THE EFFECT OF VACCINATION WITH INACTIVATED VACCINE RESPISURE AGAINST *MYCOPLASMA HYOPNEUMONIAE*

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Abstract. *Mycoplasma hyopneumoniae* remains an important pathogen in swine industry. The aim of the present work was to determine effect of vaccine Respisure against *Mycoplasma hyopneumoniae* in fields conditions. The investigation was conducted on 730 cross - bred seven days old piglets free from *Mycoplasma hyopneumoniae* infection. Three hundred sixty five piglets were vaccinated intramuscular. The other group 365 - was as a control (nonvaccinated). Piglets were divided into 2 groups for detailed investigations (22 piglets in each group). The results showed that in both groups the antibodies against *Mycoplasma hyopneumoniae* had not formed before vaccination. Antibodies against enzootic pneumonia in the vaccinated group had formed after the fourteen days of the second vaccination and remain till seventy-seven days. In nonvaccinated group antibodies against *Mycoplasma hyopneumoniae* did not develop. Vaccinated pigs achieved higher daily weight gain, growing, fattening and finishing weight, than the pigs in control group.

As immune response was done, this had a great influence on the health of pigs. The protection against *Mycoplasma hyopneumioniae* disease development decreases pneumonia lesions.

Keywords: enzootic pneumonia, pigs, vaccination.

RESPISURE VAKCINOS EFEKTAS VAKCINUOJANT NUO ENZOOTINĖS KIAULIŲ PNEUMONIJOS

Santrauka. Enzootinė pneumonija viena iš dažniausiai sutinkamų ligų kiaulininkystėje. Darbe nustatėme inaktivuotos Respisure vakcinos efektą, vakcinuojant nuo *Mycoplasma hyopneumoniae.* Tyrimams buvo atrinkta septynių dienų 730 paršelių. Sudarytos 2 grupės – kontrolinė ir bandomoji, kiekvienoje po 365 paršelius. Bandomosios grupės paršeliams vakcina buvo suleista į raumenis, kontrolinė grupė - nevakcinuota. Platesniems tyrimams buvo atrinkti 44 paršeliai (22 kiaulaitės ir 22 meiteliukai). Sudarytos taip pat 2 grupės - bandomoji ir kontrolinė, kiekvienoje po 11 meiteliukų ir 11 kiaulaičių. Tyrimai parodė, kad prieš bandymą abiejuose paršelių grupėse antikūnai prieš *Mycoplasma hyopneumoniae* nesusiformavo. Antikūnai prieš enzootinės pneumonijos sukėlėją susidarė praėjus 14 dienų po antros vakcinacijos ir išsilaikė iki 77 dienų. Vakcinuotoje paršelių grupėje nustatytas didesnis paros priesvoris, didesnis svoris kiaulių augimo, penėjimo periode bei prieš skerdimą. Be to, šioje grupėje buvo mažiau pažeisti plaučiai. Imunizavus kiaules imuninis atsakas susidarė vakcinuotoje kiaulių grupėje, todėl visi parametrai šioje grupėje buvo geresni.

Raktažodžiai: enzootinė pneumonija, kiaulės, vakcinacija.

Introduction. Enzootic pneumonia, caused by *Mycoplasma hyopneumoniae* (*M.hyopneumoniae*) occurs in all pig - rearing countries. This disease affects pigs of all ages, starting with those as young as 7-10 days of age. A high percentage of pigs are affected, but the death loss is low. The disease is characterized by a chronic non-productive cough, retarded growth rate, and inefficient utilization of feed (Kobish, Blanchard, 1993). Feed conversion may be reduced by 14 % to 20 % and rate gain by 16 % to 30 % in affected swine (Ross, Young, 1993). Typical lesions of enzootic pneumoniae occur in 30 % to 80 % of slaughter pigs (Ross, 1992).

Vaccination is an important intervention strategy used to control mycoplasma pneumonia (Thacker et al. 2000). Any protocol evaluating the efficacy of vaccination against *Mycoplasma hyopneumoniae* must involve an evaluation of the main clinical parameters, an examination of the lungs at sacrifice in the abattoir and an assessment of the principal zootechnical and economic parameters. *M.hyopneumoniae* bacterins have been useful in reducing lung lesions and improving growth performance in endemic herds (Dohoo et al.,1996, Maes et al., 1999).

