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THE COMPARATIVE THERAPEUTIC EFFICACY OF ANTIMICROBIALS IN PIGS INFECTED WITH *MYCOPLASMA HYOPNEUMONIAE*

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Abstract

Respiratory diseases are current health problem for pig. Very often they have polietiological base which triggers defined Porcine Respiratory Disease Complex (PRDC). One of the main and permanent etiologic agents in PRDC is Mycoplasma hyopneumoniae, the causative agent of enzootic pneumonia in pigs. The disease is widespread in Bulgaria, inflicting major economic damage, resulting in high morbidity, poor feed conversion, reduced average daily gains, cost of therapy and immunization. These indicators determine treatment as necessary and inevitable in control of mycoplasma infection. The purpose of this study was to compare the therapeutic potential of enrofloxacin and florfenicol in industrial pig farms in Bulgaria. The study was conducted in pig farm breeding and fattening, with laboratory proven acute form of enzootic pneumonia. It was conducted on 260 growing pigs divided into two experimental groups. The first group was treated with enrofloxacin injective at a dose of 1 ml/10 kg., for three days, and the second with florfenicol, at a dose of 1 ml/20 kg., intramuscularly twice in 48 hours. Received clinical and epidemiological data give reason to assume that the tested schemes are effective in the control of enzootic pneumonia. As a result of the treatment to stabilize by the clinical condition of the pigs, normalization of indicators of blood and limiting morbidity and mortality. The resulting high therapeutic effect in patients treated with enrofloxacin pigs - 89.6 % and respectively florfenicol - 75.6 %, presented both as equivalent antibiotic in the treatment of enzootic pneumonia.

Key words: *pigs, M. hyopneumoniae, enrofloxacin, florfenicol, therapy.*

INTRODUCTION

Porcine respiratory disease complex (PRDC) may be of various etiology in different production systems (Halbur, 1999; Motovski, 2003). It is commonly caused by a combination of one or two viruses, *Mycoplasma hyopneumoniae* (*M. hyo*) and other bacteriological agents, which results in severe respiratory diseases and, consequently, in considerable economic losses (Ganovski, D. & I. Dinev, 1996; Motovski, 2003). One of the major PRDC etiological agents isthe causative agent of enzootic pneumonia, *M. hyo*. It damages the epithelial cells in the respiratory tract and inhibits the functions of the lymphatic system (Stipkovits et al., 2001; Opriessnig et al., 2004; Thacker, 2006).

To combat PRDC, it is generally accepted that vaccination, stress reduction and the implementation of modern breeding technologies prove useful and are thus considered necessary. However, even when utmost management care is taken, pigs can still become sensitive to bacterial infections. That is why Bosch (2004) considers the use of antibacterial agents as an integral part of the complex control measures against infectious diseases. Antibacterial agents offer several advantages in the control on enzootic pneumonia; these include flexibility of use, simple introduction via feedstock and drinking water, ease of use, possibility to optimize immunoprophylaxis programs and control of bacterial infections (Maes et al., 2011).

The antibacterials potentially active against *M. hyo* include tetracyclines, macrolides, lincosamides, pleuromutilins, fluoroquinolones, amphenicols and aminoglycosides (Hannan et al., 1989; Vicca, 2005). Of these, it is tetracyclines and macrolides that are most

commonly used for treatment of respiratory infections in pigs (Timmerman et al., 2006).
The aim of the present study was to examine the therapeutic efficacy of two injectable antibiotics in growing pigs in an industrial pig farm with acute enzootic pneumonia.

