ORIGINAL RESEARCH PEER REVIEWED

Field evaluation of a new single-dose *Mycoplasma hyopneumoniae* bacterin effects on growth performance

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Summary

Objective: Evaluate the efficacy of a new single-dose bacterin against *Mycoplasma hyopneumoniae* under field conditions.

Materials and methods: Three separate farms were selected based on their history of enzootic pneumonia. On each farm, vaccinated pigs (n = 20; 10 male and 10 female) were administered a single dose of the *M hyopneumoniae* bacterin at 21 days of age while unvaccinated pigs (n = 20; 10 male and 10 female) were administered a single dose of phosphate buffered saline at the same age.

Results: Vaccination against *M hyopneumoniae* reduces the severity of lung lesions and clinical signs such as coughing, which leads to improved growth performance of the pig. Vaccinated pigs had a significantly higher ($P = .02$ for farm A, $P = .02$ for farm B, and $P = .02$ for farm C) average daily weight gain between 21 to 175 days old (0 to 154 days post vaccination) and elicited cell-mediated immunity, as measured by *M hyopneumoniae*-specific interferon-γ secreting cells, when compared with unvaccinated pigs located at all 3 farms.

Implications: The data presented in this field study demonstrated that the *M hyopneumoniae* bacterin improved growth performance effectively in 3 farms suffering from enzootic pneumonia.

Keywords: swine, enzootic pneumonia, *Mycoplasma hyopneumoniae*, vaccine

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Resumen - Evaluación de campo de los efectos de una nueva vacuna de dosis única de bacterina *Mycoplasma hyopneumoniae* sobre el rendimiento del crecimiento

Objetivo: Evaluar la eficacia de una nueva bacterina de dosis única contra *Mycoplasma hyopneumoniae* en condiciones de campo.

Materiales y métodos: Se seleccionaron tres granjas diferentes en función de su historial de neumonía enzoótica. En cada granja, a los cerdos vacunados (n = 20; 10 machos y 10 hembras) se les administró una dosis única de la bacterina *M hyopneumoniae* a los 21 días de edad, mientras que a los cerdos no vacunados (n = 20; 10 machos y 10 hembras) se les administró una sola dosis de solución salina tamponada con fosfato a la misma edad.

Resultados: La vacunación contra *M hyopneumoniae* reduce la gravedad de las lesiones pulmonares y los signos clínicos como la tos, lo que mejora el crecimiento del cerdo. Los cerdos vacunados tuvieron un aumento de peso promedio diario significativamente mayor ($P = .02$ para la granja A, $P = .02$ para la granja B, y $P = .02$ para la granja C) entre los 21 y 175 días de edad (0 a 154 días después de la vacunación) y provocó inmunidad celular, medida por células secretoras de interferón-γ específicas de *M hyopneumoniae*, en comparación con cerdos no vacunados ubicados en las 3 granjas.

Implicaciones: Los datos presentados en este estudio de campo demostraron que la bacterina contra *M hyopneumoniae* mejoró el rendimiento del crecimiento de manera efectiva en 3 granjas que padecían neumonía enzoótica.

Résumé - Évaluation terrain des effets sur les performances de croissance d'une nouvelle bactérine à dose unique contre *Mycoplasma hyopneumoniae*

Objectif: Évaluer l'efficacité d'une nouvelle bactérine à dose unique contre *M hyopneumoniae* dans des conditions de terrain.

Matériels et méthodes: Trois fermes distinctes ont été sélectionnées en fonction de leur histoire de pneumonie enzootique. Dans chaque ferme, des porcs vaccinés (n = 20; 10 mâles et 10 femelles) ont reçu une dose unique d'une bactérine contre *M hyopneumoniae* à 21 jours d'âge, tandis que des porcs non vaccinés du même âge (n = 20; 10 mâles et 10 femelles) ont reçu une dose unique de solution saline tamponnée.

Résultats: La vaccination contre *M hyopneumoniae* a réduit la sévérité des lésions pulmonaires et des signes cliniques tels que la toux, ce qui a entrainé

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une amélioration des performances de croissance des porcs. Les porcs vaccinés avaient un gain de poids quotidien moyen significativement plus élevé ($P = .02$ pour la ferme A, $P = .02$ pour la ferme B, et $P = .02$ pour la ferme C) entre 21 et 175 jours (0 à 154 jours après la vaccination) et suscité une immunité à médiation cellulaire, telle que mesurée par les cellules sécrétant l'interféron-γ spécifique de *M hyopneumoniae*, par rapport aux porcs non vaccinés situés dans les trois fermes.

Implications: Les données présentées dans cette étude de terrain ont démontré que la bactérine contre *M hyopneumoniae* améliorait efficacement les performances de croissance des animaux dans trois fermes au prise avec la pneumonie enzootique.

Mycoplasma hyopneumoniae can be an important pathogen in porcine respiratory disease complex[1] as well as the primary pathogen of enzootic pneumonia, a chronic respiratory disease in growing pigs resulting from combined infections of *M hyopneumoniae* and one or more secondary bacterial pathogens.[2] Enzootic pneumonia is characterized by a persistent nonproductive cough with a reduced growth rate, a poor feed conversion ratio, high morbidity, and low mortality.[3,4] The economic impact of *M hyopneumoniae* infections in swine farms worldwide can be considered significant.

