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VACCINATION AGAINST SWINE ENZOOTIC PNEUMONIA UNDER FIELD CONDITIONS IN DIFFERENT HOUSING AND ENVIRNOMENTAL CONDITIONS

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The efficacy of Respisure (Pfizer AH, USA), a vaccine against M. hyopneumoniae, was tested in pigs kept under good, moderate and poor housing conditions, respectively. The immune response, daily weight gain (DWG), lung lesions, and some parameters of meat important for production were examined. A total of 2.210 pigs from three different farms were used. One group of 1.105 piglets was vaccinated at the ages of 1 and 3 weeks. The second group of 1.105 piglets was used as a control. A total of 132 pigs were investigated in detail.

ELISA (Enzyme- linked immunosorbent assay) showed that antibodies against M. hyopneumoniae were formed on day 28 after the second vaccination in all vaccinated pigs. In control groups on the farms with moderate and poor housing seroconversion to M. hyopneumoniae antibodies started at 49 and 91 days of age, respectively.

Vaccination and housing conditions, taken separately, had significant effects on DWG and lean meat percentage ($p < 0.05$). Vaccination was equally effective in all studied housing conditions ($p = 0.93$). Similarly, vaccination very significantly reduced lung lesions ($p < 0.0001$).

We conclude that Respisure is a highly effective vaccine against swine EP (enzootic pneumonia). We also conclude that it is equally effective in pigs kept under good and poor housing conditions.

Key words: Immunity, housing, lean meat, lung lesions, mycoplasma

INTRODUCTION

Enzootic pneumonia is one of the most important problems in intensive swine production, due to its substantial impact on economic losses in the pork industry. The main etiologic agent is *Mycoplasma hyopneumoniae*. Even at low levels of infection, this chronic respiratory disease adds significant costs to production through reduced feed efficiency, lower daily weight gain, lack of uniformity in pig size, decreased carcass price and repeated antibiotic treatments (Clark, 1996).

Vaccination is an important intervention strategy used to control mycoplasma pneumonia (Thacker *et al*., 2000). Protective immunity, as well as lesion

reduction, induced by vaccination against *M. hyopneumoniae* has been demonstrated (Ross, 1999). Most investigations on the economic impact of respiratory diseases are based on comparison between daily weight gain and pork quality, where the latter has been assessed at the abattoir (Christensen and Mousing, 1992). Thus, Radelooff and Heinritzi (1998) showed that the flesh score was significantly higher for vaccinated groups compared to control groups.

It has been suggested that the role of management and housing conditions in the development of enzootic pneumonia (EP) may also be very important (Maes, 1998). However, this issue has not been studied systematically in the past. Therefore, the main objective of our present study was to assess the efficacy of Respisure (Pfizer) vaccine in pigs on three commercial farms with good, moderate or poor housing and environmental conditions.

MATERIALS AND METHODS

Experimental design

The field trials were conducted on three commercial pig farms located in different parts of Lithuania. These pig farms had different management conditions (Table 1). The farms had a previous history of *M.hyopneumoniae* outbreaks.

Table 1. Different conditions on the investigated farms (Lithuania, 2002)

Factors	Farm A	Farm B	Farm C
1. Housing of different aged pigs in same air space	No	No	In some cases
2. Inadequately insulated & ventilated facilities	No	No	Yes
3. Open spaces in pen dividers	No	Yes	Yes
4. Large growing- finishing rooms (>200-300 pigs)	Yes	Yes	Yes
5. Insufficient care of sick animals (isolation, handling)	No	No	Yes
6. Poor hygiene	No	No	Yes

In total, 2.210 cross- bred 7day-old piglets (males and females) were included in the trial and they were randomly split into two groups. Among these, 1.105 piglets were vaccinated against *M. hyopneumoniae* with the commercially available vaccine Respisure (Pfizer AH, USA). Two milliliters of the vaccine were injected intramuscularly behind the ear. Revaccination was carried out with the same dose 14 days later. The other group of 1.105 piglets (7 day- old) served as the control (non-vaccinated) group. For detailed investigations 132 piglets (22 vaccinated and 22 nonvaccinated from each farm) were used.

