Efficacy of a *Mycoplasma hyopneumoniae* bacterin in pigs challenged with two contemporary pathogenic isolates of *M hyopneumoniae*

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Summary

Objective: To evaluate the efficacy of a *Mycoplasma hyopneumoniae* (M hyo) bacterin against two contemporary field isolates of M hyo.

Materials and methods: Two challenge studies were performed in which pigs received two doses of either saline (nonvaccinated groups) or an M hyo bacterin (vaccinated groups), followed 3 weeks later by intratracheal inoculation of each pig with one of two M hyo field isolates. Necropsies were performed 28 or 30 days post challenge. Vaccine efficacy was determined by evaluating macroscopic lung lesions, DNA levels of M hyo in bronchial alveolar lavage fluids (BALF), M hyo-specific immunoglobulin (Ig)A and IgG antibodies, and serum antibodies measured by the Tween 20 and DAKO enzyme-linked immunosorbent assays.

Results: The percentage of macroscopic lung lesions and concentration of M hyo DNA in BALF samples post challenge with either isolate were significantly less in vaccinated than in nonvaccinated pigs ($P < .05$). M hyo-specific mucosal IgA and IgG antibody levels in BALF were significantly higher in the vaccinated pigs than in the nonvaccinated pigs.

Implications: Under the conditions of these studies, fewer M hyo organisms and fewer macroscopic lesions of pneumonia are observed when pigs are vaccinated with an M hyo bacterin prior to challenge with contemporary virulent M hyo field isolates. Vaccination is an effective tool to reduce pneumonia induced by M hyo, although more studies are needed to determine protection against a wide range of field isolates.

Keywords: swine, *Mycoplasma hyopneumoniae*, vaccine, mycoplasmal pneumonia, lung consolidation

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Resumen - Eficacia de una bacteria *Mycoplasma hyopneumoniae* en cerdos retados con dos aislados patógenos contemporáneos de *M hyopneumoniae*

Objetivo: Evaluar la eficacia de una bacteria de *Mycoplasma hyopneumoniae* (M hyo) contra dos aislados contemporáneos de campo de M hyo.

Materiales y métodos: Se realizaron dos estudios de reto en que los cerdos recibieron dos dosis de solución salina (grupos no vacunados) o una bacteria de M hyo (grupos vacunados), seguidos de la inoculación intratraqueal de cada cerdo 3 semanas después con uno de los dos aislados de campo de M hyo. Se realizaron necropsias 28 ó 30 días después del reto. La eficacia de la vacuna se determinó al evaluar lesiones macroscópicas de pulmón, niveles de DNA de M hyo en fluidos de lavado bronquio-alveolar (BALF por sus siglas en inglés), anticuerpos específicos IgA e IgG contra M hyo y anticuerpos de suero mediante el ensayo inmunoenzimático ligado a enzimas Tween 20 y DAKO.

Resultados: El porcentaje de lesiones macroscópicas de pulmón y la concentración de DNA de M hyo en las muestras de BALF después de la prueba con cualquiera de los aislados fueron significativamente menores en cerdos vacunados que en cerdos no vacunados ($P < .05$). Los niveles de anticuerpos específicos IgG e IgA de mucosa contra M hyo en BALF fueron significativamente más altos en cerdos vacunados que en cerdos no vacunados.

Implicaciones: Bajo las condiciones de estos estudios, se observaron menos organismos de M hyo y menos lesiones macroscópicas de neumonía cuando los cerdos se vacunaron con una bacterina de M hyo antes del reto con aislados contemporáneos de campo de M hyo virulentos. La vacunación es una herramienta efectiva para reducir la neumonía inducida por M hyo, aunque se necesitan más estudios para determinar la protección contra un amplio rango de aislados de campo.

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Résumé - Efficacité d'une bactérine de *Mycoplasma hyopneumoniae* lors d'une infection défi chez des porcs avec deux isolats pathogènes récents de *M hyopneumoniae*

Objectif: Évaluer l'efficacité d'une bactérine de *M hyopneunoniae* (M hyo) envers deux isolats terrain récents de M hyo.

