

The Relationship Between In Vitro Adherence Capability and Pathogenicity of *Mycoplasma hyopneumoniae* for Swine

Richard F. Ross, professor, College of Veterinary Medicine; Barbara Z. Erickson, research technician; and Theresa F. Young, assistant scientist, Veterinary Medical Research Institute

ASL-R1408

Summary and Implications

The data indicated a positive correlation exists between in vitro adherence capability and in vivo pathogenicity of *M. hyopneumoniae* clones. Results obtained with the in vitro microtiter plate adherence assay indicated that it can be used to estimate the pathogenicity of strains. However, low adherence capability and possibly pathogenicity were not stable as indicated by the shifting adherence capability of *M. hyopneumoniae* during in vivo growth. In this study, the in vivo environment apparently favored development of a population of more adherent *M. hyopneumoniae*. Further research to identify immunogenic, protective proteins involved in this phenomenon could enable development of improved *M. hyopneumoniae* vaccines.

Introduction

Mycoplasma hyopneumoniae, the cause of mycoplasmal pneumonia of swine (MPS), continues to be a major contributor to poor performance in grow-finish swine and a predisposing agent in death losses caused by a variety of secondary bacterial infections.¹ Lack of knowledge of the complex process used by mycoplasma causing MPS results in inadequate products and approaches to control the disease in swine. Identification, detection and purification of specific components of the mycoplasma which are involved in adherence or cause cell damage in the respiratory tract should provide a basis for development of better vaccines. Single colonies of pathogenic *M. hyopneumoniae* strain 232 were previously isolated and characterized in our laboratory by in vitro microtiter plate adherence assay (MPAA),² SDS-PAGE, and immunoblotting. Results indicated that *M. hyopneumoniae* contained a mixed population of cells with various adherence capabilities. High- or low-adherent clones can be identified by SDS-PAGE or immunoblotting profiles. Convalescent serum reacted strongly with a 145K antigen of the high-adherent clone and weakly with the low-adherent clone, and a high MW (>200K) antigen was found only in the low-adherent clone. However, the validation of the in vitro adherence differences among the clones from the same strain of *M. hyopneumoniae* had not been confirmed with in vivo challenge experiments. Thus, the present study was designed to determine the relationship between the in vitro adherence capability and in vivo pathogenicity of one low-adherent and one high-adherent clone isolated from *M. hyopneumoniae* strain 232.

Materials and Methods

Single colonies from *M. hyopneumoniae* pathogenic strain 232, picked from an agar-grown culture and tested in the MPAA, ranged from poorly adherent to swine cilia to strongly adherent. To evaluate the pathogenicity in pigs of low- and high-adherent clones of *M. hyopneumoniae*, four groups of disease-free pigs were challenged by intratracheal inoculation with one of four inocula: a low-adherent clone of pathogenic strain 232 (clone 60-2), a high-adherent clone of pathogenic strain 232 (clone 91-1), *M. hyopneumoniae*-infected lung homogenate, or medium (Table 1). Utilizing the in vitro MPAA, adherence activity of the low-adherent pig inoculum was approximately 13% of the activity of the high-adherent pig inoculum. The pigs were assessed daily postinoculation for clinical signs of pneumonia. At necropsy 27 or 28 days postinoculation the lungs were examined for lesions, and samples were collected for isolation of mycoplasmas and for determination of colonization by immunofluorescence.³ Ten individual colonies were picked from agar grown reisolates from each of two pigs in the *M. hyopneumoniae*-inoculated groups (groups 2, 3, & 4) and inoculated into mycoplasmal medium. The adherence ability of reisolates was compared with that of the high- and low-adherent parent clones using the MPAA. Protein profiles of the *M. hyopneumoniae* reisolates were analyzed and compared with the parent clones using SDS-PAGE and immunoblotting.⁴

Results and Discussion

Clinically, there were no significant differences between the infected groups in coughing scores as repeated measurements over time. However, the mean time of onset of coughing was significantly different between groups (Figure 1). Mean time of onset of coughing for the groups was: day 11.6 for the pigs in group 3 which received the high-adherent clone; day 15.1 for the pigs in group 4 which received the lung homogenate; and day 25.0 for the pigs in

Table 1. Experimental design.

