

Effect of a *Mycoplasma hyopneumoniae*-commercial vaccine on concurrent lung infections in pigs in the field

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Abstract

The efficacy of an inactivated aqueous vaccine against *Mycoplasma hyopneumoniae* was evaluated in two *M. hyopneumoniae*-infected farrow-to-finish commercial farms in Greece. In a prospective, randomized double-blind study, two groups on each farm received either two intramuscular doses of vaccine or two doses of adjuvant alone injected at 1 and 4 weeks of age. From each farm, 50 pigs, 25 originating from unvaccinated (placebo) group and 25 from vaccinated group, showing enzootic pneumonia lesions were sampled. Lung samples for microbiology and blood for serology were collected. The average score of lung lesions associated with enzootic pneumonia was significantly lower in vaccinated piglets compared to unvaccinated ones ($p < 0.05$). *Streptococcus spp* and *Pasteurella multocida* have been isolated from the lungs of significantly fewer vaccinated compared to non-vaccinated piglets ($p < 0.05$), while such was not observed for *M. hyopneumoniae*, *Haemophilus spp*, *Bordetella spp* and *Staphylococcus spp*. Significantly fewer vaccinated piglets were serologically positive for type H3N2 influenza virus compared to non-vaccinated pigs ($p < 0.05$). In conclusion, our results suggest that vaccination against *M. hyopneumoniae* may reduce concurrent bacterial and viral spread among pigs.

Keywords: concurrent infection; enzootic pneumonia; lung; pig

Introduction

Mycoplasma hyopneumoniae is the principal etiological agent responsible for enzootic pneumonia (EP) in pigs. Other pathogens such as *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Streptococcus suis*, *Haemophilus parasuis*, *Bordetella bronchiseptica* and *Arcanobacterium pyogenes* can aggravate the disease (Maes et al., 2000; Maes et al., 2008). All or some of the previous bacteria, in combination with certain viruses such as porcine reproductive and respiratory syndrome (PRRS) virus, Aujeszky's disease virus, or/and swine influenza (SI) virus can be involved in the development of the porcine respiratory disease complex (PRDC) (Maes et al., 2000; Georgakis et al., 2002; Maes et al., 2008). *M. hyopneumoniae* affects the mucosal clearance system by disrupting the cilia on the epithelial surface and, additionally, the organism modulates the immune system of the respiratory tract (Maes et al., 2008). Therefore, *M. hyopneumoniae* predisposes animals to concurrent infections with other respiratory pathogens including bacteria, parasites and viruses. Control of enzootic pneumonia can be accomplished by optimizing management practices and housing conditions, strategic medication and vaccination. To combat enzootic pneumonia, several chemotherapeutic agents and antibacterial regimens have been used with varying degrees of success reported (Cooper et al., 1993; Hannan et al., 1997; Stipkovits et al., 2001). However, the recent demand

for minimal use of antibiotics in animal production, particularly when these animals are directed to human consumption challenges scientists to explore alternative ways of preventing or controlling the disease (Dritz et al., 2002). Traditionally farmers have used management improvement for reducing economic losses (Desrosier, 2001; Maes et al., 2003; Haesebrouck et al., 2004). Thus for instance, a strict all-in, all-out (AIAO) production scheme is possibly the most effective way to control the disease in infected herds (Clark et al., 1990; Scheidt et al., 1990). However, modifications in management are not always achievable, and reintroduction of the organism is easy (Goodwin, 1985; Stark et al., 1992). During the past 10 years various experimental or commercial vaccines have been tested and recommended as a good measure for improving pig lung health (Pommier et al., 2000; Siugzsaite et al., 2002; Maes et al., 2003; Haesebrouck et al., 2004; Bargaen, 2004; Fano et al., 2007). Such an improvement, in conjunction to good management practices, had resulted in high economic gains (Maes et al., 2000; Maes et al., 2008). In a previous study, the efficacy of Porcilis M Hyo (Intervet, The Netherlands) in preventing enzootic pneumonia under continuous or in all-in, all-out (AIOA) farm flow field conditions had been investigated (Tzivara et al., 2007). The present study examines whether the same vaccine can affect the involvement of concurrent pathogens in

EP-affected piglets in the same farms.

