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A FIELD TRIAL TO STUDY THE EFFICACY OF *RESPISURE ONE*® VACCINE AGAINST PIGS MYCOPLASMAL PNEUMONIA

ŠIUGŽDAITE J*, JUKNA Č*, JUKNA V *, ŽILINSKAS H* and GARLAITE K**

** Lithuanian Veterinary Academy, Kaunas, Lithuania*
*** "Linas and Viza" Veterinary center, Panevėžys, Lithuania*

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The immunoprophylaxis of mycoplasmal pneumonia in swine (MPS) caused by Mycoplasma hyopneumoniae was investigated. A field trial was conducted on three commercial pig farms located in different parts of Lithuania. A Mycoplasma hyopneumoniae single dose Respisure One® vaccine was tested in 600 pigs for its effects on daily weight gain (DWG), lung lesions and meat quality. The other group of 600 piglets was left unvaccinated as a control.

The evaluation of DWG from the time of vaccination until slaughter showed that the DWG in the vaccinated group increased by 5.5% ($p<0.05$). Vaccination against Mycoplasma hyopneumoniae decreased significantly ($p<0.001$) pneumonic lung lesions in vaccinated pigs.

In this study, we examined the effects of vaccination against Mycoplasma hyopneumoniae on carcass quality, chemical composition, technological, culinary and physical properties of musculus longissimus dorsi. Our data showed that meat lightness L ($p<0.05$), drip loss ($p<0.001$), fat thickness between 6–7 thoracic vertebrae were significantly higher ($p<0.05$), than in the unvaccinated pigs.*

We concluded that Respisure One® vaccine has positive effects daily weight gain, as well as on various carcass and meat quality parameters, at the same time minimizing lung lesions characteristic for mycoplasmal pneumonia.

Key words: swine enzootic pneumonia, vaccine, meat quality

INTRODUCTION

Pig respiratory disease is the most important problem in pig producing countries. Up to 93% of swine herds worldwide are infected with mycoplasmal pneumonia, making it one of the most prevalent and costly swine diseases (Ross, 1999). Early introduction of active immunity is desirable, as transmission of *Mycoplasma hyopneumoniae (M. hyopneumoniae)* may occur from sow to piglet

before weaning, and between piglets after weaning (Dawson *et al.,* 2002; Pommier *et al.,* 2000).

In recent years, a number of efficient vaccines against *Mycoplasma hyopneumoniae* have been introduced and widely accepted by the industry. Both protective immunity and less severe lung lesions have been demonstrated in animals vaccinated against *Mycoplasma hyopneumoniae* (Dohoo *et al*., 1996; Stipkovits *et al.,* 2003). Prevalence of infection at weaning is an indicator of the number of sows in the farrowing group that are shedding *M. hyopneumoniae* and immunity level. Vaccination is an important strategy for controlling mycoplasmal pneumonia (Siugzdaite *et al.,* 2002; Stipkovits *et al.,* 2003; Siugzdaite *et al.,* 2005; Thacker *et al.,* 2000).

It is accepted that vaccination, as a way to control infection with *Mycoplasma hyopneumoniae*, results in a reduction in the number of animals with lung lesions and reduction in the severity of lesions at slaughter (Okada *et al.,* 1999; Kobish *et al.,* 1996; Siugždaite *et al.,* 2004). Vaccination also reduces the costs associated with the treatment of the disease. However, until recently vaccination regimens have involved the use of two–dose formulations given to young pigs to stimulate their protective immunity for the growing phase (Hill *et al.,* 1992). Some vaccines have been recommended for use with only as a single dose, but less data about the efficacy of such an application is available.

The objective of this study was to evaluate the efficacy of the single dose product, Respisure One® against *Mycoplasma hyopneumoniae* on body weight, daily weight gain, on the development of lung lesions, as well as on carcass and pork meat quality parameters.

MATERIALS AND METHODS

The field trial was conducted on three commercial pig farms located in different parts in Lithuania. Piglets were free from *Mycoplasma hyopneumoniae* infection. In total of 1200 Landrace breed 7 day–old piglets (males and females) were included in the trial and they were randomly divided into two groups. Among these, 600 piglets were vaccinated against *Mycoplasma hyopneumoniae* with commercially available vaccine Respisure One (Pfizer AH, Belgium). The single 2 mL (IM) dose was administered behind the ear at the age of 7 days, as recommended by the vaccine protocol. The control group (600 piglets) was injected with a placebo. For detailed investigations, 120 piglets (20 vaccinated and 20 nonvaccinated from each farm) were used. The vaccinated and control animals were housed separately from weaning to slaughter. During the fattening period they were kept in the same space, but were separated by a door. At 33 days of age the piglets were transferred to the post–weaning unit. Prevention measures (castration, iron fortification, needle teeth clipping, tail docking) and other management practices were identical for both groups.

