(RESEARCH ARTICLE)

Check for updates

Effects of discriminate and indiscriminate use of enrofloxcin on hematological parameters

Md. Mominul Islam Bhuiyan, Md. Shafiqul Islam *, Md. Rakibul Hasan and Kazi Rafiqul Islam

Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Abstract

Hematological indication is an important finding for pathophysiology analysis of biological science. This study was designed with the aim to investigate the effects of residual antibiotics on hematological parameters of broiler following discriminate and indiscriminate use. 18 broiler DOC (Cobb-500) were reared up to 31 days. On day 16, they were randomly divided into 3 groups, namely Group–A (Control group), Group-B (Discriminate group) and Group-C (Indiscriminate group). Each group contains 6 birds. The discriminate and indiscriminate group was treated with antibiotic, enrofloxacin. In Group-B withdrawal period was followed and treatment was stopped before 7 days of sacrifice. On the other hand, withdrawal period was not maintained in indiscriminate group and the antibiotic treatment was continued until the day of sacrifice. The Total erythrocyte count (million/mm3) of control, discriminate, and indiscriminate groups were 3.15±0.047, 2.66±0.091, and 2.90±0.11 respectively. The Hb (gm%) of control, discriminate, and indiscriminate groups were 8.17±0.07, 7.37±0.09, and 7.28±0.07 respectively. The Packed Cell Volume (%) of control, discriminate, and indiscriminate group were 24.17±0.48, 23.17±0.48, and 22.33±0.67 respectively. There was no significant difference on blood parameter found among the groups. Therefore, discriminate and indiscriminate use of enrofloxacin has no bad effect on hematological parameters of broiler.

Keywords: Enrofloxacin; Hematological parameters; PCV; TEC; Hb

1. Introduction

Poultry meat and eggs are extremely important human food commodity. Unfortunately edible poultry tissues may be contaminated with harmful concentration of drug residues due to continuous and improper antibiotic usage in poultry industry [1, 2]. Human consumption of toxic levels of antibiotic residues in food of animal origins (meat, milk or egg) had caused several pathological defects in man that are of public health importance [2, 3, 4, 5]. Therefore, birds treated with antibiotics are required to be held for specific withdrawal period until all residues are depleted to safe levels before their eggs and tissues can be used as food for human consumption [6]. The occurrence of veterinary drug residues in edible animal tissues remains a global problem [7].

Out of the several group of antibiotics, quinolones constitute an expanding group of synthetic antibiotics used to treat various kind of infections not only in veterinary medicine but also in the human beings. Fluoroquinolones belong to the second generation quinolones and their main target of action is the inhibition of the bacterial enzymes DNA gyrase and topoisomerase IV [8]. It has been reported that, because of the presence of quinolone residues in human food obtained from animal sources, effectiveness of quinolones in human treatment has decreased [9].

* Corresponding author: Md. Shafiqul Islam

Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Besides, antibiotic therapy is associated with toxic effect on hematopoiesis process causing a change in blood parameter of poultry [10]. Certain antibiotics show diverse effect on different elements of the blood like thrombocytopenia, anemia, leucopenia etc. [11]. Hematological investigations in monitoring the health status of birds has grown in extent, becoming an indispensable component of the protocols used for testing bioequivalence, safety and tolerance of active substances on the target species [12]. As, Hematological profile an important physiological indicator of the body associated with animal production [13], the present study was undertaken to investigate the effect of enrofloxacin antibiotic on hematological parameter of broiler following discriminate and indiscriminate use of enrofloxacin.

2. Material and methods

2.1. Experimental design

18 apparently healthy day-old "Cobb-500" broiler chicks were purchased from CP Hatchery Ltd, Valuka, Mymensingh. . On the 16th days of age chicks were randomly divided into three groups (Group A, B & C). Each group contains 6 birds. The birds of Group-A, B and C were kept in different cages. Group A was kept as untreated control & received non-medicated water. Groups B & C were administered with enrofloxacin at recommended therapeutic dose @10 mg/Kg, through drinking water. After 7 days, at the age of day 23; antibiotic supply was stopped in the group-B and withdrawal period was maintained. In group-C the antibiotic supply was continued until the day of scarifice. Birds received their freshly prepared daily medication in the morning hour of each day. The concentration of enrofloxacin in the water to give the required dose per kilogram of body weight was calculated by determining the water consumption and body weight of each bird on the day of medication.

