The comparative analysis of the influence of fluorochinolones on the blood leucogram of chickens

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Abstract. In the present research we study the changes in ratio of different kinds of leucocytes in chickens of Hisex Brown cross following the application of fluoroquinolone antibiotic drugs. In the study we used Ciprofloxacin, Ofloxacin, Levofloxacin, Moxifloxacin, Norfloxacin, Enrofloxacin. The control set was given pure drinking water throughout the experiment; each of the experimental groups was given water with one of the above-mentioned antibacterial fluoroquinolone drugs, the dosage being 200 mg/L. The leukogram contains the total number of leucocytes and the content of separate cell types and their total numbers. The study reveals significant and valid changes concerning the number of lymphocytes. On the seventh and the ninth days after the drug withdrawal all the fluoroquinolones except Ofloxacin caused lymphopenia. The valid changes in the number of pseudoeosinophils do not show clear tendencies, such changes are observed once under the influence of each drug on different days of the research. Levofloxacin, Enrofloxacin, Ofloxacin cause refractory basophilia. The application of antimicrobial fluoroquinolone drugs in the course of ten days results in changes in the ratio of different kinds of leucocytes in chicken blood, but at the conclusion of the experiment every value in the leukogram returns to physiological standard.

1. Introduction

While analyzing multiple treatment regimens for a variety of infections in poultry farms we note that fluoroquinolones have become an increasingly popular class of antibiotics. It is easy to understand why. Members of this group have proved themselves to be systemic drugs [1-3], very effective in treating acute infectious inflammatory diseases [4], they share such mechanism of action in which the bacterial DNA gyrase (the key bacterial enzyme) is inhibited [5]. Fluoroquinolones target two elements in bacterial cells, two enzymes, responsible for transforming spatial configuration of DNA [6-9]. They are also known for good tolerance by patients, for high activity against most Gram-positive and Gram-negative bacteria and many multidrug-resistant organisms [10-13]. Pharmacokinetics of fluoroquinolones shows high volume of distribution and biotransformation in the body, a longer elimination half-life, active absorption by cells and tissues of the macroorganism [14].

Fluoroquinolones are exceptionally effective against severe bacterial infections caused by infectious agents, resistant to many classes of antibiotics. Fluoroquinolones are able to penetrate into phagocytizing cells and accumulate in them in the active form. Such intracellular accumulation of antibacterial agents in the active form enhances phagocytic activity of macrophages and neutrophils and decreases viability of phagocytized bacteria [15-17].
The action of fluoroquinolones against bacterial cells is rather specific namely they are evidently active in subbacteriostatic concentration while maintaining postantibiotic effect [18]. Reports of the effect of fluoroquinolones on lymphocytes are contradictory. Ofloxacin, Norfloxacin and Pefloxacin have an antiproliferative action on cells. Nevertheless according to Riesbeck K. et al. [19] fluoroquinolones promote production of interleukin-2 also known as lymphokine which in its turn increases proliferation of lymphocytes. They also generate respiratory burst in neutrophils. As a result of fluoroquinolone administration (in rare cases) slight changes in circulatory system are observed: anemia, thrombocytopenia, eosinophilia, elevated erythrocyte sedimentation rate, leukopenia or leukocytosis [20]. Given the fact of such adverse reactions medical science must research the effect of fluoroquinolones on circulatory system.

In the present study we for the first time ever share the relative and absolute values of different kinds of leukocytes in white blood cell differential of chicken blood after treatment with six antimicrobial fluoroquinolone drugs. We documented dynamically changing values of leukogram of chicken blood dependent on administration of the drugs and it was basophils that underwent the most significant and continuous changes.

The aim of the present study was comparative analysis of the effect of fluoroquinolones on leukogram parameters of Hisex Brown chickens (Gallus gallus L.)

2. Object and methods
Throughout the experiment the chickens were retained in vivarium under recommended housing conditions. All the chickens were fed a balanced ration, getting all essential nutrients and necessary biologically active substances. The chickens in the control group were given pure drinking water, and male chickens in the experimental groups from Day 1 till Day 10 of their lives received one of the following drugs – Enrofloxacin, Ofloxacin, Ciprofloxacin, Norfloxacin, Levofloxacin, Moxifloxacin (in the dose 200 mg/L).

Blood samples were collected by cardiac puncture from 6 chickens in every experimental group on Day 1, Day 3, Day 5, Day 7 and Day 9 after the withdrawal of the drug. The collected blood was anticoagulated with 3.8% sodium citrate solution. We performed basophil count, eosinophil count, pseudoeosinophil count, lymphocyte count, monocyte count in blood smear, stained by Romanovskiy-Giemza stain. The leukogram showed the total number of leukocytes and the content of separate cell types and their total numbers. In the statistical analysis of data we calculated the mean (M) and the standard error of the mean (m). Reliability of the results was checked with the help of Mann-Whitney nonparametric test. The statistical significance was considered at p<0.05.

