

# Active immunization against enzootic pneumonia in pig farm with poor housing and environmental conditions

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### Summary

The objective of the present study was to determine the influence of inactivate vaccine against *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) in a pig farm with poor housing and environmental conditions.

The vaccine efficacy investigation was carried out at a medium-sized pig farm. A total of 740 piglets were selected for the experiment. The piglets were divided into 2 equal groups: vaccinated and non-vaccinated. Forty-four piglets were allocated in two groups of twenty-two piglets each. The first group of twenty-two piglets was vaccinated and 14 days later it was revaccinated. The control group of twenty-two non-vaccinated piglets was injected by a placebo. Blood samples with respect to antibody formation against *M. hyopneumoniae* were taken at 7, 21, 35, 49, 63, 91, 119 and 147 days-of-age and investigated by ELISA. Piglet's life weights were measured individually at the age of 7, 51, 81 and 171 days during their transfer to another unit. Back fat thickness, muscle thickness and lean meat content were measured before slaughter. Pulmonary lesions caused by *M. hyopneumoniae* were scored at slaughter by the Goodwin method. Lungs with gross lesions were investigated microbiologically.

The ELISA tests of serum showed that antibodies against *M. hyopneumoniae* in the vaccinated group formed after 14 days of the second vaccination and remained till the 147<sup>th</sup> day. In the non-vaccinated group seroconversion started on the 91<sup>st</sup> day. Daily weight gain (DWG) showed an increase of 33 grams (g) in vaccinated pigs compared with non-vaccinated ones. The percentage of lean meat was significantly greater (difference 3.26%) in the vaccinated group. Greater proportion of lung surface with pneumonic lesions was detected in non-vaccinated pigs using all methods. Eight lung samples with lesions typical of *M. hyopneumoniae* from the non-vaccinated group were investigated microbiologically and *M. hyopneumoniae* was cultured from all specimens (100%).

The study demonstrates that pigs vaccinated against *M. hyopneumoniae* with inactivate bacteria under poor housing and environmental conditions achieved higher DWG, lean meat percentage and lower lung lesions than the pigs from the control group.

**Keywords:** active immunization, enzootic pneumonia, lean meat

Pneumonia caused by *M. hyopneumoniae* is one of the most widespread and economically significant diseases affecting commercial pig production. *M. hyopneumoniae* detected in over 80% of pneumonic lungs of pigs at slaughter (7). Even at low levels of infection, this chronic respiratory disease represents significant costs to operation through reduced feed efficiency, lower daily weight gain, lack of uniformity in pig size, decreased carcass price and repeated antibiotic treatments (4).

Vaccination is an important intervention strategy used to control mycoplasmal pneumonia (6, 18, 21). Passive and active immunity against *M. hyopneumoniae* has been also established to exert an influence over vaccination intake. Maternally derived antibodies level and persistence in piglets is highly variable. The

median half-life of *M. hyopneumoniae* antibodies in piglets born to positive sows was 15.8 days (16).

Most investigations on economic impact of respiratory diseases are based on comparison between daily weight gain and slaughterhouse meat-inspection or pathology information (3).

Studies evaluating the impact of pleuritis on performance have been based on the prevalence of lesions and have shown that the presence of pleuritis at slaughter is associated with decreased weight gain (10, 13, 23).

The flesh score showed significantly better results for the vaccinated groups compared with control groups. The backfat thickness and quantity of valuable meat cuts were not significantly different between the vaccinated and control groups (17).

The objective of this field trial was to assess the efficacy of inactivate vaccine against *M. hyopneumoniae* on DWG, lung lesions and meat quality.

### Material and methods

The field trial was conducted in commercial pig farm, located in district of Lithuania. The environmental and housing conditions of this farm were poor – the pigs of different age were kept in same air space, the insulated and ventilated facilities were inadequately, open spaces in pen dividers, growing-finishing rooms were large (> 200-300 pigs), it was insufficient care of sick animals and hygiene was poor. The farm had previously history of *M. hyopneumoniae* outbreaks.

