

Efficacy of single-dose *Mycoplasma hyopneumoniae* vaccine for the control of enzootic pneumonia in pigs

Roman Pepovich

University of Forestry, Faculty of Veterinary Medicine, 1797 Sofia, Bulgaria
E-mail: rpepovich@gmail.com

Abstract

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This paper evaluates the prophylactic efficiency of the inactivated vaccine with single application for control of the enzootic pneumonia. The research was conducted at an industrial pig farm, on one hundred pigs, divided into two groups after the first week of their birth. One group was vaccinated once on 21st day with commercial inactivated *M. hyopneumoniae* vaccine (Ingelvac MycoFLEX®), and the other group was left non-vaccinated. It was demonstrated that the vaccine lowered the clinical signs and the damage of the lungs considerably and has led to improvement of the production results for the whole period of the research. Despite the economically justified result by the singular application of the vaccine against *M. hyopneumoniae*, this must be in line with the specific epidemiological situation in the farm, following a careful assessment of the infective pressure caused by *M. hyopneumoniae*, which determines the necessity of conducting more research in the future.

Keywords: pigs; *Mycoplasma hyopneumoniae*; enzootic pneumonia; vaccination

Introduction

Enzootic pneumonia caused by *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) is a chronic respiratory disease, which more often affects growing and fattening pigs (Fano et al., 2007; Maes et al., 2011; Pieters et al., 2014). The disease is widespread in all countries with well developed swine breeding, as it inflicts heavy economic losses (Georgakis et al., 2002; Maes et al., 2008; Holst et al., 2015). The improvement of the living conditions in the pig farms as well as the management, are one of the first steps, which should be taken in order to control the enzootic pneumonia (Hege et al., 2002; Thacker & Thanawongnuwech, 2002).

Immunoprophylaxis has proven as an important tool in the control against the mycoplasmic infection (Simionatto et al., 2013). Different types of vaccines and vaccination schemes have been tested, some authors have used inactivated vaccines (Martelli et al., 2014), others – live ones (Feng et al., 2013). The vaccination scheme is chosen for

every farm, depending on the type of herd, the manufacturing system and the epidemic situation. The optimal strategy for vaccination should be defined by the balance between the advantages of the delayed vaccination and the necessity of forming immunity before infecting the pigs with pathogens.

The purpose of this research is to define the efficiency of single application of the inactivated monovaccine in pigs for control of enzootic pneumonia.

Material and Methods

Animals included in the research

The research was conducted in a farrow-to-finish pig farm, with a manifestation of chronic enzootic pneumonia, confirmed by serologic analyses and observation of the lung lesions discovered during the regular slaughter. The tests were conducted on 100 pigs, having the same age and weight, divided into two test groups:

First group (designated group V1) – 50 pigs, vaccinated with commercial inactivated *M. hyopneumoniae* vaccine (In-gelvac MycoFLEX®, Boehringer Ingelheim), on 21st day of birth, in dose of 1 ml, administered intramuscularly (IM), agreed with the instructions by the manufacturer;

Second group (designated group NV) – 50 pigs, unvaccinated.

The animals from both groups were grown in the same conditions. The farm's production system included weaning (on 28th day), nursery period (lasting 73 days) and fattening period (lasting 94 days).

Clinical research

Clinical examinations have been done on every pig, during the period of the research, taking into account rectal temperature, general condition and the clinical signs, specific for the respiratory diseases in pigs (dyspnea, nasal discharge and coughing). Individual clinical parameters were evaluated in a point system defined by Lang et al. (2002), and the clinical condition of the animals was defined as very good, good, satisfying and poor.

Pathologic examinations

The animals that have died during the research were autopsied. In the end of the fattening period, we did pathologic-anatomic examinations in the slaughterhouse, as the extent of the lung lesions typical for the enzootic pneumonia was evaluated visually by the system described by Kristensen et al. (2014).

Serologic research

61 blood samples were taken from the pigs included in the research, before vaccination, 21 days after the vaccination and during the slaughter, for proving the existence of the specific antibodies against glycoprotein 74 kDa of *M. hyopneumoniae*, by using blocking ELISA (Oxoid®, England). According to the instructions by the manufacturer, we set up 2 negative and 2 positive control. The measuring of the optic density (OD) was performed monochromatically with wave length 450 nm.

