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PCR based diagnostics for profiling *Mycoplasma hyopneumoniae* shedding

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Introduction

Mycoplasma hyopneumoniae is the etiologic agent of enzootic pneumonia, a widespread disease that still affects a high percentage of the swine herds in the Midwestern US.

This agent does not contain cell wall, a feature that is responsible for its polymorphism, resistance to antibiotics that interfere with cell wall synthesis, and its sensitivity to environmental conditions and complement. Mycoplasma hyopneumoniae is not invasive; it is found on the surface of the respiratory epithelium, attached to its cili. This anatomical site is difficult to access by immune components, which partly explains the chronicity of the disease.

Traditional diagnostic tools

Knowledge of the epidemiology of Mycoplasma hyopneumoniae has been hindered by the poor diagnostic tools available for this microorganism. Culture of the organism is a cumbersome and difficult task as it grows very slowly and is commonly overgrown by M. hyorhinis. Doster and colleagues have documented that approximately 106 mycoplasmal cells are needed in a sample in order to successfully culture the microorganism.1 Immunofluorescence and immunohistochemistry are effective techniques in the early phases of the disease, as large amounts of mycoplasmal cells are needed in order to detect them.2 Slaughterhouse inspection is also a widely used technique used to diagnose enzootic pneumonia. Its drawbacks are that the etiologic agent is not detected, and that pigs affected at young ages show few lesions at slaughter.

Serology is the most widely used technique to diagnose mycoplasmosis. The ELISA tests are the most commonly used assays. Serology has the advantage in that it can be used to test live animals and serumprofiles can be performed in order to determine when the animals get infected. However, serology has its limitations; the main one being that seroconversion in natural infections is very delayed.

Several PCR assays have been described and shown to be very sensitive and specific to detect microorganisms from lung tissues. However, this one-step PCR technique is not sensitive enough to consistently detect the microorganism from nasal swabs, therefore necessitating that the pig be killed in order to achieve a diagnosis.

Nested PCR

Our group, however, has developed a nested PCR assay to detect M. hyopneumoniae from nasal swabs. This assay offers the obvious advantage of detecting the microorganism from live animals. The nested PCR consists of two PCR reactions, the first one with the outer primers, and the second one with the inner primers, which contain sequences found inside the first amplified reaction. Therefore, the assay is more sensitive and more specific than a one-step PCR.

The nested PCR assay can be used in the following ways in the swine industry:

- Diagnosis.
- Efficacy studies of antibiotics and vaccines.
- Monitoring of the breeding herd, to assess the carrier status of mycoplasma in both the incoming gilts and the sow herd. With this information, mycoplasma outbreaks in incoming naive gilts or the naive herd could be prevented. The nested PCR would also be useful in assessing the status of *Mycoplasma hyopneumoniae* presence in SPF or mycoplasma-free herds.
- Bacteriumprofiles to determine moment of infection. As mentioned above, serology is used to predict the age the animals get infected. However, this is not very accurate in the case of *M. hyopneumoniae* since seroconversion is delayed and varies with infective dose, management factors, and the test utilized. In general, seroconversion in field conditions is delayed up to 6–8 weeks post-infection. This means that, in late infections (18-week wall), the animals can go to slaughter while still seronegative. Using the nested PCR, the microorganism—not the antibodies—is detected. Dynamics of infection can be assessed, and more precise information regarding the moment of infection can be obtained. Potentially, more accurate decisions regarding the appropriate timing of medi-

