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*Influence of selected antibiotics on serological test
results at hens infected with Salmonella Enteritidis* *

Wpływ wybranych antybiotyków na wyniki badań serologicznych kurcząt
zakażonych pałeczkami *Salmonella*

SUMMARY

Salmonella rods belong to the most widespread microorganisms in the environment. Their role as a toxico-infection factor is significant and it is related to the common occurrence in poultry. That is why an important role in the transmission of the microbe is played by poultry. To limit the losses caused by *Salmonella* it is necessary first of all to identify the infection factor as quickly as possible and introduce effective control of products of poultry origin throughout the production cycle.

The purpose of the studies was to determine the effect selected antibiotics on the results of serological tests in the case birds naturally infected by *Salmonella* rods and in experimental conditions. The studies were conducted on birds from two farms in the vicinity of Lublin.

They were infected both naturally and experimentally. The following antibiotics were used in the experiment: amoxicyclin with clavulanic acid (Amoksiklaw, Lek Polska), spiramicin with kolistin (Spiracol, Grodziskie Zakłady Farmaceutyczne), linkomycin with spectinomycin (Linco-spectin, Elanco), enrofloxacin (Enrocin, Grodziskie Zakłady Farmaceutyczne), norfloxacin (Nortil, Bremmer Farma), tiamphenicol (Tirsan, Fatro), flophenicol (Nuflor, Schering-Plough Animal Health). The results of serological tests obtained in the group of experimentally infected chicken point out that the applied antibiotic therapy significantly reduced the level of antibodies, which was reflected in the low percentage of positive results achieved in ELISA test and agglutination method. In the case of naturally infected birds the obtained values showed that the antibiotic therapy had a significantly smaller influence on the level of antibodies and this influence was limited only to some antibiotics (norfloxacin, enrofloxacin, chloramphenicol).

Key words: immunity to antibiotics, antibiotic therapy, *Salmonella*, salmonellosis in poultry

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INTRODUCTION

Salmonella rods belong to the most widespread microorganisms in an environment. Their role as nutritional toxico-infection factor is still significant and associated with common occurrence in poultry [Hefez 1998, Wieliczko *et al.* 1998]. Therefore, poultry play a very important role in microbe transmission. All activities aiming to decrease the number of poultry infections with *Salmonella* rods lead to the reduction of salmonellosis foci in people. It requires such procedures that would allow releasing the birds from *Salmonella*. It aims to include the reproduction flocks within control and prophylaxis program and thus maximum reduction of salmonella rod transfer to the following poultry generations [Desmidt *et al.* 1997, Duchet-Suchaux *et al.* 1997, Błaszczak *et al.* 1999].

Reducing the losses due to *Salmonella* requires most of all quick identification of infectious factor and application of efficient control over poultry-originated products during the whole production cycle. According to legal regulations, bacteriological tests are the only method for *Salmonella* infection recognition.

Microbial diagnostics is a labor consuming and long-lasting method; therefore, diagnostic techniques based on the identification of specific antibodies in serum should find wide application. There are several serological methods for monitoring infections with *Salmonella* rods. Platelet agglutination reaction with dried antigen of *S. Pullorum* is the best known and commonly used method. Modified at present as plate agglutination method, it serves to identify other serovars (*S. Enteritidis*, *S. Typhimurium*). Immunoenzymatic ELISA technique facilitating qualitative and quantitative determination of antibodies in serum found wide application in maintenance and breeding [Barrow 1992, Barrow 1994]. The technique is characterized with great specificity and result repeatability as well as small sample volume and possibility of automation. Thus, it is applied as a test for serological control within poultry serological monitoring [Barrow 1994], making it possible to detect infected poultry flocks and gives an information on efficiency of programs for infectious disease protection, including salmonellosis.

In the case of identification of *Salmonella* carrier state in a flock, birds are subjected to the slaughter or veterinary treatment. Most applied antibiotics, besides bactericidal properties, exert a suppressive influence on an organism's immunity. The phenomenon was defined [Dohms *et al.* 1997] as a state of temporary or durable dysfunction of immunity caused by the immune system injury, leading to the increase of vulnerability to diseases. In the case of hens, it affects the decrease of antibodies number, making serological diagnostics difficult.

