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

Definitions committee members:

Maria Clavijo – ISU/PIC
Lucina Galina - Zoetis
Derald Holtkamp – ISU
Paul Yeske - Swine Vet Center
Lisa Becton - National Pork Board
Harry Snelson – AASV
Rodger Main - ISU -VDL
Emily McDowell – Pipestone Veterinary Services
Clayton Johnson - Carthage Veterinary Services
Michelle Sprague - AMVC
Eduardo Fano - Boehringer Ingelheim
Thomas Painter - Zoetis
Lauren Glowzenski – TriOak foods
Dave Baumert - Zoetis

Introduction

M. hyopneumoniae (Mhp) represents a significant burden for the U.S swine industry, with reported losses of up to $10 per pig.[1] The economic impact, coupled with ongoing research on the ecology of *Mhp* and availability of negative breeding stock has led to an increase in the frequency of successful *Mhp* elimination programs in North America.[2] The benefits of a clear and concise classification system are manifold; facilitating communication between swine producers, veterinarians, diagnosticians and breeding stock companies, monitoring the status of herds, evaluating strategies for disease control, and supporting regional control and elimination efforts. From a system-wide perspective, control of *Mhp* associated disease largely depends on minimizing transmission from sow to piglet. In fact, a high prevalence at weaning has been associated with disease in the growing phase.[3] Therefore control efforts that largely focus on controlling transmission within the breeding herd will likely have the highest impact on disease. The objective of this document is to propose an updated *Mhp* breeding herd status classification system that includes a set of diagnostic guidelines to help determine the exposure and shedding status of herds. The classification incorporates objective diagnostic criteria based on the relevant biological and ecological features of *Mhp*. A previous classification developed for *Mhp* was utilized as the foundation for this document, as well as standards and definitions developed for the porcine reproductive and respiratory syndrome virus (PRRSV) herd status classification.[4,5] This updated classification was developed by a definitions committee that included practitioners from private practice, industry, researchers and representatives from the American Association of Swine Veterinarians (AASV) and National Pork Board.

Considerations

Diagnostic criteria for category establishment

The classification is based on two diagnostic criteria to determine the *Mhp* shedding and exposure status of a herd; a) *detection of the agent in the respiratory tract,* and *b) antibody detection.* These criteria are utilized to frequently monitor a subpopulation of the breeding herd and determine its status.

Detection of the agent in lung lesions or the respiratory tract can be achieved using a variety of tests. Polymerase chain reaction (PCR) is the most commonly used and preferred test for detection of *Mhp* in tissue and samples from live pigs. While Immunohistochemistry (IHC), fluorescent antibody (FA), in situ hybridization (ISH) and bacterial

culture are utilized by diagnostic laboratories for detection of the agent within affected tissue, they are not frequently performed for monitoring populations.[6] To evaluate the infection and shedding status of live pigs, it is critical to sample *Mhp* colonization sites characterized by respiratory type epithelium, such as the subglottis portion of the larynx or the trachea. Therefore, tracheal and laryngeal swabs are the preferred ante mortem samples for *Mhp* detection, with recent investigations showing a higher sensitivity with tracheal swabs.[7-10]

To measure *Mhp* exposure, the enzyme-linked immunosorbent assay, or ELISA, is commonly utilized for detection of antibodies to *Mhp* in swine serum. In the U.S, the two most frequently used commercial tests are: the IDEXX *M. hyo* indirect ELISA, and the Oxoid *M. hyopneumoniae* EIA blocking 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 consider that current commercially available serological assays are unable to differentiate natural infection from vaccination, and thus alternative diagnostic tests should be utilized to determine status correctly.[11]

Clinical signs associated with *Mhp* infection are characterized by dry non-productive cough, exacerbated by physical exertion, fever, decreased appetite and labored breathing.[6] Microscopic lesions consist of lobular distribution of peribronchiolar and perivascular lymphocytic cuffing.[6] Alveoli and airways may contain serous fluid with a few macrophages and neutrophils. Airway epithelium is intact, and sometimes slightly hyperplastic.[6] Clinical signs and lesions are not pathognomonic of *Mhp* infection, thus, determining the shedding and exposure status should be 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 *Mhp* infection in pig populations is typically based on establishing sow herd immunity by means of effective gilt acclimation (i.e. 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 *Mhp* by breeding females.[12] However, the duration of shedding in infected pigs is quite long (~254 days).[13] Therefore, the goal of acclimating gilts to *Mhp* is to allow them to become infected early in life so they can develop protective immunity (i.e. quit shedding) before being introduced into the sow farm.[14] This reduces the number of positive piglets at weaning, which has been shown to be a predictor for *Mhp* clinical disease.[12]

In this manuscript, herds that have an acclimation program where replacement gilts are exposed to *Mhp*, either naturally or through controlled exposure (i.e. inoculation),[15-18] by a maximum of 80 days of age and diagnostic confirmation of exposure is obtained, are expected to have a low incidence of *Mhp* disease in the breeding herd and are therefore considered *Mhp* controlled herds. However, this manuscript those not require a specific gilt acclimation protocol and, thus, relies on the farm veterinarian and producer to decide how to carry out an acclimation program that better suits their production system.