MATERIALS AND METHODS

Animals included in the study

The study included animals from a pig fattening and breeding farm with enzootic pneumonia which was clinically and laboratory confirmed after a winter respiratory outbreak. Comparative evaluation of the therapeutic effect of florfenicol and enrofloxacin was performed in 260 weaners, 2 to 3 months of age, with clinical evidence of respiratory disease. Two groups of pigs were set up:
Group 1: 140 weaned pigs were treated with the florfenicol-containing drug FLORKEM®, which contains 300 mg/ml of florfenicol. The drug was administered by intramuscular injection in a dose of 1 ml/20 kg live weight, twice at a 48-hour interval.
Group 2: 120 weaned pigs were administered the drug HIPRALONA®ENRO-I, which contains 50 mg/ml of enrofloxacin. The drug was administered three times at 24-hour intervals in a dose of 1 ml/10 kg live weight. The animals in the two groups were fed and reared under the same conditions.

Paraclinical examinations

Five blood samples were collected from each group prior to therapeutic treatment and 5 days after the beginning of treatment. The blood samples were collected from *sinus ophthalmicus*, using the closed system Venoject II, with a Butterfly needle 16G and EDTA evacuated tubes for blood counts and gel evacuated tubes for biochemical tests. Whole blood counts (RBC, HGB, PCV, WBC, LYM, PLT) were performed using an automated HemaScreen analyzer (Hospitex Diagnostics, Germany). Biochemical parameters in blood sera (total protein, albumin, glucose, urea, total bilirubin) were determined by commercially available kits (Human, Germany), using a Screen Master semi-automated chemistry analyzer (Hospitex Diagnostics, Germany).

Serological tests

Sterile blood samples (serum) were collected from 30 animals (i.e. 15 samples from each of the two groups) prior to therapeutic treatment. The samples were analyzed for the presence of specific antibodies against *M. hyopneumoniae* by *bloking* ELISA, using an INGEZIM M. HYO COMPAC diagnostic kit (Ingenaza, Spain), according to the manufacturer's instructions. The results were considered positive when OD_{450} = or<cut-off of the positive control; negative, when OD_{450} = or>cut-off of the negative control; or ambiguous, when the values fell between the positive control cut-off and the negative control cut-off.

Statistical analysis

Statistical analysis was performed using the StatMost software (StatMost 3.6, Dataxiom Software, 2003). Data represent mean values with standard error of the means (Mean±SE) determine by one-way ANOVA. Results were considered statistically significantly different when $P<0.05$.

RESULTS AND DISCUSSIONS

The clinical examination prior to treatment of the pigs from both groups showed increased body temperature (40.5°C – 41.0°C), fits of dry cough breathing difficulties and visible growth retardation. The pathoanatomical examination of four pigs that died revealed changes in the lungs characteristic of catarrhal bronchopneumonia involving the apical and cardiac lobes with well-demarcated areas of light purple discoloration. This clinical morphological analysis was indicative of respiratory disease, more specifically, of enzootic pneumonia. To confirm the diagnosis, serological tests were performed using *bloking* ELISA. The assay detected antibodies against *M. hyopneumoniae* in all tested samples. There was a sharp increase in the incidence rates, which reached 29.3 % in group 1 and 48.3 % in group 2. This required injection of the studied

antibiotics to control acute enzootic pneumonia. Prior to therapeutic treatment, five blood samples were collected from each of the two groups for hematological and biochemical tests in acute clinical form of enzootic pneumonia. The results from the hematological tests in both groups of animals are presented in Table 1. In group 1, there were changes in the red blood counts, which deviated from the reference values: a decrease in erythrocyte counts to 4.07±0.16 ($p<0.05$), in hemoglobin to 54.40±6.19 ($p<0.01$) and in hematocrit to 30.06±0.48. These values were slightly higher in group 2 animals than in group 1 but were still below the reference values: 4.45±0.18 erythrocytes; 73.20±3.28 hemoglobin ($p<0.05$) and 29.34±0.41 hematocrit. There was also a decrease in MCV in both groups: 45.38±0.42 ($p<0.05$) in group 1 and 48.20±1.53 in group 2, as compared to the reference values. The test results also showed a statistically significant decrease in MCH, which was 14.18±0.22 ($p<0.01$) in group 1 and 15.82±0.32 in group 2 ($p<0.05$). In pigs with respiratory signs, the MCHC values were found to be statistically significantly lower in both groups: 270.80±2.73 ($p<0.01$) in group 1 and 258.00±3.29 ($p<0.001$) in group 2. Deviations were also observed in the white blood counts. The leucocyte counts were reduced to 6.58±0.27 in group 1 ($p<0.05$) and to 7.88±0.27 in group 2 ($p<0.05$). The percentage of lymphocytes was lower in the two groups: 22.54±0.50 ($p<0.001$) in group 1 and 23.74±0.32 ($p<0.001$) in group 2. The mean platelet counts fell within the reference values.

