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CLINICAL INVESTIGATION, AUTOPSY AND SOME BIOCHEMICAL INDICES IN BROILER CHICKENS FOLLOWING TREATMENT WITH ENROFLOXACIN ANGOIN STUDY BY COLIBACILLOSIS

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ABSTRACT

Escherichia coli commonly abbreviated E.coli is a Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms Most E.coli strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The aim of present study was to clinical investigation, autopsy and some biochemical indices in broiler chickens following treatment with enrofloxacin angoin study by colibacillosis. In this study, a broiler farm with 2 salons with 10000 birds in each was selected and in one salon enrofloxacin at a dose of 0.5 liter per 1000 liter drinking water for 3-5 days by continuously method and in the other one the same protocol was used by interrupted method. 20 blood samples before and 20 samples after drug administration was taken for biochemical factors. Data showed that enrofloxacin causes significantly increase in ALP in enro 8 h and decrease in enro 24h in compared with control group. Also, enrofloxacin yields to decrease in serum proteins non-significantly. Also, it does not change AST, ALT and creatinine in compared control group. Also, it must be noted that there was no significant difference among groups of 8 and 24 hours. MIC data showed significant inhibitory effects of enrofloxacin in both groups.

Keywords: Broiler Chickens, Enrofloxacin, Colibacillosis, MIC, Hematological and Biochemical Parameters

INTRODUCTION

Fluoroquinolones stand for major class of antimicrobial agents advocated in food animals and poultry for the treatment of a wide range of infectious diseases. Enrofloxacin, a synthetic fluoroquinolone is used large scale for the treatment of chronic respiratory diseases, Colibacillosis, Salmonellosis and Fowl cholera in poultry **(Anderson *et al*., 2003, Martinez *et al*., 2006)**. **Gurbay *et al*., (2001)** postulated that during metabolic transformation of enrofloxacin into pharmacologically active metabolite ciprofloxacin in liver **(Prescott *et al*., 2000)**, free radical intermediates are generated and resulted in lipid peroxidation. Further, **Martinez- Cayuela (1998)** opined that enrofloxacin residues may occur in meat, milk and eggs, generate free radicals owing to its metabolism and interact with other medicated drugs **(Ershov *et al*., 2001)**. Fluoroquinolones are considered relatively well tolerated than the other commonly used antimicrobial agents. However, it has been reported that they are also associated with a low incidence of adverse effects related to gastrointestinal, skin, hepatic, and central nervous system functions, and phototoxicity **(Hooper, *et al*., 1985)**. Further, enrofloxacin administration in broiler chicken resulted in significant fall in lymphocyte count **(Sureshkumar *et al*., 2012)** and reduction in haemagglutination inhibition (HI) titre and associated histopathological changes in lymphoid organs **(Sureshkumar *et al*., 2013)**. Literature evidences indicated that these adverse effects might be attributed to free radical formation **(Hayem *et al*., 1994)** and very little reports have elucidated the mechanism of toxicity of fluoroquinolones **(Gurbay *et al*., 2001)**.

Escherichia coli is a gram-negative, rod-shaped bacterium normally found in the intestine of poultry and most other animals. Although most serotypes are nonpathogenic, a limited number produce extraintestinal infections. Avian pathogenic *E.coli* (APEC) strains are commonly of the O1, O2, and O78 serogroups, but many others have also been associated with cellulitis and colibacillosis. There is considerable diversity of serogroups among clinical isolates, with a high percentage of APEC isolates being untypeable. Therefore, no single *E. coli* serogroup used as a bacterin can provide full protection against all of the serogroups that cause infections. Virulence factors include possession of large virulence plasmids and the abilities to resist phagocytosis and serum killing, acquire iron in low iron conditions, and adhere to host structures. APEC are

generally nontoxigenic and poorly invasive **(Ishii and Sadowsky, 2008)**.

Large numbers of E.coli are maintained in the poultry house environment through fecal contamination. Initial exposure to APEC may occur in the hatchery from infected or contaminated eggs. Although most E.coli isolated from colibacillosis are well equipped with virulence factors that distinguish them from fecal commensal strains, systemic infection often involves predisposing environmental factors or infectious causes. Thus, mycoplasmosis, infectious bronchitis, Newcastle disease, hemorrhagic enteritis, and turkey bordetellosis, or exposure to poor air quality and other environmental stresses, may precede colibacillosis. Systemic infection occurs when large numbers of APEC gain access to the bloodstream from the respiratory tract or intestine. Bacteremia progresses to septicemia and death, or the infection extends to serosal surfaces, pericardium, joints, and other organs **(Eckburg *et al*., 2005)**.