Serology

Blood samples from both the vaccinated and control groups were taken at 7 days of age (prior to vaccination), and at 21, 35, 49, 63, 91, 119 and 147 days of age on each farm, with the exception of farm A, where the last samples were collected on day 91. The antibody response of all piglets was examined by blocking ELISA (Dako, Denmark) (Feld *et al*., 1992). The serum samples were diluted 1:10 and incubated for 1.5 hours in microwells pre-coated with the *M. hyopneumoniae* antigen. Then, without emptying the wells, a peroxidase- conjugated mouse monoclonal antibody against a *M. hyopneumoniae*- specific epitope on the 74 kDa protein was added. The higher the antibody titers were in the serum, the less peroxidase-conjugated antibody was bound to the well. After 15 minutes of incubation the microwells were washed and a chromogenic substrate (1.2 phenylenediamine dihydrochloride) was added. A golden – brown color developed in all wells, and after 10 minutes the reaction was stopped by adding sulfuric acid. Higher levels of the antibody in a given pig serum specimen were reflected by a lower intensity of the color in the well. The absorbance (OD) of each microwell was read at 490 nm, and the absorbences of specimen wells were compared with the absorbence of a control well with the buffer. Specimens were considered positive if their OD values were less than 50% of the OD value of the control well.

Daily weight gain (DWG) studies

Pigs were weighed individually when vaccinated, at weaning, when moved to the finishing unit and before slaughter. The pigs from vaccinated and control groups were compared during suckling, post weaning and grower- finishing periods. DWG in each group was calculated as the difference between the mean weight at the start and at the end of the finishing period divided by the number of fattening days of a group.

Pig management

The vaccinated and control animals were housed separately from weaning to slaughter. During the fattening period they used the same air, but were separated by doors. Prevention measures (castration, iron injection, needle teeth clipping, tail docking) and other management practices were identical for both groups on all three farms.

Analysis of meat

Back fat thickness, muscle thickness and lean meat content were recorded by an ultrasound PIGLOG 105 measurement (SFK- Technology, Denmark) before slaughtering. The estimate of the lean meat content was made at two predetermined anatomical sites. Fat 1was estimated between the last third and fourth lumbar vertebrae (7cm from the midline). Fat 2 and muscle thickness was estimated between the last third and fourth ribs, 10 cm and 7cm from the midline.

Evaluation of lung lesions

The lungs of vaccinated and non-vaccinated pigs were examined at the time of slaughter by the same person on all three farms. Lung lesions were scored by

percent according to Goodwin *et al.* (1968). This score ranges from 0 (no lesions) to 55 (extensive lung lesions in all lobes). The size of the lungs was recorded onto a lung diagram and surface areas showing pneumonia for each lobe were given a score from 1 to 5. Total score by percent is 55. This consists of: left apical lobe 10%, right apical lobe 10%, right cardiac lobe 10%, left cardiac lobe 10%, cranial edge of left diaphragmatic lobe 5%, cranial edge of right diaphragmatic lobe 5% and for intermediate lobe 5%.

Bacteriological evaluation

Lungs with gross lesions were selected for microbiological investigation. All mycoplasma cultivation procedures were performed according to the methods used in the mycoplasma section at the Danish Veterinary Laboratory in Copenhagen (Friss, 1974; Friss, 1975). To isolate mycoplasma, tissues were homogenized with a grinder using 5 ml of Friis medium. Lung suspensions were inoculated in 10-100 000 fold dilutions in Friis medium. 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 *M. hyopneumoniae* and strain Ms 42 of *Mycoplasma flocculare (M. flocculare).*

Statistical analyses

Means, standard deviations, and coefficients of variation were calculated for all data. The unpaired t-test (SIGMAPLOT 2000) was used to test differences between the means in different groups.