2.2. Collection of blood samples

Blood samples from all groups (Control, discriminate & indiscriminate) were collected into sterile heparinized and non-heparinized vials during sacrifice and were immediately stored into refrigerator for further use.

2.3. Hematological Parameters

Total erythrocyte count (TEC), Hemoglobin content (Hb %) and Packed cell volume (PCV) were studied for hematological investigation. For determination of hematological parameter, blood samples were collected at the end of experiment (31th day) from all groups. Immediately after collection of blood, blood was transferred to sterile test tube containing anticoagulant at a ratio of 1: 10.

2.3.1. Determination of Hemoglobin Concentrations (Hb)

With the help of a dropper the N/10 hydrochloric acid (HCL) was taken in a graduated tube up to 2 marks. Then Citrated well-homogenized blood sample was then drawn into the Sahli pipette up to 20 cm mark. The tip of the pipette was wiped with sterile cotton and the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid solution. The pipette was rinsed 2-3 times by sucking fluid into tube. This blood and acid were thoroughly mixed by stirring with a glass stirrer into the diluting tube. There was a formation of acid hematin mixture in the tube by hemolysed blood RBC & HCL. The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. Then distilled water was added drop by drop, solution was mixed well with a glass stirrer until the color of the mixture resembled to the standard color of the comparator. The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus & expessed in gm percent.

2.3.2. Determination of Total Erythrocyte Count (TEC)

The tip of a dry clean red pipette was dipped into the blood sample and blood was sucked upto 0.5 mark opf the pipette. Outside of the tip of the pipette was wiped with cotton. Then the pipette was immediately filled with the red cell diluting fluid (Hayem's solution) up to 101 marks. The rubber tube was stretched at the other end of pipette and both ends were held with thumb and finger. The contents of the pipette were mixed throughlty by shaking with 8-knot motion for 3-5 minutes. The counting chamber was placed with cover glass under microscope using low power (10x) objectives. After discarding 2 or 3 drops of fluid from the pipette, a small drop was placed to the edge of the cover glass on the counting chamber as the entire area under the cover glass was filled by the fluid. One-minute time was spared to allow the cells to settle over the chamber uniformly. The cells were counted from the recognized 80 small squares (16 x 5) under high power objectives (40x). After completion of counting, the total number of RBC was calculated as number of cells counted x 10,000 and the result was expressed in million/µl of blood.

2.3.3. Determination of Packed Cell Volume (PCV)

The citrated well mixed blood sample was drawn into special loading pipette (Wintrobe pipette) and pipette was inserted up to the bottom of a clean, dry Wintrobe hematocrit tube. The rubber bulb of the pipette was pressed continuously to expel the blood out of the pipette. As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of blood to avoid foaming. The tube was exactly filled to the 10 cm mark and then placed in the centrifuge machine and was centrifuged for 30 minutes at 3000 rpm. After 30 minutes the tubes were taken out of centrifuge machine and PCV was read directly from the calibration on the right side of the tube.

The result was expressed in percentage.

PCV% = (Height of the red cell volume in cm)/(Height of total blood in cm)×100.

3. Results

### 3.1. Determination of different Haematological Parameters of broiler

Blood is a very good media to carry the antibiotic to the cells and there must be a certain level of concentration in the blood stream. If antibiotic persists in the blood circulation for a long time, they might alter the normal volume and other parameters.

3.2. Hemoglobin concentration

The highest mean Hb (%) was obtained from Group-A, that was 8.17±0.07. The mean TEC of discriminate and indiscriminate groups were 7.37±0.09 and 7.28±0.07 respectively. The differences among means of three different groups were statistically significant (p<0.05). The multiple pairwise comparisons showed that the difference of means between Group-B and Group-C were not statistically significant. But other two pairs, Group-A & Group-B and Group-A & Group-C showed significant differences among means.

Table 1 Hemoglobin (gm %) of Three Individual Groups.

