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CLINICAL INVESTIGATION AND SOME BIOCHEMICAL INDICES IN BROILER CHICKENS WITH COLIBACILLOSIS FOLLOWING TREATMENT WITH FLORFENICOL

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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 and some biochemical indices in broiler chickens with colibacillosis following treatment with florfenicol. In this study, a broiler farm with 2 salons with 10000 birds in each was selected and in one salon florfenicolat 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 administration of florfenicol in broiler chickens with colibacillosis increases the serum values of ALP significantly (p=0.042). As well as, florfenicol caused no changes in serum values of AST, ALT, protein and creatinine in compared control group (p>0.05).Data showed that administration of florfenicol in broiler chickens with colibacillosis decreases the serum values of ALP significantly (p=0.032). Also, it decreases the level of serum protein non-significantly (p=0.068). As well as, florfenicol increases serum values of AST, ALT (p=0.040) and creatinine non-

significantly (p=0.073) in compared control group.Our results showed that there is no significant changes in measured parameters in florfenicol 8h and 24h (p>0.05).It showed that administration of florfenicol make no changes in percentage of Eosinophil, Monocytes, