



Original Scientific Article

CLINICAL TESTING OF COMBINED VACCINE AGAINST ENZOOTIC PNEUMONIA IN INDUSTRIAL PIG FARMING IN BULGARIA

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ABSTRACT

In the pig farm with signs of a respiratory disease complex and laboratory confirmed enzootic pneumonia, the prophylactic efficacy of the combination vaccine (*M. hyo*+PCV2), a single injection administered intramuscularly 21 days after birth, at a dose of 2 ml was tested. The clinical condition, pathological changes in the lungs and some epidemiological and economic results were reported. It was found that vaccinated pigs are in a better clinical condition in comparison with the control group. Morbidity in the rearing period was reduced from 16.3% in the control group to 6.0% in vaccinated pigs, and in the fattening period, respectively, from 30.6% in the control group to 10.0% in the vaccinated group. Pathological features in the lung characteristic for the enzootic pneumonia in the vaccinated pigs were reduced from 25.5%±7.24 to 4.0%±2.44, and PCVI - from 13.0%±4.66 to 0%. Vaccination of pigs has been received and a higher average daily gain in groups for rearing (0.624 kg) and for fattening (0.723 kg) was recorded.

Key words: Enzootic pneumonia, *Mycoplasma hyopneumoniae*, pigs, vaccination

INTRODUCTION

Porcine enzootic pneumonia (EP) is a chronic respiratory disease caused by *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*, *M. hyo*) (13). This is one of the most common respiratory diseases in swine and causes considerable economic

losses worldwide as a result of poor feed efficiency, growth retardation, higher morbidity and mortality, emergency slaughter and prophylaxis and treatment costs (2, 5, 11).

Immunoprophylaxis is a key tool in the overall infection control program. Although the existing vaccines do not prevent lung colonization by *M. hyo*, what they do provide is partial protection against pathological changes in organs (9). Vaccination has a marked positive effect: improvement of clinical signs, reduced severity of pathological lesions in the lungs, improvement in mean daily gain, shorter fattening period, and last but not least, lower mortality (3, 10, 12, 14, 15).

Eggen et al. (4) studied the effect of different combinations of *M. hyo* and porcine circovirus type 2 (PCV2) vaccines in swine and reported that

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following single-dose vaccination, the lung lesions decreased with 53% to 61% and the antibody titer against *PCV2* continued to increase up to 10 weeks of age. The authors suggest that there is no immunological interference between *M. hyo* and *PCV2* in the tested combinations and that there is little difference in the antibody titer against *PCV2* between the groups given a single combined vaccine and those given the two vaccines separately.

The aim of the present study was to assess and analyze the prophylactic effect of a combined vaccine against enzootic pneumonia in industrial pig farming in Bulgaria.

MATERIAL AND METHODS

Animals

The study was carried out on a pig-breeding and fattening farm where manifestations of porcine respiratory disease complex (PRDC) – with enzootic pneumonia as the main etiological agent – had been noted. The study included 100 animals at the same age and live weight. They were equally divided into two groups:

Group 1: Pigs (n=50) were given a single dose of the combined inactivated vaccine (*M. hyo*+*PCV2*) on day 21 after birth. The vaccine was administered intramuscularly (IM) at a 2 ml dose, according to the manufacturer's instructions.

Group 2: Pigs (n=50) were not vaccinated and served as a control group.

The animals from the two groups were reared under the same conditions.

Clinical examinations

All animals were subjected to clinical examinations during the experimental period. The examinations included overall condition (febrility, conjunctivitis, appetite, discomfort, nutritional status) and clinical signs characteristic of porcine respiratory infections (respiratory distress, cough, discharge from nostrils). The clinical condition of the animals was evaluated on the basis of a four-point scale: very good (++++); good (+++); satisfactory (++) and poor (+).

Pathological lesions

Autopsy was performed on animals that died during the experiment. At the end of the fattening period the lungs of all animals were examined and the size, type and severity of the lung lesions were scored. The severity of macroscopic changes in lung-specific enzootic pneumonia was determined by quantification of the damage described by Kristensen et al. (8). Individual lobes represent

a percentage of the total area of the lungs: right apical lobe (10%); right cardiac lobe (10%); right diaphragmatic lobe (35%); right accessory lobe (5%); left apical lobe (5%); left cardiac lobe (5%); left diaphragmatic lobe (30%).