The aim of the study was to evaluate the influence of selected antibiotics on serological test results in the case of birds naturally infected with *Salmonella* rods and under experimental conditions.

MATERIAL AND METHODS

Studies were carried out using birds originating from two farms near Lublin and naturally and experimentally infected. Birds naturally infected. Studies included forty-two 52-week-old Isa Brown hens hatching consumption eggs. Before the experiment, hens were subjected to diagnostic examinations, on the base of which birds were divided into free from internal parasites. Infection due to *Salmonella* rods was controlled by means of serological tests and bacteriological analyses of feces.

Hens were divided into nine groups of five birds each. Seven groups were treated with antibiotics according to the producer's recommendations, two were the positive (K+) and negative (K-) controls.

Birds experimentally infected. The experiment included forty-five 3-week-old Ross broilers. Before the experiment, hens were subjected to diagnostic examinations, on the base of which birds were divided into free from internal parasites.

Birds were divided into nine groups of five broilers each. Seven groups were infected and treated with antibiotics according to the producer's recommendations, two were the positive (K+) and negative (K-) controls. Antibiotic treatment was started on the third day after infection.

Antibiotics used in the experiments included amoxycilin with clavulanic acid (Amoksiklaw, Lek Poland), spiramycin with kolistin (Spiracol, Grodziskie Zakłady Farmaceutyczne), linkomycin with spectinomycin (Linco-spectin, Elanco), enrofloxacin (Enrocin, Grodziskie Zakłady Farmaceutyczne), norfloxacin (Nortril, Bremmer Farma), tiamphenicol (Tirsan, Fatro), florphenicol (Nuflor, Schering-Plough Animal Health). Preparation of bacterial suspension for poultry infection with *Salmonella Enteritidis* was earlier described in literature [Rzedzicki *et al.* 2001].

Serological tests. Blood for tests was taken from wing vein to Eppendorf's tubes and placed at 37°C for 2 hours. Then, clot was separated from the tube's walls and maintained at 4°C for 12 hours. After that, the material was centrifuged for 20 minutes at 2500 × g at 4°C.

Platelet agglutination reaction with fresh blood drop. Blood samples from particular birds were tested using died antigen SP Antigen (Intervet).

Plate agglutination reaction. Particular serum samples were tested using died antigen achieved from *Salmonella Enteritidis* (Enterognost) according to the method worked out by Chart *et al.* [Tokarzewski 2001].

ELISA kit. Tests for the presence of anti-*Salmonella* antibodies in particular birds were performed using immuno-enzymatic ELISA test (PIWet, Puławy) according to the producer's recommendation [Tokarzewski 2001]. Results were read in a reader for micro-plates (Labsystem Multiscan Plus, Finland) at 405 nm wavelength.

Samples were classified as positive when the mean absorbance was at least 60% of the value for S+ serum and as doubtful when mean value was within the range 40–60% of S+.

RESULTS AND DISCUSSION

Serological examination of birds before experiments revealed that all of them had antibodies against *Salmonellas*.

Serum and blood tests performed after completing antibiotic therapy marked as „zero” day.

In tests of serum and blood from naturally infected hens and carried out by means of direct agglutination method on the 10th day of the experiment, positive results were achieved in 17% of tested hens, which was 40% of positive results obtained using ELISA test. As compared to the results achieved before therapy using antibiotics, the percentage of birds reacting positively decreased by over 80% both in tests with Enterognost and SP Antigen. Percentage of birds showing positive results in particular groups ranged from 40% in group treated with amoxycilin with clavulanic acid to 20% using other antibiotics; in the case of chloramphenicol, no positive results were observed. The number of hens in which OD values for serum shaped at the level of 60% in birds treated with amoxycilin with clavulanic acid and linkomycin with spectinomycin to 20% in the case of chloramphenicol. Forty percent of positive results were recorded in the groups of birds treated with enrofloxacin, norfloxacin, spiramycin with kolistin and tiamphenicol. As compared to results achieved before the medical therapy, in general, the percentage of hens, whose OD values were at the level of positive results, decreased by 85%.