Two-way ANOVA (SPSS 11.5) was used to assess the separate effects of vaccination and environment on DWG, lung lesion reduction and lean meat percentage. In all tests, $p<0.05$ was used as the significance level.

RESULTS

The serological results showed, that, on farm A, antibodies against *M. hyopneumoniae* were not present in serum before vaccination in either the vaccinated or control group. On farms B and C, maternally- derived antibodies were found in 27.0% and 15.9% animals, respectively. These antibodies completely disappeared after 21 days. Antibodies against *M. hyopneumoniae* in the vaccinated groups were presented at detectable levels 28 days after the second vaccination on all 3 farms. Antibodies could be detected in all vaccinated pigs until the last day of collecting samples: on farm A - till 91days, on farms B and C till - 147 days. In the control group on farm A all serum samples were seronegative until the last day of collection. On farm B, seroconversion in the control group started at day 49, and by the end of study it was found in 10 samples out of 22 (45.5%). On farm C, seroconversion started at 91 days and at day 147 positive cases constituted 47.1% (Table 2).

Table 2. Presence of antibodies against *M.hyopneumoniae* on the investigated farms in Lithuania, 2002

Farm	Pig group	Results	7 days	21 days	35 days	49 day s	63 days	91 days	119 days	147 days
A	Vaccinate	Positive (n)			22	22	19	22		
		Doubt (n)					3			
		Negative (n)	22	22						
	Control	Positive (n)								
		Doubt (n)			2	1				
		Negative (n)	22	21	19	20	21	19		
B	Vaccinated	Positive (n)	3		22	22	22	22	22	22
		Doubt (n)								
		Negative (n)	19	22						
	Control	Positive (n)	9			5	7	7	9	10
		Doubt (n)								
		Negative (n)	13	22	22	17	15	15	13	12
C	Vaccinate	Positive (n)	5		10	22	22	20	20	20
		Doubt (n)								
		Negative (n)	17	22	12					
	Control	Positive (n)	2					7	7	8
		Doubt (n)	3							
		Negative (n)	17	22	22	20	17	10	10	9

The DWG in the vaccinated group compared to the control group was greater by 35g, 37g and 36g on farms A, B and C, respectively. These differences were statistically significant (Table 3).

Two-way ANOVA showed that both vaccination and housing conditions had highly significant effects on DWG [$F(1,108)=30.48$, $p<0.001$; $F(2,108)=234.81$, $p<0.001$]. The interaction between vaccination and housing conditions was not statistically significant [$F(2,108)=0.074$, $p=0.93$].

Measurements of lean meat percentage and other parameters on all farms are shown in Table 4. The percentage of lean meat was greater in the vaccinated groups on all farms (by 0.2%, 0.9% and 3.3% respectively).

Two-way ANOVA showed that both vaccination and housing conditions had significant effects on lean meat content [$F(1,108)=4.82$, $p<0.05$; $F(2,108)=29.18$, $p<0.001$]. The interaction between vaccination and housing conditions was not significant [$F(2,108)=1.83$, $p=0.17$].

Table 3. Effect of vaccine on daily weight gain from growing to finishing

Farms	Parameters	Vaccinated group	Control group	*p* value
FARM A	Daily weight gain (DWG)	0.5783	0.5431	0.000001
	Initial weight (kg) 7 days	2.9065	2.754	0.07
	Weight (kg) after weaning (33 days)	9.9783	10.142	0.07
	Weight (kg) going to fattening (107 days)	55.5304	44.6421	0.0000001
	Finishing weight (kg) (206 days)	110.13	104.275	0.000001
FARM B	Daily weight gain (DWG)	0.6612	0.6239	0.000001
	Initial weight (kg) 7 days	2.4955	2.3455	0.164
	Weight (kg) after weaning (28 days)	7.6955	7.4727	0.055
	Weight (kg) going to fattening (107 day)	48.2591	46.8591	0.0000001
	Finishing weight (kg) (185 days)	111.49	105.45	0.000001
FARM C	Daily weight gain (DWG)	0.753	0.7172	0.05
	Initial weight (kg) 7 day	2.95	3.46	0.01
	Weight (kg) after weaning (51 day)	11.39	13.67	0.01
	Weight (kg) going to fattening (107 day)	24.1	24.8	0.05
	Finishing weight (kg) (171 days)	103.21	99.57	0.000001

