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# Using diagnostic tools to monitor *Mycoplasma hyopneumoniae* in the field

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## Introduction

During the last decade, many strategies have been developed to reduce respiratory disease in swine. However, these diseases still have a major economic impact worldwide.

*Mycoplasma hyopneumoniae* has been one of the most important pathogens, associated with both enzootic pneumonia and recently with the Porcine Respiratory Disease Complex (PRDC). These two syndromes are responsible for most of the respiratory disease that affects modern swine production, resulting in a large economic impact.

Although control of the disease is possible through vaccination, medication, or management, timing of these strategies is critical for their effectiveness.

## Diagnostic techniques for M. hyopneumoniae

Some new diagnostic techniques for *M. hyopneumoniae* have been developed in the last years, allowing us to understand more about this pathogen. There are, however, many unresolved questions.

Procedures used in diagnosing *M. hyopneumoniae* have included Giemsa-stained touch preparations, isolation and culture of the microorganism, evaluation of gross and microscopic lesions, serological tests, immunofluorescence, immunohistochemistry, and, finally, PCR and N-PCR

Diagnosis of *M. hyopneumoniae* has traditionally been difficult. The organism is very fastidious, so that isolation from infected sites is so difficult that it is rarely attempted and may require as long as one month for a positive result (2). On the other hand, if the microorganism is not isolated, it may still be present, either in low numbers or masked by other contaminating bacteria.

The lesions are characteristic, but not pathognomonic. They are usually considered sufficient evidence for the diagnosis of Mycoplasmal pneumonia. Reliance on pathology however, is a relatively insensitive diagnostic method. It has been reported that 19% of culture-positive lungs did not have gross or microscopic lesions (3). Also, other microorganisms (e.g., Aujesky's virus together with *Pasterella multocida*), can cause similar macroscopic lesions (4). Microscopic lesions are frequently used for diagnosis; however, it may be difficult to differentiate both the acute and the chronic forms of the disease from other inflammatory events in the lung (5).

Detection of Mycoplasma antigens by immunofluorescence or immunohistochemistry is routinely done. These are specific diagnostic methods but suffer from lack of sensitivity, especially when applied to post-mortem specimens. Immunofluorescence is performed on smears or frozen tissue sections, while the immunoperoxidase test can demonstrate organisms in fixed sections. These tests are effective only when adequate amounts of antigen are present, such as in the early stages of infection. In late stages of infection, the amount of mycoplasmal organisms decreases and the test usually gives a false negative result (6). Immunohistochemistry has two additional disadvantages; it is time consuming and expensive. Additionally, diagnostic antibodies are difficult to obtain and they are not available commercially. There are, therefore, important limitations to these commonly used direct visualization techniques. False negative results are of special concern, since they are frequent in both early and late phases of the disease. Another important consideration is that pathology and direct visualization require post-mortem samples, which makes them inadequate for monitoring, especially of healthy herds.

Detection of Mycoplasma antibodies by complement fixation (CF), indirect hemagglutination and enzyme-linked immunosorbent assay (ELISA) tests have all been used (6). The CF test is an inexpensive but low-sensitivity procedure which detects antibodies 2-3 weeks after inoculation. Studies have indicated that swine may become CF-negative during the latter periods of *M. hyopneumoniae* infection, while the microorganism can still be isolated (7). False negative results are quite common and even false positives may occur. The indirect hemagglutination test detects serum antibodies especially at the early stages of infection due to the higher capacity of IgM antibodies for agglutination. However, this test is relatively insensitive during the later phases of the disease. It is also technically cumbersome making it impractical as a field-test (7).

At present, the ELISA is considered the most useful serological test. It detects all classes of immunoglobulins, gives quantitatively measurable results, and is very sensitive. Multiple ELISA tests have been developed, ranging from those that use sodium dodecyl sulfate to obtain antigens, to those that used neutral detergent (Tween 20). Additionally, blocking ELISA against specific protein targets have been used in recent years (8).

However these serological tests have some limitations and are difficult to interpret. Serology detects the onset of seroconversion, not the onset of infection. Time to seroconversion after exposure to *M. hyopneumoniae* is quite variable (2-8 weeks) and not all animals seroconvert at the same time. It is been possible to observe seroconversion as late as 6–8 weeks post-infection (9). This makes interpretation of negative results difficult. Additionally, the ELISA does not give us information if the antibodies are the result of a natural infection or of vaccination.

## Development of PCR diagnosis for M. hyopneumoniae

Polymerase chain reaction (PCR) technology is ideally suited for *M. hyopneumoniae* diagnosis because it is rapid and specific, does not depend on viable bacteria, and can be done on live or dead animals. Several PCR tests to detect specifically *M. hyopneumoniae* have been described (10, 11, 12, 13, 14, 15). A conventional PCR (one step) technique has been reported to detect *M. hyopneumoniae* from lung samples and nasal swabs (13). However, this direct test has not been, until now, consistently able to detect the microorganism from nasal swabs. On the other hand, it works very well from samples taken at necropsy or from lung lavages, but these demands make the technique difficult to apply under field conditions. This is mayor drawback, since animals must be euthanized in order to establish a diagnostic, thereby limiting its use as a monitoring toll.

A nested PCR (N-PCR) was developed in order to detect the organism from nasal swabs collected from live animals (15). The technique has been extensively validated using cultures of related organism, and field material (nasal swabs and samples from euthanized animals). Additionally, N-PCR, in contrast to serology, shows a higher proportion of infected animals in the early stages of the disease and provides more accurate information on the infection dynamics (16). On the other hand, the N-PCR has the problem of false positives, which limits its use to a population test. Another mayor drawback of most PCR techniques is that they do not give information about the amount of microorganism, only if the animals are positive or negative. This is important, since it limits our ability to interpret a positive animal in terms of if it is infectious or not and, therefore, if it poses a risk to the population or not. Preliminary evidence from our laboratory suggests that nonclinical, serologically negative carriers of *M. hyopneumoniae* may exist.

There is a need for accurate PCR diagnostics using nasal samples from live animals. The N-PCR has fulfilled this need, but has shortcomings with false positives and lack of microbial quantification. We are presently quite advanced in developing both a semi-quantitative PCR and a one-step PCR diagnostic test. These tests will allow a more informed interpretation of monitoring profiles, while at the same time considerably reducing problems with false positives typical of nested techniques.

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