Several strategies may be implemented to successfully prevent and control *M hyopneumoniae* including optimized management practices and vaccination.[5] While all-in/all-out production and multisite operations are great management tools, vaccination remains an important and cost-effective method for reducing the impact of *M hyopneumoniae* infection. The *M hyopneumoniae*-free status of herds is difficult to maintain especially in pig-dense areas, since the airborne spread of this pathogen may occur over several kilometers.[6]

In Korea, approximately 70% of total piglets farrowed in 2018 were vaccinated with *M hyopneumoniae* (**http://www.kahpha.or.kr**). Therefore, vaccination is one of the tools used to control *M hyopneumoniae*. The objective of this study was to evaluate the efficacy of a new single-dose *M hyopneumoniae* whole-cell bacterin (Hyogen, CEVA Santé Animale) based on strain BA 2940–99, oil adjuvanted with paraffin and *Escherichia coli* J5 LPS with thiomersal as excipient under field conditions in accordance with the registration guidelines of the Republic of Korea's Animal, Plant and Fisheries Quarantine and Inspection Agency (**http://www.qia.go.kr**).

Materials and methods

The protocol for this field study was approved by the Seoul National University Institutional Animal Care and Use Committee (approval number SNU-180621-13).

Farm histories

The clinical field trial was conducted on 3 Korean swine farms (denoted as Farms A, B, and C) between August 2018 and February 2019. Status of porcine reproductive and respiratory syndrome (PRRS) was stable with no active PRRS virus circulation (high-parity sows were the only seropositive animals in the herd). Porcine circovirus type 2 (PCV2) was circulating in the postweaning and growing period without overt clinical signs of porcine circovirus-associated disease on the 3 farms.

Farm A was a conventional 400-sow farrow-to-finish swine farm where the owner complained about a dry recurrent cough beginning at 40 days of age accompanied by growth retardation. Real-time polymerase chain reaction (PCR) testing[7] of pneumonic and atelectatic lung samples from pigs at 49 days of age was conducted for *M hyopneumoniae* at the Veterinary Diagnostic Center, College of Veterinary Medicine, Seoul National University in May 2018. The testing returned positive results for 5 of the 7 lung samples submitted for *M hyopneumoniae*. The combined occurrence of clinical signs, detection of *M hyopneumoniae* by PCR, and histopathological lesions (peribronchiolar and perivascular lymphoid tissue hyperplasia) were indicative of an ongoing infection with *M hyopneumoniae*.

Farm B consisted of a conventional 150-sow farrow-to-finish swine farm managed in a 2-week batch system and included a history of enzootic pneumonia. Infection with *M hyopneumoniae* was evident by severe dry coughing, histopathological peribronchiolar lymphoid tissue hyperplasia, and detection of *M hyopneumoniae* in lung samples by real-time PCR[7] in all three of the 38-day-old pigs tested.

Farm C, a conventional 450-sow farrow-to-finish swine farm, was suggested to our clinical study team by its practitioner to participate in this field trial on *M hyopneumoniae* vaccine efficacy. A pilot survey was implemented to assess the circulation of *M hyopneumoniae* within the herd, as the producers had complained of severe dry coughing and retardation of growth between 10 and 50 days of age. Lung samples from 74-day-old pigs were submitted to the Veterinary Diagnostic Center, College of Veterinary Medicine, Seoul National University in June 2018. Three of the 5 lung sample submissions were positive for *M hyopneumoniae* using real-time PCR testing.[7] The histological lesions were characterized by peribronchiolar lymphoid tissues hyperplasia and bronchopneumonia. *Pasteurella multocida* was isolated in 4 of the 5 lung samples. These results were indicative of enzootic pneumoniae by *M hyopneumoniae* with secondary *P multocida* infection.

Study design

The experimental design of the field study strictly adhered to the registration guidelines set by the Republic of Korea's Animal, Plant and Fisheries Quarantine and Inspection Agency. Guidelines require that 20 piglets (10 male and 10 female) be selected and assigned to each group of vaccinated and unvaccinated animals. To minimize sow variation, four to six 7-day-old piglets were randomly selected from each sow and assigned to either the vaccinated or unvaccinated group using the random number generator function in Excel (Microsoft Corporation). The pigs in the vaccinated groups were injected intramuscularly in the right side of the neck with 2 mL of the *M hyopneumoniae* bacterin (Hyogen, CEVA Santé Animale, Lot No.1405582B) at 21 days of age, while an equal volume of phosphate buffered saline (0.01M, pH 7.4) was injected in the same anatomical location for pigs of the unvaccinated groups. At 24 days of age, all vaccinated and unvaccinated pigs were transferred to the nursery facility and kept in co-mingled groups until the end of the trial. In the nursery, pigs were then randomly distributed into 4 total pens to include 10 pigs/pen, all within one room. A similar proportion of each treatment was included in each pen. All pens were identical in design and equipment which included free access to a feed and water trough in accordance with standard farm procedures. The 3 farms did not use feed or water medication effective against *M hyopneumoniae*. Antibiotics (ie, penicillin) were given to vaccinated and unvaccinated pigs to help control respiratory

diseases during the course of the study. Blood and nasal swabs were collected at study days 0 (21 days of age), 21 (42 days of age), 49 (70 days of age), 77 (98 days of age), and 105 (126 days of age).