On the 17th day of experiment, a significant increase of number of birds reacting positively both to SP Antigen and Enterognost, was recorded. Positive results were 46% of the tested birds, which was 89% of positive results achieved in ELISA test. In particu-

lar groups, the obtained positive results ranged from 80% after linkomycin with spectomycin treatment, through 60% in the case of amoxycilin with clavulanic acid, spiramycin with kolistin and tiamphenicol, to 20% after treatment with enrofloxacin, norfloxacin and chloramphenicol. In Elisa test, a increase of the number of birds reacting positively was recorded in the groups treated with linkomycin with spectomycin (80%), spiramycin with kolistin (60%), tiamphenicol (60%) and chloramphenicol (20%). The decrease of percentage (by 20%) of birds reacting positively was recorded in the group treated with enrofloxacin. The values remained at similar levels in other groups. In the following experiment period (on the 24th day), the percentage of positive results in agglutination method test decreased to 40%, which was 50% of positive values achieved by means of ELISA test, and it decreased up to 40–20% in the groups treated with linkomycin with spectomycin, spiramycin with kolistin and tiamphenicol. It was the same in the group given with amoxycilin with clavulanic acid and norfloxacin. A slight decrease of OD value percentage at the positive result level was observed in the group of birds treated with linkomycin with spectinomycin (40%), spiramycin with kolistin (20%), chloramphenicol (20%), and tiamphenicol (20%). After enrofloxacin treatment, the increase of the values by 20% was recorded, and in the case of norfloxacin these values were unchanged.

Tests performed by means of plate (Enterognost) and platelet agglutination (SP Antigen) methods revealed that achieved positive results of serum and blood are the same in 100% and they are 33.3% of positive results obtained in ELISA test.

Table 1. Results of serological tests after antibiotic therapy at the group of chickens experimentally infected with *Salmonella rods*

Tabela 1. Wyniki badań serologicznych uzyskane po antybiotykoterapii w grupie kurcząt zakażonych eksperymentalnie pałeczkami *Salmonella*

Tests	Groups								
	Amoxycilin with clavulanic acid	Linkomycin with spectomycin	Spiramycin with kolistin	Enrofloxacin	Norfloxacin	Florphenicol	Tiamphenicol	Group K+	Group K-
	d/b (%)	d/b (%)	d/b (%)	d/b (%)	d/b (%)	d/b (%)	d/b (%)	d/b (%)	d/b (%)
SP Antigen	1/15 (6)	0/15 (0)	0/15 (0)	0/15 (0)	0/15 (0)	0/15 (0)	0/15 (0)	15/15 (100)	0/15 (0)
Enterognost	1/15 (6)	0/15 (0)	0/15 (0)	0/15 (0)	0/15 (0)	0/15 (0)	0/15 (0)	15/15 (100)	0/15 (0)
ELISA	2/15 (13)	1/15 (6)	0/15 (0)	0/15 (0)	0/15 (0)	0/15 (0)	0/15 (0)	15/15 (100)	0/15 (0)

In the group of experimentally infected chickens, in direct agglutination method studies, beginning from the 10th day, no positive results were recorded. On the 24th day after treatment, positive results were obtained only in the case of birds treated with amoxycilin with clavulanic acid. Comparing the results of the test performed by means of

agglutination method using two antigens (Enterognost and SP Antigen), it was proved that the achieved positive results for blood and serum were the same in 100% and they are 33.3% of positive results obtained in ELISA test.

OD values achieved in ELISA test on the 10th and 17th days after treatment, were at the level of negative values. At the same time, OD values for positive control group including infected and not treated birds on the 10th day of the experiment, were also negative, but on the 17th day they increased as compared to the previous test giving positive values. In subsequent test on the 24th day of the experiment, an increase of OD values in experimental groups as compared to the previous test was observed. OD values in particular the groups were at different levels depending on the applied medicine. Positive results were achieved in the groups of birds treated with amoxicilin with clavulanic acid (40%), and linkomycin with spectomycin (20%).

