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Molecular diagnostics for mycoplasma

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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 is the primary agent of enzootic pneumonia, and it as been associated 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.

Diagnosis of *M. hyopneumoniae* has traditionally been difficult. Among the procedures routinely used for its diagnosis are the following:

- Isolation and culture of the microorganism
- Evaluation of gross and microscopic lesions
- Serological tests
- Immunofluorescence
- Immunohistochesmestry
- PCR and N-PCR

Mycoplasma hyopneumoniae is a very fastidious microorganism because of its culture requirements and extremely slow growth, which often result in overgrowth by other bacteria present in the respiratory tract of pigs (2). Additionally, the lesions are characteristic, but not pathognomonic (3, 4), and it has been reported that 19% of culture-positive lungs do not have gross or microscopic lesions (5).

Time to seroconversion after exposure to *M. hyopneumoniae* is quite variable (2-8 weeks) (6) and not all animals seroconvert at the same time. Serology detects the onset of seroconversion, not the onset of infection and does not give us information if the antibodies are the result of a natural infection or of vaccination. This makes interpretation of negative results difficult.

Detection of *M. hyopneumoniae* by conventional immunological methods is routinely done, but cross-reactions with *Mycoplasma flocculare* and *Mycoplasma hyorhinis* reduce the specificity of detection (7). Additionally, these methods suffer a lack of sensitivity, especially in late stages of infection (8).

Some new diagnostic techniques for *M. hyopneumoniae,* such as in situ hybridization (9) and PCR, have been developed in the last years, allowing us to understand more about this pathogen. There are, however, many unresolved questions.

N-PCR and *M. hyopneumoniae* diagnosis

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 specifically detect *M. hyopneumoniae* have been described (10, 11, 12, 13, 14, 15, 16, 17, 18, 19). A conventional PCR (one step) technique has been reported to detect *M. hyopneumoniae* from lung samples and nasal swabs (12). This technique works very well from samples taken at necropsy or from lung lavages, but it has not been consistently able to detect the microorganism from nasal swabs from live animals. This is mayor drawback, since animals must be euthanized in order to establish a diagnosis, thereby limiting its use as a monitoring tool.

In order to overcome this drawback, a nested PCR (N-PCR) able to detect the organism from nasal swabs collected from live animals was developed (16). The technique has been extensively validated using cultures of related organisms and field material (nasal swabs and samples from euthanized animals). Additionally, the N-PCR has been contrasted to serology and lesions, showing a higher proportion of infected animals in the early stages of the disease and providing more accurate information on the infection dynamics (20, 21).

N-PCR has multiple advantages compared to the other diagnostic test, especially because it can be used on live or dead animals, it is rapid and specific, it does not depend on viable bacteria, and it can be automated. Most importantly, it can give us an idea when the infection took place. On the other hand, the N-PCR has the problem of false positives, which limits its use to a population test. It does not differentiate if the bacteria are alive or not and

does not give information on the number of microorganisms involved.

All of this limits our ability to interpret positive results. Does a positive animal mean that it is shedding? Or it is just getting colonized? Or it is eliminating the bacteria from their body? Additionally, we don't know if it is infectious or not and, therefore, if it represents a risk to the population or not.

Preliminary evidence from our laboratory suggests that animals shed different quantities of mycoplasma, that may be this is a non-linear event. We have shown that it is possible to have nonclinical, serologically negative carriers of *M. hyopneumoniae*, and that N-PCR-negative animals can be colonized.

A recently developed TaqMan PCR gives us a more automated process, but sensitivity at this point is low.

Conclusions

The important question to answer is, What does a PCR result mean? Obviously, we need more than one sample to be able to obtain precise information of what it is going on in the farm and how the bacteria is moving.

There is a need for accurate diagnoses from live animals. The N-PCR has fulfilled this need, but it has shortcomings.

The use of a semi-quantitative PCR or a real-time PCR would allow a more informed interpretation for profile monitoring, while at the same time considerably reducing problems with false positives typical of nested techniques.

More research is needed in order to understand this microorganism.