Mortalities

Pigs that died were subjected to gross pathological examination within 24 hours at a local veterinary practitioner's clinic. All major organs such as brain, lung, subinguinal lymph node, small and large intestine, liver, kidney, and tonsils were collected from each pig. In the case of lung lesions, samples were collected from the edge of these lesions. Polymerase chain reaction assays were used to detect specific nucleic acids for PCV2, PRRS virus, swine influenza virus, and *M hyopneumoniae*.[8-11] All other bacterial isolation and identifications were carried out by using routine methods.

Clinical observations

Pig physical condition was monitored daily, and pigs were scored weekly for clinical respiratory disease from study days 0 to 105. Scores ranged from 0 to 6: 0 = normal; 1 = mild dyspnea, tachypnea, or both when stressed; 2 = mild dyspnea, tachypnea, or both when at rest; 3 = moderate dyspnea, tachypnea, or both when stressed; 4 = moderate dyspnea, tachypnea, or both when at rest; 5 = severe dyspnea, tachypnea, or both when stressed; 6 = severe dyspnea, tachypnea, or both when at rest. Observers were blinded to vaccination status.

Growth performance

Pigs were weighed at study days 0 (21 days of age), 49 (70 days of age), 91 (112 days of age), and 154 (175 days of age). Average daily gain (ADG) was determined for study days 0 to 49, study days 50 to 91, and study days 92 to 154 (Table 1). The ADG during these various stages was calculated as the difference between the starting and final weight divided by the duration of the stage. Data for dead or removed pigs were included in the calculation.

Quantification of *M hyopneumoniae* DNA in nasal swabs

Sterile polyester swabs (Fisher Scientific Inc) were used to swab the nasal mucosa of both nostrils, reaching deeply into the turbinates. Swabs were stored in 5 mL plastic tubes (Fisher Scientific Inc) containing 1 mL of sterile saline solution. A commercial kit (QIAamp DNA Mini Kit, QIAGEN) was used to extract DNA from nasal swabs to quantify the *M hyopneumoniae* genomic DNA copy numbers by real-time PCR as previously described.[7] To construct a standard curve, real-time PCR was performed in quadruplicate in 10-fold serial dilution of chromosomal DNA from *M hyopneumoniae* strain SNU98703, with concentrations ranging from 10 ng/µL to 1 fg/µL. One femtogram of chromosomal DNA from *M hyopneumoniae* is considered to be approximately one genome equivalent.[12] A negative control was included in each run using double distilled water as the template.

Enzyme-linked immunosorbent assay

Blood samples were collected from each pig by jugular venipuncture. Serum samples were tested for *M hyopneumoniae* antibodies using a commercial enzyme-linked immunosorbent assay (ELISA; IDEXX Laboratories Inc). Serum samples were considered positive for *M hyopneumoniae* antibodies if the sample-to-positive (S:P) ratio was ≥ 0.4 in accordance with the manufacturer's instructions.

Enzyme-linked immunospot assay

Blood samples were collected from each pig by jugular venipuncture. The enzyme-linked immunospot (ELISpot) assay was conducted to measure the number of *M hyopneumoniae*-specific interferon-γ secreting cells (IFN-γ-SC) in peripheral blood mononuclear cells (PBMC).[13] *Mycoplasma hyopneumoniae* antigens were prepared as previously described.[14] The IFN-γ positive spots on the membranes (MABTECH) were imaged, analyzed, and counted using an automated ELISPOT Reader (AID ELISPOT Reader, AID GmbH). The results were expressed as the number of IFN-γ-SC per million PBMC. The ELISpot assay was completed in duplicate.

Pathological evaluation

Lung samples were collected in pigs from each group at study day 147 (168 days of age). Lung pathology evaluation was done by two pathologists (authors

Table 1: Mean (SD) average daily gain (ADG) in pigs vaccinated for *Mycoplasma hyopneumoniae* or unvaccinated pigs on 3 Korean swine farms*

Farm	Group (n)	ADG (SD), g/day			
		D 0-49	D 50-91	D 92-154	D 0-154
A	VacA (20)	402 (19)[a]	745 (30)	763 (21)	643 (10)[a]
	UnVacA (20)	382 (22)[b]	739 (39)	743 (61)	627 (25)[b]
B	VacB (20)	390 (27)[a]	755 (44)	764 (40)	643 (13)[a]
	UnVacB (20)	367 (24)[b]	739 (53)	755 (40)	627 (22)[b]
C	VacC (20)	387 (28)[a]	727 (26)[a]	765 (28)	634 (11)[a]
	UnVacC (20)	366 (26)[b]	704 (34)[b]	760 (44)	620 (22)[b]

* The clinical field trial was conducted on 3 farms (A, B, and C). To minimize sow variation, four to six 7-day-old piglets were randomly selected from each sow and assigned to either the vaccinated or unvaccinated group using the random number generator function in Excel (Microsoft Corporation). Groups VacA, VacB, and VacC were vaccinated with a one-dose *M hyopneumoniae* bacterin (Hyogen, CEVA Santé Animale) at study day 0 (21 days of age). Groups UnVacA, UnVacB, and UnVacC were injected with phosphate buffered saline at study day 0 (21 days of age).

[ab] Within a column, values with different superscript letters are significantly different within each farm. ADG was compared between the two groups within each farm using a Student *t* test.

