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Investigation of seroepidemiology of *Mycoplasma hyopneumoniae* infection and establishment of on-farm eradication protocol

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Mycoplasma hyopneumoniae 감염의 혈청역학조사 및 농장에서의 근절방안 설정

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Abstract : The purposes of this study are to examine seroprevalence of *Mycoplasma hyopneumoniae* infection in pigs of different age groups, and retrospectively determine if nursery depopulation (ND) could influence the seroprevalence of *M hyopneumoniae* infection in nurseries. Sera of 4, 8, 12, 16, and 20 weeks old pigs from 7 farms were first selected from a serum bank to examine serologic profiles for *M hyopneumoniae* infections. Availability of representative sera in the serum bank was a major criterion for farm selection. The sera were tested for *M hyopneumoniae* antibodies by an enzyme linked immunosorbent assay (ELISA) using Tween-20 extracted antigen. Serum samples were also selected from 15 of 34 swine farms that previously participated in a ND study. In order to evaluate *M hyopneumoniae* infection following ND, ELISA was performed with sera of 8~10 weeks old nursery pigs collected prior to and after ND for up to 12 months from the 15 farms. Serological profiles showed positive ELISA titers for 2 of 7 farms at 8 weeks, 4 of 7 farms at 12 weeks, 6 of 7 farms at 16 weeks, 6 of 6 farms at 20 weeks of age. Prior to ND, 11 of the 15 farms had positive titers in sera of 8~10 weeks old pigs. Sera of 8~10 weeks old pigs collected from 7 of the 11 farms (63.6%) were ELISA antibody negative for up to 12 months following ND. In conclusion, seroconversion to *M hyopneumoniae* was detected commonly between 10~16 weeks of age, indicating the occurrence of natural infection during the nursery age. The ND appeared to be an effective method to prevent *M hyopneumoniae* infection within the nursery pig in some farms.

Key words : *Mycoplasma hyopneumoniae*, seroepidemiology, nursery pig, eradication protocol.

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Introduction

Swine enzootic pneumonia (SEP) is one of the major respiratory disease of the pig caused primarily by *Mycoplasma hyopneumoniae*. The incidence and severity of SEP are enhanced by a complexed interaction between *M hyopneumoniae*, environmental factors, management practices, and secondary pathogens such as *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* [1,2]. Recently, a porcine respiratory disease complex (PRDC) has been described as one of the most significantly problems in Korea as well as in the United States swine industry[3]. The PRDC is caused by infection with multiple agents, including porcine reproductive and respiratory syndrome (PRRS) virus, *M hyopneumoniae*, swine influenza virus and others.

To confirm the presence of *M hyopneumoniae* and identify secondary infections, lung lesion checks can be performed at slaughter. However, these measures can underestimate the presence of the disease because lung lesions from early infections may have resolved by the time of slaughter, while the economic losses have already occurred. Serological tests can also be performed on live animals to detect antibodies to *M hyopneumoniae*. The other way clinical diagnosis of enzootic pneumonia can be verified by serological analysis. So far, this verification has been performed by immunofluorescence (IF)[4], and cultivation[5]. Recently, however, an antigen-ELISA[6] and a polymerase chain reaction (PCR)[7] for demonstration of *M hyopneumoniae* in porcine lungs have been developed.

In the present study these four different methods for demonstration of *M hyopneumoniae* in lungs were evaluated by the use of experimentally infected pigs. At an individual level, the sensitivity for this ELISA was estimated to 98~100% and the specificity to 93~100%. The nasal swabs were additionally used for demonstration of *M hyopneumoniae* and the PCR was found also superior to the other methods.

Prevention of *M hyopneumoniae* infection has been demonstrated by either performing modified medicated early weaning (MMEW) or isolated weaning techniques[8]. Because of the potential excessive cost per pig weaned, MMEW technology has not been routinely used on commercial farms. Some researchers have conducted studies investigating the effect of mycoplasma vaccine on the performance of pigs with inconsistent results[9,10] and emphasized the importance of antibody serum profile for *M hyopneumoniae* infection in the herd[11].

Recently, nursery depopulation (ND) method has been demonstrated as an effective strategy to control problem in nursery pigs following infection with PRRS virus. In the present study, we examined the serologic profiles for *M hyopneumoniae* infection with pigs of different age groups, and compared the antibody levels in 8~10 weeks old pig sera collected prior to and after ND to determine whether ND could effect to the seroprevalence in the nurseries.

Materials and Methods

Swine farms and sera : Farms selection was based on the following criteria : farm sera were stored in a serum bank ; the farm performed a ND ; representative serum samples were collected prior to and after ND for up to 12 months ; and no previous history of vaccination against *M hyopneumoniae*. The serum bank includes sera submitted for PRRS virus antibody profiles during the last 5 years. Ten sera of 4, 8, 12, 16 and 20 weeks old pigs from 7 different swine farms were first selected and examined for antibody levels to *M hyopneumoniae* infection.