Signs are nonspecific and vary with age, organs involved, and concurrent disease. Young birds dying of acute septicemia have few lesions except for an enlarged, hyperemic liver and spleen with increased fluid in body cavities. Birds that survive septicemia develop subacute fibrinopurul entairsacculitis, pericarditis, perihepatitis, and lymphocytic depletion of the bursa and thymus (unusually pathogenic salmonellae produce similar lesions in chicks). Although airsacculitis is a classic lesion of colibacillosis, it is unclear whether it results from primary respiratory exposure or from extension of serositis. Sporadic lesions include pneumonia, arthritis, osteomyelitis, peritonitis, and salpingitis.

Unlike pathogenic *E.coli* associated with illnesses in other animal species, avian isolates are generally nonhemolytic on sheep (5%) blood agar. Isolation of a pure culture of E coli from heart blood, liver, or typical visceral lesions in a fresh carcass indicates primary or secondary colibacillosis. Consideration should be given to predisposing infections and environmental factors. Pathogenicity of isolates is established using multiplex PCR panels for plasmid-mediated virulence genes or when parenteral inoculation of young chicks or poults results in fatal septicemia or typical lesions within 3 days. Pathogenicity can also be detected by inoculation of the allantoic sac of 12-day-old chicken embryos. Resulting gross lesions include cranial and skin hemorrhages in addition to encephalomalacia in embryos inoculated with virulent isolates **(Blattner *et al*., 1997)**.

Treatment strategies include attempts to control predisposing infections or

environmental factors and early use of antibacterials indicated by susceptibility tests. Most isolates are resistant to tetracyclines, streptomycin, and sulfa drugs, although therapeutic success can sometimes be achieved with tetracycline. In fact, 90% of clinical isolates are resistant to tetracycline, with 60% of isolates resistant to five or more antibiotics. Fluoroquinolone use is now banned in many countries, including the USA. Commercial bacterins administered to breeder hens or chicks have provided some protection against homologous E.coli serogroups **(Blattner *et al*., 1997; Eckburg *et al*., 2005)**.

Sulfonamides are produced by chemical synthesis. They have bacteriostatic activity against a broad spectrum of pathogens. They interfere with RNA and DNA, which are necessary for cell growth and replication. Sulfonamides, such as trimethoprim, are effective against Staphylococcus species, Streptococcus species, Pasteurella, Salmonella, and *E coli* **(Löhren *et al*., 2009; Sirdar *et al*., 2012)**.

The aim of present study was to clinical investigation, autopsy and some biochemical indices in broiler chickens following treatment with enrofloxacin angoin study by colibacillosis.

MATERIALS AND METHODS

In present study, a broiler farm with 2 similar salon with 10000 chick in each with colibacillosis were selected in which vaccination program, nourishment conditions and quality of day old chickens were the same. Animals were fed based on their physiological and culturing demands and were fed with different formulated feed. In farm with colibacillosis, 20 blood samples before and 20 blood samples after administration of drugs were obtained and some biochemical and hematological factors such as total protein, ALP, Uric acid, Albumin, glucose, RBS, heterophils and hematocrit were measured. In one salon enrofloxacin administrated at a dose of 0.5 liter per 1000 liter drinking water for 3-5 days by continuously method and in the other one the same protocol was used by interrupted method. MIC and MBC methods were used in concomitant with disc diffuse method to evaluate the resistance of agents to enrofloxacin. Data were analyzed using SPSS ver. 18. ANOVA was used to compare groups and Tukey's Post Hoc Test and t-test were used to show accurate difference among groups. $P<0.05$ considered as significant difference.

RESULTS

Comparison of enro 8h with control group

Data showed that administration of enrofloxacin in broiler chickens with colibacillosis increases the serum values of ALP non-significantly ($p>0.05$). Also, it decreases the level of serum protein non-significantly ($p>0.05$). As well as, enrofloxacin caused no changes in serum values of AST, ALT and creatinine in compared control group ($p>0.05$).

Comparison of enro 24h with control group

Data showed that administration of enrofloxacin in broiler chickens with colibacillosis decreases the serum values of ALP significantly ($p<0.05$). Also, it decreases the level of serum protein non-significantly ($p>0.05$). As well as, enrofloxacin caused no changes in serum values of AST, ALT and creatinine in compared control group ($p>0.05$).

Comparison of enro 8h and 24h

Our results showed that there is no significant changes in measured parameters in enro 8h and 24h ($p>0.05$) **(Table 1, 2)**.

Results of hematological parameters

It showed that administration of enrofloxacin make no changes in percentage of Eosinophil, Monocytes, Lymphocytes, HCT and WBC but decreased the percentage of Heterophil significantly ($p<0.05$).

Results of MIC

Results obtained from MIC showed that enrofloxacin inhibits bacterial growth in different dilutions and times (8h and 24h) in which it was observed significant differences in compared control group ($p<0.05$).