Oh and Chae) at the Seoul National University (Seoul, Republic of Korea). Macroscopic lesion scores were estimated, and a score was given to reflect the amount of pneumonia in each lobe. For the entire lung, up to 100 points were assigned as follows: 10 points each to the right cranial lobe, right middle lobe, left cranial lobe, and left middle lobe; 27.5 points each to the right caudal lobe and left caudal lobe; and 5 points to the accessory lobe.[15] Eight pieces of lung tissues (two pieces from the right cranial lobe, two from the right middle lobe, one from the ventromedial part of the right caudal lobe, one from the dorsomedial part of the right caudal lobe, one from the midlateral part of the right caudal lobe, and one from the accessory lobe) were collected from each pig. Three tissue sections of the eight lung pieces were examined blindly by two veterinary pathologists (Oh and Chae). Lung sections were scored for presence and severity of type 2 pneumocyte hypertrophy and hyperplasia, alveolar septal infiltration with inflammatory cells, peribronchial lymphoid hyperplasia, amount of alveolar exudate, and amount of inflammation in the lamina propria of bronchi and bronchioles ranging from 0 to 6: 0 = normal; 1 = mild multifocal; 2 = mild diffuse; 3 = moderate multifocal; 4 = moderate diffuse; 5 = severe multifocal; 6 = severe diffuse.[16]

Statistical analysis

Prior to statistical analysis, real-time PCR data were transformed to $\log_{10}$ values to reduce variance and positive skewness. The normality of the distribution of the examined variables was evaluated by the Shapiro-Wilk test. Continuous data (ADG, real-time PCR, ELISA, and ELISpot) were analyzed with a Student *t* test to determine the significance of group differences at each time point. Discrete data (clinical signs and pathology lesions) were analyzed by Mann-Whitney test to determine the significance of group differences at each time point. A *P* value < .05 was considered significant.

Results

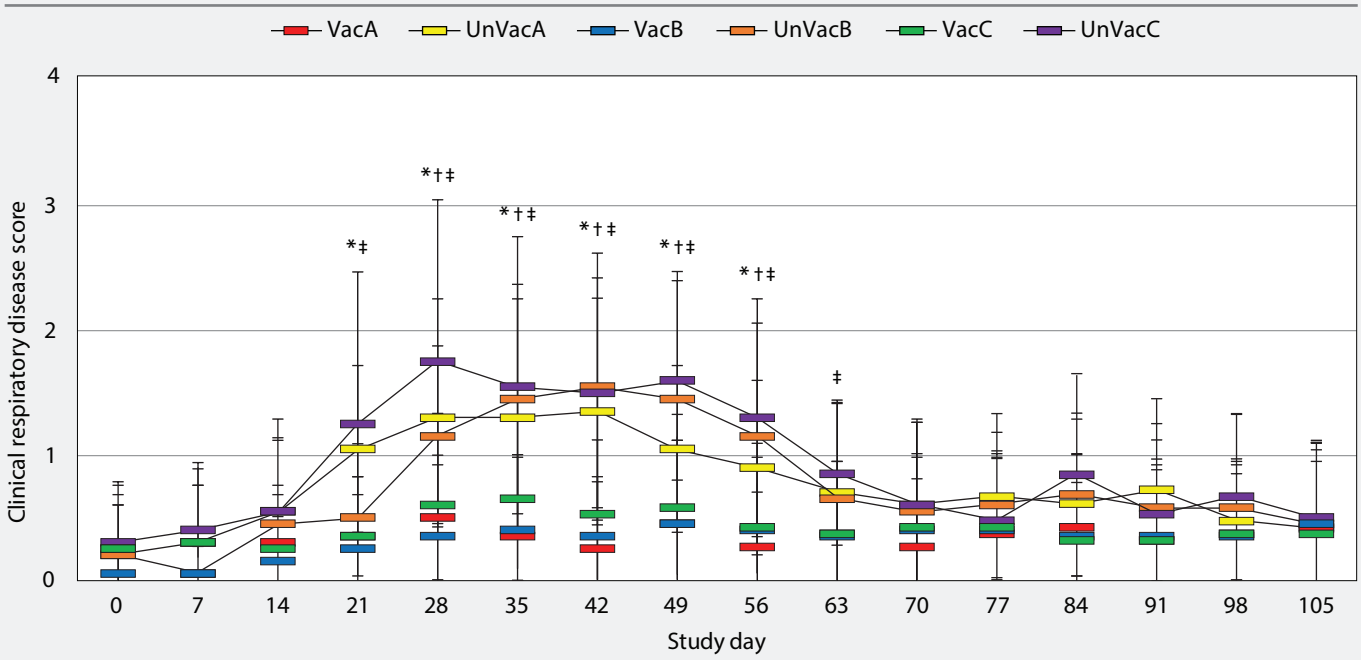
Mortality

One vaccinated pig from farm A died of bronchopneumonia resulting from a combination of PCV2 that was detected with PCR and *Glasserella parasuis* that was isolated from the lung at study day 51 (72 days of age). Three unvaccinated pigs from farm A died of pleuropneumonia caused by a combination of *Actinobacillus pleuropneumoniae* and other bacteria. *Actinobacillus pleuropneumoniae* and *P multocida* were isolated from lung tissue at study days 74 (95 days of age) and 77 (98 days of age), and *A pleuropneumoniae* and *Streptococcus suis* were isolated from lung tissue at study day 93 (114 days of age). Farm C had 1 vaccinated pig die of salmonellosis at study day 42 (63 days of age) and 2 unvaccinated pigs died of bronchopneumonia caused by a combination of PCV2 that was detected with PCR and *P multocida* that was isolated from lung tissue at study day 72 (93 days of age) and 92 (113 days of age), respectively. But PCV2-associated lesions were not observed in lymph nodes from these 2 pigs.

