Evaluation of *Mycoplasma hyopneumoniae* Inactivated Vaccine in Pigs under Field Conditions

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ABSTRACT. An inactivated vaccine prepared from broth culture supernatant of *Mycoplasma hyopneumoniae* with an aluminum adjuvant was evaluated in three herds (herd A: specific pathogen-free herd, herd B: high health status herd with no clinical signs of respiratory infection, herd C: low health status herd with serious epidemiological and economical problems). A total of 212 pigs from the three herds were divided into two groups. One group was injected twice with the vaccine at 4-week intervals and the other was a control group. No adverse reactions were noted following the vaccinations either systematically or locally in any of the vaccinated pigs from any of the herds. In herd A, the vaccination provided antibody response within 4 weeks after the second vaccination and antibody responses continued for more than 12 weeks. In herds B and C, the number of pigs with lung lesions, mean percentage of lung lesions, and the numbers of *M. hyopneumoniae* recovered from pigs at slaughter in the vaccinated group were significantly ($P<0.05$) reduced compared to the control group. Furthermore, vaccination resulted in improved average daily weight gain (ADG), improved feed conversion ratio (FCR), and improved days to market weight in herd C, whereas no difference in growth performance was shown in herd B. It is suggested that the inactivated vaccine prepared from broth culture supernatant of *M. hyopneumoniae* is effective in reducing clinical signs and lung lesions. Also, vaccination resulted in improved growth performance in herds where clinical signs and economic losses were significant.—
KEY WORDS: field trial, *Mycoplasma hyopneumoniae*, mycoplasmal pneumonia of swine, swine, vaccine.

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Mycoplasmal pneumonia of swine (MPS), caused by *Mycoplasma hyopneumoniae*, is one of the most common and economically important diseases of swine [14]. The primary mycoplasmal infection often becomes complicated by secondary bacterial infections of *Pasteurella multocida* [4] and *Actinobacillus pleuropneumoniae* [20], resulting in more severe lung lesions and production losses, such as retarded growth and poor feed conversion. Decreased production costs and improved growth performance are important for pig producers. Therefore, the control of MPS is critical for reducing economic losses in any swine operation [13]. Antibiotic therapies, repopulation with specific pathogen-free (SPF) pigs, and improved management have been used for many years to control MPS [1, 7].

Active immunization of pigs has been recommended for preventing MPS [6]. Recently, vaccination has been shown experimentally to induce protection against *M. hyopneumoniae* [9, 15], and vaccines have been used to control naturally occurring MPS [5]. These vaccines have been accomplished by the use of mainly whole cells of *M. hyopneumoniae* inactivated bacterin. In Japan, a formalin inactivated culture supernatant vaccine for MPS has been approved and is commercially available for reducing lung lesions in endemic herds. The purpose of this study was to evaluate the effects of the vaccine against *M. hyopneumoniae* on clinical parameters and on the productive performances of pigs from different types of herds.

MATERIALS AND METHODS

Herds: Three herds were selected for the trial on the basis of clinical respiratory status. Herd A was located in Shizuoka prefecture and was an SPF herd. Herd B, located in Hokkaido, was a high health status herd, with a previous history of only *M. hyopneumoniae*, *P. multocida* capsular type A and *H. parasuis* related respiratory diseases, but there was no clinical signs of respiratory infection. Herd C was located in Kagoshima prefecture and was a low health status herd with a history of *M. hyopneumoniae*, *P. multocida* capsular type A, *Actinobacillus pleuropneumoniae*, porcine reproductive and respiratory syndrome virus and pseudorabies virus, and there was serious associated epidemiological and economical problems.

Vaccine: The vaccine tested in this study was marketed as Mycobuster (Scientific Feed Laboratory Co., Ltd., Tokyo, Japan), and was an inactivated vaccine prepared from broth culture supernatant of *M. hyopneumoniae* 1986–1–1 strain with an aluminum hydroxide adjuvant.

Experimental design: A total of 212 pigs (herd A: 56 Large White or Duroc pigs, herd B: 94 Large White × Landrace × Duroc pigs, and herd C: 62 Large White × Landrace pigs) were used in this experiment. Pigs of each herd were randomly assigned to a two-treatment group. Pigs in the vaccinated group were injected intramuscularly twice with 2 m*l* of the vaccine. The first vaccination was given at

3 to 7 weeks of age and repeated 4 weeks later. Pigs in the control group were served as unvaccinated controls. No medical treatment was given for pneumonia during the study.