The traditional methods for laboratory diagnosis of mycoplasma are complement fixation assay, indirect hemaggliutination assay, IF (Ðiugþdaitë, Keviðas, 2001). For detection of antibodies to *Mycoplasma hyopneumoniae* in porcine serum a specific and sensitive serological test is a blocking ELISA (Feld et al. 1992).

The purposes of the study was to determine effect of vaccination with inactivated vaccine Respisure against *Mycoplasma hyopneumoniae* in fields conditions.

Materials and methods. The 730 cross - bred 7 days old piglets from one Jonavos district farm were divided into 2 groups: 365 were vaccinated against *M. hyopneumoniae* with commercially available vaccine of 2 ml behind the ear, as recommended by vaccine protocol. The first dose was administered during the first week of life. After 14 days followed the second vaccination in the same vaccine doses. The other group 365 piglet (7 days)

was as the control (nonvaccinated). Piglets were free from *Mycoplasma hyopneumoniae* infection. The 44 cross - bred 7 days old piglets (22 females and 22 males) were used for detailed investigations. Twenty-two piglets were vaccinated, the other group 22 (11 males and 11 females) piglet was nonvaccinated. The blood samples of both groups of piglets were taken before vaccination, after vaccination, after 35, 49, 63, 77 days. After vaccination, the immune status of all piglets was determined by blocking ELISA (Dako, Denmark). Results were read at 490 nm, and the absorbency of specimen wells were compared with the absorbency of a buffer control well. Positive specimens give OD - values, which was less than 50 % of the OD - value of the buffer control well.

The piglets of 33 days old were transferred to the post - weaning unit. Pigs were weighed individually when moved between buildings and subsequent to slaughter.

Prevention measures (castration, iron injection, needle teeth clipping, tail docking) and other management practices were identical for both groups.

Vaccinated and control (44 piglets) groups were compared during the weaning/ growing / finishing unit. Liveweight was studied at the age 7, 33, 107 and 206 days.

The average of liveweight was determinate at the same time for 686 piglets in the vaccinated and nonvaccinaed groups.

Daily weight gain (DWG) in each group was calculated as the difference between mean weight at the start and at the end of finishing period divided by the number of fattening days of a group.

The lungs of vaccinated and nonvaccinted groups of pigs were examined at the slaughter to determine the incidence of macroscopic lesions. Lung lesion was scored by percent (Goodwin et al.1968). The extents of lungs' lesions were recorded onto a lung diagram and surface areas showing pneumonia for each lobe were given a score expressed as a fraction 5. Total score by percent is 55. This consist of: left apical lobe10 %, 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 for intermediate lobe 5 %.

Lung lesions also evaluated score . The maximum score for each lobe was 5 and for the all lungs 35 (Hannan et al.1984). Percentage reduction in lung lesions score relative to control group was calculated for vaccinated group by formula - mean control group minus vaccinate group multiply of 100 and divided to mean control group.

Lungs with gross lesions were selected for microbiological investigations. All mycoplasma cultivation procedures were performed according to the methods used at the mycoplasma section at the Danish Veterinary Laboratory, Copenghagen (Friis 1974; Friis 1975).

Statistical analyses were performed using " Microsoft Excel " program. Significance was set at $P < 0.05$.

Results. The results of blocking ELISA showed that in vaccinated and nonvaccinated groups of pigs the antibodies against *M. hyopneumoniae* before vaccination had not formed. The mean OD - value of vaccinated group was 119. 52 %, in control group - 119.12 %. Antibodies against *M. hyopneumoniae* in the vaccinated group had for the 14 days of the second vaccinated, OD - values of positive pigs in vaccinated group was 14. 24 % in nonvaccinated group - 90. 21 %. Antibody concentration in vaccinated group remains till 77 days (OD - value 46.6 %). In control group all sera samples were seronegative - OD - value 128. 48 %. Table 1 shows the presence of antibodies for *M. hyopneumoniae*.