A total 740 piglets were selected for the experiment. The piglets were divided into 2 equal groups, vaccinated and non-vaccinated. Further, forty-four 7-day-old piglets were randomly selected and split into groups of males and females. Twenty-two (11 females and 11 males) 7 days old healthy piglets were vaccinated against enzootic pneumonia with commercially available vaccine Respisure (Pfizer AH, USA) as recommended manufacture. The other group of 22 same size and age piglets was as a control (non-vaccinated) group. Piglets were injected by intramuscular route of placebo (saline solution, 0.9% NaCl) and re-injected 14 days later.

The blood samples for respect *M. hyopneumoniae* antibodies of both groups were taken before vaccination, 21, 35, 49, 63, 91, 119 and 147 days of age. Concentration of *M. hyopneumoniae* specific antibodies in serum was determined by use of ELISA (Dako, Denmark) (8). The higher the antibody concentration in the pig's serum specimen, the lower the intensity of the color in the well. The absorbance (OD) in each microwell was read at 490 nm, and the absorbance of specimen wells was compared with the absorbance of a buffer control well. Positive specimens gave OD-values, which were less than 50% of the OD-value of the buffer control well.

The vaccinated and non-vaccinated animals were housed separately during weaning and post-weaning periods. Fifty one day old piglets were transferred to the post-weaning unit. Pigs were weighted individually when moved among units and at slaughter. Vaccinated and non-vaccinated groups were compared during weaning/growing/finishing unit. Life weight was measured at age 7, 51, 81 and 171 days.

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

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.

Back fat thickness, muscle thickness and lean meat content were recorded by ultrasound PIGLOG 105 measurement (SFK-Technology, Denmark) before slaughter. To estimate the lean meat content of the swine must be made at two predetermined anatomical sites. Fat 1 between 3<sup>rd</sup> and 4<sup>th</sup> last lumbar vertebrae (7 cm from midline). Fat 2 and muscle thickness between 3<sup>rd</sup> and 4<sup>th</sup> last rib (10 cm) and 7 cm from midline.

The lungs of vaccinated and non-vaccinated pigs were examined at the slaughter, to determine the incidence of macroscopic lesions. Lung lesions were scored by percent according Goodwin et al. (9). 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. The total score by percent is 55. This consist of: left apical lobe 10%, right apical 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%.

Lungs with gross lesions were selected for microbiology investigation. All mycoplasma cultivations procedures were performed according to the methods used at the mycoplasma section at the Danish Veterinary Laboratory in Copenhagen. For mycoplasma isolation tissues were homogenized grinder using 5 ml of Friis medium. Lungs 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*).

The data values proposed statistically. The arithmetic average values (X), standard deviation (S) and coefficients of variation (cv) were calculated for all data. The significance of differences between the average values was determined by using SIGMAPLOT 2000 program.  $P < 0.05$  was an indicator of the data significance.

### Results and discussion

The sera samples investigated by ELISA showed that before vaccination in both selected groups the antibodies against *M. hyopneumoniae* were found 15.91%. These are maternal derived antibodies. After 14 days results showed that all sera samples were negative 100%.

Seroconversion in the vaccinated group started on 35<sup>th</sup> day – 45.45% samples were positive.

100% positive results in vaccinated group were found on 49<sup>th</sup> day. An OD value in positive pigs group was – 37.8%. The concentration of positive antibodies remained till 147 day (OD value – 48.9).

In the control group seroconversion started on 91<sup>st</sup> day. Results showed 41,17% positive samples. On day 147 positive cases were amounted to 47.06%. Table 1 shows the presence of antibodies for *M. hyopneumoniae*.

The DWG showed an increase of 33 grams (g) in the vaccinated pigs ( $P < 0.001$ ). The final weight of the vaccinated animals was significantly greater ( $P < 0.001$ ).