Table 4. Investigated variables of relevance for meat quality

	Parameters	Vaccinated group	Control group
FARM A	Fat 1, (mm)	16.8	15.9
	Fat 2, (mm)	15.7	16.7
	Muscle, (mm)	51.9	50.6
	Lean meat, (%)	55.0	54.8
FARM B	Fat 1,(mm)	17.2	16.6
	Fat 2, (mm)	16.2	16.8
	Muscle, (mm)	55.5	53.9
	Lean meat, (%)	55.7	54.8
FARM C	Fat 1, (mm)	19.4	21.4
	Fat 2,(mm)	20.2	23.1
	Muscle, (mm)	42.4	39.8
	Lean meat, (%)	51.3	48.0

Non – vaccinated pigs had a greater proportion of lung surface pneumonic lesions. This difference was statistically significant ($p<0.05$) irrespective of which of the three methods was used. The differences of the Goodwin estimates between vaccinated versus non-vaccinated animals on farms A, B, and C were 5.9%, 5.8% and 12.1% respectively (Table 5).

Table 5. Mean lung lesions at slaughter for each farm

Farm	Treatment	Goodwin method by percentage (%)
Farm A	Vaccinated	3.2
	Control	9.1
p value		0.01
Farm B	Vaccinated	3.3
	Control	9.1
p value		0.004
Farm C	Vaccinated	1.6
	Control	13.7
p value		0.001

Two- way ANOVA of the Goodwin scores showed that vaccination had a highly significant effect on lung lesion reduction [$F(1,108)=31.87$, $p<0.0001$]. In contrast, housing conditions did not have a significant effect on lung lesion reduction [$F(2,108)=0.48$, $p<0.618$]. The interaction between vaccination and environment was not significant [$F(2,108)=2.33$, $p=0.103$].

On the farm (A) with good housing conditions, *M. hyopneumoniae* could be isolated from 5 out of 22 (22.7%) lungs with gross lesions. On the farm (B) with moderate housing conditions, 6 out of 9 (66.7%), and on the farm (C) with poor housing conditions 8 out of 8 (100%).

DISCUSSION

A *M. hyopneumoniae* bacterin (Respisure, Pfizer AH) was used to test the effects of vaccination on pigs kept under different housing conditions. This commercial bacterin induced protection against mycoplasmal pneumonia in all vaccinated pigs, which is consistent with published results (Diekman *et al*., 1999; Maes *et al*., 1999; Thacker *et al*., 2000). Antibodies against *M.hyopneumoniae* were detected using blocking ELISA. Maternal antibodies were detected on farms with moderate and poor housing conditions in both vaccinated and non-vaccinated animals, and these antibodies disappeared by day 21. Similar results have been reported by Morris *et al.*(1994).

In the vaccinated groups, antibodies against *M. hyopneumoniae* formed after 14 days following the second vaccination on all three farms and they were de-

tectable until the last day of sampling (on farm A – day 91, on farms B and C – day 147).

These antibodies did not develop in the non-vaccinated pig group on the farm with good housing. On the farms with moderate and poor housing *M. hyopneumoniae* antibodies were detected, and an outbreak of *M. hyopneumoniae* occurred during the fattening period. The largest percentage (47.1%) of seropositive pigs occurred on the farm with the poorest housing and environmental conditions. This was demonstrated by isolating *M. hyopneumoniae* from lungs with gross lesions selected non-vaccinated pigs. On this farm, *M. hyopneumoniae* was found in 100% of the tested cases, in comparison with 22.7% and 66.6% cases on the farms with good and moderate housing conditions, respectively.

