Diagnosis of *Mycoplasma hyopneumoniae*

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Mycoplasma hyopneumoniae lacks a cell wall, has a very small amount of genetic material, and is one of the smallest bacteria in nature. This organism is ubiquitous within swine herds throughout the world. By itself, *M hyopneumoniae* is minimally pathogenic; however, when it infects pigs concurrently with other bacteria and viruses, it remains one of the most problematic organisms to the swine industry. It is recognized as the causative agent of enzootic pneumonia and is a primary contributor to the porcine respiratory disease complex.[1] Concurrent infection with *M hyopneumoniae* and viral pathogens, including porcine reproductive and respiratory syndrome virus (PRRSV) or porcine circovirus type 2 (PCV2), increases the severity and duration of mycoplasmal pneumonia.[2,3] Because of the importance of *M hyopneumoniae* in respiratory disease, eradication is often a goal, making accurate diagnosis of the organism important.

It is crucial when considering diagnostic assays to decide whether you are interested in assessing the timing of infection with *M hyopneumoniae* and its role in disease, or determining herd status, ie, whether or not the herd is free of *M hyopneumoniae*. Differences in the two goals will determine which diagnostic assays should be performed and how extensive the testing must be.

The pathogenesis of disease due to *M hyopneumoniae* makes accurate diagnosis of the organism challenging. *Mycoplasma hyopneumoniae* is a mucosal pathogen that attaches to the epithelial cilia of the pig's lower airways, causing clumping and loss of cilia and epithelial cell death, which results in diminished function of the mucociliary apparatus.[4,5] In addition to the physical impact of the organism on the defense mechanisms of the respiratory tract, *M hyopneumoniae* also alters the host immune response. A number of *Mycoplasma* species, including *Mycoplasma hyorhinis,* are able to evade immune clearance by varying expression of surface proteins at the gene level. This is accomplished either by turning genes on and off or by changing the length of surface proteins through the addition or subtraction of repeating genetic sequences.[6] While less is known about the variability of surface proteins in *M hyopneumoniae* compared to other *Mycoplasma* species, there is evidence that similar genetic alteration of surface proteins may occur.[7] A major component of mycoplasmal pneumonia is immunopathologic in nature, although the underlying immune and inflammatory mechanisms remain unknown. The ability of *M hyopneumoniae* to modulate the immune response makes diagnosis of mycoplasmosis difficult.

Clinical disease

Clinical disease induced by *M hyopneumoniae* infection results in a mild, dry, nonproductive cough, with onset 7 to 14 days postinfection.[8,9] Co-infection with other pathogens, especially *Pasteurella multocida,* PRRSV, or PCV2, may increase the severity of the disease associated with *M hyopneumoniae* infection.[2,10–12] The presence of fever usually indicates either concurrent infection with *M hyopneumoniae* and a secondary pathogen, or clinical disease associated with swine influenza virus (SIV), which may be difficult to differentiate without microscopic examination of lung tissue or isolation of the virus.[13]

Macroscopic and microscopic examination

Lesions associated with mycoplasmal pneumonia are chronic in nature. Macroscopically, *M hyopneumoniae* induces consolidation in the cranioventral lobes of the lungs. Lesions may range from deep purple to tan-gray in appearance, and, in the absence of secondary infections, tend to be focal and fairly well demarcated. Microscopically, lesions are characterized by peribronchiolar and perivascular infiltration of mononuclear cells, consisting of lymphocytes and monocytic cells. Interstitial pneumonia may also be observed, with the airways filled with cellular debris. Neither macroscopic nor microscopic lesions are specific for *M hyopneumoniae*, and other respiratory pathogens, including bacterial invaders and SIV, must be ruled out.

Culture

Culture and isolation are used to detect and identify most bacterial organisms. While culture is considered the "gold standard" for detecting *M hyopneumoniae*, isolation of the organism is difficult due to its requirement for specialized media and its slow growth properties, often requiring 4 to 8 weeks to grow to measurable levels.[14] Difficulty in culturing the organism is increased by the additional requirement for swine serum negative for antibodies to *M hyopneumoniae*. Due to the slow growth in culture, contamination by other swine mycoplasmas or bacteria may preclude the growth of *M hyopneumoniae*. All of these factors make isolation and growth of the organism expensive and difficult. Thus, culture is not recommended as a diagnostic technique. Failure to isolate the organism under field conditions should not be used to confirm or deny the presence of the organism within a herd.