Matériels et méthodes: Deux infections défis ont été effectuées au cours desquelles des porcs ont reçu deux doses de saline (groupes non-vaccinés) ou une bactérine de M hyo (groupes vaccinés), suivi 3 semaines plus tard de l'inoculation intra-trachéale de chaque porc avec l'un ou l'autre des isolats terrain de M hyo. Des nécropsies ont été effectuées 28 ou 30 jours post-inoculation. L'efficacité du vaccin a été déterminée par évaluation des lésions pulmonaires macroscopiques, la quantité d'ADN de M hyo dans les liquides de lavage broncho-alvéolaire (BALF), les anticorps spécifiques de types IgA et IgG dirigés contre M hyo, et les anticorps sériques mesurés par méthodes immuno-enzymatiques au Tween 20 et DAKO.

Résultats: Le pourcentage de lésions pulmonaires macroscopiques et la concentration d'ADN dans les échantillons de BALF post-inoculation avec l'un ou l'autre des isolats étaient significativement moindre chez les animaux vaccinés comparativement aux animaux non-vaccinés ($P < .05$). Les niveaux d'anticorps de type IgA et IgG dirigés spécifiquement envers M hyo et retrouvés dans les échantillons de BALF étaient significativement plus élevés chez les porcs vaccinés que chez les porcs non-vaccinés.

Implications: Dans les conditions expérimentales étudiées, des quantités moindres de M hyo et de lésions macroscopiques de pneumonie sont observées lorsque les porcs sont vaccinés avec une bactérine de M hyo avant l'inoculation défi avec des isolats terrain virulents récents. La vaccination est un moyen efficace pour réduire la pneumonie induite par M hyo, bien que des études supplémentaires soient nécessaires afin de déterminer la protection contre une grande variété d'isolats de terrain.

Mycoplasma hyopneumoniae (M hyo) is the causative agent of enzootic pneumonia, but more importantly, it is a primary component of the porcine respiratory disease complex. The impact of disease caused by M hyo leads to significant economic losses in the swine industry today. *Mycoplasma hyopneumoniae* is an extracellular pathogen that resides primarily on the ciliated respiratory epithelium of the lower respiratory tract, where it induces a delayed humoral immune response in the pig. Currently available commercial bacterins are unable to prevent M hyo colonization in the lungs, but significantly reduce the severity of pneumonia in vaccinated pigs.[1,2] Most experimental studies evaluating the efficacy of M hyo bacterins have relied on challenge models with older reference strains of M hyo. Many of these studies have used a model developed at Iowa State University, that consists of intratracheal inoculation of a diluted lung homogenate containing pig-passaged strain 232,[1,2] which was derived from a strain first isolated in the 1960s.[3] Recently, a difference in virulence among M hyo isolates has been demonstrated.[4-6] In addition, genotypic and phenotypic variation among isolates has been shown to exist, although specific implications of these differences have not yet been identified.[7-12]

It is currently unknown whether differences in virulence factors or other putative immunogens affect the ability of current vaccines to protect against different field isolates of M hyo. As a result, there is a need to measure the efficacy of M hyo vaccines against a variety of field isolates.

In the study reported here, each pig was assigned to one of two treatment groups that received either an M hyo bacterin or saline. Half of each treatment group was then challenged with one of two different contemporary M hyo field isolates. The M hyo isolates used in this study, 95MP1509 and 00MP1301,[6] are genetically distinct from each other and from strain 232.[12] The parameters used to measure efficacy included macroscopic lung lesions, M hyo level in the lungs as assessed by real-time polymerase chain reaction (PCR), M hyo-specific immunoglobulin (Ig)A and IgG antibodies in bronchial alveolar lavage fluids (BALF) measured by enzyme-linked immunosorbent assays (ELISAs), and M hyo-specific serum antibodies assessed by Tween 20 and DAKO ELISAs.

Materials and methods

Study animals

Ninety-three weaned and castrated male crossbred pigs, 9 to 14 days of age, were transported from a commercial farrowing unit to the Iowa State University Animal Research Facilities in Ames, Iowa. The source herd had no history of vaccination against M hyo and was serologically negative for M hyo and porcine reproductive and respiratory syndrome virus. No clinical signs of swine influenza virus were present in the pigs at the time of arrival. All animal procedures and care were conducted under the supervision and regulations of the Iowa State University Institutional Animal Care and Use Committee.