Clinical observations: The pigs were observed daily for reaction to the vaccination, by examining clinical signs of pneumonia and other abnormalities. In order to assess growth performance, all animals were weighed at monthly intervals to determine average daily weight gain (ADG), and the total feed intake on a pen basis was recorded at monthly intervals to determine feed conversion ratio (FCR).

Serological tests: Blood samples were collected from all pigs at regular time intervals. Serum antibody titers against *M. hyopneumoniae* were measured using a complement fixation (CF) test. The CF test was performed by the method described previously [11] with SEP-CF antigen (Scientific Feed Laboratory Co., Ltd., Tokyo, Japan).

Pathological investigations: At the end of this trial, all pigs were slaughtered at market weight (herd A: 95 kg, herd B: 90 kg, and herd C: 110 kg) and the day to market weight was recorded. Lung lesions from all slaughtered pigs were examined using an image processor (Qube 600, Nexus Co., Ltd., Tokyo) as previously described [3]. Samples from lung lesions of all pigs were collected for bacteriological examination. In pigs having no lesions, samples were taken routinely from apical lobes of the right lung.

Isolation of organisms: Mycoplasma cultures were performed from samples with lung lesions, which were homogenated to make a 10% suspension (w/v) in a BHL medium [21] as previously described [16]. Cultures were identified as *M. hyopneumoniae* by a metabolism inhibition test [19]. The samples from lung lesions were cultured for *P. multocida*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, and other bacteria using Tryptic Soy (TS) agar (Difco Lab., Detroit, MI., U.S.A.) containing 10% sheep blood, TS agar (Difco) containing 100 μg/m*l* β-NAD, 5% horse serum and 5% fresh yeast extract, and DS agar (Difco) containing 0.1 μg/m*l* of gentamicin and 30 μg/m*l* of vancomycin, incubated at 37°C and examined 24 and 48 hr later. Suspected colonies were identified by using conventional biochemical tests [12, 17].

Statistical analysis: CF antibody titer, the numbers of *M. hyopneumoniae* recovered, extent of lung lesions, the day to market weight, and ADG among the groups within herds were statistically compared by Student's *t*-test.

RESULTS

Clinical signs: No adverse reactions were noted following the vaccinations, either systematically or locally in any of the pigs vaccinated in any of the herds. No clinical signs of pneumonia, such as serious coughing, were observed in any of the pigs of herds A or B throughout the course of the trial. In herd C, a serious cough was noted in one pig of the vaccinated group and 4 pigs of the control group. Four of the 32 pigs in the control group of herd C died due to *A. pleuropneumoniae* infection. There were no deaths due to respiratory diseases, but two of the 30 pigs died from anal prolapse in the vaccinated group of herd C.

Serological tests: All pigs in the vaccinated groups of herds A, B, and C were seroconverted by 4 weeks after the second vaccination. They all developed CF antibody titers ranging from 1:4 to 1:16, and geometric (GM) antibody titers were 5.9 ± 0.2, 6.3± 0.2, and 5.5 ± 0.2, respectively. In herd A, serum antibody titers lasted until 16 weeks after the second vaccination in the vaccinated group, but no detectable levels of antibodies were found in any of the serum samples collected from the control group. On the other hand, in herds B and C, antibody titers of the vaccinated group increased slowly and antibody responses were detected 8 weeks after second vaccination in all pigs of the control group. There were significant differences ($P<0.05$) between the vaccinated group and the control group at 4 weeks after the second vaccination in herd B and from 4 to 8 weeks after the second vaccination in herd C (Table 1).