In addition to the changes in the hematological profile of the pigs before treatment with the tested antibiotics, there were also changes in some of the biochemical indicators (Table 2). The results showed a decrease in the total protein content in both groups: 59.98±0.44 ($p<0.05$) in group 1 and 61.50±0.71 ($p<0.05$) in group 2. The indices that remained within the reference limits in both groups were albumin (19.46±0.28 in group 1and 18.32±0.33 in group 2) and glucose (4.72±0.29 in group 1 and 4.04±0.17 in group 2 ($p<0.05$). There were small differences in the blood urea in the two groups; the values were within the reference limits in group 1 (7.06±0.29) and slightly increased (8.60±0.33) in group 2 ($p<0.05$). The total blood bilirubin was statistically significantly increased in the diseased pigs as compared to the reference values and was 9.24±0.31 ($p<0.001$) and 7.50±0.53 ($p<0.001$), in group 1 and 2, respectively.

The results from the comparative clinico-epidemiological study of the two antibiotics are presented in Table 3. In the florfenicol treatment group (group 1), the mortality was reduced to 4.3 % and the lethality, to 14.6 %. The percentage of emergency slaughtered pigs in this group amounted to 9.7 % at the end of the experiment. In comparison, in the group treated with enrofloxacin (group 2), there was lower mortality (3.3 %) and twice as low lethality (6.9 %) as compared to group 1. A similar trend was observed in the percentage of emergency slaughtered pigs: it was reduced to 3.4 % in group 2.

Table 1. Hematological profile prior to therapeutic treatment of weaners with enzootic pneumonia

Parameters	Units	I group Florkem® (n=5) Mean±SE	II group Hipralona® ENRO-I (n=5) Mean±SE	References (mean)
1. Erythrocytes – RBC	$10^{12}/1$	4.07±0.16*	4.45±0.18	5 – 8 (6.5)
2. Hemoglobin – HGB	g / l	54.40±6.19**	73.20±3.28*	100 – 160 (130)
3. Hematocrit – PCV	%	30.06±0.48	29.34±0.41	32 – 50 (42)
4. MCV	fl	45.38±0.42*	48.20±1.53	50 – 68 (60)
5. MCH	pg	14.18±0.22**	15.82±0.32*	17 – 21 (19)
6. MCHC	g / l	270.80±2.73**	258.00±3.29***	300 – 340 (320)
7. Leukocytes – WBC	$10^{9}/1$	6.58±0.27*	7.88±0.27*	11 – 22 (16)
8. Lymphocyte – LYM	%	22.54±0.50***	23.74±0.32***	39 – 62 (53)
9. Thrombocytes – PLT	$10^{9}/1$	267.20±3.71	240.20±2.22	100 – 900 (520)

*$p < 0,05$ **$p < 0,01$ ***$p < 0,001$ *(References: Nemi, C. J., 1993. Essentials of Veterinary Hematology, p. 23)*

Table. 2. Biochemical profile prior to therapeutic treatment of weaned pigs suffering from enzootic pneumonia

Parameters	Units	I group Florkem® (n=5) Mean±SE	II group Hipralona®ENRO-I (n=5) Mean±SE	References (mean)
1. Total Protein – TP	g / l	59.98±0.44*	61.50±0.71*	65 – 85 (75)
2. Albumin	g / l	19.46±0.28	18.32±0.33	19 – 24 (21.5)
3. Glucose	mmol / l	4.72±0.29	4.04±0.17*	4.44 – 6.38 (5.41)
4. Blood Urea	mmol / l	7.06±0.29	8.60±0.33*	2.6 – 8.0 (5.3)
5. Bilirubin Total – T Bili	µmol / l	9.24±0.31***	7.50±0.53***	0.0 – 3.1 (1.55)