Clinical signs

Vaccinated pigs from farm A had significantly lower (*P* = .004) clinical respiratory scores when compared with unvaccinated pigs at study days 21 to 56. Farm B vaccinates also had significantly lower (*P* < .001) clinical respiratory scores when compared with unvaccinated pigs, but at study days 28 to 56. On farm C, vaccinated pigs had significantly lower (*P* = .002) clinical respiratory scores when compared with unvaccinated pigs at study days 21 to 63 (Figure 1).

Figure 1: Mean (SD) clinical respiratory disease scores of *Mycoplasma hyopneumoniae* vaccinated (Vac) or unvaccinated (UnVac) pigs on 3 Korean swine farms (A, B, and C). The study design is described in Table 1. Mean respiratory scores were scored on a scale from 0 to 6: 0 = normal; 1 = mild dyspnea, tachypnea, or both when stressed; 2 = mild dyspnea, tachypnea, or both when at rest; 3 = moderate dyspnea, tachypnea, or both when stressed; 4 = mild dyspnea, tachypnea, or both when at rest; 5 = severe dyspnea, tachypnea, or both when stressed; and 6 = severe dyspnea, tachypnea, or both when at rest. Significant difference (*P* value < .05; Mann-Whitney test) is indicated between vaccinated and unvaccinated groups within each farm (*farm A, †farm B, and ‡farm C).



Growth performance

The body weight of pigs at study day 0 (21 days of age, time of vaccination) did not differ significantly between the vaccinated and unvaccinated groups on all 3 farms. Vaccinated pigs from all farms (A-C) had significantly higher ($P = .007$ for farm A, $P = .01$ for farm B, and $P = .03$ for farm C) ADG at study days 0 to 49 (21-70 days old) when compared with unvaccinated pigs from the same farm. Additionally, farm C vaccinated pigs had a significantly higher ($P = .031$) ADG at study days 50 to 91 (71-112 days old) when compared with the unvaccinated pigs. Overall (study days 0-154), the difference between vaccinated and unvaccinated groups was significant ($P = .02$ for farm A, $P = .02$ for farm B, and $P = .02$ for farm C) on all 3 farms (Table 1).

Quantification of *M hyopneumoniae* in nasal swabs

On farm A, vaccinated pigs had a significantly lower ($P = .009$) number of genomic copies of *M hyopneumoniae* in their nasal swabs when compared with unvaccinated pigs at study day 21. On farm B, there was a numerical, but not statistically significant ($P = .05$), difference in the number of *M hyopneumoniae* genomic copies on the nasal swabs of vaccinated and unvaccinated pigs. Farm C vaccinated pigs had a significantly lower ($P = .02$ at study day 21, $P = .03$ at study day 49, and $P = .001$ at study day 77) number of *M hyopneumoniae* genomic copies in their nasal swabs when compared with unvaccinated pigs at study days 21, 49, and 77 (Figure 2).

Serology

On farm A, vaccinated pigs had a significantly higher *M hyopneumoniae* ELISA S:P ratio at study days 49 ($P = .001$) and 77 ($P = .006$) when compared with unvaccinated pigs. On farm B, vaccinated pigs had a significantly higher ($P = .001$) *M hyopneumoniae* ELISA S:P ratio at study days 21, 49, and 77 when compared with unvaccinated pigs. On farm C, vaccinated pigs had a significantly higher ($P = .001$) *M hyopneumoniae* ELISA S:P ratio at study days 49 and 77 when compared with unvaccinated pigs (Figure 3).

ELISpot

On farm A, vaccinated pigs had a significantly higher ($P < .001$) number of *M hyopneumoniae*-specific IFN-γ-SC at study day 49 in their PBMC when compared with the unvaccinated pigs. On farm B, vaccinated pigs had a significantly higher number of *M hyopneumoniae*-specific IFN-γ-SC in their PBMC at study days 21 ($P = .01$) and 49 ($P = .001$) when compared with the unvaccinated pigs. On farm C, vaccinated pigs had a significantly higher ($P = .002$) number of *M hyopneumoniae*-specific IFN-γ-SC at study days 49 and 77 in their PBMC when compared with the unvaccinated pigs (Figure 4).

Pathology

Vaccinated pigs had significantly lower ($P < .001$) macroscopic and microscopic lung lesion scores when compared with the unvaccinated pigs on the 3 farms at study day 154 (Table 2).

Discussion

In the present field trial, vaccination against *M hyopneumoniae* reduced the severity of lung lesions and clinical signs, including coughing, which resulted in improved growth performance. Controlling *M hyopneumoniae* and its associated diseases in the field can be challenging. Vaccination against *M hyopneumoniae* using commercial vaccines is the most common strategy within Asian swine production systems. The major advantages of vaccination include reduction of clinical signs and pneumonic lung lesions and improvement of daily weight gain in field trials.[17-20] No statistically significant difference was observed in the growth performance (ADG) over the nursery period between groups. This confirmed that vaccine did not have a detectable negative impact on growth performance shortly after injection. Overall (study days 0 to 154), the difference in growth performance between vaccinated and unvaccinated pigs was significant on all 3 farms where *M hyopneumoniae* was circulating.