Live body weight was measured before vaccination, at the age of 64, 95, 126 and 157 days. Daily weight gain (DWG) was calculated in each group as the difference between mean body weight at the start and at the end of finishing period divided by the number of fattening days for a group. The pigs from the

vaccinated and nonvaccinated groups were compared during the weaning, growing and finishing periods.

The nasal swab samples were collected to determine *Mycoplasma hyopneumoniae* nasal prevalence during experiment. The lungs of vaccinated and nonvaccinated pigs were examined at the time of slaughter by the same qualified person. Lung lesions were scored by percent according to Goodwin *et al.* (1968). The extent of the lung lesions was recorded onto a lung diagram. Surface areas showing pneumonia for each lobe were given a score from1 to 5. Total score by percent was 55. This consisted by left apical lobe 10%, right apical lobe 10%, left cardiac lobe 10%, right cardiac lobe 10%, cranial edge of left diaphragmatic lobe 5%, cranial edge of right diaphragmatic lobe 5% and intermediate lobe 5 %.

Lungs with gross lesions were selected for microbiological investigation. All mycoplasma cultivation procedures were performed according to the method described by Friis (Friis, 1975). For the isolation of mycoplasma, the tissue was homogenized in a tissue grinder using 5 ml of selective Friis medium. Lung suspension was inoculated in 10–100 000 fold dilutions in Friis. Inoculation was carried out at + 35-37°C in a roller drum. Cultures with acid shift were subcultured 3-5 times and inoculated on Friis agar. Isolated strains of mycoplasma were identified by the disc growth inhibition test (DGI), using antisera against the type "J" strain of *Mycoplasma hyopneumoniae* and strain Ms 42 of *Mycoplasma flocculare*.

At the end of the fattening period ten pigs were randomly selected from each group for slaughter. The left half of the carcass was dissected as described in Methods for the Control of Pig Fattening and Slaughtering (2003). Meat samples (500 g) for meat quality evaluation were taken at the last ribs from *musculus longissimus dorsi* after carcass and lung evaluation. Meat samples from the slaughterhouse were transported to the Laboratory of Meat Characteristics and Quality Assessment at Lithuanian Veterinary Academy in a portable refrigerator at +6-8°C. Meat quality analysis was performed 36 hours after slaughter at +4°C. Meat quality indices were determined: the amount of dry matter with automatic scales for dry matter SMO-01 by drying samples at 105°C temperature, meat pH with pH meter Inolab 3 with contact electrode pH (ISO 2917:1999 Meat and meat products Measurement of pH), color (with Minolta Chroma Meter) by measuring the lightness L*, redness a* and yellowness b*, intramuscular fat with the system of automatic extraction Soxterm SE 416 macro (ISO144:1973 Meat and meat products determination of total fat content), ash by burning organic matter at 700}C (ISO 936:1998 Meat and meat products determination of total ash) and protein (Kjeldahl method). Drip loss was determined by pack method storing meat for 24 h at +4°C temperature (Honikel, 1987), water-holding capacity was determined by Grau and Hamm method (Offer and Knight, 1988), cooking loss (vacuumed and boiled at 70°C for 30 min. with a circulation bath) and shear force by Warner-Bratzler test. The amount of tryptophan and oxyproline in the meat were determined by the spectrofotometric method. The amount of cholesterol was determined by liquid chromatography. Other parameters were examined as follows: tryptophan using *p* dimethylamino-benzaldehyde, according to the

method described by Miller (1967); hydroxyproline – as described in the Methodological Guidelines (1978).

The data were analyzed statistically. The arithmetic average values (X), standard deviation (SD), and coefficient of variation (CV) were calculated for all data. Dispersive analysis (ANOVA) was used to assess the separate effect of vaccination on lung lesions reduction. The significance of differences between groups was calculated using Student's t-test, where $p<0.05$ was considered statistically sinificant.

RESULTS

Our data show that the DWG, one of the most important biological parameters, showed a significant difference ($p<0.05$) in the vaccinated group compared to the nonvaccinated group. The DWG in the vaccinated group was 637.2 g and in the nonvaccinated group was 604.1 g. Values of average body weight during the experiment are shown in Table 1.