Polymerase Chain Reaction (PCR)

We have achieved the confirmation of *M. hyopneumoniae* in lungs via PCR with one primer couple from the „Jena Bioscience” company: MHP950-1L (5'-AGG AAC ACC ATC GCG ATT TTT A-3') and MHP950-1R (5'-ATA AAA ATG GCA TTC CTTTT A-3'). They are responsible for the synthesis of a DNA amplification product of 910 bp in size that is specific for *M. hyopneumoniae*. We have isolated the bacterial DNA with Tissue & Cell Genomic DNA Mini

Kit (Geneshun Biotech co. Ltd, China). For the reaction we have used 3.0 µl of DNA, 10 pmol of each primer, 12.5 µl 2×PCR Master Mix, and distilled water up to 25 µl. We have performed the amplification in „LKB” thermocycler with the following temperature sequence: initial denaturation – 95°C/5 min; 35 cycles of amplification including denaturation – 95°C/30 sec; annealing – 53.5°C/40 sec; elongation – 72°C/1 min; final elongation – 72°C/7 min, followed by cooling to 4°C. We controlled the obtained DNA and the PCR products by gel electrophoresis at 120 V for 40 min. We have made the result interpretations on UV-transilluminator and documented them with „VisiDoc” photo camera.

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±SE), after application of the one-way ANOVA statistic. Statistically significant differences were accepted at $p < 0.05$.

Results and Discussion

The results of our research show that during the nursery period the vaccinated pigs had a higher average daily weight gain, which is 0.052 kg more than the control group. The average weight gain for the whole nursery period was 3.847 kg higher in pigs from V1 group in comparison with the pigs from NV group. The morbidity rate rapidly rises in the NV group which was 6.6% higher in comparison with the pigs from the V1 group. The mortality rate at pigs from V1 group was 2% higher than the NV group with proven pathologic changes in the lungs typical for monoinfection with *M. hyopneumoniae* (Table 1). Presumably it is caused by the high mother-transferred antibodies, which have negative influence on the vaccination. This was confirmed by the results of serological testing of both groups. In the fattening period, we reported higher average daily weight gain in vaccinated pigs which exceeded with 0.073 kg in comparison with non-vaccinated pigs. During this period the pigs from V1 group had an average weight gain of 7.0 kg higher than the NV group. The morbidity rate was 12.2% higher in the NV group. Neither of the groups had death cases (Table 2).

The vaccination of the pigs leads to suppression of the clinical appearances of the disease which corresponds with a better clinical condition. During the first period of nursery (8-12 kg), 44% of the pigs from V1 group are in very good clinical condition in comparison to 22.4% of the pigs from NV group. This tendency continues until the end of the nursery period (12-40 kg), in which 42% of the pigs from

Table 1. Production and epidemiological results at nursery period

Group / Parameters	Average daily weight gain (kg)	Average weight gain (kg)	Morbidity rate (%)	Mortality rate (%)
V1	0.564±0.006**	41.200±0.65*	10	2
NV	0.512±0.008*	37.353±0.83	16.6	0
Difference	+0.052	+3.847	-6.6	+2

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

Table 2. Production and epidemiological results at fattening period

Group / Parameters	Average daily weight gain (kg)	Average weight gain (kg)	Morbidity rate (%)	Mortality rate (%)
V1	0.680±0.004***	64.0±0.58*	18.4	0
NV	0.607±0.006*	57.0±0.74*	30.6	0
Difference	+0.073	+7.0	-12.2	0

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

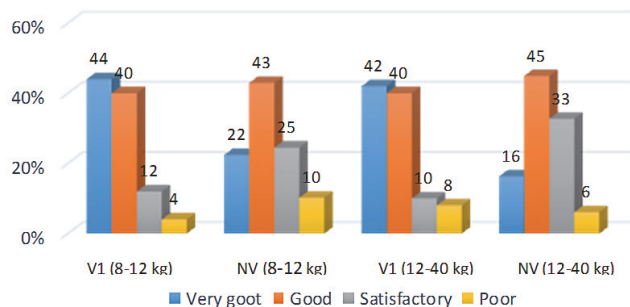


Fig. 1. Clinical condition of pigs during the nursery period

V1 group are in very good clinical condition, in comparison to the NV group in which only 16.3% kept their good clinical condition (Fig. 1). The most distinctive difference in the clinical condition of the pigs was registered during the fattening period. In the early stages of fattening (40-70 kg) the results showed that 32.6% of the pigs in the V1 group were in very good condition, compared to 12.2% of the pigs in the NV group. At the end of the fattening period (70-110 kg), in very good clinical condition were 42.9% of the pigs in the V1 group. In the NV group the portion of the pigs with very good clinical condition was minimized to 10.2% (Fig. 2). The positive effects of the vaccine against mycoplasmal infection are obvious and it is expressed in minimizing the manifestation of clinical symptoms of the infection and with a rise of the average daily growth. Our results are similar with the results received from Wallgren et al. (2000), Pallares et al. (2001), Dawson et al. (2002) and Maes et al. (2003).