Serology

Serology is the most common tool used to determine the presence or absence of an organism within a herd. However, as with most diagnostic assays associated with *M hyopneumoniae* infection, interpretation of serological results may be challenging.[15–18] Three ELISA assays are currently used in the United States for detecting serum antibodies to *M hyopneumoniae*. Indirect ELISA assays include the Tween-20 assay[19]

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and the HerdChek *Mycoplasma hyopneumoniae* ELISA assay (Idexx Laboratories, Westbrook, Maine). The DAKO *Mycoplasma hyopneumoniae* ELISA (DAKO Corporation, Carpenteria, California) is a blocking ELISA based on an internal protein that appears antigenic. A study assessing the predictive value of these three assays was recently performed in our laboratory.[15] All three assays had excellent specificity in recognizing samples negative for antibodies, and thus an extremely low rate of false-positive results, making the positive predictive value of the three tests high. However, the sensitivity of the three assays was low and ranged from 37 to 49%. This low sensitivity results in a high percentage of false-negatives and a low negative predictive value. Of the assays assessed, the DAKO ELISA was the most consistent in identifying infected pigs. Sorensen et al[20] also found high specificity and low sensitivity in *M hyopneumoniae* ELISA assays. In addition, recent research in our laboratory[17] found that the assays varied in their ability to detect antibodies induced in pigs experimentally infected with differing field isolates of *M hyopneumoniae*. The low sensitivity of these assays may be partly attributed to the fact that the organism colonizes the airways, resulting in minimal interaction with the systemic immune system and thus variable serological results. In addition, antigenic variation of *M hyopneumoniae* surface proteins results in variable antibody responses.[21] Variability in the induction of *M hyopneumoniae* serum antibodies makes interpretation of serologic results challenging, and the high percentage of false-negative results must be considered when the results of these assays are interpreted.

Antibody levels following vaccination with *M hyopneumoniae* bacterins may vary depending on the vaccine, the infection status of the pig, and the serological assay used.[15,22] No correlation between vaccine-induced serum antibody levels and protection from colonization and disease has been determined.[22,23] Concurrent infection with PRRSV,[12] SIV,[13] or PCV2[2] appears to enhance *M hyopneumoniae* antibody levels. However, while concurrent infection with PRRSV increases serum antibody levels in response to vaccination with *M hyopneumoniae*, mycoplasma vaccine efficacy is reduced.[24]

Polymerase chain reaction

Accurate detection of *M hyopneumoniae* has significantly increased with development of polymerase chain reaction (PCR) assays.[25,26] The various collection sites and potential uses of PCR to accurately detect infection have been investigated.[9,27–29] On the basis of these studies, lung tissue and bronchial washings appear to be among the most reliable collection sites, while detection of the organism in samples from the nasal cavity appears more variable. Polymerase chain reaction assays utilizing a single set of primers do not appear to be sensitive enough to accurately detect low numbers of *M hyopneumoniae*. Thus, nested PCR assays, utilizing two sets of primers and detecting as few as four to five organisms, are most commonly used by diagnostic laboratories. While detection of such low numbers of organisms may assist in detecting *M hyopneumoniae* on a herd basis, the potential for contamination of the environment with *M hyopneumoniae* may be problematic. It has been documented that PCR assays are capable of detecting the organism in the air of production units housing pigs infected with *M hyopneumoniae*.[30] In addition, recent research in our laboratory[31] has found that the ability to detect various *M hyopneumoniae* field isolates differed, and this could be attributed to genetic differences among the isolates. These results suggest that further research is required to determine the accuracy of detection of *M hyopneumoniae* by PCR under field conditions.

Fluorescent antibody and immunohistochemistry assays

Mycoplasma hyopneumoniae is usually detected in lung tissue either by fluorescent antibody (FA) or immunohistochemistry (IHC) assay.[2,32] In situ hybridization of fixed tissues has been used less frequently.[2,32–34] Frozen tissues are required to detect *M hyopneumoniae* antibodies by FA assay, making sample collection problematic in the field. As in situ hybridization and IHC assays can be performed on fixed tissues, these assays are more practical for samples collected on the farm. It is critical that the samples collected include airways with ciliated epithelial cells.

Conclusions

Accurate diagnosis of disease caused by *M hyopneumoniae* may be frustrating. Confirmation of negative herd status for the organism remains problematic in many cases. The presence of the organism alone is not always correlated with disease or pneumonia. However, if *M hyopneumoniae* is present and the herd is exhibiting clinical respiratory disease, it may be assumed that the organism is contributing to the pathology through either primary or secondary means. The development of PCR assays has greatly enhanced our ability to detect *M hyopneumoniae*. However, more research is required to determine the ability of the various assays to detect different *M hyopneumoniae* isolates. The rationale for use of the various diagnostic assays should be determined, as the assays required to establish appropriate timing of intervention strategies in a positive herd differ from those needed to confirm negative herd status. Serology alone would be a poor choice to confirm that a herd is negative for *M hyopneumoniae*, while PCR assays are usually not required to determine timing of vaccination or therapy, which should be based more on occurrence of clinical disease. Thus, the sensitivity and specificity of each of the assays must be considered both for accurate interpretation of the clinical signs and serological and PCR test results, and according to the information needed by the veterinarian and producer.

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