Materials and methods

Experimental animals

The study was conducted on two farms in the area of Central Greece. Both units had a history of enzootic pneumonia. Farm A had a capacity of 180 sows and utilized a continuous flow system, while farm B had a capacity of 300 sows and was applying AIAO system. In both farms, weaning was taking place at approximately 4 weeks of age. Subsequently pigs were transferred to nurseries up to the age of 65-75 days, then to growing rooms until the age of 100-120 days, and eventually to a finishing unit until a slaughter weight (95-100 kg) was achieved. In farm A, breeding animals were vaccinated for *Escherichia coli*. In farm B breeding animals were vaccinated for Aujeszky's disease, atrophic rhinitis, parvovirus, erysipelas, *E. coli* and *Clostridium perfringens* type A and C, while fattening pigs were vaccinated once at 90 days of age for Aujeszky's disease. Both farms had pens with slatted floors, and ventilation was automatically controlled in nurseries, or by windows and electrical ventilators in the growing and finishing units. The feed provided to the pigs was home-mixed and based on corn, barley, wheat and soy, depending on availability. Feed and water were available *ad lib*. The farms were tested free of Porcine Reproductive and Respiratory Syndrome (PRRS) virus. They were tested positive for Porcine Circovirus Type 2 (PCV 2), but without any clinical or pathological findings of Porcine Multisystemic Wasting Syndrome (PMWS). All adults were treated with ivermectin twice a year.

Description of vaccine

Porcilis M Hyo (Intervet International, The Netherlands) contains an inactivated bacterin as a whole cell concentrate of Mh strain 11 in a dl-alpha-tocopheryl based aqueous adjuvant (Diluvac Forte). According to the manufacturer, each pig should receive intramuscular injections of 2 ml of vaccine from one week of age, with a second injection three weeks later.

Experimental design

Two months prior to the initiation of the trial, the high prevalence of enzootic pneumonia in each farm was confirmed in sera and lungs as described elsewhere (Tzivara et al, 2007). No antibiotics were included in the feed during the trial period.

A prospective, randomized double-blind study was performed over a total of ten months, with a total of some 500 Cotswold crossbred piglets on the two units. These pigs were randomly allocated to one of two different treatments on each farm. Group A received an intramuscular dose of vaccine at the age of four to thirteen days (average age at injection \pm Standard Deviation [SD] = 7.7 \pm 2 days \pm SD), and a second dose fourteen to twenty five days later (mean interval \pm SD = 21.4 \pm 2.6 days), at the day of weaning. Those in Group B received an intramuscular injection of the adjuvant alone, on the same dates. The adjuvant preparation was indistinguishable from the vaccine and administered at the same sites in the same volume. During their first injection, all piglets were clinically healthy and marked accordingly. Administration of medication or castration was not allowed within 48 hours prior to or post vaccination. Both groups had the same composition with regard to genders at the start of

the trial, at weaning, and they were all time kept under the same airspace.

Observations and sampling

Lung microbiology and serology of EP-affected pigs had been attempted in the present study, in addition to clinical signs, morbidity, mortality and body weight, the latter being presented elsewhere (Tzivara et al, 2007). Thus, at slaughter, lung lesions were recorded and scored as described by Goodwin and Whittlestone (1967) in 50 pigs (approximately 10% of the total pigs used in each farm), 25 originating from group A and 25 from group B. The selection of pigs was limited to those showing lesions in at least 2 front lobes (apical and cardiac) of either side of the lungs. From those pigs, lung samples for microbiology and blood for serology were collected.