Serological analyses

Before vaccination, a total of 16 blood samples were taken (8 samples per group) and were analyzed by blocking ELISA (Oxoid) for the presence of antibodies against *M. hyopneumoniae* glycoprotein 74 KDa. Two negative and two positive controls were included, according to the manufacturer's instructions. Optical density (OD) was measured monochromatically at 450 nm.

DNA extraction and Polymerase Chain Reaction (PCR)

DNA was extracted from 20–50 mg lung tissue samples. The samples were incubated with 20 µL of proteinase K (10 mg/mL) and 400 µL of reagent B (10 mM Tris, 1 mM EDTA and 0.1 M NaCl) at 50° C for at least 3 h or overnight. Following centrifugation for 3 min at 3000 rpm, 400 µL of the supernatant were mixed with 400 µL of reagent C (Tris-saturated phenol : isoamyl alcohol (24:1) in a 1:1 ratio), vortexed and centrifuged at 13 000 rpm for 5 min. The supernatant was pipetted into another microtube and an equivalent amount of chlorophorm/isoamyl alcohol (at a 24:1 ratio) was added. After vortexing and centrifugation for 5 min at 13 000 rpm, the supernatant was again pipetted into a new microtube and mixed with 100% ethanol (Merck, Germany) in a 1:2 ratio; and 5 µL of 7 M ammonium citrate (Sigma, USA) were added. The samples were then left to precipitate for 30 min at -20° C, and were centrifuged at 13 000 rpm for 15 min. The resulting supernatant was discarded and the pellet was washed with cold 70% ethanol and centrifuged at 13 000 rpm for 2 min. The resulting pellet was dried for 7 min and dissolved in 20–50 mL distilled water.

PCR reactions were performed with the primers and protocol described by Villarreal et al. (15) with modifications (annealing at 53.5°C for 40s). The reaction mixtures contained: 3.0 µL of DNA, 10 pmol of each primer, 12.5 µL of 2x PCR Master Mix (Geneshun Biotech co. ltd, China) and distilled water to a volume of 25 µL.

The quantity and quality of the extracted DNA and the PCR products were analyzed by 2% agarose gel electrophoresis (Geneshun Biotech co. ltd, China), with 10 mg/mL ethidium bromide (Sigma, USA) staining and a 100 bp DNA Ladder (Geneshun Biotech Co. ltd, China), at 120 V for 40 min.

Statistical analysis

All results were processed statistically by the use of computer software StatMost (StatMost 3.6, Dataxiom Software, 2003). The results are presented as mean with standard error ($mean \pm SE$), after application of the one-way ANOVA statistic. Statistically significant differences were accepted at $p < 0.05$.

RESULTS

During the suckling period –from birth to weaning– no clinically infected pigs were observed. One pig from the control group died and the

pathoanatomical examination showed lesions typical for EP and *Actinobacillus pleuropneumoniae* (APP). In the group of animals which were administered the combined vaccine (*M. hyo*+PCV2), there was a 0.538 kg ($p < 0.01$) higher weight gain (Table 1).

All the animals from group 1 ($n = 50$) as well as 49 animals from group 2 continued into the growing period (Table 2), which continued for 73 days. During the growing period, there were no deaths, but the morbidity of the control group increased sharply to reach 16.3% in comparison to 6% in the group of the vaccinated animals. In this period, the vaccinated animals showed higher mean daily gain (up to 0.624 kg) than the non-vaccinated ones (0.512 kg).

Table 1. Business and economic outcomes in experimental piglets vaccinated with combination vaccine (*M. hyo*+PCV2) and unvaccinated

Parameters	Units	I group (<i>M. hyo</i> +PCV2)	II group (Control)
1. Pigs in the group	number	50	50
2. Average live weight at birth	kg	1. 200 \pm 2.23*	1. 200 \pm 1.89
3. Stay in group “piglets”	days	35	35
4. Diseased	number	0	0
	%	0	0
5. Died from PRDC	number	0	1
	%	0	2
6. Average live weight at the end of the period	kg	9.090 \pm 2.58**	8.552 \pm 3.02
	%	106.3	100
7. Derived growth period	kg	7.890	7.352
	%	107.3	100
8. Average daily gain	kg	0. 225	0. 210
	%	107.2	100
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$			

Table 2. Business and economic outcomes in the experimental weaners pigs vaccinated with combination vaccine (*M. hyo*+PCV2) and unvaccinated