The DWG was significantly higher in vaccinated pigs. The average DWG difference was 36g/day. In other studies the increase in daily weight gain of vaccinated animals ranged from 40g to 60g (Bilic *et al*., 1994; Charlier *et al*., 1994; Martinod, 1996; Schatzmann *et al*., 1996). Two- way ANOVA showed that housing conditions had a highly significant effect on DWG with no significant interaction between vaccination and environment.

A greater proportion of lung surface with characteristic of *M. hyopneumoniae* lesions was detected in nonvaccinated pigs on all three-farms. The largest reduction in lung lesions was on the farm, where housing and environmental conditions were poor. However, statistical analysis showed that housing conditions did not have a significant effect on lung lesion reduction. This could be the result of other factors (absence of respiratory diseases, stronger immune response, genetic predisposition, etc.) that were not controlled in this experiment.

Indicators of carcass quality important for production were evaluated before slaughter in our investigation. The main parameter for meat evaluation was lean meat percentage. The lean meat percentage and muscle thickness were significantly greater in all vaccinated pigs. According to existing literature, the flesh score shows significantly better results for vaccinated groups compared with controls (Radeloff and Heinritzi, 1998). Housing conditions had a highly significant effect on this parameter, but the interaction between vaccination and housing conditions was not statistically significant.

In conclusion, these studies confirm that vaccination with the inactivated vaccine, Respisure, induced an immune response, improved the DWG, lean meat percentage, and decreased lung lesions. Good housing and environmental conditions also had a significant effect on the DWG and lean meat content. However, the absence of a statistically significant interaction between vaccination and environment suggest that vaccination effects did not depend on the actual housing and environmental conditions.

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Garliate Kristina *et al*. Vaccination against swine enzootic pneumonia
under field conditions in different housing and environmental conditions

VAKCINACIJA PROTIV ENZOOTSKE PNEUMONIJE SVINJA NA TERENU, POD RAZLIČITIM SMEŠTAJNIM I AMBIJENTALNIM USLOVIMA

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SADRŽAJ

U ovom radu su izneti rezultati testiranja efikasnosti vakcine protiv *M. hyopneumoniae* (Respisure, Pfizer AH, USA), kod svinja držanih u dobrim, umerenim (srednjim) i lošim smeštajnim uslovima. Ispitivani su imunološki odgovor na vakcinu, dnevno povećanje telesne mase, lezije na plućima kao i neki parametri vezani za produkciju mesa. U ogled je bilo uključeno 2210 svinja, podeljenih u tri grupe, sa tri različite farme. Prva grupa prasadi (1105 jedinki) je vakcinisana u starosti od 1 do 3 nedelje dok je druga grupa iste veličine (1105 prasadi) služila kao kontrola. Za detaljna ispitivanja korišćeno je još 132 svinje.

ELISA testom je dokazano da se antitela protiv *M. hyopneumoniae* kod svih vakcinisanih svinja formiraju 28 dana, nakon druge vakcinacije. U kontrolnoj grupi, koja je držana na farmi u osrednjim i lošim uslovima serokonverzija antitela na *M. hyopneumoniae* je počela 49. do 91. dana starosti.

Vakcinacija i uslovi držanja, gledani odvojeno, imaju značajan uticaj na dnevni prirast i procenat posnog mesa ($p<0,05$). Vakcinacija ima isti efekat u svim uslovima držanja ($p=0,93$) i značajno redukuje lezije na plućima ($p<0,0001$).

Autori zaključuju da je "Respisure" veoma efikasna vakcina protiv enzootske pneumonije koja ima isti imunogeni efekat bez obzira na uslove držanja svinja.