Group	No. of Pigs	Inoculum
1	8	Medium
2	7	Low-adherent clone (60-2)
3	7	High-adherent clone (91-1)
4	7	Lung homogenate

group 2 which received the low-adherent clone. Four of the seven pigs in group 2 were never observed coughing. The pigs in group 1 which received medium only were never observed coughing during the experimental period. Necropsy 27 or 28 days postinoculation indicated that all of the pigs from the three *M. hyopneumoniae*-inoculated groups had lesions of MPS, and mycoplasmas were isolated and identified in all of their lungs. Pigs receiving medium only were negative for lesions and mycoplasmas in the lungs. Pigs receiving the low-adherent clone had significantly less lobes with pneumonia per pig and significantly lower immunofluorescence scores in comparison to pigs in the other two *M. hyopneumoniae*-inoculated groups.

Utilizing the MPAA, in vitro adherence in all of the subcloned reisolates was enhanced after pig passage; however, the average adherence of the subcloned reisolates from the low-adherent group was still the lowest among the three *M. hyopneumoniae*-inoculated groups (Table 2). Only one of the 20 subclones from the low-adherent reisolates had low adherence, with the other 19 subclones being moderately to strongly adherent.

The protein profiles of the reisolates from each of the three *M. hyopneumoniae*-inoculated groups were all identical to that of the high-adherent parent clone, with the exception of the one low-adherent subclone which was found to be similar, but not identical, to the low-adherent parent clone.

The shifting adherence capability of *M. hyopneumoniae* during in vivo growth was evidence that the low adherence capability was not stable. Possibly this phenomenon relates to the high frequency switching of surface antigens (including those involved in adherence) reported previously in other mycoplasma species. Apparently, the in vivo environment supported development of a population of more adherent *M. hyopneumoniae*. Further investigation to identify immunogenic, protective proteins involved in this phenomenon could permit development of improved *M. hyopneumoniae* vaccines.

References

- ¹Ross, R.F. (1992) *In: Diseases of Swine*, 7th ed., pp. 537-543.
- ²Zhang, Q., Young, T.F., and Ross, R.F. (1994) *Infection and Immunity* 62:1616-1622.
- ³Amanfu, W., Weng, C.N., Ross, R.F., and Barnes, H.J. (1984) *American Journal of Veterinary Research* 45:1349-1352.
- ⁴Young, T.F. and Ross, R.F. (1987) *American Journal of Veterinary Research* 48:651-656.

Figure 1. Mean day of onset of coughing. Means with different superscripts are significantly different ($P < .05$). Group 2 - pigs inoculated with a low-adherent clone of *M. hyopneumoniae*; group 3 - pigs inoculated with a high-adherent clone of *M. hyopneumoniae*; group 4 - pigs inoculated with *M. hyopneumoniae* lung homogenate. Four of the seven pigs in group 2 were never observed coughing. The pigs in group 1 which received medium only were never observed coughing during the 27-day observation period.

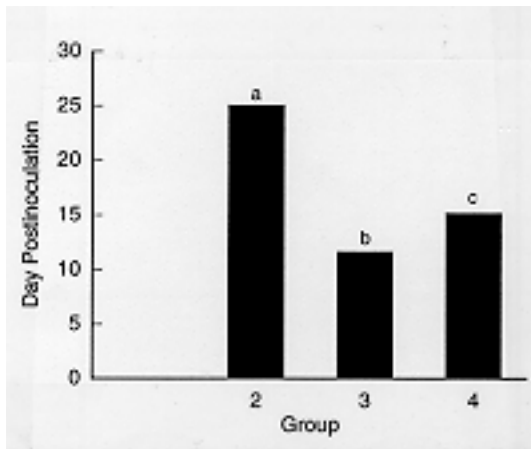


Table 2. Mean % adherence of single colony isolates from pig-passaged mycoplasmas.^a

Group	Inoculum	No. of Colonies Tested	Mean % Adherence \pm S.D.
2	Low-adherent clone (60-2)	20	78 \pm 18 ^b
3	High-adherent clone (91-1)	20	115 \pm 18 ^d
4	Lung homogenate	19	93 \pm 22 ^c

^aControls: Mean % adherence was 100 for the high-adherent parent clone and 18 \pm 4 for the low-adherent parent clone.

^{b,c,d}Means \pm S.D. with different superscripts are significantly different ($P < .05$).