Experimental design

Eighty-four pigs were distributed into two separately housed challenge groups. Each group was randomly assigned to seven pens of six pigs according to a generalized block design. Within each pen, three pigs were vaccinated with the M hyo bacterin and three pigs received saline (nonvaccinates). Two groups of three pigs, used as procedural negative-controls, were housed separately in a different facility and received no treatment or challenge. Food and water were supplied ad libitum throughout the study. At approximately 3 and 5 weeks of age, pigs were injected intramuscularly (IM) in the right and left neck, respectively, with 2 mL of an M hyo bacterin (RespiSure; Pfizer Animal Health, New York, New York) or with 2 mL of 0.9% saline as per treatment assignment and were designated as vaccinated and nonvaccinated pigs, respectively. At approximately 8 weeks of age, each challenge group of pigs was inoculated intratracheally with an M hyo broth culture containing between 10^7 and 10^8 color changing units per mL of either field isolate 95MP1509 or 00MP1301 on 3 consecutive days, designated 0, 1, and 2 days post challenge (DPC), as previously described.[1] Both 95MP1509 and 00MP1301 were isolated from pigs submitted to the Iowa State University Veterinary Diagnostic Laboratory in 1995 and 2000, respectively. Pigs challenged with isolate 95MP1509 were necropsied 28 DPC, and pigs challenged with 00MP1301were necropsied 30 DPC. The investigators were blinded to the vaccination status of the pigs until all results from all study parameters were collected. Blood for serological testing was collected 1 day prior to each vaccination, 1 day prior to challenge, and at necropsy. All serum samples were frozen at -20°C until the end of the study and then batch-tested by the

Tween 20 ELISA[13] and the DAKO M hyo blocking ELISA (DAKO Corporation, Carpenteria, California).

Serum antibody tests

For the Tween 20 ELISA, all samples were tested in duplicate and the average optical density (OD) of the two was used in analysis. The positive control was normalized to an OD of 0.40, and the samples were then adjusted accordingly. Optical density ≥ 0.24 was defined as positive, OD < 0.20 as negative, and OD ≥ 0.20 and < 0.24 as suspect.

For the DAKO ELISA, a sample was defined as positive if the percent inhibition of the sample was ≤ 50% of the buffer control, and negative if the percent inhibition of the sample was > 50% of the buffer control according to the manufacturer's directions.

Necropsy

All animals, including the procedural negative-control pigs, were euthanized using an AVMA-approved euthanasia solution administered intravenously (Fatal-Plus; Vortech Pharmaceuticals, Ltd, Dearborn, Michigan) followed by exsanguination.

Lung lesions consistent with M hyo pneumonia were sketched on a standard diagram and assessed for the proportion of lung surface with lesions using a Zeiss SEM-IPS image analysis system (Carl Zeiss, Inc, Thornwood, New York) as previously described.[1]

Bronchial swabs were cultured for swine respiratory pathogens, including *Bordetella bronchiseptica, Pasteurella multocida, Actinobacillus* species, and *Haemophilus* species. Samples were inoculated onto blood agar plates, each streaked with a *Staphylococcus epidermidis* nurse colony for support of *A pleuropneumoniae* and *Haemophilius* species. In addition, bronchial swabs were inoculated into brain-heart infusion trypticase soy mycoplasma medium[14] for isolation of *M hyorhinus* and Friis medium[15] for isolation of M hyo. Species identification of mycoplasma cultures was confirmed using a multiplex PCR for M hyo, *Mycoplasma flocculare,* and *Mycoplasma hyorhinis* as previously described.[16]