The measurements of fat 1 and fat 2 were lower in the vaccinated groups of pigs respectively 9.1% and 12.63%. Muscles in the vaccinated group were bigger by 6.64%. The percentage of lean meat was signifi-

Tab. 1. Presence antibodies of *Mycoplasma hyopneumoniae* in pigs from 7 till 147 days of age

Pigs groups	Results	7 day	21 day	35 day	49 day	63 day	91 day	119 day	147 day
Vaccinate	Number of samples (n)	22	22	22	22	22	20	20	20
	Positive (n)	5	0	10	22	22	20	20	20
	Doubt (n)	0	0	0	0	0	0	0	0
	Negative (n)	17	22	12	0	0	0	0	0
Control	Number of samples (n)	22	22	22	20	17	17	17	17
	Positive (n)	2	0	0	0	0	7	7	8
	Doubt (n)	3	0	0	0	0	0	0	0
	Negative (n)	17	22	22	20	17	10	10	9

Tab. 2. Means of daily weight gain, weight after weaning, post weaning and finishing weight, fat 1, fat 2, muscles and lean meat

Parameters	Control (non-vaccinated)	Vaccinated	Difference $\pm$ , compared with control
Daily weight gain (DWG)	0.8376	0.8706	+0.033***
Initial weight (kg) 7 day	3.46	2.95	-0.51**
Weight (kg) after weaning (51 day)	13.67	11.39	-2.28**
Weight (kg) going to fattening	24.10	24.80	+0.7*
Finishing weight (kg)	99.57	103.21	+3.64***
Fat 1, mm	21.35	19.40	-1.95
Fat 2, mm	23.12	20.20	-2.92*
Muscle, mm	39.76	42.40	+2.64
Lean meat, %	48.02	51.28	+3.26*

Explanations: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

cantly higher ( $P < 0.05$ ) in the vaccinated group. Table 2 provides a summary of animal's mean weight in different periods, data of fat 1, fat 2, muscle and lean meat thickness comparing non-vaccinated group with vaccinated.

A statistically significant ( $P < 0.0001$ ) greater proportion of lung surface with enzootic pneumonia lesions was detected in the non-vaccinated pigs. Less lung lesions in vaccinated group indicated the efficacy of the vaccine. The difference was 12.1 points less in vaccinated pig lungs group comparing with non-vaccinated.

Eight samples of lung lesions typical of *M. hyopneumoniae* from the non-vaccinated group were investigated microbiologically. *M. hyopneumoniae* was isolated from all lung tissues samples (100%).

Investigated farm could represent the common middle size farm in Lithuania with all-in/all-out system with poor housing and environmental conditions. We believe that conclusions drawn from these results are probably applicable to other medium size Lithuanian herds chronically infected with *M. hyopneumoniae*.

A *M. hyopneumoniae* bacterin (Respire, Pfizer AH) was used for investigation. A commercial *M. hyopneumoniae* bacterin induced protection against mycoplasmal pneumonia in pigs, which correlates with existing literature (5, 14, 20). Antibodies against *M. hyo-*

*pneumoniae* were detected using blocking ELISA. Passive antibodies in both groups were detected on day 7 and they were not detected at day 21. Similar results were described by Morris et al. (16) (15.8 days). Also same tendency was described by Wallgren et al. (22) and Leon et al. (12).

In the vaccinated group antibodies formed after 14 days of the second vaccination. *M. hyopneumoniae* – specific antibodies increased and were detectable in all vaccinated group samples 100% on day.

In the non-vaccinated pig group the *M. hyopneumoniae* antibodies started to form on day 91 (7 cases from 10) and increase till day 147

(8 cases from 17). These results were proved and by cultivation culture. *M. hyopneumoniae* was isolated from 8 pigs (100%) of the non-vaccinated group.

The DWG was one of the most important biological factors that demonstrated a significant difference and increase in 33 g. This increase is smaller than the increase observed in field trials with Stellamune *Mycoplasma* which at times exceeded 40 g (1, 2, 17). In other studies the daily weight increase of the vaccinated animals exceeded the non-vaccinated in almost 60 g (20). In our studies the weight of vaccinated pigs during the fattening period was significantly greater compared to the non-vaccinated pigs ( $P < 0.001$ ).