Herd status classification

The proposed herd-status classification focuses on the breeding herd. Four herd-status categories are proposed for breeding herds: positive uncontrolled (I), positive controlled (II), provisionally negative (III), and negative (IV) (Table 1). Category III is further divided into two subcategories: unvaccinated (IIIA) and vaccinated (IIIB).

Positive uncontrolled (I): breeding herds in this category meet at least one of the two diagnostic criteria. The following herds fall into Category I; a) breeding herds going through an *Mhp* outbreak, and b) herds that have not performed the necessary testing described below and the status is unknown.

Positive controlled (II): In these herds, the agent is not present in P1 sows and the herd is serologically positive. For the purpose of classifying herds, P1 sows are those that have farrowed their first litter. Herds in this category are assumed to have an on-going *Mhp* gilt acclimation program where gilts are exposed at an early age, however this is not a requirement. This status will be considered the end goal for those herds that do not wish to pursue elimination and decide to only control *Mhp*. To classify into this category, four consecutive negative monthly samplings of 30 tracheal swabs, tested by PCR, from P1 sows up to 30 days post-weaning should be obtained, suggesting successful early acclimation and cessation of shedding by the end of the first parity (Figure 1). However, this status cannot rule out the possibility that there is continued *Mhp* transmission in the herd. It is presumed that Category II herds have a

low level of infection in piglets at weaning and thus have the ability to make improvements on pig flow (i.e. commingling sources) or medications (i.e. stopping antimicrobial treatments at weaning).

Provisionally negative (III): In these herds, the agent is not detected within the breeding herd population, however the population may be serologically positive. Category III is sub-divided into two subcategories:

> Provisional negative unvaccinated (IIIA): Herds in this subcategory have completed a whole herd elimination program. To be classified as IIIA, herds need to meet one of two diagnostic requirements; a) Two consecutive negative samplings of 60 tracheal swabs of last population exposed prior to introducing negative replacement gilts, or b) two consecutive monthly negative samplings of 30 serum samples, tested by ELISA or 30 tracheal swabs, tested by PCR, from naive replacement gilts after a minimum of 120 days post entry, to allow sufficient time for *Mhp* to be detected if still present in the herd (Figure 1).

- Provisional negative vaccinated (IIIB): Herds in this subcategory have completed a whole herd *Mhp* elimination program and have fulfilled the diagnostic requirements for subcategory IIIA but continue to vaccinate naive breeding females for *Mhp* or b) herds that have been stocked negative but implement *Mhp* vaccination. Herds may decide to continue vaccinating and remain in Category IIIB indefinitely. Clinical signs and lesions suggestive of *Mhp* in the breeding herd would trigger a diagnostic investigation (Figure 1).

Negative (IV): In these herds, the agent is not detected in any type of sample, the population is serologically negative. Herds undergoing elimination efforts should have been category IIIA and the breeding herd has been completely rolled over to fall into category IV. Newly established *Mhp*-negative herds and those that went through complete depopulation and repopulation efforts fall within category IV. To maintain negative status, a minimum of 30 monthly negative ELISA results from various parity sows should be obtained (Figure 1).

Discussion

Standardized nomenclature and a simple classification system are fundamental for *Mhp* management and will 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 by swine systems to understand their health baseline status and set realistic goals for control or elimination. At the industry level, this classification could lead to the establishment and success of future *Mhp* regional elimination efforts. From a business perspective, contractual arrangements could include premiums for weaned pigs from Category II, III or IV breeding herds. Furthermore, sustained utilization of this classification by the industry will allow for the identification of areas of improvement and knowledge gaps that warrant novel research.

Table 1. *Mycoplasma hyopneumoniae* breeding herd-status classification criteria.

Herd category		Criteria		Description and diagnostic recommendations
		Agent detection in respiratory tract	Antibody detection in serum	
Positive uncontrolled (I)		Positive	Positive	*Mhp* is detected within lesions, in the respiratory tract. Most herds will be serologically positive, while does 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. To be categorized as II, 4 consecutive negative monthly samplings of 30 tracheal swabs of P1 sows should be obtained.
Provisionally negative (III)	Unvaccinated (IIIA)	Negative	Positive	Herds that have completed a whole herd elimination program. 1. Two consecutive negative samplings of 60 tracheal swabs of last exposed population before introducing negative replacement gilts 2. Two consecutive monthly negative samplings of 30 serum samples or 30 tracheal swabs from negative replacement gilts after a minimum of 120 days post entry.