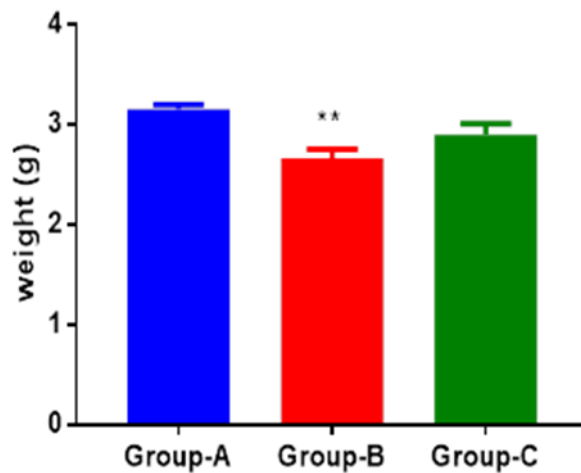| Name of group	Average blood parameters value (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	8.17 ± 0.07		
Group-B(Discriminate group)	7.37± 0.09	<0.001	***
Group-C(Indiscriminate group)	7.28 ± 0.07		
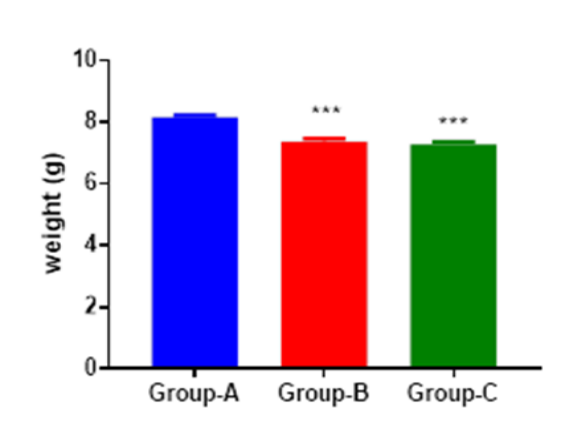
Figure 1 Hb (%) of three individual groups

3.3. Total Erythrocyte Count (Million/mm3) of Three Individual Groups

The highest mean TEC was obtained from Group-A which was 3.15±0.047. The mean TEC of Group-B and Group-C were 2.66±0.091 and 2.90±0.11 respectively. The differences among means of three different groups were statistically significant (P<0.05). The multiple pairwise comparisons showed that the difference of means between Group-A & Group-C, and Group-B & Group-C were not statistically significant. But mean TEC between Group-A and Group-B showed significant differences among means.

Table 2 Total Erythrocyte Count (Million/mm3) of Three Individual Groups.

| Name of group	Average blood parameters value (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	3.15 ± 0.047		
Group-B(Discriminate group)	2.66± 0.091	0.004	**
Group-C(Indiscriminate group)	2.90 ± 0.11		

Figure 2 TEC of three individual groups

3.4. Packed Cell Volume (%) of three Individual Groups

PCV count of Group-A, B & C were 24.17±0.48, 23.17± 0.48 and 22.33 ± 0.67 respectively. Packed cell volume (PCV) among the three groups (Group- A, B and C) did not show any significant difference (P < 0.05).

Table 3 Packed Cell Volume (%) of Three Individual Groups.

Name of group	Average blood parameters value (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	24.17 ± 0.48		
Group-B(Discriminate group)	23.17± 0.48	0.092	ns
Group-C(Indiscriminate group)	22.33 ± 0.67		
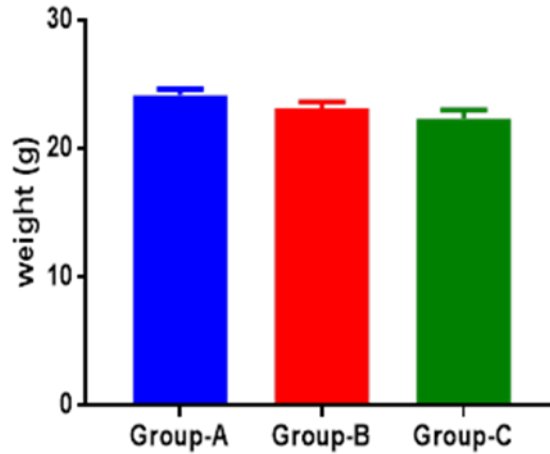
Figure 3 PCV of three individual groups

4. Discussion

Bangladesh is a developing country which largely depends on the use of poultry meat as a dietary animal protein source. The poultry industry has to face various diseases every year. Antibiotics are used in the treatment and prevention of these diseases as well as a growth promoting agent. The presence of antibiotic residues in poultry meat has received colossal worldwide attention from public health agencies. This is due to the importance and significance of antibiotic residues on public health. Many reports indicated that microbial resistance [14]. These residues ultimately pose serious health threats to human beings when meat from such animals is consumed [2]. Furthermore, these residues may also be formed if proper withdrawal times of drugs have not been established. All these situations produce some health effects in humans on consumption of contaminated meat [15].