Table 1. The growing body weight (kg) of vaccinated and nonvaccinated pigs during experiment

Groups	Weight before vaccination	Weight after weaning	Weight at the age of 64 days	Weight at the age of 95 days	Weight at the age of 126 days	Weight before slaughter
Vaccinated	2.90 ± 0.11	9.30 ± 0.17***	23.30 ± 0.88	47.50 ± 1.04	73.30 ± 1.32	98.50 ± 0.65
Non-vaccinated	2.80 ± 0.8	7.90 ± 0.07	22.10 ± 0.18	46.60 ± 0.24	71.00 ± 1.10	93.40 ± 1.27
Difference	+ 0.1	+ 1.4	+1.2	+ 0.9	+ 2.3	+ 5.1

***p< 0.001

A statistically significant ($p<0.001$) larger proportion of lung surface with pneumonic lesions was detected among the nonvaccinated pigs. Lower percentage rate of lungs belonging to the vaccinated pigs had pneumonic lesions indicating the efficiency of the used vaccine (Table 2).

Samples of lung lesions typical of *Mycoplasma hyopneumoniae* from the vaccinated and nonvaccinated groups were investigated microbiologically. *Mycoplasma hyopneumoniae* was isolated from vaccinated pigs in 5% of samples, and in nonvaccinated pigs in 20% of samples.

In this study, we examined the effect of vaccination against *Mycoplasma hyopneumoniae* on carcass quality, as well. In the vaccinated pigs the fat thickness between 6–7 thoracic vertebrae was significantly higher ($p<0.05$), than in the control animals (Table 3).

Table 2. Effect of vaccination with Respisure One on lung lesions

Lugs all lobes	The influence of group (%)	p value
Right apical lobe	16.70	p >0.05
Left apical lobe	20.00	p >0.05
Right cardiac lobe	55.60	**p <0.01
Left cardiac lobe	40.00	*p <0.05
Cranial edge of left diaphragmatic lobe	7.70	p >0.05
Cranial edge of right diaphragmatic lobe	27.30	p >0.05
Intermediate lobe	10.00	p >0.05
All gross lung lesions	65.70	***p <0.001

*p<0.05; **p<0.01; ***p<0.001

Table 3. Effect of vaccination with Respisure One on carcass quality

Parameters	Nonvaccinated group	Vaccinated group	Difference compared with nonvaccinated
Carcass yield, %	72.30±0.54	71.60±0.60	– 0.7
Ham weight, kg	13.50±1.59	14.00±0.81	+0.5
Ham yield, %	32.50±1.40	32.40±0.50	– 0.1
Shoulder part, kg	13.40±0.80	13.70±0.83	+0.3
Middle part, kg	13.90±1.17	14.80±0.91	+0.9
Carcass side length, cm	102.80±1.28	104.80±0.55	+2.00
Fat thickness between 6-7 thoracic vertebrae	20.00±2.13*	28.50±2.97*	+8.5
Fat thickness above 10 rib, mm	15.80±4.88	18.03±3.23	+2.23
Fat thickness above last rib, mm	13.00±4.14	19.00±3.80	+6.00
Fat thickness above crest, mm	21.30±6.24	28.00±3.16	+6.7
Fat thickness lumbar vertebrae, mm	21.08±5.55	17.80±4.77	– 4.0

*p<0.05

Our data show, that the meat color lightness L* ($p<0.05$) and drip loss ($p<0.001$) in vaccinated pigs group were significantly improved (Table 4).

Table 4. Effect of vaccination on longissimus dorsi muscle physical-chemical properties

Parameters	Groups		Difference compared with nonvaccinated
	Nonvaccinated	Vaccinated	
Dry matter (%)	28.20±0.99	27.20±0.96	– 1.0
pH	5.50±0.07	5.40±0.03	– 1.0
Color :			
L*	51.90±0.81*	56.70±1.19	+4.8
a*	15.00±0.54	14.40±0.37	– 0.6
b*	6.10±0.58	7.70±1.39	+1.6
Water holding capacity, (%)	49.10±0.90	47.50±1.97	– 1.6
Drip loss, %	4.00±0.31***	8.70±0.67	+4.7
Cooking loss, %	26.10±1.36	28.00±0.95	+2.7
Tenderness, kg/cm^2	1.30±0.09	1.00±0.17	– 0.3
Fat (%)	2.40±0.97	1.80±0.55	– 0.6
Protein (%)	22.70±0.70	22.30±0.67	– 0.4
Ash (%)	1.10±0.01	1.10±0.01	0
Tryptophan (mg %) (T9)	362.90±27.72	349.40±23.62	– 13.5
Hydroxyproline (mg %) (O)	55.60±1.33	51.51±2.25	– 4.1
Tryptophan/hydroxyproline (T/O)	6.50±0.57	6.80±0.64	+0.3
Cholesterol, mg	49.56±4.33	48.23±2.33	– 1.33