Microbiologic examination of lungs

Isolation of *M.hyopneumoniae* was attempted in FDML liquid and on FDMS solid media (Mycoplasma Experience, UK), following the instructions of the media supplier. Briefly, a small tissue sample aseptically collected from the periphery of lung lesions was spread on the surface of FDMS and the same sample was immersed in the FDML. The two were incubated anaerobically for up to nine days at 37⁰ C. After the recommended incubation time, cultures were examined under a stereoscopic microscope for the identification of *M. hyopneumoniae* characteristic colonies. All characteristic colonies were subcultured in FDML media, kept at -70⁰ C and identified to species by a Nested-PCR according to Calsamiglia et al. (1999) after DNA extraction using the recommendations of Sambrook et al. (1989). For isolating and identifying other microorganisms possibly colonizing pig lungs, an aseptically collected small piece of lung from the periphery of lung lesions was immersed in Nutrient Broth (Oxoid-LTD, Basingstoke, UK). The inoculated broth was incubated for 16 hours at 37⁰ C. After a thorough mixing of the culture, a drop of it was inoculated on Columbia blood agar (CBA) (Oxoid LTD, Basingstoke, UK) and a drop onto MacConkey agar (Oxoid LTD, Basingstoke, UK). The inoculated solid media were incubated aerobically for 24 -36 hours at 37⁰ C. Different colonies were subcultured on CBA, and pure cultures were examined by Gram. All microorganisms were identified following the methods of Barrow and Feltham (1999).

Serologic examination of selected piglets

All serum samples were examined for antibodies against *M. hyopneumoniae* using the ELISA kit HYOPTTEST-II (Bommeli A.G., Switzerland), and with the commercial kits for Aujeszky's disease (HerdChek Anti PRV gE), Swine Influenza (HerdChek H1N1 and H3N2), and PRRS (HerdChek PRRS 2XR) supplied by IDEXX Laboratories (IDEXX Laboratories Inc Westbrook, ME, USA). The same samples were also examined for antibodies against OMP antigen of *A. pleuropneumoniae* by the Bacteriological R&D Department of Intervet International (Boxmeer-Netherlands) with Standard Operating Procedures. For serum neutralization (SN), sera were inactivated for 30 min at 56⁰C and were examined for SN antibodies by preparing 2-fold dilutions in a 96-well plate. The dilutions were mixed with an equal volume of Aujeszky's disease virus containing approximately 100 TCID₅₀. After 1 hour incubation at 37⁰C, approximately 25,000 cells of the SK-6 cell line were seeded

Table 1. Microorganisms isolated from 100 lungs severely affected with EP typical lesions

	Number of pigs with lungs positive for			
	Farm A (continuous)		Farm B (AIAO)	
	Vaccinated (N=25)	Control (N=25)	Vaccinated (N=25)	Control (N=25)
<i>Mycoplasma hyopneumoniae</i> *	5	5	6	5
<i>Streptococcus</i> spp	5 ^a	13 ^b	10	9
<i>Pasteurella multocida</i>	7 ^a	18 ^b	11	14
<i>Haemophilus</i> spp	2	2	1	0
<i>Bordetella</i> spp	3	2	5	5
<i>Staphylococcus</i> spp	0	1	0	0
Negative	9	4	5	4

a, b Different superscripts in the same line show significant difference (p<0.05)

* Culture + PCR

Table 2. Serology of 100 pigs with lungs severely affected with EP typical lesions

Antibodies* against:	Serologically positive pigs in			
	Farm A (continuous)		Farm B (AIAO)	
	Vaccinated (N=25)	Control (N=25)	Vaccinated (N=25)	Control (N=25)
<i>Mycoplasma hyopneumoniae</i>	25	23	22	21
Aujeszky's disease (gE-)	0	0	0	0
Aujeszky's disease (SN test)	0	0	25	25
PRRS European & American	0	0	0	0
Swine Influenza H1N1	0	0	0	0
Swine Influenza H3N2	25	24	19 ^a	24 ^b
<i>A. pleuropneumoniae</i>	25	25	25	25

* Detection of antibodies against all pathogens was performed by commercial ELISA methods (for Aujeszky's disease gE-ELISA and serum neutralization (SN)-test were used)

a, b Different superscripts in the same line show significant difference (p<0.05)

in each well. The reciprocal of the highest dilution which inhibited the viral cytopathic effect in 50% of the cell cultures after 5 days was considered as the SN titer (Kritis et al., 1999).