The highest percentage of positive results in both experimental groups was achieved after treatment with amoxicilin with clavulanic acid as well as linkomycin with spectomycin (Fig. 1, Tab. 1). After treatment with spiramycin with kolistin, OD values were negative in the group of chickens experimentally infected; in the group of naturally infected birds, a slight decrease of positive result percentage after treatment and then gradual increase in following research periods was observed (Fig. 1).

In the available literature data, no information on the influence of either combined or particular antibiotics on immune processes at animals were found. Taking into account the action mechanisms most of all consisting in protein biosynthesis disturbance, it can be supposed that the achieved data are the results of their immunomodulatory effects. It can also result from antibiotics' medical action itself consisting in the inhibition of bacterial reproduction in an organism, thus leading to the weakening of antigen stimulus interaction strength. Then, a destructive influence of antibiotics on bacteria, including their reproduction, is added to the negative interaction with active immune cells, which leads to the disturbance of antigen information transfer mechanisms and inhibition of lymphoblast transformation into plasmatic cells able to synthesize antigens directed towards *Salmonellas* [Rzedzicki *et al.* 1992].

Studies performed by Desmidt *et al.* [1982] using group of chickens experimentally infected with *Salmonella* rods and then treated with enrofloxacin revealed the decrease of antibody titre in the studied group. Goren [1992] achieved similar results. Wray [1985], in analogous experiments, did not observe any changes of antibody level. In the authors' own studies, treatment of experimentally infected birds with antibiotics from quinolon group (enrofloxacin and norfloxacin) exerted an obviously inhibiting influence on antibody level, which manifested low OD values within negative range achieved in all research periods. A clear decrease of OD values in a large percentage of birds up to negative and doubtful values, as well as a slight increase of positive results in the group of naturally infected poultry was observed in two subsequent tests (Fig. 1).

The influence of quinolons on birds' immune mechanisms is a subject of few studies and at present is not fully known. Different opinions of scientists probably result from the fact that quinolons at medical doses do not significantly change the functions of phagocytosing cells, interferon- γ production and lymphocyte proliferation [Dębowy 1997, Shalit 1991]. However, in the case of fluoroquinolones, obvious an immunosuppressive action is observed. It is the medicine group very often applied at birds' salmonellosis therapy. These preparations are characterized with low toxicity and high safety of dosage. Anti-

bacterial action consists in inhibition of activity of A gyrase sub-unit of DNA in the enzyme that catalyzes ATP-dependent formation of double DNA spiral through blocking the nucleotide building-in, which leads to DNA over-spiral formation (twisting) [Griggs *et al.* 1994, Dębowy 1997]. Instead of gyrase, cells of higher organisms have a similarly functioning enzyme – DNA topoisomerase type II [Hardman 1996, Dębowy 1997]. Fluoroquinolones may have an inhibiting effect to its activity [Shalit 1991].

Chloramphenicol from phenicol group is the antibiotic with a strong immunosuppressive effect. Its antibacterial action is associated with the inhibiting influence of mRNA through binding to 50S sub-unit at 70S bacterial ribosome, and in consequence inhibition of peptidyltransferase activity occurs. Immunosuppressive effects of the antibiotic is most often mediate as a result of bone marrow cell proliferation inhibition, mainly of erythropoetic line [Kania 1998]. Moreover, it was found that chloramphenicol influenced the decrease of antibody level in serum and diminished the phagocyte activity of granulocytes [Ibrahim 1987, Artsimovich 1988]. Studies using birds [Freeman 1999] revealed a significant decrease of antibody level after giving chloramphenicol to chicks immunized with sheep's erythrocytes [Laval *et al.* 1988].

An obviously inhibiting influence of phenicols on antibody level in both experimental groups was observed in the studies. OD values in the group of chickens experimentally infected remained at the same level during the whole study; in naturally infected hens, OD values decreased after treatment to the negative and doubtful result level at a large percentage of birds (Fig. 1). Furthermore, significant differences between other percentage of positive results were observed in the groups of birds treated with chloramphenicol and tiamphenicol. In the case of tiamphenicol, the number of positive results in particular study periods was higher than in birds treated with chloramphenicol (Fig. 1). It would point out that tiamphenicol exerts a weaker suppressive effect on functioning of particular elements of immune system than chloramphenicol.