Figure 1

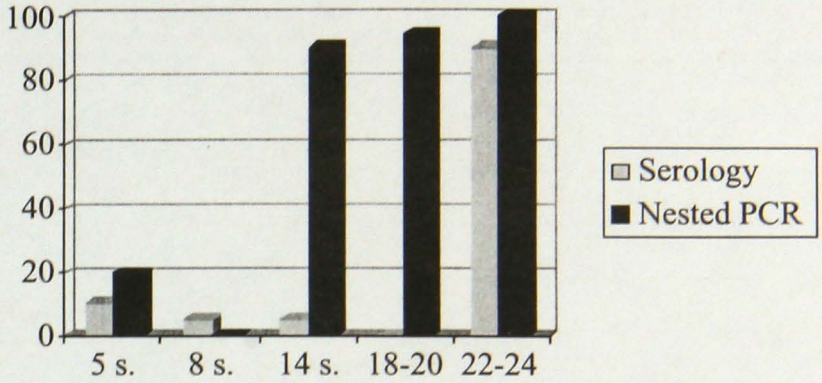
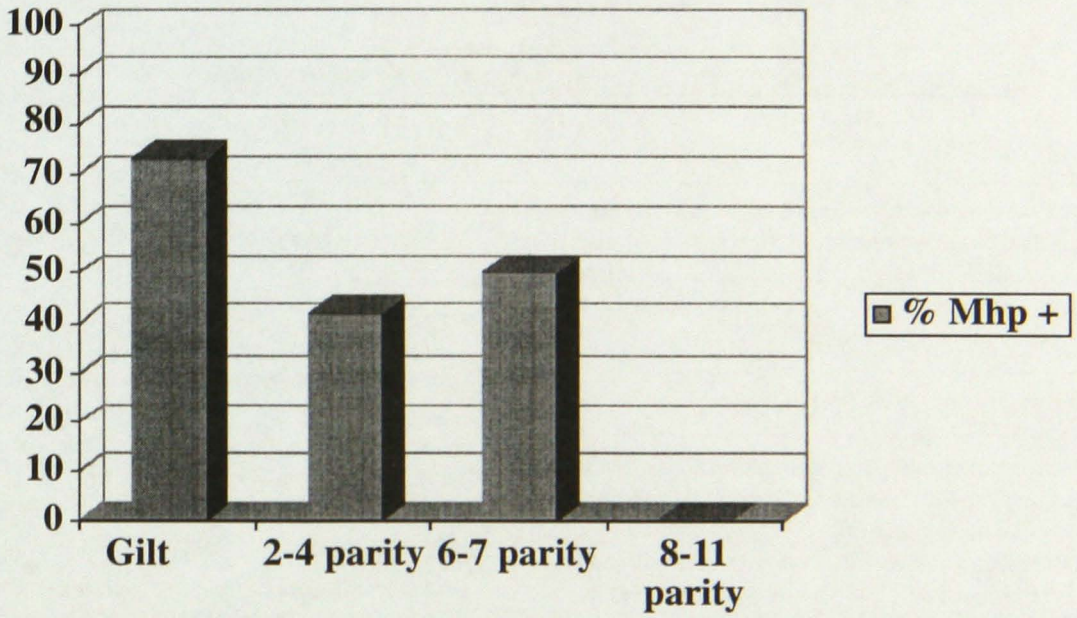