Figure 2: Mean (SD) number of *Mycoplasma hyopneumoniae* (Mhp) genomic copies in nasal swabs from vaccinated (Vac) and unvaccinated (UnVac) pigs on 3 Korean swine farms (A, B, and C). The study design is described in Table 1. Significant difference (*P* value < .05; Student *t* test) is indicated between vaccinated and unvaccinated groups within each farm (*farm A and ‡farm C).

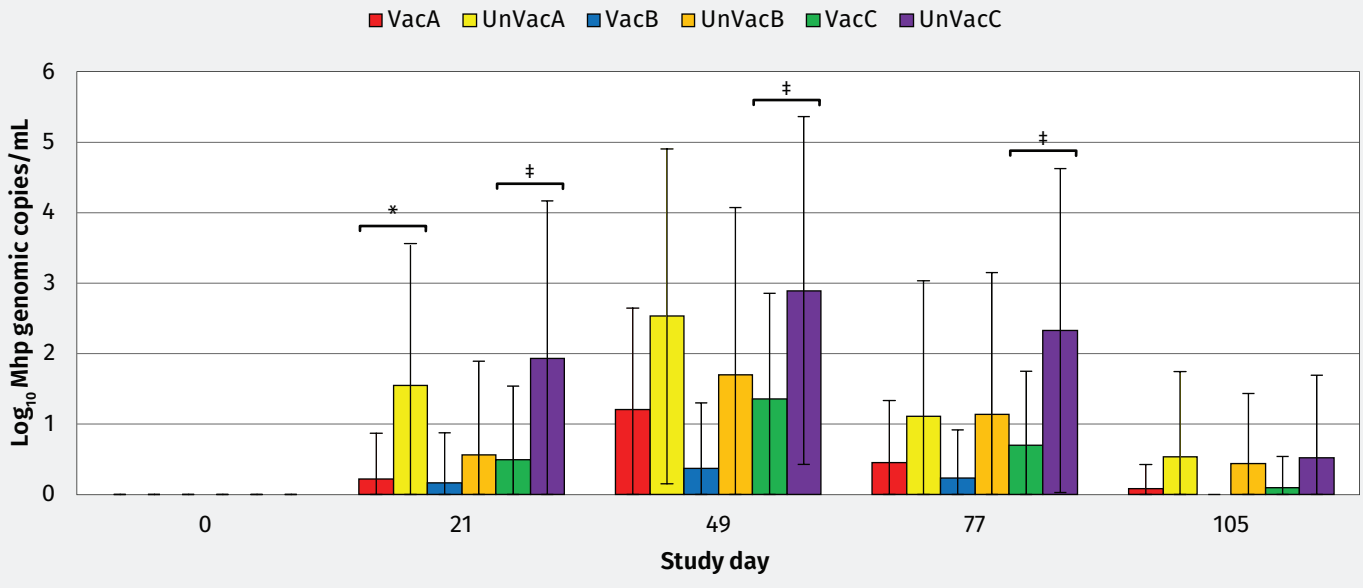


Figure 3: Mean (SD) sample-to-positive (S:P) ratio in serum samples from *Mycoplasma hyopneumoniae* (Mhp) vaccinated (Vac) and unvaccinated (UnVac) pigs on 3 Korean swine farms (A, B, and C). The study design is described in Table 1. Significant difference (*P* value < .05; Student *t* test) is indicated between vaccinated and unvaccinated groups within each farm (*farm A,†farm B, and ‡farm C).