Pathological investigations: In herd A, no pigs from either group showed any lung lesions at slaughter. In herd B, lung lesions from MPS were observed in 22 of the 57 pigs (38.6%) of the vaccinated group and in 24 of the 37

Table 1. Complement fixation antibody titers against *M. hyopneumoniae*

Herd	Group	No. of pigs	Complement fixation antibody titer					
			0[a)]	4	8	12	18	20
A	Vaccinated	29	2.0 ± 0.0[b)]	2.0 ± 0.0	5.9 ± 0.2*	8.6 ± 0.2*	NT[c)]	9.5 ± 0.2*
	Control	27	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	NT	2.0 ± 0.0
B	Vaccinated	57	2.0 ± 0.0	2.3 ± 0.2	6.3 ± 0.2*	10.1 ± 0.2	NT	21.2 ± 0.3
	Control	37	2.0 ± 0.0	2.2 ± 0.2	2.7 ± 0.3	8.2 ± 0.4	NT	18.2 ± 0.4
C	Vaccinated	28	2.1 ± 0.1	2.3 ± 0.2	5.5 ± 0.2*	13.5 ± 0.3*	22.4 ± 0.4	NT
	Control	28	2.1 ± 0.1	2.3 ± 0.1	2.7 ± 0.2	7.8 ± 0.4	21.5 ± 0.5	NT

a) Weeks after first vaccination.
b) Values represent mean ± standard deviation. Antibody titer lower than 1:4 was converted to 2.0.
c) Not tested.
* Significantly different compared with control group in each herd ($P<0.05$).

Table 2. The prevalence and mean percentage of lung lesions at slaughter

Herd	Group	No. of pigs	Lung lesions		
			No. of pigs positive	Mean percentage of positives (mean ± SD)	Reduction rate[a)] (%)
A	Vaccinated	29	0 (0%)	–	–
	Control	27	0 (0%)	–	
B	Vaccinated	57	22 (38.6%)	0.6 ± 1.4*	86.1
	Control	37	24 (64.9%)	4.4 ± 6.3	
C	Vaccinated	28	15 (53.6%)	1.8 ± 3.7*	79.0
	Control	28	28 (100%)	8.4 ± 9.3	

a) Reduction rate in lung lesions compared to control.
* Significantly different compared with control group in each herd ($P<0.05$).

Table 3. Isolation of organisms from the lung specimens at slaughter

Herd	Group	No. of pigs	*M. hyopneumoniae*		*M. hyorhinis*	*P. multocida*	*A. pleuro-pneumoniae*	*H. parasuis*
			No. of pigs positive	Mean titer of positives[a)]				
A	Vaccianted	29	0		0[b)]	0	0	0
	Control	27	0		0	0	0	0
B	Vaccinated	57	28 (49.1%)*	1.7 ± 1.9[c)]	0	1 (1.8%)	0	1 (1.8%)
	Control	37	32 (86.5%)	4.0 ± 2.2	0	8 (21.6%)	0	3 (5.4%)
C	Vaccinated	28	24 (85.7%)	1.8 ± 1.3*	2 (7.1%)	0	1 (3.6%)	0
	Control	28	25 (89.3%)	3.5 ± 1.6	3 (10.7%)	3 (10.7%)	0	0

a) Log CCU/0.2 m*l*.
b) No. of pigs positive.
c) Values represent mean ± standard deviation.
* Significantly different compared with control group in each herd ($P<0.05$).

pigs (64.9%) of the control group. Mean percentage of lung lesions in the vaccinated group (0.6 ± 1.4%) was significantly ($P<0.05$) lower at a reduction of 86.1%, when compared with the control group (4.4 ± 6.3%). In herd C, lung lesions from MPS were observed in all pigs of the control group, but were also observed in 15 of the 28 pigs (53.6%) of the vaccinated group. Mean percentage of lung lesions in the vaccinated group (1.8 ± 3.7%) was significantly ($P<0.05$) lower at a reduction of 79.0%, when compared with the control group (8.4 ± 9.3%) (Table 2). Pleuritis and abscesses were found in almost all pigs in herd C and no significant differences were found among the two groups.