BALF was collected using 25 mL of sterile phosphate buffered saline (PBS) as previously described.[17] BALF samples were assayed by ELISA for local IgA and IgG antibodies to M hyo[18] and for M hyo DNA by real-time PCR. For the real-time PCR, a 1-mL aliquot of BALF from each pig was centrifuged at 16,000*g* for 10 minutes. The resulting pellet was resuspended in 200 µL of PBS, and M hyo DNA was isolated using the QIAamp DNA mini kit (Qiagen, Valencia, California). In addition, DNA was isolated from M hyo strain 232 by phenol-chloroform extraction[19] for use in generating a standard curve for semi-quantitative analysis. The real-time PCR, which targets the repeated element MHYP1–03–950 (accession no. AF004388), was performed with minor modifications as previously described.[20] The concentration used for the probe was 250 nM. An internal positive control was added to control for potential inhibition caused by the DNA sample (Applied Biosystems, Foster City, California). The 10× exogenous internal positive-control mix and 50× exogenous internal positive-control DNA were diluted two-fold further than recommended by the manufacturer to prevent interference within the assay. All PCR reactions were performed in duplicate on a Rotor-Gene RG-3000 (Corbett Research, San Francisco, California). Results were quantified by comparison to a standard curve generated for each run using Rotor-Gene 5 software (Corbett Research) and expressed as ng per µL DNA. Duplicate samples were then averaged.

Data analysis

The two challenge groups were treated as separate studies for the purposes of randomization and statistical analysis. In all analyses, the individual pig was the experimental unit.

The arcsine square root transformation was applied to the total percent lung involvement prior to analysis, and the transformed percent lung scores were analyzed with a general linear mixed model with fixed term treatment and random term block.

For each antibody-test procedure, OD values were back-transformed to obtain geometric mean titers prior to analysis. Transformed serum antibody values were analyzed with a general linear repeated measures mixed model with fixed effects treatment, time point, and treatment-by-time-point interaction, and random effects block and animal-within-block by treatment. Positive-negative status for both Tween 20 and DAKO ELISAs was analyzed with a generalized linear repeated measures mixed model (GLIMMIX) with fixed effects treatment, time point, and treatment-by-time-point interaction, and random effects block and animal-within-block by treatment. Suspect results for the Tween 20 assay were considered negative for the analysis. The M hyo IgA and IgG mucosal antibody titers and real-time PCR concentrations from BALF were analyzed with a generalized linear mixed model with fixed term treatment and random term block. If the model (via the GLIMMIX macro) did not converge when positive-negative status was analyzed, then Fisher's exact test was used to analyze that variable. All analyses were performed using SAS/STAT guide version 8 (SAS institute, Inc, Cary, North Carolina). The 5% level of significance ($P < .05$) was used to assess statistical differences.

Results

Mortality

One pig in the nonvaccinated group (95MP1509 challenge) and two pigs in the vaccinated group (00MP1301 challenge) died of causes not related to the study.

Serology

All pigs were classified as seronegative for M hyo antibodies by the Tween 20 and DAKO ELISAs at beginning of the study and prior to vaccination. The nonvaccinated pigs remained seronegative for M hyo prior to challenge, and the procedural negative-control pigs remained serologically negative at all time points throughout the trial (data not shown). The percentages of serologically positive pigs as measured by the Tween 20 and DAKO assays are summarized in Table 1. The OD levels measured by the Tween 20 assay were significantly higher ($P < .001$) in vaccinated pigs than in nonvaccinated pigs from both the 95MP1509 and 00MP1301 challenge groups prior to challenge and at necropsy (Table 2).

Macroscopic lung lesions

Analysis of the percentage of macroscopic lung lesions is summarized in Table 3. The mean percent lung involvement was significantly lower in vaccinated pigs than in nonvaccinated pigs in both challenge studies ($P < .001$).

The distribution of lung lesions by severity is summarized in Table 4. The macroscopic lung lesion scores for the procedural negative-control pigs averaged 0.36% for the 00MP1301 challenge study and 1.1% for the 95MP1509 study.

M hyo real-time PCR

Detection of M hyo DNA in BALF samples collected at necropsy is summarized in Table 5. Following challenge with either

isolate, the level of M hyo DNA detected in vaccinated pigs was significantly lower ($P < .001$) than the level detected in the nonvaccinated pigs.

IgA and IgG antibodies in BALF at necropsy

Significantly higher OD levels corresponding to M hyo-specific mucosal IgA and IgG antibodies were detected in vaccinated pigs than in nonvaccinated pigs in both challenge groups ($P < .001$; Table 6). The IgA and IgG OD values for all procedural negative-control pigs were low (OD < 0.07 for all samples; data not shown).