Pneumonic lesions are very important for evaluation of economic loss due to enzootic pneumonia. A greater proportion of lung surface with pneumonic lesions was detected by Goodwin in the non-vaccinated pig group – 13.7 points and in the vaccinated group respectively 1.6 points. According existing literature authors also indicating that in the vaccinated pig's lungs lesions decreasing significantly (11, 14).

The reduced percentage in mortality of the vaccinated pigs showed the influence of the vaccination. The reduction of mortality was showed by Maes et al (14).

The quality of meat was evaluated before slaughter. The amount of the lean meat and muscle thickness was

bigger in vaccinated pigs. According literature the flesh score showed significantly better results in the vaccinated groups compared to the control groups (17).

In conclusion, this study has confirmed that vaccination even when housing and environmental conditions were poor, induced immune response, improved DWG, lean meat percentage and decreased lung lesions.

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**BARRETT E. J., FARLAM J., PROUDMAN C. J.: Badania terenowe skuteczności kombinacji ivermektyny i prazykwantelu u koni zarażonych robakami obłymi i robakami płaskimi. (Fidel trial on the efficacy of a combination of ivermectin and praziquantel in horses infected with roundworms and tapeworms). Vet. Rec. 154, 323-325, 2004 (11)**

Badania nad skutecznością kombinacji ivermektyny i prazykwantelu w leczeniu koni zarażonych słupkowcami i tasiemcami przeprowadzono na 62 koniach zarażonych równocześnie tymi dwoma gatunkami pasożytów wybranych z grupy 233 koni wydalających jaja słupkowców z kałem i reagujących dodatnio w teście ELISA na obecność antygenu 12 kDa i 13 kDa tasiemców. Dawka lecznicza wynosiła 0,2 mg ivermektyny i 1,5 mg prazykwantelu na 1 kg masy ciała. Skuteczność terapii określono na podstawie liczby jaj słupkowców wydalanych z kałem po 2, 8 i 10 tyg. po leczeniu oraz liczby jaj tasiemców po 2 i 12 tyg. oznaczonych metodą flotacji oraz na podstawie wysokości miana przeciwciał oznaczonych metodą ELISA po 12 tyg. Leczenie obniżyło do 0 średnią liczbę jaj słupkowców w kale po 10 tyg. i statystycznie obniżyło miano przeciwciał dla antygenów o masie 12 i 13 kDa. Ryzyko wystąpienia kolki po 12 tyg. po leczeniu zmniejszone o połowę.

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**MANSFIELD K. L., BURR P. D., SNODGRASS D. R., SAYERS R., FOOKS A. R.: Czynniki wpływające na odpowiedź serologiczną psów i kotów po szczepieniu przeciwko wścieklicznie. (Factors affecting the serological response of dogs and cats to rabies vaccination). Vet. Rec. 154, 423-426, 2004 (14)**

Dane zebrane w grupie 14 035 psów i 2038 kotów szczepionych przeciwko wścieklicznie, u których określono nasilenie odpowiedzi humoralnej w teście FAVN (test seroneutralizacji z użyciem przeciwciał znakowanych fluoresceiną) lub Bio-Test wykazały istnienie wyraźnych różnic w nasileniu odporności humoralnej. Odporność u części psów była niższa od wymaganej przez UK Pet Travel Scheme. Prawdopodobieństwo, że miano przeciwciał przeciwko wirusowi wściekliczyny będzie niższe od 0,5 jml/ml zależy od czasu, jaki upłynął od szczepienia do pobrania próbki krwi do badania, rodzaju zastosowanej szczepionki i miejsca pochodzenia zwierząt. U kotów oprócz tych czynników duże znaczenie odgrywała pleć. Zarówno badania wykonane w VLA (Veterinary Laboratories Agency) w Weybridge, jak i BioTest wykazały, że u 4,12% psów i 2,85% szczepionych kotów miano przeciwciał wynosiło poniżej 0,5 jml/ml. Istotny spadek wysokości miana notowano pomiędzy 8-9. tygodniem po szczepieniu.

G.