The data from the pathological examinations at the regular slaughter with the percentage of observed changes specific for *M. hyopneumoniae* in lungs in vaccinated and control pigs are presented in Table 3. It is shown that in the animals in NV group the changes in lungs are typical for enzo-

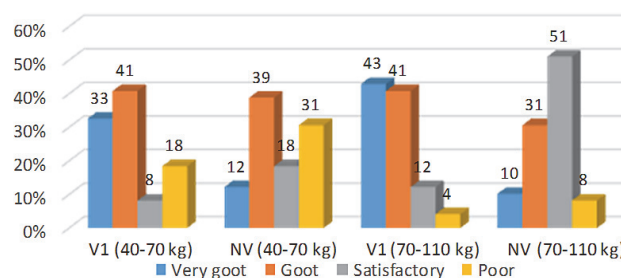


Fig. 2. Clinical condition of pigs during the fattening period

otic pneumonia in average of 25.5%±7.24. In V1 group the pathologic changes which are specific for enzootic pneumonia in lungs are reduced to 5.5%±2.16. Our results confirm the information from Villarreal et al. (2011a) concerning the

Table 3. Percentage of affected lobules from total lung area, caused by *M. hyopneumoniae*

Pigs (n)	Group V1 lung lesions (%)	Group NV lung lesions (%)
1	0	50
2	10	75
3	5	30
4	0	30
5	0	20
6	20	0
7	10	0
8	10	20
9	0	10
10	0	20
Mean ±SE	5.5%** ±2.16	25.5%** ±7.24

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

efficiency of vaccinating pigs with vaccines against *M. hyopneumoniae*, which significantly decreased the macroscopic and microscopic lesions of the lungs in pigs infected with highly virulent strain of *M. hyopneumoniae*.

The results from the serologic examination show that before the vaccination in both groups (V1 and NV) were proven specific antibodies against *M. hyopneumoniae* in the tested blood serums. Three weeks after vaccination in pigs from V1 group vaccination titres are registered in 29.4% of the test samples. In the NV group there weren't established any positive titres which is indication for depletion of the passive mother immunity. The analysis of the serologic results received before slaughter shows that vaccinated pigs did not prove to have positive titres of antyodies against *M. hyopneumoniae* which was shown at the end of the fattening period in pigs in this group with exhausted humoral immunity. In the NV group in 33.3% of test samples establishing specific antyodies against *M. hyopneumoniae*, which in our opinion can be from the fact that there was a late infection with *M. hyopneumoniae*. From the received results of the serologic tests we can come to the conclusion that the higher titres of the mother's antyodies induced from the infection or vaccination had negative effects on the vaccination of the pigs which confirms the information of Jayappa et al. (2001) and Hodgins et al. (2004).

The results from the PCR analysis of the lungs in both test groups (V1 and NV) showed that in pigs from V1 group the percentage of participation of *M. hyopneumoniae* in the affected lung regions was 42.9%, while in the NV group – 65.3%. Proving *M. hyopneumoniae* in the lungs in both test groups (V1 and NV) shows that vaccination does not significantly reduce the transmission of this respiratory pathogen, but leads to reduction of the seriousness of lung lesions caused by *M. hyopneumoniae*, which confirms the information from Meyns et al. (2006), Pieters et al. (2010), Villarreal et al. (2011b).

Conclusions

The current study demonstrates the good prophylactic effect of the inactivated *M. hyopneumoniae* vaccine in controlling of enzootic pneumonia. Single vaccination of pigs on 21st day of age significantly reduces the occurrence of clinical symptoms and severity of lung lesions and it can lead to improvement of production results. In conclusion, we accept the economic effect of a single application of the vaccine against *M. hyopneumoniae* but we have to take to consideration the epidemiologic situation in the farm after careful evaluation of the infection pressure caused by *M. hyopneumoniae*.

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