Isolation of organisms: In herd A, *M. hyopneumoniae*, *M. hyorhinis*, *P. multocida*, *A. pleuropneumoniae*, and *H. parasuis* were not isolated from the lungs of any pigs. In herd B, *M. hyopneumoniae* was isolated from 28 (49.1%) and 32 (86.5%) of the vaccinated and control pigs, respectively. The difference in the numbers of *M. hyopneumoniae* isolated from lungs between the vaccinated group ($10^{1.7}$ color-changing units (CCU) /0.2 m*l*) and control group ($10^{4.0}$ CCU/0.2 m*l*) was significant ($P<0.05$). *P. multocida* was isolated from 1 pig (1.8%) in the vaccinated group and 8 pigs (21.6%) in the control group. *H. parasuis* was isolated from 1 pig (1.8%) in the vaccinated group and 3 pigs (5.4%) in the control group. *M. hyorhinis* and *A. pleuropneumoniae* were not isolated from any pig in either group. In herd C, *M. hyopneumoniae* was isolated from 24 (85.7%) and 25 (89.3%) of the vaccinated and control pigs, respectively. The difference in the numbers of *M. hyopneumoniae* isolated from lungs between the vaccinated group ($10^{1.8}$ CCU/0.2 m*l*) and control group ($10^{3.5}$ CCU/0.2 m*l*) was significant ($P<0.05$). *M. hyorhinis* was isolated from a few pigs in each group. *P. multocida* was isolated from 3 pigs (10.7%) in the control group, but was not isolated from any pig of the vaccinated group. *A. pleuropneumoniae* was isolated from only one pig in the vaccinated group and was identified to be serotype 2. *H. parasuis* was not found from any pig in either group (Table 3).

Growth performance: In herds A and B, no statistical differences for ADG and the day to market weight were shown between the vaccinated and control groups. On the other hand, in herd C, differences of ADG and the day to

Table 4. The effect of vaccination on the growth performance

Herd	Group	No. of pigs	ADG[a)] (g, mean ± SD)	Day to market weight (Days, mean ± SD)	FCR[b)]
A	Vacciated	29	763.2 ± 62.3	147.6 ± 8.7	NT[c)]
	Control	27	786.8 ± 61.3	144.5 ± 9.3	NT
B	Vaccinated	57	968.0 ± 12.8	140.4 ± 11.4	NT
	Control	37	969.6 ± 15.6	135.6 ± 10.8	NT
C	Vaccinated	28	640.3 ± 58.8*	194.8 ± 12.3*	3.04
	Control	28	595.7 ± 57.2	207.1 ± 16.3	3.17

a) ADG: average daily weight gain.
b) FCR: feed conversion ratio.
c) Not tested.
* Significantly different compared with control group in each herd ($P<0.05$).

market weight between the vaccinated group and the control group were significant ($P<0.05$). ADG during the experiment was 640.3 ± 58.8 g for the vaccinated group and 595.7 ± 57.2 g for the control group. ADG in the vaccinated group was 44.6 g greater than that of the control group. The day to market weight (110 kg) was 194.8 ± 12.3 days for the vaccinated group and 207.1 ± 16.3 days for the control group. Pigs of the vaccinated group attained market weight 12.3 days sooner than pigs of the control group. FCR was 3.04 for the vaccinated group and 3.17 for the control group. While no statistical calculations could be performed since individual animal feed intake was not recorded, vaccination resulted in an improvement of FCR (Table 4).

DISCUSSION

Clinical evidence from the vaccination of all three herds showed that the inactivated vaccine against *M. hyopneumoniae* infection proved to be safe under field conditions.

In the SPF herd (herd A), CF antibody titers against *M. hyopneumoniae* increased 4 weeks after the second vaccination in the vaccinated group, but no increase was detected in any of the sera of the control group. Furthermore, *M. hyopneumoniae* was not isolated from the lungs of any pigs, and no lung lesions developed in any pigs at slaughter. These results suggested that there was no infection of *M. hyopneumoniae* but the vaccination induced an antibody response, which continued for more than 3 months.

As an infection would occur at any time in conventional herd, it was important to demonstrate protection throughout the life of the pig. In another study, lung lesions were reduced in vaccinated pigs, when challenge-exposed with *M. hyopneumoniae* six-months after vaccination. These results suggested that the duration of immunity induced by the vaccination lasted for at least 6 months (unpublished data).

In the conventional pig herds in this study (herds B and C), vaccination resulted in a significant ($P<0.05$) reduction of lung lesions and the numbers of *M. hyopneumoniae* recovered at slaughter, although it could not completely prevent infection with *M. hyopneumoniae*. Clinical trials with the vaccine showed a reduction in the prevalence of pigs with lung lesions and in the extent of the lung lesions.