In order to compare seroprevalence of *M hyopneumoniae* infection in nurseries prior to and after ND, 15 of 34 farms used in a previous study[12] were selected. Farm selection was again based on the availability of sera in the serum bank. Ten sera were selected from 8~10 weeks old nursery pigs prior to ND, and 2~4, 6~8, and 10~12 months after ND from the 15 farms.

Enzyme-linked immunosorbent assay (ELISA) : Mycoplasma ELISA antigen was prepared as described previously [13]. Briefly, *M hyopneumoniae* was grown in Friis mycoplasma broth[5] at 35℃ for 36 to 48 hours and the cells were pelleted by centrifugation at 10,000 rpm for 30 min. The pellet was washed 3 times and suspended in phosphate buffered saline (PBS, pH 7.2). The suspension was mixed with

an equal volume of 2% Tween-20 in PBS. The mixture was stirred for 90 min at 37℃ and centrifuged at 10,000 rpm for 1 hour. The supernatant was then passed through a 0.2m membrane filter and stored at -70℃ in aliquots.

For ELISA, 96-well microplates (Immulon II, Dynatech Lab. Inc, Alexandria, VA) were coated by incubating at 4℃ overnight with 100μl of antigen in carbonate-bicarbonate buffer (pH 9.6). Then the antigen was removed, and the plates were incubated at 37℃ for 3 hours with a blocking solution containing 1% bovine serum albumin (BSA) and 3% rabbit serum in PBS.

Before use, the plates were washed three times with PBS containing 0.05% Tween-20. Fifty μl of each test serum diluted 1 : 50 in the blocking solution was added to duplicate wells. The plates were incubated at 37℃ for 30min. After washing, 50μl of peroxidase conjugated rabbit antiswine IgG (1 : 3,000, Organon Teknica-Cappel Co. PA) was added to each well and incubated at 37℃ for 30 min. The wells were washed again, and 50μl of substrate containing *o*-phenylenediamine (Sigma Chemical Co., St.Louis, MO) and H_2O_2 was added. After 10 min at room temperature, the reaction was stopped by adding 0.5N sulfuric acid. The optical density (OD) values were measured by an ELISA reader at 490nm. The OD values of 0.300 or greater was regarded as positive in this study.

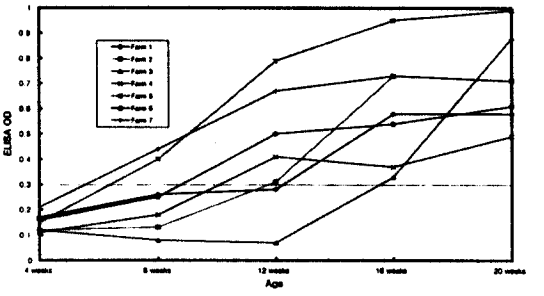

Fig 1. Antibody profiles of *M hyopneumoniae* infection in the 7 farms. Mean ELISA OD values of 10 sera were graphed for each age group. Positive Cutoff OD value was 0.300.

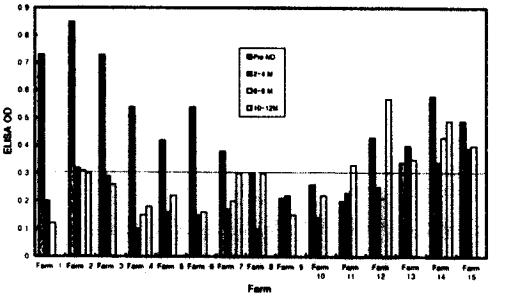

Fig 2. Mean ELISA OD values of 10 sera from 8~10 weeks old pigs collected prior to and 2~4, 6~8 and 10~12 months after nursery depopulation(ND) in the 15 farms. Farms 1~7 were positive antibody to *M hyopneumoniae* prior to ND and negative after ND.

Results

The serologic profiles of *M hyopneumoniae* infection in pigs of different age groups showed positive pigs in 2 of 7 farms at 8 weeks, 4 of 7 farms at 12 weeks, 6 of 7 farms at 16 weeks and 6 of 6 farms at 20 weeks of age (Fig 1). Pigs in all 7 farms had no positive antibody titers at 4 weeks of age.

Results of the ELISA with sera from 8~10 weeks old nursery pigs collected prior to and after in 15 of the selected farms are shown in Fig 2.

Sera from 4 of the 15 farms sampled prior to ND were antibody negative (Farms 8~11). Three of the 4 farms remained negative during the study, and one (Farm 12) became seropositive. Sera from 8~10 weeks old nursery pigs in 11 of the 15 farms were ELISA antibody positive prior to ND. Following ND, sera collected from 7 (63.6%, Farms 1~7) of the 11 farms remained negative for up to 12 months. Sera from 4 (Farms 12~15) of the 11 farms were antibody positive prior to and after ND.