Statistical analysis

The statistical analysis was performed using the Statistix Package (Statistix 8, Analytical Software, Tallahassee, Florida). Proportions were evaluated in 2x2 contingency tables.

Results

Lung tissue damage scoring

After the initiation of the trial, the average lung lesion scores for vaccinated and non-vaccinated pigs in farm A were 3.65 and 8.73, respectively and in farm B, 2.72 and 8.2 respectively (p<0.05).

Isolation of microorganisms

The species of microorganisms isolated from the lungs of trial piglets are presented in Table 1. In farm A, *Streptococcus* spp and *Pasteurella multocida* had been isolated from the lungs of significantly fewer vaccinated compared to non-vaccinated piglets (p<0.05). Such was not observed in farm B. The rate of isolation of *M. hyopneumoniae*, as well as of *Haemophilus* spp, *Bordetella* spp and *Staphylococcus* spp was low in both farms, and almost similar between both experimental groups.

Serologic examination

As presented in Table 2, more than 80% of the pigs with affected lungs that were selected had antibodies against *M.*

hyopneumoniae. A similarly high rate of antibodies against swine influenza H3N2 and *A. pleuropneumoniae* had been detected in both farms. In farm B, significantly fewer vaccinated piglets were serologically positive for type H3N2 influenza virus compared to non-vaccinated pigs (p<0.05). No antibodies were detected in both groups of both farms for wild type Aujeszky's disease virus, for both types 1 and 2 of PRRS virus and for type H1N1 of swine influenza virus (Table 2).

Discussion

Vaccination of pigs against *M. hyopneumoniae* has been suggested to improve economic gains, but it can only be most effective, thus cost effective only if management practices are optimized (Maes et al., 2000; Maes et al., 2008). All-in, all-out (AIAO) production is probably the most important factor in the control of enzootic pneumonia since it allows the producer to create uniform populations of pigs and to clean the facilities between groups of pigs. This way it can interrupt the cycle of pathogen transmissions from older to younger pigs (Clark et al., 1990). Therefore, AIAO production results in better performance and less lung lesions in slaughter pigs. In our investigation, lung score was significantly improved in vaccinated piglets, correlating well with the significant reduction in colonization of the same lungs by common bacteria such as, *P. multocida* and *S. suis*. Such beneficial effect was more evident in the farm having continuous flow, supporting the observation that the advantage after Mh vaccination is greater in farms with low rather than high health status, (Okada et al., 1999; Tzivara et al., 2007). Vaccination has significantly improved the serologic profile of piglets to H₃N₂ influenza virus in the farm using the AIAO system. Considering that this virus is frequently involved in the development of PRDC, the vaccination against *M. hyopneumoniae* seems to indirectly assist toward the prevention of this complex. Other important porcine viral

infections seem to be reduced by such vaccination (Silin, 2001). Researchers believe that *M. hyopneumoniae* infection and the resulting lung damage “open the gate” (Silin, 2001) to quantitative and qualitative parameters of immunity negatively affecting resistance to important viruses. Although colonization of lung by *M. hyopneumoniae* was almost the same between vaccinated and unvaccinated piglets (Table 1), colonization of lungs by important secondary bacteria was significantly reduced. Perhaps, the investigation should have considered the enumeration of *M. hyopneumoniae* cells, which could be a determining factor in the transmission rates or even severity of lung lesions, promoting secondary infections.

In conclusion, our results suggest that vaccination against *M. hyopneumoniae* may reduce concurrent bacterial and viral spread among pigs and in this way, it may reduce significantly the complications of EP and PRDC.

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