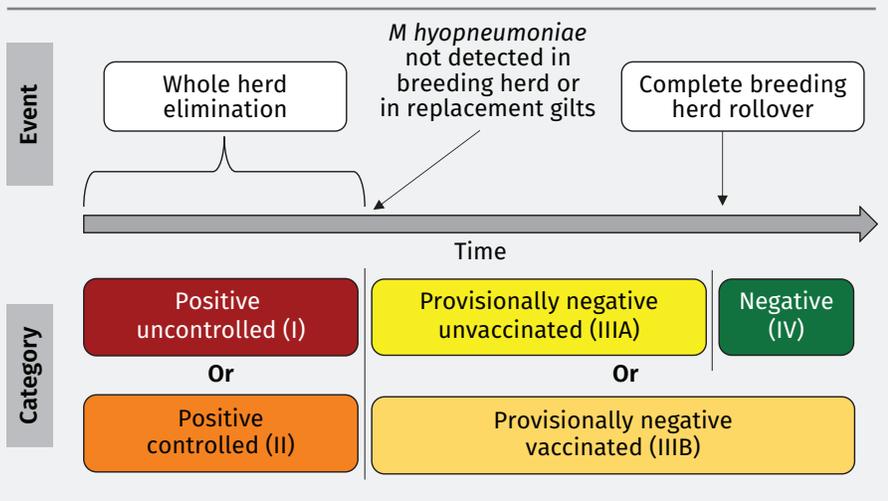
define disease status. However, neither of these are pathognomonic of *M hyopneumoniae* infection and, thus, objective and measurable diagnostic criteria were favored, such as agent and antibody detection.

Garza-Moreno et al<sup>8</sup> published a review article titled “Acclimation strategies in gilts to control *Mycoplasma hyopneumoniae* infection.” Within this review article, a subsection included an *M hyopneumoniae* classification proposal. Several critical pieces of information were not considered in the Garza-Moreno et al<sup>8</sup> classification, affecting its usefulness and likelihood of implementation by the swine industry. Similar to the Galina and Clavijo<sup>33</sup> classification, it considered subjective parameters such as clinical signs and lesions to define status. Furthermore, it did not provide specific diagnostic requirements, such as sample size, target population, and frequency of testing, which are critical for the accurate determination of the herd status and the ability to shift between statuses. The classification required postmortem samples (ie, lungs) for agent detection, rather than antemortem sample types, hindering adoption by the industry due to the impracticality and cost of

**Figure 2:** Schematic representation of *Mycoplasma hyopneumoniae* classification use when the goal is to control *M hyopneumoniae*.



**Figure 3:** Schematic representation of *Mycoplasma hyopneumoniae* classification use when the goal is to eliminate *M hyopneumoniae*.



ethanizing replacement gilts or sows to determine the health status of a herd. Finally, the Garza-Moreno et al<sup>8</sup> classification lacked industry input for its development.

Sustained use of this new classification system by the industry will allow for the identification of knowledge gaps that warrant research and will promote refinements in diagnostic and gilt acclimation protocols. One critical area is the implementation of novel pathogen-specific sampling guidelines for timely and accurate detection of the agent at varying prevalence levels, sample types, sample sizes, and production settings. Sampling guidelines for detection of *M hyopneumoniae* in a wean-to-finish site have been recently published and support the use of larger sample sizes.<sup>23</sup> However, for the development of this classification, the feasibility of collecting larger sample sizes that would afford

a higher degree of confidence in determining disease status in low-prevalence scenarios was weighed against the consequences of a missed detection. Due to the increase in cost and labor required to detect disease in low-prevalence scenarios, the working group determined that a more feasible approach should be favored, encouraging adoption by the industry. Nonetheless, given the biology of *M hyopneumoniae*, swine practitioners and producers should be aware of the risk of not detecting the agent when using low sample sizes. Furthermore, it is expected that as novel information emerges, the diagnostic criteria and terminology presented here will need to be reassessed.

Standardized nomenclature and a simple classification system are fundamental for *M hyopneumoniae* management and can enable more effective communications between key industry stakeholders, such as researchers, diagnosticians,

packers, practitioners, and producers. At the herd level, this classification can be used as a roadmap for *M hyopneumoniae* management by swine producers and veterinarians to effectively characterize the health status of farms and set realistic goals for control or elimination and improve pig flow management. Veterinarians can use this tool to classify farms within a system and update their biosecurity pyramid and improve flow of personnel, multi-site commingling, transport, and feed delivery events. At the industry level, this classification would facilitate efforts to monitor the *M hyopneumoniae* status of breeding herds and their downstream pig flow and potentially lead to the establishment and successful execution of *M hyopneumoniae* regional control and elimination efforts in the future. For example, the novel *M hyopneumoniae* classification could be adopted by surveillance initiatives, such as the Morrison Swine Health Monitoring Project, that report temporal patterns of pathogen-specific outbreaks and provides the proportion of enrolled breeding herds by disease status.<sup>14</sup> More recently, efforts are underway to develop a US Swine Health Improvement Plan, modeled after the National Poultry Improvement Plan, that has the objective of developing and implementing certification programs for important swine pathogens, such as *M hyopneumoniae*.<sup>38</sup> Finally, from a business perspective, contractual arrangements could include premiums for weaned pigs from category II, III, or IV breeding herds, thus, directly incentivizing the implementation of efforts to produce *M hyopneumoniae*-negative pigs.

## Implications

- Standardized terminology and diagnostic criteria for *M hyopneumoniae* are needed.
- A classification system was developed using *M hyopneumoniae* biological features.
- A valuable tool for disease management and communication across stakeholders.

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## Conflict of interest

None reported.

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