Discussion

Although several serological tests including complement fixation[14] and indirect hemagglutination[15,16] tests have been used for the detection of antibodies to *M hyopneumoniae*, ELISA using Tween-20 extracted antigen was the choice in this study. In comparative studied among different serologic tests[16~18] the ELISA was found to be highly sensitive and specific. No significantly cross reaction was reported when

sera with high levels of antibodies against *M flocculare* were tested in the ELISA with *M hyopneumoniae* [18].

In this study, seroconversion to *M hyopneumoniae* infection was first detected at 8 weeks of age in 2 of the 7 farms, and most of the farms became seropositive by 10~16 weeks of age. These results are similar to those reported by Sheldrake *et al* [19] who found that seroconversion was more likely at of 12~16 weeks of age. Considering the time of 4 or more weeks required for pigs to seroconvert following *M hyopneumoniae* challenge[16], natural infection on those farms could have occurred during the nursery age. This seroconversion appears to correlate with the time at which passive antibodies became negative. Morris *et al* [20] suggested that the time at which passive antibodies waned was 1~2 months, depending on the initial antibody concentration in the piglets.

Transmission of *M hyopneumoniae* occurs commonly from carrier sows to their pigs, from infected pigs to other pigs in the farrowing and nursery rooms, and between pigs in the growing and finishing units[21]. It has been reported that gilts are more apt to transmit *M hyopneumoniae* to their pigs during lactation period than are sows[22]. Therefore, piglets originating from herds with a large number of gilt litters would have a higher risk of infection prior to weaning. The serologic profiles in this study show that *M hyopneumoniae* infection was occurring mostly in pigs during nursery phase. These data indicate that newly infected nursery pigs and young gilts are the highest risk groups in swine farms for *M hyopneumoniae* transmission.

In order to control *M hyopneumoniae* infection on a farm, all-in all-out management of nursery pigs, vaccination and/or medication of the potential risk group would be helpful. Especially vaccination of gilts in the gilt pool at 5 and 2 weeks prior to farrowing may assist in reducing the spread of the organism during lactation.

Recently, Dee and Joo[23] demonstrated ND to be an effective method to eliminate PRRS virus in endemically infected farms. The elimination was successful on farms showing serological pattern of high seroprevalence of PRRS antibodies in 8~10 weeks old pigs and of no or low seroprevalence in sows and recently weaned piglets. In this study with ND, 7 farms had seropositive nursery pigs prior to ND but seropositive pigs were not detected after ND for up to 1 year. It is not clear how the seroconversion was stopped and the seronegative status was maintained, However, similar to the example of PRRS, ND may caused prevention of the spread of *M hyopneumoniae* infection from older, previously infected nursery pigs to those recently weaned. Also, vertical transmission in the litters of young sows may have been prevented, thereby reducing the risk of introducing infected piglets into the nursery.

As touching on the introduction part, ND method has

Table 1. Nursery depopulation and clean-up protocol for the elimination of *M hyopneumoniae*

Day	Procedure
1	Empty all nurseries, begin off-site weaning, pump out slurry pits, clean and wash rooms with hot (> 95℃) water and disinfect in formaldehyde-based product[a]. Allow disinfectant water to remain in pits overnight.
2	Pump out pits, repeat washing procedure and disinfect in phenol-based product[b]. Allow disinfectant to remain in pits.
3~11	Allow facility to remain empty.
12	Pump out slurry pits, repeat washing procedure and disinfect with Formaldehyde-based product.
13	Allow facility to remain empty.
14	Resume conventional flow of pigs into clean nurseries.

[a] Active ingredients: formaldehyde 2.28 percent, ammonium chloride 3.08 percent, propanediol 19.20 percent. One part of the product was mixed with 128 parts of water.

[b] Active ingredients: sodium *o*-phenylphenate 11.3 percent, sodium *o*-benzylchlorophenate 9.4 percent and sodium *p*-tertiary amylphenate 2.3 percent. One part of the product was mixed with 256 parts of water.

been demonstrated as an effective protocol to control problem in nursery pigs following infection with PRRS by Dee and Joo[23]. This protocol should be also effective for the elimination of *M hyopneumoniae* as a resume was shown in Table 1. And this protocol may be very effective to reduce the *M hyopneumoniae* if use in the swine farm of Korea.

It is not known for how long the nurseries will remain free of *M hyopeumoniae*. This depopulation and cleaning protocol may need to be repeated, perhaps annually. However, the benefits of the program appear to outweigh the financial cost and short-term inconvenience of off-site weaning.

From these results, it is suggested that the technique of ND may be effective towards preventing the lateral transmission of *M hyopneumoniae* in nurseries. While it appears that seroconversion has been prevented in certain cases, further studies are necessary to assess the ability of ND to eliminate the organism. In addition to the practicing ND, prefarrowing vaccination of lower parity sows may reduce the risk of transmission from sows to piglets and give a higher success in the elimination of *M hyopneumoniae* in the nurseries.

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