Figure 2

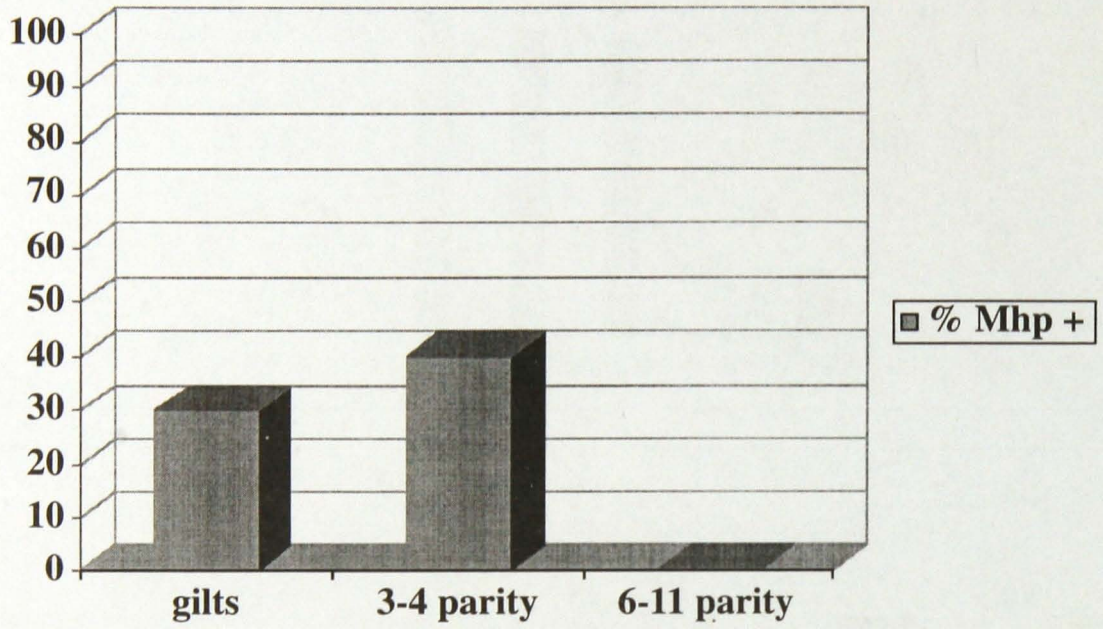


cations, vaccinations, or management strategies could be made. An example is shown in **Figure 1**, where ten pigs from each age group were nasally sampled and the nested PCR performed. Also, blood was drawn from these age groups to measure antibody titers against *Mycoplasma hyopneumoniae*.

In this case, nurseries were Mhp-negative, both by PCR and serology. In the finishing, a large percentage of animals were PCR-positive in all the ages tested, but seroconversion did not occur until 22–24 weeks of age. This indicates that the delay infection-seroconversion was eight weeks. The finishing barns were all-in/all-out by rooms, and the microorganism was probably recirculating between rooms, since the animals were clean in the nurseries. Measures could be taken to minimize transmission between rooms. A medication protocol at entry of the finishing or vaccination in the nurseries (four weeks before moving them to the finishers) could be established. Only by knowing when infection occurs can one address when and which strategies will be most successful.

- Transmission studies. The transmission routes of *Mycoplasma hyopneumoniae* are still not clear. Direct contact seems to be the most common way—if not the only one—by which this microorganism is transmitted. The source of transmission varies depending on the production system. In conventional herds with continuous flow systems, the main source of transmission of *Mycoplasma hyopneumoniae* is from the older pigs to the younger pigs coming into a room. With the introduction of all-in/all-out management practices, horizontal transmission between different age groups is minimized, and the main source of infection of a group of pigs is probably the mother. When and which sows transmit the microorganism is not known. It has been proposed that young sows have

Figure 3



more shedding of the microorganism and transfer less passive immunity than older sows. However, this has not been demonstrated. In this study we wanted to establish if there is a parity distribution in the shedding of the microorganism.

Materials and Methods

A 2,000 sow herd with a three site production system was visited two times. The first time, there was an outbreak of mycoplasmosis in the nurseries. At the same time, a vaccination program for the gilts was just beginning. Nasal swabs of 16 vaccinated gilts, 10 non-vaccinated gilts, and 24 sows of parities 2–11 were taken and the nested PCR was performed (**Figure 2**).

Twelve of 16 vaccinated gilts were positive, whereas 7 of 10 of the non-vaccinated gilts were found positive. Therefore, no differences between vaccinated and non-vaccinated gilts were found.

Three months later, the farm was visited for the second time. The mycoplasmosis outbreak had moved from the nurseries to the finishing barns. Meanwhile, the gilt vaccination protocol was still in place. Nine gilts, thirteen 2–4 parity sows, and eight 6–8 parity sows were sampled (**Figure 3**).

Conclusions

From these results we concluded:

- There is a relationship between parity and Mhp shedding, but the results suggest that sows shed during very long periods of time, up to sixth parity.
- The nested PCR is not quantitative. There may be differences between the amount of shedding among different parity groups which would not have been detected with this test. A way around this problem might be to perform serial dilutions of the samples and run the nested PCR, but this has not yet been investigated.
- In the second visit, a lower proportion of carrier animals in the sow herd was observed, and this was accompanied by a delay in the presentation of the disease. This is in agreement with our hypothesis that delayed presentation of the disease is associated with a lower percentage of colonized animals at weaning. Low pressure of infection causes the organisms to spread very slowly, reaching the sufficient infection level for an outbreak to occur in the late finishing stages.

References

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2. Amanfu W, Weng CN, Ross RF, et. al.: 1984. Diagnosis of mycoplasmal pneumonia of swine: sequential study by direct immunofluorescence. *Am J Vet Res* 45:1349–1352.