Analysis of haematological parameters showed quantitative changes in some blood indices of broiler were observed under a prolonged exposure to indiscriminate and therapeutic concentrations of enrofloxacin antibiotic. There was established a marked changes in the hemoglobin level and in the number of total erythrocyte count towards reduction (Table-2) in the case of enrofloxacin antibiotic (P< 0.01) in discriminate antibiotic group (Group-B) & indiscriminate antibiotic group (group-C) compared with the control group (group-A) whereas the packed cell volume did not show any significant changes.

Total Erythrocyte Count (TEC) was highest in control and lowest in indiscriminate group. Total Erythrocyte Count was recorded in three individual group didn't show any significant difference among means. The present finding is similar to previous experiment of Ognean et al. [12] who reported that Total Erythrocyte Count decreased in broilers after erythrocyte treatment (recommended dose and double dose of recommended erythromycin),

The Packed Cell Volume (PCV) was highest in the control group. But indiscriminate and discriminate groups showed lower mean than the control group. The PCV (%) of three groups was not statistically significant and multiple pairwise comparisons during one way ANOVA showed that there was no statistical significance among discriminate, indiscriminate and control groups. The result of the study is the reflection of Al-Mayah and Al-Ahmed [11] who reported that antibiotic treatment during 22-27 days of age numerically decrease Packed Cell Volume in comparison with control group but the differences are not significant.

5. Conclusion

Fluoroquinolones constitute an expanding group of synthetic antibiotics, widely used in the treatment of infections in both human and veterinary medicine. A number of these drugs have been licensed to be administered in broiler chickens for the prophylaxis and treatment of respiratory, renal and digestive infections in different regions of the world. Fluoroquinolones are one of the few classes of antimicrobial agents with activity against the full range of pathogens involved in broiler chickens, such as *Campylobacter jejuni, Salmonella*, *Shigella* or *Escherichia coli*, being commonly used. Currently, the widespread use of these antimicrobials such as enrofloxacin in the poultry industry has become a matter of concern because it has led to the emergence of resistance in *Salmonella serovars, Campylobacter* spp. and *E. coli* .This

situation raises public health concerns regarding reduction in the clinical efficacy of fluoroquinolones in human medicine. In addition, the use of fluoroquinolones in food-producing animals may leave drug residues in foods. These residues represent a risk to public health, including stimulation of bacterial resistance, alterations on intestinal micro flora and hypersensitivity reactions. Extra label use of these drugs or unintentional contamination of feed for poultry (cross-contamination during premix manufacture or during feed transport) may be the source of violative drug residues in meat for human consumption. Therefore, the depletion of these drugs in meat and other edible tissues should be assessed. Considering the above-mentioned issues and the fact that published information regarding enrofloxacin depletion in poultry meat and offal is scarce (Lolo et al., 2005) the present study was designed. Administration of enrofloxacin was started at 16 days of age and continued for 31st days of age. Mean body weight was highest for indiscriminate group. The mean differences among the three groups were statistically significant (<0.05). The TLC was performed for identification of antibiotic residues. The analysis revealed that all the samples were positive in indiscriminate group with an exception of fat tissue (66.7%). There was no positive sample in control group. The TEC of control, discriminate, and indiscriminate group were 3.15 ± 0.047, 2.66± 0.091, and 2.90 ± 0.11 respectively. The hemoglobin % of control, discriminate, and indiscriminate group were 8.17 ± 0.07, 7.37± 0.09, and 7.28 ± 0.07 respectively. And Packed Cell Volume (PCV) of control, discriminate, and indiscriminate group were 24.17 ± 0.48, 23.17± 0.48, and 22.33 ± 0.67 respectively. The differences among means of blood parameters of three individual groups were statistically significant (P<0.05). The results indicate the presence of residues, whereas the usage of this contaminated meat causes resistance in consumers and seems to be a public health threat. Thus, there is a need to educate the farmers about the ill effects of residual drugs on human health and withdrawal time in poultry birds. National authorities should also adopt more judicious approaches to ensure prudent use of antibiotics in food animals.