Parameters	Units	I group (<i>M. hyo</i> +PCV2)	II group (Control)
1. Pigs in the group	number	50	49
2. Average live weight at beginning of period	kg	9.090 \pm 2.58*	8.552 \pm 3.02
3. Stay in weaners	days	73	73
4. Average live weight at the end of the period	kg	54. 634 \pm 3.07*	45. 905 \pm 3.64
	%	119	100
5. Diseased	number	3	8
	%	6	16.3
6. Died from PRDC	number	0	0
	%	0	0
7. Derived growth period	kg	45.544	37.353
	%	121.9	100
8. Average daily gain	kg	0. 624	0. 512
	%	121.9	100
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$			

During the fattening period (94 days), the mean live weight was 8.729 kg higher in the group of vaccinated pigs than in the control (non-vaccinated) group. There were also no deaths. However, clinical signs of a respiratory infection were evident in 10% of the vaccinated pigs and in 30.6% of the control ones. The live weight measured at regular slaughter of animals from both groups was shown to be 19.6 kg ($p < 0.05$) higher in the vaccinated animals as compared to the non-vaccinated ones (Table 3).

Details about the overall and the clinical condition of the pigs during the growing and the fattening period are given in Table 4 and Table 5.

Table 6 presents the results from the pathoanatomical examinations and the observed lesions in the vaccinated and non-vaccinated pigs. The pathoanatomical findings in the lungs of both groups of animals were similar: for EP, APP and PCVI. In the vaccinated animals, the number of lesions characteristic of EP was reduced from 25.5 % \pm 7.24 ($p < 0.01$) to 4% \pm 2.44 ($p < 0.01$), and of PCVI, from 13 % \pm 4.66 ($p < 0.05$) in the control group to 0% ($p < 0.05$) in the vaccinated group. The lesions typical for APP amounted to 11.5% \pm 7.67 in the control group, but were reduced to 2.0% \pm 1.10 owing to the vaccination.

Table 3. Business and economic outcomes in the experimental fattening pigs vaccinated with combination vaccine (*M. hyo*+PCV2) and unvaccinated

Parameters	Units	I group (<i>M. hyo</i> +PCV2)	II group (Control)
1. Pigs in the group	number	50	49
2. Average live weight at beginning of period	kg	54.634 \pm 3.07*	45.905 \pm 3.64
3. Stay in group fattening	days	94	94
4. Average live weight at the end of the period	kg	122.6 \pm 2.06*	103.0 \pm 1.98
	%	119	100
5. Derived growth period	kg	68.0	57.0
	%	119.3	100
6. Average daily gain	kg	0.723	0.607
	%	119.1	100
7. Diseased	number	5	15
	%	10	30.6
8. Died from PRDC	number	0	0
	%	0	0
9. Slaughtered «economic foreshore»	number	1	4
	%	2	8
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$			

Table 4. Total and clinical state of the experimental pigs in the two groups during the period of «rearing» vaccinated with combination vaccine (*M. hyo*+PCV2) and unvaccinated

Clinical condition of the experimental pigs	Rearing - first period (8 – 12 kg)				Rearing - second period (12 – 40 kg)			
	I group (<i>M. hyo</i> +PCV2) n=50		II group (Control) n=49		I group (<i>M. hyo</i> +PCV2) n=50		II group (Control) n=49	
	number	%	number	%	number	%	number	%
Very good (+++)	28	56	11	22.4	22	44	8	16.3
Good (++)	17	34	21	42.9	23	46	22	44.9
Satisfactory (+)	3	6	12	24.5	4	8	16	32.7
Poor (+)	2	4	5	10.2	1	2	3	6.1
Mean \pm SE	12.5 \pm 6.19	25.0*** \pm 12.39	12.3 \pm 3.30	25.0*** \pm 6.74	12.5 \pm 5.80	25.0 \pm 11.61	12.3 \pm 4.21	25.0 \pm 8.60
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$								

Table 5. Total and clinical state of the experimental pigs in the two groups during the period of “fattening” vaccinated with combination vaccine (*M. hyo*+PCV2) and unvaccinated