*$p<0.05$; ***$p<0.001$

On the basis of the present study we can suggest about the positive vaccination effects on growing body weight, daily weight gain, fat thickness between 6 and 7 thoracic vertebrae, color intensity and drip loss.

DISCUSSION

Respisure One® administered to pigs at 7 days of age significantly reduced lung lesions consistent with *Mycoplasma hyopneumoniae* infection under field conditions.

Pneumonic lesions are very important for the evaluation of economic loss due to mycoplasmal pneumonia. In our study, dispersive analysis (ANOVA) of the Goodwin scores showed that vaccination had a significant effect on lung lesion reduction ($p<0.001$). In the previous studies, using two vaccine doses of *Mycoplasma hyopneumoniae*, the severity of the lung lesions was reduced by 4% (Siugzdaite *et al*., 2003; Garlaite *et al*., 2004).

The DWG showed an increase of 33.1 grams (g) in vaccinated pigs ($p<0.05$). The vaccination against enzootic pneumonia increased the final weight of vaccinated animals (5.1 kg). This increase is smaller than the increase observed in fields trials with *Stellamune Mycoplasma* (40 g) (Charlier *et al.,* 1994). In other studies the daily weight gain of the vaccinated animals increased by almost 30-60g in comparison with that of unvaccinated pigs (Thacker *et al.,* 2000; Garlaite *et al.,* 2004). It is more difficult to demonstrate the effects of vaccination on DWG than on lung lesions scores (Okada *et al.,* 1999). No analysis of the costs and benefits of vaccination was made in this study. As observed by Maes *et al.* (1996) the cost of a two dose vaccination was outweighed by the improved DWG of the pigs and reduced medication costs, as well as reduced labour cost associated with the administration of a single dose of vaccine to young pigs, and improvements in DWG.

The quality of carcass and meat was evaluated before slaughter. Concerning the chemical composition of the meat, no difference was found in the tryptophan/hydroxyproline (T/O) ratio between the unvaccinated and vaccinated groups. The influence of vaccination was determined for meat lightness L*. This parameter in vaccinated pigs was significantly higher ($p<0.001$). Meat drip loss ($p<0.001$) and fat thickness between 6–7 thoracic vertebrae ($p<0.05$) were significantly higher, compared with the control group. Bidner (2003) reported that meat drip losses increase with intensive growing and stress. However, much more data should be generated in respect to the effects of vaccination on meat quality.

On the basis of the past study with Respisure vaccine (Pfizer AH, USA) (*Siugzdaite et al.,* 2003) and the present study with Respisure One (Pfizer AH, Belgium) we can conclude that the vaccination did not show negative effects on carcass and meat quality.

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Address for correspondence:
Jûratë Siugždaite
Lithuanian Veterinary Academy,
Lithuania, Tilžës 18, LT-47181,
Kaunas, Lithuania
E-mail: jurate.siugzdaite@lva.lt

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TERENSKO ISPITIVANJE EFIKASNOSTI VAKCINE RESPISURE ONE PROTIV MIKOPLAZMATSKE PNEUMONIJE SVINJA

ŠIUGŽDAITE J, JUKNA Č, JUKNA V, ŽILINSKAS H i GARLAITE K

SADRŽAJ

U ovom radu su prikazani rezultati ispitivanja imunoprofilakse mikoplazmatske pneumonije svinja izazvane sa *Mycoplasma hyopneumoniae*. Terensko ispitivanje je sprovedeno na tri farme svinja u različitim oblastima Litvanije. Eksperimentalna grupa od 600 prasadi je vakcinisana vakcinom Respisure One, dok je kontrolnu grupu predstavljalo 600 nevakcinisanih jedinki. Po završetku tova, prosečan dnevni prirast je bio veći za 5,5% ($p<0,05$) u vakcinisanoj grupi. Procenat pojavljivanja lezija na plućima je takođe bio manji u ovoj grupi ($p<0,001$). Posmatrani parametri kvaliteta mesa su bili bolji u vakcinisanoj grupi prasadi.