References

1. Ross, R. F. Mycoplasmal Disease. In: Straw B, Ed. *Diseases of Swine*. 8th ed. Ames, IA: Iowa State University press: 1999-495-501
2. Friis N F. Some recommendations concerning primary isolation of *Mycoplasma suipneumoniae* and *Mycoplasma flocculare*. A survey. *Nordic Veterinary Medicine*. 1975; 27: 337-339
3. Fuentes M and Pijoan C. Pneumonia in pigs induced by intranasal challenge exposure with Pseudorabies virus and *Pasteurella multocida*. *Am J Vet Res*. 1987; 48:1446-1448.
4. Sorensen VP, Ahrens K, Barfod A. *Mycoplasma hyopneumoniae* infection in pigs: duration of the disease and evaluation of four diagnostic tests. *Vet Microbiol*. 1997; 54: 23-34.
5. Armstrong CH, Scheidt AB, Thacker HL, Runnels LJ, Freeman MJ. Evaluation of criteria for the postmortem diagnosis of mycoplasmal pneumonia of swine. *Can J Comp Med*. 1984; 48:278-281.
6. Sitjar M, Noyes EP, Simon X, Pijoan C. Relationshps among seroconversion to *Mycoplasma hyopneumoniae*, lung lesions, and production parameters in pigs. *Swine Health Prod*. 1996; 4:276-277.
7. Bolske G, Strandberg M, Bergstrom K, Johansson K. Species-specific antigen of *Mycoplasma hyopneumoniae* and cross-reaction with other porcine mycoplasmas. *Curr. Microbiol*. 1987; 15:233-239.
8. Amanfu W, Weng CN, Ross RF, Barnes H J. Diagnosis of mycoplasmal pneumonia of swine: sequential study by direct immunofluorescence. *Am J Vet Res*. 1984; 45(7):1349-1352.
9. Kwon D, Chae C. Detection and localization of *Mycoplasma hyopneumoniae* DNA in lungs from naturally infected pigs by in situ hybridization using a digoxigenin-labeled probe. *Vet Pathol*. 1999; 36:308-313.
10. Harasawa R, Koshimizu K, Takeda O, Uemori T, Asada K, Kato I. Detection of *Mycoplasma hyopneumoniae* by the polymerase chain reaction. *Mol Cell Probes*. 1991; 5: 103-109.
11. Stemke G, Phan R, Young T, Ross R. Differentiation of *Mycoplasma hyopneumoniae, M. flocculare,* and *M. hyorhinis* on the basis of amplification of a 16S rRNA gene sequence. *Am J Vet Res*. 1994; 55:81-84.
12. Mattsson JG, Bergstrom K, Wallgreen P, Johansson KE. Detection of *Mycoplasma hyopneumoniae* in nose swabs from pigs by in vitro amplification of the 16S rRNA gene. *J Clin Microbiol*. 1995; 33: 893-897.
13. Artiushin S, Stipkovits L and Minion FC. Development of polymerase chain reaction primers to detect *Mycoplasma hyopneumoniae*. *Mol Cell Probes*. 1993; 7: 381-385.
14. Blanchard B, Kobisch M, Bove JM, Saillard C. Polymerase chain reaction for *Mycoplasma hyopneumoniae* in tracheobronchiolar washings from pigs. *Mol Cell Probes*. 1996; 10: 15-22.
15. Stark, KD, Nicolet J, Frey J. Detection of *Mycoplasma hyopneumoniae* by air sampling with a nested PCR assay. *Appl Environ Microbiol*. 1998; 64:543-548.
16. Calsamiglia M, Pijoan C, Trigo A. Application of a nested polymerase chain reaction assay to detect *Mycoplasma hyopneumoniae* from nasal swabs. *J Vet Diagn Invest*. 1999; 11:246-251.
17. Caron J, Ouardani M, Dea S. Diagnosis and differentiation of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* infections in pigs by PCR amplification of the p36 and p46 genes. *J Clin Microbiol*. 2000; 38:1390-1396.
18. Verdin E, Saillard C, Labbe A, Bove JM, Kobisch M. A nested PCR assay for the detection of *Mycoplasma hyopneumoniae* in tracheobronchiolar washings from pigs. *Vet. Microbiol*. 2000; 76:31-40.
19. Verdin E, Kobisch M, Bove JM, Garnier M, Saillard C. Use of an internal control in a nested-PCR assay for *Mycoplasma hyopneumoniae* detection and quantification in tracheobronchiolar washing from pigs. *Mol Cell Probes*. 2000; 14:365-372.
20. Calsamiglia M, Pijoan C, Bosch G. Profiling *Mycoplasma hyopneumoniae* in farms using serology and a nested PCR technique. *Swine Health Prod*. 1999; 7(6): 263-268.
21. Calsamiglia M, Collins J, Pijoan C. Correlation between the presence of enzootic pneumonia lesions and the detection of *Mycoplasma hyopneumoniae* in bronchial swabs by PCR. *Vet Microbiol*. 2000; 76:299-303.