Clinical condition of the experimental pigs	Fattening - first period (40 – 70 kg)				Fattening - second period (70 – 105 kg)			
	I group (M. hyo+PCV2) n=50		II group (Control) n=49		I group (M. hyo+PCV2) n=50		II group (Control) n=49	
	number	%	number	%	number	%	number	%
Very good (++++)	20	40	6	12.2	26	52	5	10.2
Good (+++)	23	46	19	38.8	22	44	15	30.6
Satisfactory (++)	2	4	9	18.4	1	2	25	51
Poor (+)	5	10	15	30.6	1	2	4	8.2
Mean ±SE	12.5 ±5.26	25.0 ±10.53	12.3 ±2.92	25.0 ±5.98	12.5 ±6.68	25.0 ±13.37	12.3 ±4.92	25.0 ±10.03
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$								

Table 6. Results of pathology examinations at regular slaughter of pigs in experimental groups

№ of pigs	I group vaccinated with “M. hyo+PCV2”			II group unvaccinated “Control”		
	% of changes in the lungs			% of changes in the lungs		
	EP	APP	PCVI	EP	APP	PCVI
1	0	0	0	50	0	0
2	0	0	0	75	30	0
3	25	0	0	30	0	0
4	5	0	0	30	0	20
5	5	0	0	20	10	25
6	5	0	0	0	0	0
7	0	5	0	0	0	40
8	0	0	0	20	75	0
9	0	5	0	10	0	25
10	0	10	0	20	0	20
Mean ±SE	4.0 %** ±2.44	2.0 % ±1.10	0 %* ±0	25.5 %** ±7.24	11.5 % ±7.67	13.0 %* ±4.66
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$						

DISCUSSION

The efficacy of a single dose of the combined inactivated vaccine (*M. hyo*+PCV2) on day 21 after birth of the pigs was demonstrated by the date of the vaccination in a farm, where manifestations of PRDC with enzootic pneumonia as the main etiological agent had been noted.

The results related to the overall and the clinical condition of the pigs during the growing and the fattening period indicate that, as a whole, the vaccinated pigs were in a better condition and with

better health than those in the control group. The vaccination led to a reduction in the percentage of animals with signs associated with poor clinical outcomes.

The results of the serological tests before vaccination show that the studied serum 16 (8 sera from both groups) in pigs in the experimental group did not show specific antibodies against *M. hyopneumoniae*, and in the control group 6 samples reacted positive (75%). When examining the antibody titers against *M. hyopneumoniae*, three weeks after application of the combined vaccine it

was found that of the test samples 17 - 13 (76.5%) reacted positive. These animals had an intense and protective immune response. In the sera of 3 pigs (17.6%) vaccine antibodies were not detected, in a pig from group 1 (5.9%) the results are questionable. In the control group of 17 analyzed samples, 16 samples (94.1%) had negative titers and in 1 sample (5.9%) they were dubious. Analysis of the results of serological tests on samples obtained at the end of the fattening period, shows that in pigs vaccinated with a combination vaccine, out of 6 samples in 2 (33.3%) there were antibodies against *M. hyo*. In the control group the results are similar. In conclusion, we can say that high titers of maternal antibodies induced by infection or vaccination, have had a negative effect on vaccination of pigs, which is confirmation of the data exported from Jayappa et al. (7) and Hodgins et al. (6).

Since established pathomorphological changes are characteristic and typical for enzootic pneumonia, but not specific, then contact differentiating them from changes caused by other participants in the PRDC, especially swine influenza (SI) was established through conducting a molecular biological research. Of the 14 examined lungs with lesions typical of EP by a conventional PCR, the DNA of *M. hyopneumoniae* was detected in 5 samples (35.7%). 4 samples (28.6%) are suspicious and 5 samples (35.7%) were negative.

Aiguo et al., (1) demonstrated the improvement of productivity in a Chinese farm with a continuous production system by implementing combination vaccine (*M. hyo*+PCV2). The vaccines are mixed before using and thus they save labor and reduce stress for the piglets. Similar to our results, they established that total mortality and the culling rate during the nursery and fattening period was reduced (5.26% in the vaccinated group), as well as the clinical signs.

Our results demonstrate that the combined vaccine against *M. hyo* and PCV2 has good prophylactic efficacy not only in terms of reduction of clinical signs and good overall condition of the animals, but in higher mean daily gain in the growing (0.624 kg) and the fattening period (0.723 kg). The vaccination also contributed to the decrease in the degree and severity of the pathoanatomical lesions characteristic of EP and PCVI in the lungs of slaughtered pigs. These results are in agreement with other investigations (3, 10, 12, 14) who report a good prophylactic effect of vaccination, despite the fact that it does not render pigs completely immune against *M. hyo* infection.

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