Table 1. **Enzootic pneumonia antibodies in pigs from 7 till 77 days of age**

Pigs groups	Results	7 day	21 day	35 day	49 day	63 day	77 day
Vaccinated	Positive			22	22	19	22
	Doubt					4	
	Negative	22	22				
Control	Positive						
	Doubt						
	Negative	22	21	19	20	21	19

The main causes of mortality in control group in the growing and fattening periods were gastric ulcer. The DWG of vaccinated pigs was 28 g more than that of the control pigs. The initial weight between both groups was without a big difference. The weight in growing period in vaccinated group of animals was 9. 55 kg greater. The difference was significant ($P < 0.001$). Table 2 shows the effect of vaccine on daily gain, weight after weaning, post weaning weight and finishing weight.

The initial weight of 343 vaccinated piglets were 2.97 kg, in nonvaccinated –2. 9 5 kg, weight after weaning in vaccinated group - 9.4 kg, in control – 9.6 kg. The weight going to fattening period in vaccinated group was 7.5 kg greater, than in nonvaccinated group. Finishing weight in vaccinated group was 110.2 kg and in nonvaccinated group –105.3 kg.

A significantly greater proportion of lung surface with pneumonic lesions was detected in the nonvaccinated pigs. Less percentage of the lungs of vaccinated pigs had pneumonic lesions, indicating the efficacy of the vaccine. Lungs' lesions evaluation was made by 2 methods (Table 3).

Mycoplasma hyopneumoniae was not isolated from specimens collected from vaccinated pig group. However,

cultured from lungs specimens of 5 of 22 (22.73 %) nonvaccinated pig group.

Table 2. **Effect of vaccine on daily weight gain, weight after weaning, post weaning and finishing weight**

Parameters	Pigs groups Vaccinated	Control
Daily weight gain DWG (kg /day)	0. 492	0.520
Initial weight (kg)	2. 971	2. 915
Weight (kg) after weaning	9. 623	9.782
Weight (kg) going to fattening	46. 230	55. 780
Finishing weight (kg)	104.2	110.10

Table 3. **Determination of lung surface lesions**

Pigs groups	Goodwin method by percentage	Hannan method by score
Vaccinated	3. 25	1. 871
Control	9. 00	3. 092

Discussion. The effect of vaccination with inactivated vaccine Respisure (Pfizer AH) against *M. hyopneumoniae* was investigated. A commercial *M. hyopneumoniae* bacterin induces protection against mycoplasmal pneumonia in pigs, which correlates with existing literature (Diekman et al. 1999; Maes et al., 1999; Thacker et al., 2000). According to the guidelines of the manufacturer, the piglets received the first dose at about 1 week of age and the second dose after 2 weeks. Piglets younger than 3 days of age were not vaccinated because it is unclear whether the immune response in the pigs is optimal (Hammerberg et al. 1989). In the vaccinated group antibodies had formed after 14 days of the second vaccination. Serum concentrations of *M. hyopneumoniae* - specific antibodies increased over time in the vaccinated group. In this pig group on day 77 concentrations of specific serum antibodies were detectable. In nonvaccinated pig group during the experiment

M. hyopneumoniae specific antibodies did not develop. As the antibodies had not formed in control group *M. hyopneumoniae* infection occurred. The *M. hyopneumoniae* was isolated from 5 (22. 73 %) pigs lungs of nonvaccinated group.

The DWG is the most important biological factor which during growing showed significant difference in vaccinate pig group and increase in 28 g. This increase is less than the increases observed in field trials with Stellmune Mycoplasma, which in time exceed 40g (Dohoo, Montgomery, 1996). In our studies the weight of vaccinated pig group during growing, fattening and finishing period was significantly greater.

A greater proportion of lung surface with pneumonic lesions was detected in nonvaccinated pig group, by Goodwin method 9 %, and by Hannan 3.09 score. In vaccinated group respectively 3.25 % and 1.81 score. The lung lesions observed in nonvaccinated pig group on day 206 was consistent with lesions of bacterial pneumonia. The lesions induced *by M. hyopneumoniae* were positive for 5 of the 22 (22.73 %) nonvaccinated pigs. *M. hyopneumoniae* was not detected in vaccinated pig group. Percentage reduction in lung lesion by Goodwin was 66 % and by Hannnan - 57.4 %.

Conclusion.

1. Vaccination against *M. hyopneumonaie* induced immune response, improve average daily gain, reduced the pneumonia lesions.
2. *M. hyopneumoniae* was isolated from lungs specimens with lesions from nonvaccinate pigs.

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2002 07 03