It was reported that *P. multocida* did not produce pneumonic lesions when inoculated alone, but pigs infected with *M. hyopneumoniae* developed more severe lung lesions when they were simultaneously inoculated with *P. multocida* [4, 18]. Recently, Amass *et al.* [2] found that pigs, which recovered from or had been vaccinated against infection with *M. hyopneumoniae* were resistant to experimental *P. multocida* infection. This study confirms the results of these previous reports on field studies. In herds B and C, the number of pigs from which *P. multocida* was isolated was lower in the vaccinated group than in the control group. The results of the present study showed that the inactivated vaccine prepared from broth culture supernatant of *M. hyopneumoniae* not only reduced clinical signs and lung lesions of MPS, but also reduced colonization and lesions caused by dual infection with *M. hyopneumoniae* and *P. multocida*. Furthermore, in herd C, vaccination also reduced mortality due to *A. pleuropneumoniae* infection, but did not reduce the severity of lesions of porcine pleuropneumonia. This is in disagreement with a previous study [10] showing that vaccination against *M. hyopneumoniae* reduces the severity of lesions of MPS and porcine pleuropneumonia. These differences may have been due to the severity of the *A. pleuropneumoniae* infections.

While this vaccine results in the marked benefits of fewer clinical signs and reduced lung lesions, the primary aim of a vaccine for the control of MPS would be improvement of growth performance. In herd C, with serious epidemiological and economical problems, the results of reduced clinical signs and lung lesions in vaccinated pigs were shown to be an advantage in growth performance. A significant reduction ($P<0.05$) in day to market weight was observed in the vaccinated group compared to the control group, as well as a significant increase in ADG and a lower

FCR. In this trial, there was a significant difference in the growth performance of vaccinated pigs and the controls in herd C, while no such significant difference in herds A and B was demonstrated. In herds where clinical signs and economic losses were significant, improvement in growth performance with vaccination was sufficient. However, in herds with no infection of *M. hyopneumoniae* or high health status, improvement in growth performance by vaccination seems to give few benefits. This may be attributed to the difference in the severity of lung lesions. A previous study demonstrated a negative relationship between ADG and the percentage volume of lung affected by pneumonia, and a positive relationship between the day to market weight and the extent of lesions [8]. Mild lung lesions shown in herd B may not have influenced the growth performance.

In conclusion, it is suggested that inactivated vaccine prepared from broth culture supernatant of *M. hyopneumoniae* not only reduces the prevalence of pigs with pneumonia, the extent of lung lesions, and colonization of *M. hyopneumoniae*, but also improves ADG, FCR, and day to market weight in herds where clinical signs and economic losses are significant. It is expected that the use of this vaccine may help to control MPS in conventional pig herds.