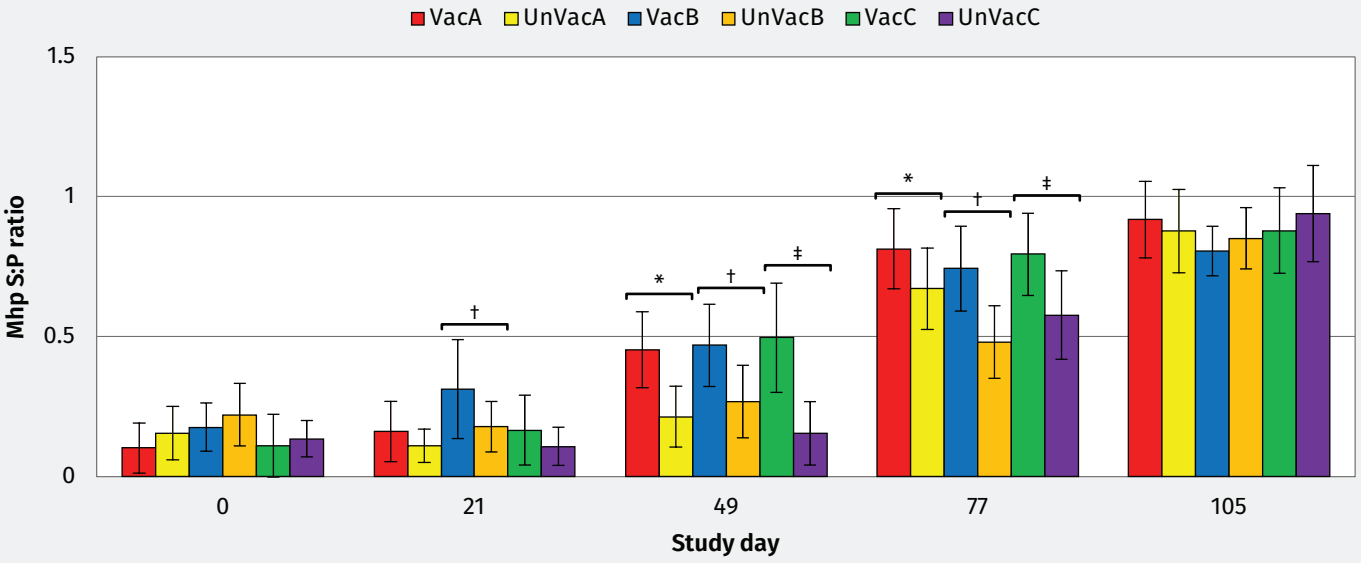


Figure 4: Mean (SD) *Mycoplasma hyopneumoniae* (Mhp)-specific interferon-γ secreting cells (IFN-γ-SC) in peripheral blood mononuclear cells (PBMC) in vaccinated (Vac) and unvaccinated (UnVac) pigs on 3 Korean swine farms (A, B, and C). The study design is described in Table 1. Significant difference (*P* value < .05; Student *t* test) is indicated between vaccinated and unvaccinated groups within each farm (*farm A, †farm B, and ‡farm C).

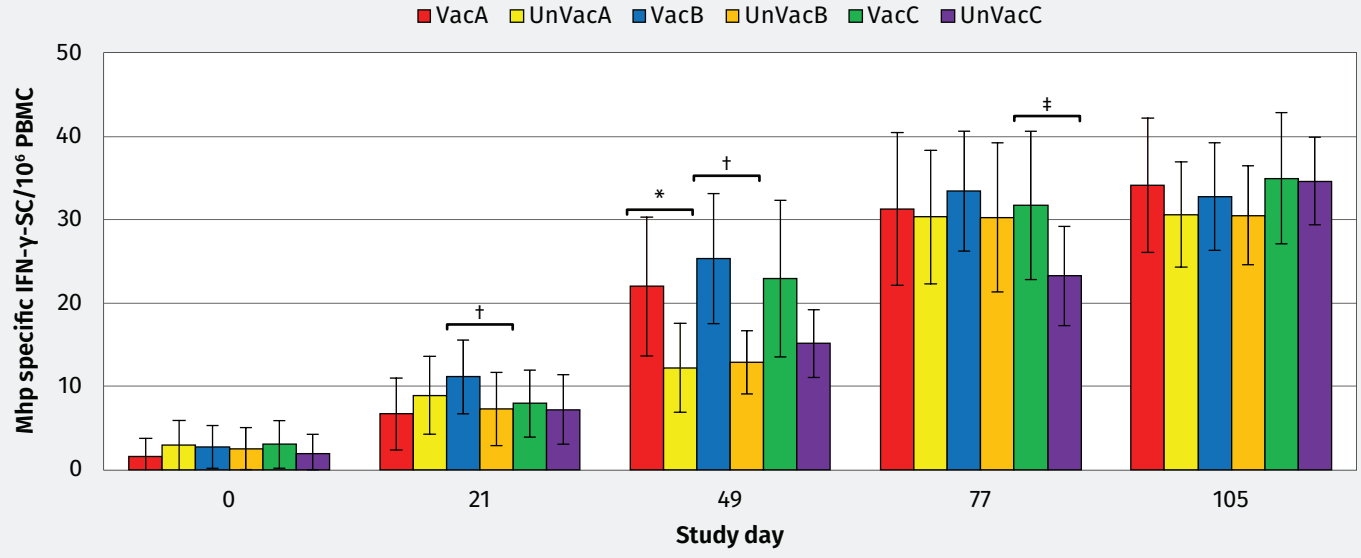


Table 2: Mean (SD) lung lesion scores*

Farm	Group (n)	Macroscopic lesion scores	Microscopic lesion scores
A	VacA (20)	12 (13.57)[a]	0.7 (0.32)[a]
	UnVacA (20)	50 (15.05)[b]	1.7 (0.36)[b]
B	VacB (20)	14 (11.48)[a]	0.9 (0.32)[a]
	UnVacB (20)	46 (21.10)[b]	1.7 (0.38)[b]
C	VacC (20)	14 (12.51)[a]	0.6 (0.27)[a]
	UnVacC (20)	46 (25.51)[b]	1.9 (0.41)[b]

* Study design described in Table 1.

[ab] Within a column, values with different superscript letters are significantly different within each farm. Macroscopic and microscopic lesion scores were compared between the two groups within each farm using a Mann-Whitney test.

The mycoplasma organism is a small bacterium without a cell wall. It is a unique pathogen in that it does not invade the body, but instead colonizes the mucosal surface of the respiratory tract damaging the cilia.[21,22] Therefore, the serum antibody response to the bacteria may be variable and not a great measurement of protective immunity. No correlation between vaccine-induced serum antibody levels and protection from colonization and disease has been determined.[13,23] Although protective immunity against *M hyopneumoniae* is not fully understood, cell-mediated immunity is likely to play an important role in the protection against *M hyopneumoniae* infection as described in previous studies.[13,23] In this study, *M hyopneumoniae*-specific IFN-γ-SC gradually increased from day 21 and reached a peak at day 49. During this period, vaccinated groups improved ADG and reduced respiratory signs significantly compared with unvaccinated groups on the 3 farms. These results indicate that *M hyopneumoniae*-specific IFN-γ-SC may provide protective immunity. However, since increased levels of IFN-γ-SC coincide with the increased amount of mycoplasmal loads in nasal shedding, further studies are needed to determine the functional role of cell-mediated immunity as a protective immunity.