**p < 0,05 **p < 0,01 ***p < 0,001 (References: Angelov, G. et al., 1999. Klinichno-laboratorni izsledvaniya v veterinarnata meditsina, p. 106-146)*

Table. 3. Results from the clinico-epidemiological study of weaned growing pigs suffering from enzootic pneumonia

Parameters	Units	I group (n=140)	II group (n=120)
1. Treated pigs	Number	41	58
2. Age of pigs	Months	2.5	2.5
3. Medicament	-	Florkem®	Hipralona®ENRO-I
4. Active substance	-	Florfenicol 300 mg/ml	Enrofloxacin 50 mg/ml
5. Dose	ml/kg b.w.	1 ml / 20kg b.w.	1 ml / 10 kg b.w.
6. Method of administration	-	Intramuscular	Intramuscular
7. Course of treatment	Days	Twice in 48 hours	three times in 24 hours
8. Duration of the experiment	Days	21	21
9. Diseased by EP	Number	41	58
	%	29.3	48.3
10. Mortality by EP	Number	6	4
	%	4.3	3.3
11. Lethality	%	14.6	6.9
12. Emergency slaughtered	Number	4	2
	%	9.7	3.4
13. Clinically recovered	Number	31	52
14. Therapeutic efficiency	%	75.6	89.6

Table. 4. Hematological profile on the 5th day of therapeutic treatment of weaned pigs suffering from enzootic pneumonia

Parameters	Units	I group Florkem® (n=5) Mean±SE	II group Hipralona® ENRO-I (n=5) Mean±SE	References (mean)
1. Erythrocytes – RBC	10^{12} / l	6.11±0.06	5.97±0.22	5 – 8 (6.5)
2. Hemoglobin – HGB	g / l	107.60±1.91	102.60±2.94	100 – 160 (130)
3. Hematocrit – PCV	%	33.66±0.98	36.08±0.78	32 – 50 (42)
4. MCV	fl	52.54±0.65	48.60±1.72	50 – 68 (60)
5. MCH	pg	14.34±0.29**	16.40±0.31	17 – 21 (19)
6. MCHC	g / l	283.20±3.25*	253.20±2.92***	300 – 340 (320)
7. Leukocytes – WBC	10^{9} / l	17.52±0.42	19.72±0.36	11 – 22 (16)
8. Lymphocyte – LYM	%	43.64±0.81***	48.90±1.44***	39 – 62 (53)
9. Thrombocytes – PLT	10^{9} / l	456.20±18.55	367.20±9.01	100 – 900 (520)

**p < 0,05 **p < 0,01 ***p < 0,001 (References: Nemi, C. J., 1993. Essentials of Veterinary Hematology, p. 23)*

Table. 5. Biochemical profile on the 5th day of therapeutic treatment of weaned pigs suffering from enzootic pneumonia

Parameters	Units	I group Florkem® (n=5) Mean±SE	II group Hipralona® ENRO-I (n=5) Mean±SE	References (mean)
1. Total Protein – TP	g / l	78.08±0.90	74.76±0.76	65 – 85 (75)
2. Albumin	g / l	28.72±0.52***	30.54±0.51***	19 – 24 (21.5)
3. Glucose	mmol / l	4.92±0.14	4.50±0.44	4.44 – 6.38 (5.41)
4. Blood Urea	mmol / l	6.80±0.23	7.64±0.27	2.6 – 8.0 (5.3)
5. Bilirubin Total – T Bili	μmol / l	8.64±0.35***	7.30±0.32***	0.0 – 3.1 (1.55)

**p < 0,05 **p < 0,01 ***p < 0,001 (References: Angelov, G. et al., 1999. Klinichno-laboratorni izsledvaniya v veterinarnata meditsina, p. 106-146)*