REFERENCES

1. Alexander, T. J. L. and Harris, D. L. 1992. Methods of disease control. pp. 808–836. *In*: Disease of Swine. 7th ed. (Leman, A. D., Straw, B. E., Mengeling, W. L., Allaire, S. D. and Taylor, D. J. eds.), Iowa State University Press, Ames.
2. Amass, S. F., Clark, L. K., Alstine, W. G. V., Bowersock, T. L., Murphy, D. A., Knox, K. E. and Albregts, S. R. 1994. Interaction of *Mycoplasma hyopneumoniae* and *Pasteurella multocida* infections in swine. *J. Am. Vet. Med. Assoc.* 204: 102–107.
3. Asai, T., Okada, M., Ono, M., Irisawa, T., Mori, Y., Yokomizo Y. and Sato, S. 1993. Increased levels of tumor necrosis factor and interleukin 1 in bronchoalveolar lavage fluids from pigs infected with *Mycoplasma hyopneumoniae. Vet. Immunol. Immunopathol.* 38: 253–260.
4. Ciprian, A., Pijoan, C., Cruz, T., Camacho, J., Tortora, J., Colmenares, G., Lopez, R.R. and de la Garcia. M. 1988. *Mycoplasma hyopneumoniae* increases the susceptibility of pigs to experimental *Pasteurella multocida* pneumonia. *Can. J. Vet. Res.* 52: 434-438.
5. Dayalu, K. I. and Ross, R. F. 1990. Evaluation of experimental vaccines for control of mycoplasmal pneumonia of swine. *IOM Letters* 1: 486.
6. Etheridge, J. R. and Lloyd, L. C. 1982. A method for assessing induced resistance to enzootic pneumonia of pigs. *Res. Vet. Sci.* 33: 188–191.
7. Hannan, P. C. T., Bhogal, B. S. and Fish, J. P. 1982. Tylosin tartrate and tiamutilin effects on experimental piglet pneumonia induced with pneumonic pig lung homogenate containing mycoplasmas, bacteria and viruses. *Res. Vet. Sci.* 33: 76–88.
8. Hill, M. A., Scheidt, A. B., Tecrow, R. F., Clark, L. K., Knox, K. E. and Jordan, M. 1992. Association between growth indicators and volume of lesions in lungs from pigs at slaughter. *Am. J. Vet. Res.* 53: 2221–2223.
9. Kobisch, M., Quillien, L., Tillon, J. P. and Wroblewski, H. 1987. The Mycoplasma hyopneumoniae plasma membrane as a vaccine against porcine enzootic pneumonia. *Ann. Inst. Pasteur Immunol.* 138: 693–705.
10. Martinod, S. 1996. Protection against *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* infections using a mycoplasma inactivated vaccine Respisure under field conditions. *Proc. 14th IPVS Congress* 221.
11. Mori, Y., Yoshida, Y., Kuniyasu, T. and Hashimoto, K. 1983. Improvement of complement fixation test antigen for the diagnosis of *Mycoplasma hyopneumoniae* infection. *Natl. Inst. Anim. Health. Q. (Jpn.)* 23: 111–116.
12. Namioka, S. 1978. *Pasteurella multocida*- Biochemical characteristics and serotypes. *Methods Microbiol.* 10: 271–292.
13. Pointon, A. M., Byrt, D. and Heap, P. 1985. Effect of enzootic pneumonia of pigs on growth performance. *Aust. Vet. J.* 62: 13–18.
14. Ross, R. F. 1992. Mycoplasmal diseases. pp. 537–551. *In*: Disease of Swine, 7th ed. (Leman, A. D., Straw, B. E., Mengeling, W. L., Allaire, S. D. and Taylor, D. J. eds.), Iowa State University Press, Ames.
15. Ross, R. F., Zimmerman-Erickson, B. J. and Young, T. F. 1984. Characteristics of protective activity of *Mycoplasma hyopneumoniae* vaccine. *Am. J. Vet. Res.* 45: 1899–1905.
16. Shibata, I., Okada, M., Uruno, K., Samegai, Y., Ono, M., Sakano, T. and Sato, S. 1998. Experimental dual infection of cesarean-derived, colostrum-deprived pigs with *Mycoplasma hyopneumoniae* and pseudorabies virus. *J. Vet. Med. Sci.* 60: 295–300.
17. Sirois, M. and Higgins, R. 1991. Biochemical typing of *Actinobacillus pleuropneumoniae. Vet. Microbiol.* 27: 397–401.
18. Smith, I. M., Hodges, R. T., Betts, A. O. and Hayward, A. H. S. 1973. Experimental infections of gnotobiotic piglets with *Pasteurella septica* (sero-group A) alone or with *Mycoplasma hyopneumoniae. J. Comp. Pathol.* 83: 307–321.
19. Taylor-Robinson, D. 1983. Metabolism inhibition tests. pp. 411–417. *In*: Methods in Mycoplasmology, vol. 1. Mycoplasma Characterization (Razin, S. and Tully, J. G. eds.), Academic Press, New York.
20. Yagihashi, T., Nunoya, T., Mitsui, T. and Tajima, M. 1984. Effect of *Mycoplasma hyopneumoniae* infection on the development of *Haemophilus pleuropneumoniae* pneumonia in pigs. *Jpn. J. Vet. Sci.* 46: 705–713.
21. Yamamoto, K., Koshimizu, K. and Ogata, M. 1986. *In vitro* susceptibility of *Mycoplasma hyopneumoniae* to antibiotics. *Jpn. J. Vet. Sci.* 48: 1–5.