The clinical impact of reducing nasal mycoplasmal shedding by vaccine may be controversial. The vaccine used in this study reduced the genomic copies of *M hyopneumoniae* on the nasal swabs from vaccinated pigs. Similarly, some studies indicate that other commercial vaccines may also reduce the number of organisms in the respiratory tract and may decrease the infection level in a herd.[24] Contradictory to these findings, additional field studies have shown that vaccination does not significantly reduce the transmission of this respiratory pathogen.[25] In addition, vaccines do not prevent colonization.[17-19,26] Consequently, vaccination alone will not be sufficient to eliminate *M hyopneumoniae* from infected pig herds. The producer must still pay attention to stocking density, ventilation, biosecurity, and the control of other diseases to be successful in the long-term control of mycoplasma.

Different sampling sites were used to detect *M hyopneumoniae* infection by PCR on experimentally and naturally infected pigs. Laryngeal swabs were a reliable sample for early detection of *M hyopneumoniae*, followed by broncho-alveolar lavage fluid and nasal swabs in live experimentally infected pigs, especially during the acute period.[27] In contrast, the most sensitive sampling sites in live naturally infected pigs were tracheo-bronchial swabbing and tracheo-bronchial washing, as compared to oral-pharyngeal brushing and nasal swabbing.[28] This may partly explain the relative inaccuracy of the nasal swabbing method.[28] In the present study, sterile swabs were inserted into nasal turbinates deeply and rotated hard enough on the inside of the nose to collect the samples properly for the detection of *M hyopneumoniae*. In addition, nasal swabs are practical samples for the detection of *M hyopneumoniae* under field conditions.

Mycoplasma hyopneumoniae is a slow-growing bacterial organism with a long period between infection and clinical impact.[29] Early infection during the life of a pig is important for the organism to grow and develop clinical disease in pigs. *Mycoplasma hyopneumoniae* prevalence at weaning can be an important indicator of disease severity in growing pigs.[30] Thus, control measures directed at lowering *M hyopneumoniae* prevalence at weaning could have a significant impact in disease presentation in grow-finishing pigs. This enhances the criticality that early control of *M hyopneumoniae* infection by vaccination is essential to control mycoplasma pneumonia. Early vaccination of piglets (< 3 weeks of age) is more common in single-site herds in Korea. Early vaccination has the advantage that immunity can be induced before the pigs become infected, and that fewer pathogens are present to possibly interfere with an immune response. In this field trial, commercial *M hyopneumoniae* vaccine was also administered to piglets at 3 weeks of age as recommended by company claims.

Single-dose *M hyopneumoniae* vaccination at 3 weeks of age significantly improved growth performance in pig farms suffering from *M hyopneumoniae* infection. This field trial was conducted on 3 farms and included housing conditions and a health status reflecting those of conventional facilities in Korea. The results of this study demonstrate that the newly introduced *M hyopneumoniae* vaccine provided good protection against *M hyopneumoniae* on farms.

Implications

Under the field conditions of this study:

- *Mycoplasma hyopneumoniae* bacterin effectively improved growth performance.
- *Mycoplasma hyopneumoniae* bacterin reduced pathological lung lesions.

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Conflict of interest

None reported.

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*Non-refereed reference.

Conversion tables

Weights and measures conversions

Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.35
1 lb (16 oz)	0.45 kg	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.3 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in^2	6.45 cm^2	in^2 to cm^2	6.45
0.16 in^2	1 cm^2	cm^2 to in^2	0.16
1 ft^2	0.09 m^2	ft^2 to m^2	0.09
10.76 ft^2	1 m^2	m^2 to ft^2	10.8
1 ft^3	0.03 m^3	ft^3 to m^3	0.03
35.3 ft^3	1 m^3	m^3 to ft^3	35.3
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.26 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	0.95 L	qt to L	0.95
1.06 qt	1 L	L to qt	1.06

Temperature equivalents (approx)

°F	°C
32	0
50	10.0
60	15.5
61	16.1
65	18.3
70	21.1
75	23.8
80	26.6
82	27.7
85	29.4
90	32.2
102	38.8
103	39.4
104	40.0
105	40.5
106	41.1
212	100.0

°F = (°C × 9/5) + 32

°C = (°F - 32) × 5/9

Conversion calculator available at: amamanualofstyle.com/page/si-conversion-calculator

Conversion chart, kg to lb (approx)

Pig size	Lb	Kg
Birth	3.3-4.4	1.5-2.0
Weaning	7.7	3.5
	11	5
	22	10
Nursery	33	15
	44	20
	55	25
	66	30
Grower	99	45
	110	50
	132	60
Finisher	198	90
	220	100
	231	105
	242	110
	253	115
Sow	300	136
	661	300
Boar	794	360
	800	363

1 tonne = 1000 kg

1 ppm = 0.0001% = 1 mg/kg = 1 g/tonne

1 ppm = 1 mg/L