Bacterial isolation from bronchial swabs at necropsy

Streptococcus suis was isolated from nine and three pigs in the 95MP1509 and 00MP1301 challenge groups, respectively, but no clinical disease associated with this bacterium was observed. No other swine bacterial pathogens were isolated from any pigs at necropsy. Culture for M hyo was positive in 19 of 20 nonvaccinated pigs and 18 of 21 vaccinated pigs challenged with 95MP1509, and for 20 of 21 nonvaccinated and 16 of 19 vaccinated pigs challenged with 00MP1301. Isolation

Table 1: Frequency of pigs serologically positive for *Mycoplasma hyopneumoniae* (M hyo) by the Tween 20 and DAKO enzyme-linked immunosorbent assays (ELISAs)*

Challenge isolate	Treatment	No. of pigs	Test	Pigs positive (%)			
				Vacc 1	Vacc 2	Challenge†	Necropsy‡
95MP1509	Saline	20	Tween 20	0	0	0	60
			DAKO	0	0	0	100
	M hyo bacterin	21	Tween 20	0	0	76	100
			DAKO	0	5	100	100
00MP1301	Saline	21	Tween 20	0	0	0	67
			DAKO	0	0	0	100
	M hyo bacterin	19	Tween 20	0	0	74	100
			DAKO	0	5	100	100

* Pigs were injected at 3 and 5 weeks of age with an M hyo bacterin or saline, challenged with M hyo at approximately 8 weeks of age, and necropsied 28 days post challenge (isolate 95MP1509) or 30 days post challenge (isolate 00MP1301). Vacc 1 = first vaccination, Vacc 2 = second vaccination. Blood samples were collected 1 day before each vaccination, 1 day before challenge, and at necropsy. For the Tween 20 ELISA, the positive control was normalized to an OD of 0.4, and samples were adjusted accordingly: positive, OD ≥ 0.24; negative, OD ≥ 0.20; suspect, OD ≥ 0.20, and < 0.24 with suspect ODs considered negative for analysis. For the DAKO ELISA: positive, percent inhibition ≤ 50% of the buffer control; negative, percent inhibition > 50% of the buffer control. Positive-negative status for both tests was analyzed with a generalized linear repeated measures mixed model (GLIMMIX) with fixed effects treatment, time point, and treatment-by-time-point interaction, and random effects block and animal-within-block by treatment. If the model (via the GLIMMIX macro) did not converge when positive-negative status was analyzed, then Fisher's exact test was used to analyze that variable at each time point.

† For each challenge isolate, the percentage of pigs seropositive by the Tween 20 and DAKO tests was higher for vaccinates than for saline controls ($P < .001$).

‡ For each challenge isolate, the percentage of pigs seropositive by the Tween 20 assay was higher for vaccinates than for saline controls ($P < .01$).

Table 2: *Mycoplasma hyopneumoniae* (M hyo) Tween 20 serum antibody titers in pigs described in Table 1*

Challenge isolate	Treatment	No. of pigs	Tween 20 serum antibody titers (mean OD ± SE)†			
			Vacc 1	Vacc 2	Challenge	Necropsy
95MP1509	Saline	20	0.022 ± 0.000	0.016 ± 0.000	0.031 ± 0.000	0.273 ± 0.004
	M hyo bacterin	21	0.018 ± 0.000	0.021 ± 0.000	0.323 ± 0.008	0.962 ± 0.017
00MP1301	Saline	21	0.025 ± 0.000	0.017 ± 0.000	0.026 ± 0.000	0.292 ± 0.006
	M hyo bacterin	19	0.023 ± 0.000	0.014 ± 0.009	0.340 ± 0.009	0.935 ± 0.028

* Vacc 1 = day of first vaccination; Vacc 2 = day of second vaccination. Blood samples were collected 1 day before each vaccination, 1 day before challenge, and at necropsy.