For paraclinical examination, a new set of blood samples were collected from the two groups of animals on the 5th day of treatment. The results (table 4) showed a trend for the red blood counts to return to normal in both groups: erythrocytes (6.11±0.06 in group 1 and 5.97±0.22 in group 2); hemoglobin (107.60±1.91 in group 1 and 102.60±2.94 in group 2); hematocrit (33.66±0.98 in group 1 and 36.08±0.78 in group 2). The MCV values were back to normal in group 1 (52.54±0.65), whereas in group 2 they were slightly below the reference values (48.60±1.72). MCH remained slightly below the reference values: 14.34±0.29 in group 1 (*p<0.01*) and 16.40±0.31 in group 2. A similar trend was observed in MCHC, which was statistically significantly reduced in both groups: 283.20±3.25 in group 1 (*p<0.05*) and 253.20±2.92 in group 2 (*p<0.001*). Moreover, both antibiotics led to a normalization in the leucocyte counts (17.52±0.42 in group 1 and 19.72±0.36 in group 2), as well as in the percentage of lymphocytes (43.64±0.81 (*p<0.001*) in group 1 and 48.90±1.44 (*p<0.001*) in group 2). The comparison of the platelet counts prior to and after treatment showed an upward trend in both groups (456.20±18.55 in group 1 and 367.20±9.01 in group 2) but without exceeding the reference values.

The results from the biochemical tests carried out after the therapeutic treatment (Table 5) showed that the total protein content in the blood samples collected from both groups was within the reference range: 78.08±0.90 in group 1 and 74.76±0.76 in group 2. The albumin content was slightly above the norm in both groups: 28.72±0.52 (*p<0.001*) in group 1 and 30.54±0.51 (*p<0.001*) in group 2. Regarding the blood glucose, there were no significant differences between the samples collected prior to and following the treatment with either antibiotic; it was 4.92±0.14 in group 1 and 4.50±0.44 in group 2. It was also obtained that in both groups the blood urea was within the reference range: 6.80±0.23 in group 1 and 7.64±0.27 in group 2. The total bilirubin values were significantly increased in both groups after the antibiotic treatment, the values being 8.64±0.35 (*p<0.001*) in group 1 and 7.30±0.32 (*p<0.001*) in group 2.

At the end of the experiment (on day 21), the clinical condition of the pigs in both groups was improved and stable. The rectal temperature, albeit varying from one pig to another, was within the normal range (38.0–38.5°C). There were no clinical signs of respiratory disease (such as cough, dyspnea, anorexia or depression) in the antibiotically treated pigs. These observations were in accordance with the obtained data showing normalization of the clinical status of the treated pigs. The results from this study demonstrate the high therapeutic efficacy *in vivo* of both enrofloxacin (89.6 %) and florfenicol (75.6 %) in the control of acute enzootic pneumonia.

Our results are in agreement with the reports of Hannan et al. (1989) and Vicca (2005) about the good therapeutic efficacy of antimicrobial drugs from the amphenicol and fluoroquinolone group against *M. hyopneumoniae*. The good therapeutic effect achieved by enrofloxacin (89.6 %) against acute enzootic pneumonia suggests that, despite reports on cases of acquired antibiotic resistance in *M.*

hyopneumoniae to some fluoroquinolones (Vicca et al., 2004; Vicca, 2005), it could be considered not a serious problem in the therapy of enzootic pneumonia.

Antimicrobials, as a strategic choice of therapy against enzootic pneumonia, give good results when applied appropriately and at the appropriate moment. Parenteral therapy is preferred in the case of acute disease, whereas treatment with the forage (*per os*), as a preventive measure in metaphylaxis.