Compliance with ethical standards

Acknowledgments

Disclosure of conflict of interest

The authors state no conflict of interest.

Statement of ethical approval

The experimental broilers were used ethically and at the end of the experiment sacrificed humanely following the ethical and welfare guidelines set by the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University [approval 5 number: AWEEC/ BAU/2021(09)].

References

[1] Donoghue DJ. Antibiotic residues in poultry tissues and eggs: Human health concerns? Poultry Science. 2003; 82(4): 618-621.

[2] Shareef AM, Jamel ZT, Yonis KM. Detection of antibiotic residues in stored poultry products. Iraq Journal of Veterinary Science. 2009; 23(1): 45-48.

[3] Dipeolu MA, Alonge DO. Residues of Streptomycin antibiotic in meat sold for human consumption in some states of SW Nigeria. Archivos de Zootecnia. 2002; 51: 447-480.

[4] Nisha AR. Antibiotic residues: A global health hazard. Veterinary World. 2008; 1(12): 375-377.

[5] Muhammad G, Anal AK, Athar M, Saleem M, Bajracharya SL. Testing milk and meat for antibiotic residues. Pakistan J. Food Sci. 1997; 7: 35-8.

[6] Olatoye IO, Ehinwomo AA. Oxytetracycline Residues in Edible tissues of cattle slaughtered in Akure, Nigeria. International Journal of Food Safety. 2009; 11: 62-66.

[7] Geidam YA, Usman MHI, Anosike F, Adeyemi Y. Oxytetracycline and Procaine Penicillin residues in tissues of slaughtered cattle in Maiduguri, Bornostate, Nigeria. Terrestrial and Aquatic Environmental Toxicology. 2009; 3(2): 68-70.

[8]   Manceau J, Gicquel M, Laurentie M, Sanders P. Simultaneous determination of enrofloxacin and ciprofloxacin in animal biological fluids by high-performance liquid chromatography Application in pharmacokinetic studies in pig and chicken. J. Chromatography B. 1999; 726(1-2): 175-184.

[9]   Donkor E, Newman MJ, Tay SCK, Dayie NTKD, Bannerman E, Olu-Taiwo M. Investigation into the risk of exposure to antibiotic residues contaminating meat and egg in Ghana. Food Contr. 2011; 22: 869–873.

[10]   Stolker A. Brinkman UT. Analytical strategies for residue analysis of veterinary drugs and growthpromoting agents in food-producing animals-a review. J. Chromatogr. A. 2005; 1067: 15-53.

[11]   Al-Mayah AA, Al-Ahmed JA. Influence of antibiotics treatment on hematological aspect in chicken. Int. J. Poul Sci. 2005; 4: 323-325.

[12]   Ognean L, V Chiurciu, C Cernea, Trîncă, Oroian R. The evaluation of therapeutic doses of erythromycin on the main hematological parameters of broiler chickens. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Vet. Med. 68: 277-283.

[13]   Aboubakr M, Elbadawy M. Efficacy of flagymox® (amoxicillin and metronidazole combination) in controlling clostridium perfringens infection in broiler chickens. World Journal of Pharmacy and Pharmaceutical Sci. 2016; 6: 80-95.

[14]   Yorke JC, Froc P. Quantification of nine quinolones in chicken tissues by HPLC with fluorescence detection. Journal of Chromatography A. 2000; 882: 63-67.

[15]   Kožárová I, Mate DM, Hussein KT. High-performance liquid chromatographic determination of sulfadimidine residues in eggs. Acta Veterinaria. 2004; 54: 5-6.

[16]   Lolo M, Pedreira S, Miranda JM, Vazquez BI, Franco CM, Cepeda A, Afente C. Effect of cooking on enrofloxacin residues in chicken tissue. Food.Addit.Contam. 2006; 23(10): 88-93.