† Back-transformed geometric mean titer ± standard error (SE). Serum antibody titers were log transformed and analyzed with a general linear repeated measures mixed model with fixed effects treatment, time point, and treatment-by-time-point interaction, and random effects block and animal-within-block by treatment. The individual pig was the experimental unit. For each challenge isolate, titers were significantly higher ($P < .001$) in the M hyo bacterin groups than in the saline groups 1 day before challenge and at necropsy.

rate for any bacterial species did not differ among the treatment groups. All procedural negative-control pigs from each challenge study were culture-negative for all bacterial species, except one pig from which M hyo was cultured.

Discussion

This study investigated the efficacy of an M hyo bacterin against two contemporary M hyo isolates which had previously been shown to produce more macroscopic lung lesions than the prototypic challenge strain 232.[6] Currently, there are no vaccines able to prevent pigs from becoming infected with M hyo. In this study, M hyo was isolated at the same rate from pigs regardless of their treatment, although organism levels were higher and lung lesions were more severe in the saline controls than in the vaccinates. In addition, a stronger local and systemic immune response in the vaccinated pigs was observed, demonstrating that while the bacterin in this study did not prevent M hyo from infecting the pigs, it did protect them against disease.

One of the pigs in the procedural negative controls cultured positive for M hyo. This was likely due to post-harvest contamination, as the procedural control pigs were housed in a different facility than the challenged pigs and exhibited no clinical signs of pneumonia at necropsy. A low percentage of macroscopic lesions was observed in the lungs of some procedural control pigs, consistent with background levels normally observed in our challenge models using conventional pigs.

Serology is a common tool used for monitoring herd status of M hyo, as well as for determining vaccination compliance. It has been suggested, in a study using field

Table 3: Parametric analysis of *Mycoplasma hyopneumoniae* (M hyo) percent lung lesions in pigs described in Table 1

Challenge isolate	Treatment	No. of pigs	Lung involvement (%)	
			LS means ± SE*	Range
95MP1509	Saline	20	19.3 ± 3.1	3 - 47
	M hyo bacterin	21	5.1 ± 1.7	0 - 43
00MP1301	Saline	21	7.4 ± 1.4	1 - 28
	M hyo bacterin	19	1.2 ± 0.6	0 - 19

* The arcsine square root transformation was applied to the total percent lung involvement prior to analysis. The transformed percent lung scores were analyzed with a general linear mixed model with fixed term treatment and random term block. Results are presented as back-transformed least squares means ± SE. For both challenge isolates, values were lower for M hyo vaccinates than for saline controls ($P < .001$).

Table 4: Frequency distribution of percent lung-lesion scores at necropsy in pigs described in Table 1

Challenge isolate	Treatment	No. of pigs	Frequency distribution of lung-lesion score (% of pigs per range)*					
			< 2%	2% to < 5%	5% to < 10%	10% to < 20%	20% to < 30%	≥ 30%
95MP1509	Saline	20	0	5	20	30	20	25
	M hyo bacterin	21	48	24	5	5	14	5
00MP1301	Saline	21	14	29	19	29	10	0
	M hyo bacterin	19	68	21	0	11	0	0

* Row totals may exceed 100% because of rounding.

Table 5: *Mycoplasma hyopneumoniae* (M hyo) DNA levels detected by real-time polymerase chain reaction in bronchial alveolar lavage fluids (BALF) at necropsy in pigs described in Table 1

Challenge isolate	Treatment	M hyo DNA (ng/µL)	
		GMT ± SE*	Range
95MP1509	Saline	2.415 ± 0.622	0.49 - 11.29
	M hyo bacterin	0.378 ± 0.095	0.02 - 2.62
00MP1301	Saline	1.188 ± 0.502	0.01 - 6.14
	M hyo bacterin	0.110 ± 0.049	0.00 - 2.32

* Back-transformed geometric mean titer (GMT) ± SE. A log transformation was applied to titer values and titers were analyzed with a generalized linear mixed model with fixed effect treatment and random effects block. Values for M hyo vaccinates were lower than those for nonvaccinates ($P < .001$).