CONCLUSIONS

In the group of experimentally infected chickens the achieved serological test results point out that the applied antibiotic therapy had a significantly inhibiting influence on antibody level, which was expressed with a low percentage of positive results obtained in ELISA test and agglutination method. In the case of naturally infected poultry, the achieved results pointed out that the applied antibiotic therapy had a significantly smaller influence on antibody level and the effect was reduced only to some antibiotics (norfloxacin, enrofloxacin, chloramphenicol).

REFERENCES

- Artsimovich N. G., Nastoishchaia N. N., Lyman' N. P., Mul'tanovskaia V. N., Navashin P. S. 1988: Effect of antibiotics on indices of immunologic reactivity in mice. *Antibiot. Khimioter.* 33, 838–842.
- Barrow P. A. 1992: Further observations on the serological response to experimental *Salmonella typhimurium* in chickens measured by ELISA. *Epidemiol. Infect.* 108, 231–241.

- Barrow P. A. 1994: Serological diagnosis of *Salmonella* serotype *enteritidis* infections in poultry by ELISA and other tests. *Int. J. Food Microbiol.* 21, 55–68.
- Błaszczak B., Binek M.: Nosicielstwo w stadzie oraz obecność *Salmonella enteritidis* w jajach i zarodkach kurzych. *Medycyna Wet.* 55, 39–41, 1999.
- Desmidt M., Uyttendaele E., de Groot P. A., Ducatelle R., Haesebrouck F. 1992: Lipopolisaccharide versus whole germ ELISA and possible consequences of antibiotic treatment on seroconversion to *Salmonella enteritidis*. The role of antibiotics in the control of food-borne pathogens. *FLAR* no. 6. The prevention and control of potentially pathogenic microorganisms in poultry and poultry meat processing, 103–110.
- Desmidt M., Ducatelle R., Haesebrouck F. 1997: Pathogenesis of *Salmonella enteritidis* phage type four after experimental infection of young chickens. *Vet. Microbiol.* 56, 99–109.
- Dębowy J.: Chinolony we współczesnej farmakoterapii weterynaryjnej. *Medycyna Wet.* 53, 307–312, 1997.
- Dohms J. E., Saif Y. M., Bacon W. L. 1978: Metabolism and passive transfer of immunoglobulins in the turkey hen. *Am. J. Vet. Res.* 39, 1472–1481.
- Duchet-Suchaux M., Mompert F., Berthelot F., Beaumont C., Léchopier P., Pardon P. 1997: Differences in frequency, level, and duration of cecal carriage between four outbred chicken lines infected orally with *Salmonella enteritidis*. *Avian. Dis.* 41, 559–567.
- Freeman F. M., Young I. G. 1999: Chloramphenicol-induced amnesia for passive avoidance training in the day-old chick. *Neurobiol. Learn. Mem.* 71, 80–93.
- Goren E., Untitled letter in World Health Organisation on national and local schemes of *Salmonella* control in poultry. Ploufragen, France. 1992
- Griggs D. J., Hall M.C., Jin Y. F., Piddock L. J. V. 1994: Quinolone resistance in veterinary isolates of *Salmonella*. *J. Antimicrobial Chemotherapy* 33, 1173–1189.
- Hafez H. M. 1998: Zakażenia drobiu pałeczkami *Salmonella*: diagnostyka i zwalczanie. Materiały Konferencji: „Salmonelozy drobiu”. Puławy, 7.
- Hardman J. G., Goodman Gilman A., Limbird L. E. 1996: Goodman & Gilman's The pharmacological basis of therapeutics., 9/e, McGraw-Hill, New York.
- Ibrahim M. S. Maged Z. A., Haron A., Khalil R. Y., Attallah A. M. 1987: Antibiotics and immunity: effect of antibiotics on natural killer, antibody dependent cell-mediated cytotoxicity and antibody production. *Chemioterapia*. 6, 426–430.
- Kania B. F. 1998: Chloramfenikol i jego przydatność terapeutyczna u zwierząt. *Medycyna Wet.* 54, 22–28.
- Laval A., Viso M., Berhanu A., Kerveillant-Lenoire S.: Immunomodulator effects of 2 antibiotics, chloramphenicol and kitasamycin, in the chicken. *Ann. Rech. Vet.* 19, 259–266, 1988.
- Rzedzicki J., Kowalska M. 1992: Immunosupresyjne działanie niektórych leków u zwierząt. *Medycyna Wet.* 48, 291–293.
- Rzedzicki J., Kołodziejczyk A., Tokarzewski S., Boś M. 2001: Wpływ antybiotykoterapii na wyniki badań bakteriologicznych i serologicznych kur zakażonych pałeczkami *Salmonella*. *Annales UMCS Sectio DD*, 56, 73–84.
- Shalit I. 1991: Immunological aspects of new quinolones. *Eur. J. Clin. Microbiol. Infect. Dis.* 10, 262–266.
- Tokarzewski S. 2001: Stymulacja odporności żółtkowej u ptaków oraz wpływ antybiotyków na jej przekazywanie, Dysertacja doktorska Lublin.
- Wieliczko A., Mazurkiewicz M. 1998: Salmonelozy u kur. Materiały Konferencji „Salmonelozy drobiu”, Puławy, 81–82.
- Wray C. 1985: Is salmonellosis still a serious problem in veterinary practice? *Vet. Rec.* 116, 485–489.