Despite the fact that antimicrobials do not protect pigs against infection and do not completely eliminate the microorganisms in the respiratory tract of diseased pigs, this type of therapeutic agents can alleviate the clinical signs of enzootic pneumonia as well as the severity of pathological lesions in the lungs. Most importantly, they can reduce the mortality from the disease and, in turn, the economic losses. Further studies are needed to determine whether administration of the tested antibiotics could prevent the transmission of *M. hyopneumoniae* from infected pigs to healthy ones.

CONCLUSIONS

Both tested schemes for therapy in acute enzootic pneumonia caused by *M. hyo* with enrofloxacin in a dose of 1 ml/10 kg b.w. administered intramuscularly three times in 24 hours and florfenicol in a dose of 1 ml/20 kg b.w. administered intramuscularly twice at 48 hours, proved to be effective in the control of disease, result of complete clinical recovery of pigs, normalization in indicators of blood and most importantly - reducing mortality.

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REFERENCES

Angelov, G., N. Ibrishimov, S. Milashki, 1999. Klinichno-laboratorni izsledvaniya v veterinarnata meditsina. Sofia, p. 106-146.

Ganovski, D. & I. Dinev, 1996. Respiratornite zabolyavaniya pri svinete. ASB, IS – Shumen, NT ot KMU "Vsichko za svinevadstvoto", 29-30 may, p. 134–138.

Motovski, A., 2003. Respiratoren bolesten kompleks. VM-novini, № 3-4, p. 24-28.

Bosch, G., 2004. Single-dose treatment against respiratory bacteria. Pig international, March, Vol. 34, N 3, p. 30-32.

Halbur, P. G., 1999. Porcine respiratory disease complex. Proc. of the North Carolina Healthy Hogs Seminar (North Carolina Swine Veterinary group), p. 1–14.

Hannan, P.C.T., O'Hanlon, P.J. & Rogers, N.H., 1989. In vitro evaluation of various quinolone antibacterial agents against veterinary mycoplasmas and porcine respiratory bacterial pathogens. Research in Veterinary Science 46, p. 202-211.

Maes, D., Pasmans, F., Haesebrouck, F., 2011. Porcine Mycoplasmas: the Never Ending Story. Proc. of the 5-th Asian Pig Veterinary Society Congress, Pattaya, Thailand, p. 7–15.

Nemi, C., Jain, 1993. Essentials of Veterinary Hematology. Philadelphia: Lea & Febiger, p. 23.

Opriessnig, T., Thacker, E. L., Yu, S., Fenaux, M., Meng, X. J., Halbur, P.G., 2004. Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with *Mycoplasma hyopneumoniae* and porcine circovirus type 2. Vet. Pathol. 41, p. 624-640.

Stipkovits, L., D. Miller, R. Glavits, L. Fodor, D. Burch, 2001. Treatment of pigs experimentally infected with *Mycoplasma hyopneumoniae, Pasteurella multocida*, and *Actinobacillus pleuropneumoniae* with various antibiotics. Can. J. Vet. Rec., Vol. 65, p. 213–222.

Thacker, E. L., 2006. Mycoplasmal diseases. In: Leman, A.D., Straw, B.E., D'Allaire, S., Mengeling, W.L., and Taylor, D.J., (Ed.), Diseases of Swine, 9th ed. The Iowa State University Press, Ames, IA, p. 701-717.

Timmerman, T., Dewulf, J., Catry, B., Feyen, B., Opsomer, G., de Kruif, A. & Maes, D., 2006. Quantification and evaluation of antimicrobial-drug use in group treatments for fattening pigs in Belgium. Preventive Veterinary Medicine, Vol. 74, Issue 4, p. 251–263.

Vicca, J., Stakenborg, T., Maes, D., Butaye, P., Peeters, J., de Kruif, A., Haesebrouck, F., 2004. In vitro susceptibilities of *Mycoplasma hyopneumoniae* field isolates. Antimicrob. Agents Chemother. 48, p. 4470–4472.

Vicca, J., 2005. Virulence and antimicrobial susceptibility of *Mycoplasma hyopneumoniae* isolates from pigs. Faculty of Veterinary Medicine. Gent University. Thesis, p. 27-47.