3. Results
Different statistically reliable changes in leukogram were noted after administration of each of the fluoroquinolones. On Day 1 after the drug withdrawal the relative number of lymphocytes decreased in groups that received Levofloxacin and Ciprofloxacin by 28% and 17% respectively. On Day 3 Norfloxacin caused a decrease in lymphocytes by 30%, Moxifloxacin caused a decrease in lymphocytes by 20% as compared to the control group, on Day 5 the number of lymphocytes fell by 19% and 17% respectively (table 1).

On Day 7 all the studied fluoroquinolones except Ofloxacin caused a reliable absolute and relative lymphopenia. The final blood samples, collected on Day 9 after the drug withdrawal, showed a significant decrease in lymphocyte number only for the group that received Moxifloxacin. Developing lymphopenia may be caused by drug-induced leukopenia since it is known that a number of antibacterial drugs have a cytopenic effect. This may be a sign of immune deficiency inasmuch as this condition is about the same as that which is caused by blocked lymphopoiesis.

We did not note any marked tendency as for change in pseudoeosinophil number, the relative number of pseudoeosinophils increased only in group that received Moxifloxacin on Day 3 and Day 9 of the experiment. Short-term pseudoeosinophilia (neutrophilia) may be caused by toxic effects of medicinal drugs.
Monocyte number increased insignificantly on Day 7 due to administration of fluoroquinolones, but it was still physiologically normal. Brief increase in monocyte number could be caused by short-term pseudoeosinophilia as monocytosis is known to arise from the same conditions that lead to neutrophilia.

Table 1. Relative values of the leukogram for chick blood when using Norfloxacin and Moxifloxacin ($N=6$, $M=\pm m$), %.

| days | groups | monocytes | lymphocytes | eosinophils | pseudo-eosinophils | basophils |
|------|--------|-----------|-------------|-------------|-------------------|-----------|
| c    | 2.7±0.71 | 38.8±1.42 | 8.8±0.95    | 47.2±2.77   | 32.2±1.95         |
| 1    | n 1.3±0.21 | 41.3±2.32 | 10.5±1.48   | 44.7±2.12   | 24.9±0.83 b       |
| m    | 3.1±1.06 | 40.5±1.31 | 6.1±0.73    | 46.6±2.27   | 25.7±0.81 b       |
| c    | 1.3±0.21 | 49.8±2.17 | 9.4±1.61    | 36.7±2.17   | 29.8±0.88         |
| 3    | n 2.7±0.42 b | 34.0±1.59 a | 14.1±2.03   | 45.0±2.08 b | 29.5±1.42         |
| m    | 3.8±0.48 a | 36.5±1.26 a | 10.8±1.41   | 45.1±2.59 b | 24.2±0.95 a       |
| c    | 2.8±0.87 | 41.8±1.96 | 9.2±1.11    | 44.2±1.79   | 29.3±0.99         |
| 5    | n 2.2±0.48 | 33.8±1.71 b | 11.5±1.15   | 50.2±2.11 b | 23.8±1.07 a       |
| m    | 1.8±0.41 | 34.3±0.76 b | 12.1±0.68   | 49.3±0.49   | 24.2±0.68 a       |
| c    | 1.2±0.17 | 39.5±2.14 | 6.8±0.48    | 50.5±1.91   | 33.1±0.81         |
| 7    | n 2.7±0.76 | 30.7±2.06 a | 6.8±0.79    | 55.5±2.35   | 30.2±1.07         |
| m    | 1.8±0.31 | 31.8±1.56 b | 8.5±0.67    | 55.2±2.02   | 26.9±1.86 b       |
| c    | 3.0±0.36 | 40.5±2.41 | 7.2±0.71    | 47.0±2.21   | 36.8±1.28         |
| 9    | n 3.3±0.56 | 34.8±3.02 | 8.7±0.81    | 50.5±2.59   | 32.6±0.81 a       |
| m    | 4.5±1.15 | 27.5±2.45 a | 8.0±1.34    | 56.7±1.45 a | 31.8±0.89 a       |

Note: a: $p<0.01$ (Mann–Whitney U-test); b: $p<0.05$ (Mann–Whitney U-test). “c” – control, “n” – Norfloxacin, “m” – Moxifloxacin.

Ofloxacin and Enrofloxacin caused mild eosinophilia from Day 5 after the withdrawal of the drug till the final day of the experiment (Table 2).