Table 6: *Mycoplasma hyopneumoniae*- (M hyo-) specific IgA and IgG antibodies in bronchial alveolar lavage fluids (BALF) at necropsy in pigs vaccinated at 3 and 5 weeks of age with an M hyo bacterin or saline, challenged at approximately 8 weeks of age with M hyo, and necropsied 28 days post challenge (DPC) (isolate 95MP1509) or 30 DPC (isolate 00MP1301)

Challenge isolate	Treatment	BALF M hyo antibodies (GMT ± SE)*	
		IgA	IgG
95MP1509	Saline	0.236 ± 0.017	0.377 ± 0.025
	M hyo bacterin	0.486 ± 0.035	0.970 ± 0.062
00MP1301	Saline	0.257 ± 0.018	0.399 ± 0.041
	M hyo bacterin	0.458 ± 0.020	0.932 ± 0.041

* Back-transformed geometric mean titer (GMT) of optical density (OD) ± SE. For each antibody isotype, a log transformation was applied to OD titer values and titers were analyzed with a generalized linear mixed model with fixed effect treatment and random effect block. For both challenge studies, immunoglobulin IgA and IgG OD titers were higher ($P < .001$) in the M hyo vaccinates than in the saline controls.

sera,[21] that the immune response in pigs infected with M hyo is not uniform and that these differences may impact the sensitivity of M hyo-specific ELISAs. Therefore, two different M hyo ELISAs were used to evaluate M hyo serum antibodies in this study. Both the Tween 20 ELISA and the DAKO ELISA detected M hyo-specific serum antibodies in vaccinated pigs from each challenge group after two vaccinations. Antibody levels in the vaccinated pigs increased post challenge, indicative of an anamnestic response. Direct comparisons of ODs between the vaccinates and nonvaccinates were made using Tween 20 results, as OD values in this assay correlate with M hyo-specific antibody levels. In contrast, the DAKO ELISA is a competitive inhibition assay and OD values do not directly correlate with antibody levels. In this study, the DAKO ELISA was more sensitive at earlier time-points after challenge with either field isolate, in agreement with results of a study[22] using sera from pigs experimentally challenged with M hyo strain 232.

Anecdotal reports from the field suggest that variation in M hyo isolates may play a role in M hyo vaccine failure. Results from the studies presented here indicate that the M hyo vaccine provided protection against mycoplasmal pneumonia in pigs challenged with more recent M hyo field isolates. This challenge model is a more severe exposure than would occur in a natural field infection. Because challenge with bacterial cultures may be less reproducible than challenge with lung inoculum, pigs were inoculated on 3 consecutive days as has been previously described.[23] In addition, vaccinated and nonvaccinated pigs were co-mingled. Therefore, any effect of population immunity on shedding of M hyo was lost. Although these studies were analyzed separately and therefore were not directly compared, the 95MP1509 challenge resulted in more pigs in the saline treatment group with higher gross lesion scores (> 10%) than was observed in the saline treatment group in the 00MP1301 challenge. In addition, 19% of vaccinated pigs in the 95MP1509 group had pneumonia affecting > 20% of the lung, while in the 00MP1301 group, lesions affected < 5% of the lung in most (89%) of the vaccinated pigs. The severity of the challenge model may partially explain the high lung-lesion scores observed in pigs challenged with isolate 95MP1509. Alternatively, the greater percent of lung lesions may demonstrate less protection against this isolate.

The importance of genetic variation in cross-protection between M hyo isolates is not known. Since the virulence factors for M hyo have not been identified, the protective antigens required in a vaccine remain undefined. Neither field isolate used in this study has been used in commercial vaccine production. In addition, it is not known how genetically similar these two field isolates are to the strains of M hyo currently used in M hyo bacterins. No M hyo genes or gene products have been correlated with protection against disease caused by M hyo; therefore, no suitable targets for sequencing exist for comparison of field isolates in relation to virulence or protection from disease.

This study measured vaccine efficacy following experimental challenge with two recent virulent M hyo field isolates. Further studies need to be performed evaluating genetic and antigenic variation among M hyo isolates to better understand any impact these differences may have on vaccine efficacy.

Implications

- Under the conditions of these studies, vaccination is an effective tool to reduce M hyo organisms and macroscopic lesions of pneumonia when pigs are challenged with contemporary virulent field isolates of M hyo.
- More studies assessing M hyo vaccines need to be performed using a diverse range of M hyo field isolates.

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