STRESZCZENIE

Pałeczki *Salmonella* są jednym z najbardziej rozpowszechnionych drobnoustrojów w środowisku. Ich rola jako czynnika toksykoinfekcji pokarmowych jest stale istotna i łączy się z powszechnym występowaniem u drobiu. Dlatego bardzo ważną rolę w transmisji zarazka odgrywa drób. Ograniczenie strat wywołanych przez salmonelle wymaga przede wszystkim szybkiej identyfikacji czynnika zakaźnego oraz wprowadzenia efektywnej kontroli produktów pochodzenia drobiowego w czasie całego cyklu produkcyjnego.

Celem prowadzonych badań było określenie wpływu wybranych antybiotyków na wyniki badań serologicznych w przypadku ptaków naturalnie zakażonych pałeczkami *Salmonella* oraz w warunkach eksperymentalnych.

Badania przeprowadzono na ptakach pochodzących z dwóch ferm z okolic Lublina, zakażonych naturalnie oraz zakażonych eksperymentalnie.

W doświadczeniu wykorzystano następujące antybiotyki: amoksycylina z kwasem klawulanowym (Amoksiklaw, Lek Polska), spiramycyna z kolistyną (Spiracol, Grodziskie Zakłady Farmaceutyczne), linkomycyna ze spektynomycyną (Linco-spectin, Elanco), enrofloksacyna (Enrocin, Grodziskie Zakłady Farmaceutyczne), norfloksacyna (Nortril, Bremmer Farma), tiamfenikol (Tirsan, Fatro), florfenikol (Nuflor, Schering-Plough Animal Health).

W grupie kurcząt zakażonych eksperymentalnie uzyskane wyniki badań serologicznych wskazują, iż zastosowana terapia antybiotykowa miała istotnie hamujący wpływ na poziom przeciwciał, co wyrażało się niskim odsetkiem wyników dodatnich uzyskanych w badaniach testem ELISA oraz metodą aglutynacji. W przypadku ptaków zakażonych w warunkach naturalnych uzyskane wartości wskazywały, iż zastosowana antybiotykoterapia miała istotnie mniejszy wpływ na poziom przeciwciał, a wpływ ten ograniczał się tylko do niektórych antybiotyków (norfloksacyna, enrofloksacyna, chloramfenikol).

Słowa kluczowe: antybiotykooporność, antybiotykoterapia, *Salmonella*, salmonelloza drobiu