Table 2. Relative values of the leukogram for chick blood when using Ofloxacin and Enrofloxacin ($N=6$, $M=\pm m$), %.

| days | groups | monocytes | lymphocytes | eosinophils | pseudo-eosinophils | basophils |
|------|--------|-----------|-------------|-------------|-------------------|-----------|
| c    | 3.8±0.48 | 40.5±3.73 | 10.1±1.15   | 38.3±2.82   | 7.3±1.21          |
| 1    | o 1.6±0.33 a | 41.7±2.36 | 11.2±1.08   | 37.2±2.61   | 8.3±0.71          |
| e    | 2.3±0.42 b | 42.1±2.01 | 9.6±1.56    | 35.8±2.36   | 10.2±0.71         |
| c    | 3.3±0.56 | 40.2±0.92 | 17.1±2.43   | 36.3±2.59   | 3.1±0.45          |
| 3    | o 2.8±0.31 | 44.1±2.05 | 10.7±1.49   | 38.1±1.81   | 4.3±0.43          |
| e    | 3.5±0.56 | 47.2±1.42 a | 11.5±1.38   | 32.1±1.21   | 5.8±0.48 a        |
| c    | 1.5±0.22 | 50.2±1.89 | 7.1±0.68    | 38.2±2.25   | 3.0±0.36          |
| 5    | o 2.3±0.33 | 45.8±2.06 | 9.1±0.45 b  | 36.7±1.94   | 6.1±0.61 a        |
| e    | 2.7±0.42 | 44.8±1.19 | 9.5±0.76 b  | 37.7±1.41   | 5.3±0.49 a        |
| c    | 1.6±0.33 | 48.7±2.04 | 8.5±0.76    | 39.1±2.19   | 2.1±0.26          |
| 7    | o 3.3±0.33 a | 38.8±2.24 | 12.2±0.94 b | 38.6±2.17   | 7.1±0.52 a        |
| e    | 3.3±0.49 b | 43.0±1.93 | 12.0±0.57 b | 36.0±1.15   | 5.7±0.71 a        |
| c    | 2.5±1.22 | 51.2±1.17 | 9.8±0.25    | 34.7±1.28   | 1.8±0.31          |
| 9    | o 3.2±0.48 | 44.2±2.39 | 12.1±0.62 b | 28.4±2.32   | 4.1±0.36 a        |
| e    | 2.7±0.33 | 40.1±2.31 a | 12.3±0.76   | 32.8±3.53   | 4.1±0.52 a        |

Note: a: $p<0.01$ (Mann–Whitney U-test); b: $p<0.05$ (Mann–Whitney U-test). “c” – control, “o” – Ofloxacin, “e” – Enrofloxacin.
Enrofloxacin, Ofloxacin and Levofloxacin had a great effect on basophil number in experimental groups and on Day 1 after the withdrawal of the drugs it was 2.5 times the basophil number of the control group. On Day 3 marked basophilia was observed in every experimental group. It was still present on Day 7. However by Day 9 of the experiment basophil number returned to baseline in groups that received Ciprofloxacin and Norfloxacin. In other experimental groups basophil percentage was higher than that of the control group, but it was physiologically normal.

Basophilia may be accompanied by eosinophilia (as indicated above) and one can consider it a result of developing allergic reaction.

In the conducted experiment we have noted a number of changes in the leukogram of chickens receiving fluoroquinolones. It is worth mentioning that there are few publications on the effect of antimicrobials on leukogram of chickens. There are more publications on the effect of biologically active substances on hematological parameters of chickens. We have already published our studies of significant changes in the number of different types of leukocytes in chickens in experimental staphylococcosis, salmonellosis and colibacillosis, treated with Ciprofloxacin [21-23]. Therefore we can draw a conclusion that leukocytic reactions to fluoroquinolones in healthy chickens and experimentally infected chickens are different.

4. Conclusion
Consequently our investigation into the effect of fluoroquinolones on relative numbers of different types of leukocytes in chickens that we have carried out in our studies enhances the knowledge of physiologic reactions of birds to the action of antimicrobials. The data we have obtained will allow to ascertain the most susceptible to drugs elements of leukocyte system in poultry. Our data add to the available information on leukogram of chickens of very young ages. As a matter of fact studies of blood cells of chickens being treated with antimicrobials are scarce, and age scaling aspects of the effect of such drugs have practically never been studied.

In Hisex Brown chickens lymphocytes and basophils are the most susceptible to antimicrobials of fluoroquinolone family elements of leukocyte system. Enrofloxacin, Norfloxacin and Moxifloxacin caused substantial lymphopenia (lymphocyte number decreased by 22% as compared to the control group). Persistent basophilia occurred in chickens that received Levofloxacin, Enrofloxacin and Ofloxacin (their basophil number was 2.5 times the basophil number of the control group). The basophil count in these experimental groups kept high as long as the experiment continued. Only by Day 9 it returned to physiological norm, though it was still higher than that in the control group. The most significant changes in leukograms were observed on Day 3 and Day 7 after the withdrawal of the drug. Four out of six administered fluoroquinolone drugs exerted a substantial effect on leukogram of chickens, namely Enrofloxacin, Ofloxacin, Levofloxacin and Moxifloxacin. We have discovered that Ciprofloxacin does not cause any substantial changes in leukogram and its effect on leukocytes in chickens lasts the shortest time.

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