Vaccinated (IIIB)	Negative	Positive	Herds that have completed an elimination and have satisfied diagnostic criteria for IIIA but continue to use vaccination or herds that have been stocked negative but decide to implement *Mhp* vaccination. Clinical signs and lesions suggestive of a would trigger a diagnostic investigation.
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 results from various parity sows should be obtained.

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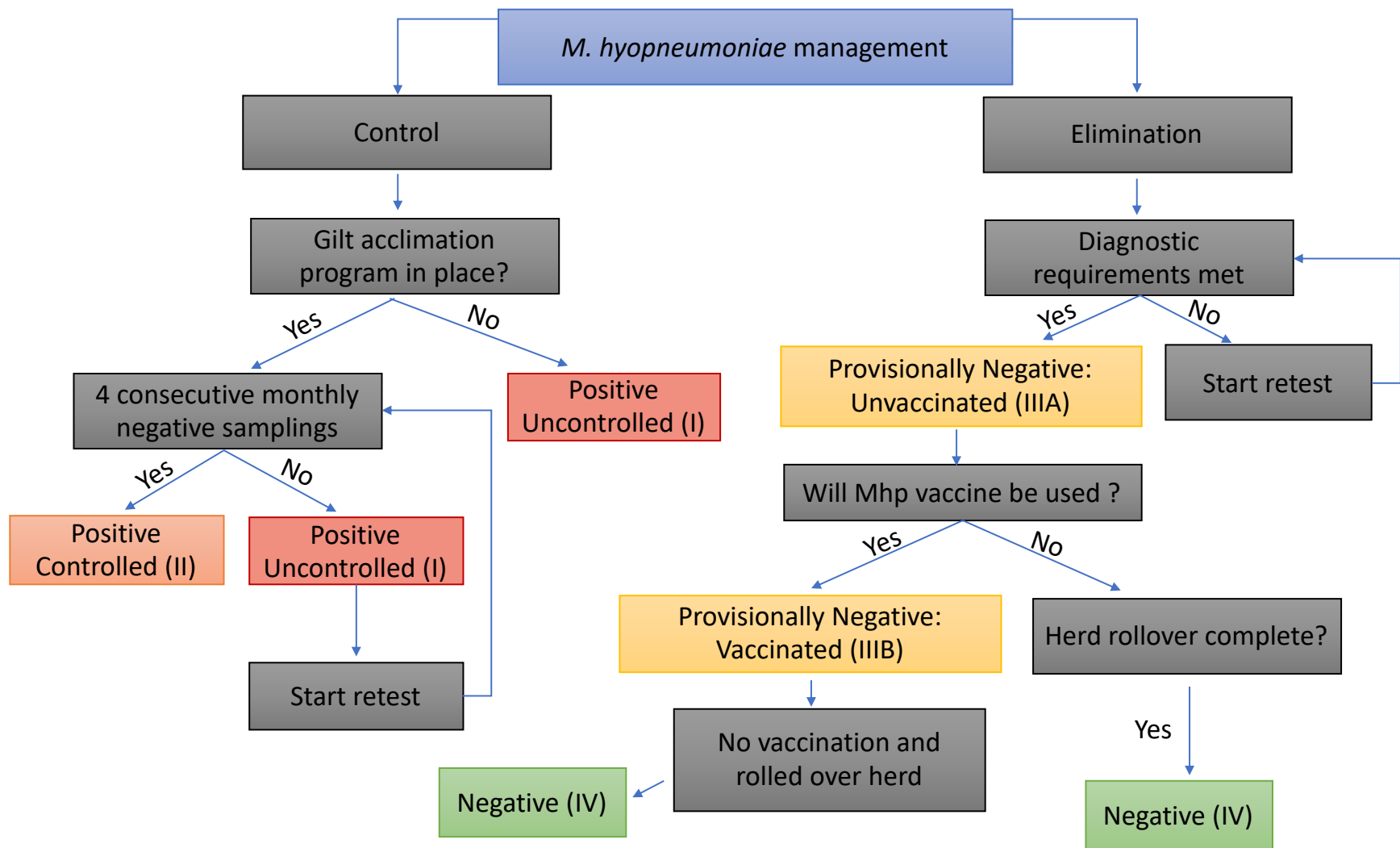
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