Table 1: Comparison data obtained from biochemical factors

Sample No.	ALP (U/L)	ALT (U/L)	AST (U/L)	Protein (g/dl)	Creatinine (mg/dl)
1 enro 8h before	52	57	170	6.5	0.47
2 enro 8h after	35	57	200	5.8	0.56
3 enro 8h after	59	60	160	4.9	0.51
4 enro 8h after	65	55	180	4.4	0.46
5 enro 8h after	75	55	175	3.9	0.51
6 enro 8h after	56	56	170	4.8	0.48
7 enro 8h after	58	57	184	4.3	0.52
8 enro 8h after	60	55	180	4.8	0.53
9 enro 8h after	59	56	178	4.9	0.51
10 enro 24h before	69	56	160	4.9	0.32
11 enro 24h after	52	60	170	4.2	0.46
12 enro 24h after	59	73	180	4.6	0.42
13 enro 24h after	53	70	190	4.2	0.32
14 enro 24h after	53	55	170	4.4	0.45
15 enro 24h after	55	65	175	4.3	0.39
16 enro 24h after	52	63	180	4.1	0.40
17 enro 24h after	58	63	175	4.4	0.42
18 enro 24h after	57	66	180	4.4	0.41
19 enro 24h after	55	65	177	4.3	0.42
20 enro 24h after	59	67	176	4.4	0.47

Table 2: comparison data obtained from hematological factors

Group	HCT%	Eosinophil%	Monocytes %	Lymphocytes %	Heterophil %	WBC/mm3
enro 8h	38	3	4	79	14	20000
enro 24h	33	3	3	80	14	16000
Control	37	4	3	71	22	21000

DISCUSSION AND CONCLUSION

In vivo, enrofloxacin is de-ethylated into its primary metabolite ciprofloxacin by liver microsomal enzymes of the cytochrome P450 family **(Stratton, 1998)**. Further, ciprofloxacin is metabolized by alteration to the piperazine side chain **(Sorgel, 1989)**. Several authors have speculated that consequent to the metabolism of enrofloxacin and ciprofloxacin, free radicals are generated, and resulted in lipid peroxidation **(Martinez-Cayuela, 1998; Gurbay *et al*., 2001)**. These hypotheses are very well portrayed in the present findings as evidenced by significant decrease in GST, GSH and CAT level in liver, muscle and serum of broiler chicken administered with enrofloxacin. **Vaccaro *et al*. (2003)** reported that oxidation of ciprofloxacin by CYP450 leads to formation of reactive intermediates. As a sequel, a series of subsequent deleterious reactions occurred **(Nie *et al*., 2008)**.

The significant fall in antioxidants enzymes after enrofloxacin medication followed by its restoration during the withdrawal period could be attributed to the depletion of the enrofloxacin residues from liver and muscle during the withdrawal period. This proposition is substantiated by **Chattha *et al*. (2008)**, who observed that enrofloxacin residues were washed out in 9 days whilst its major metabolite ciprofloxacin was washed out in 8 days in chicken meat. Further, **Petrovic *et al*. (2006)** showed that a 4 days of withdrawal period after enrofloxacin administration resulted in decrease in enrofloxacin residues to a tolerable level in the broiler meat and liver. While, San **Martin *et al*. (2010)** demonstrated that based on the European Union maximum residue limits (EU MRLs) of 100 µg/Kg (muscle) and 200 µg/Kg (liver), the withdrawal time was 5 days, and when Japan MRL was considered (10 µgKg,), the withdrawal time was found to be 8 days in broiler chicken. From these reports it is evident that enrofloxacin residues were high during the earlier days of the withdrawal period, whereas on day 9 post treatment it was below 10 µgKg. Same trend was reflected in the antioxidant status of the liver, muscle and serum as evidenced by corresponding restoration in the antioxidants level on 9th day of the withdrawal period.

The result of this study also accorded to the findings of **Chansiripornchai (2007)** that Chickens received antibiotics 1 hr before challenges, immediately after challenges or 1 hr after challenges. The current experiment revealed the efficacy of enrofloxacin to treat colibacillosis and fowl cholera, the results may cause by the merit of rapid tissue penetration of enrofloxacin. A time to maximum (Tmax) of enrofloxacin (Baytril®) in serum, lung and muscle is 0.5, 2 and 2 hrs, respectively. Also, a maximum tissue concentration (Cmax) of enrofloxacin (Baytril®) in serum, lung and muscle is 0.77, 1.76 and 1.75 µg/ml or µg/g, respectively **(Franz, 2006)**. The Tmax of enrofloxacin is faster than the incubation period of colibacillosis around 24 hrs **(Chansiripornchai, 2007; 2009)**.

In conclusion, the pathological lesions and mortality of the *E. coli* infected birds which 8hr treated with enrofloxacin after infection had not significant lower than those of the infected birds that treated with enrofloxacin 24 hrs after infection.

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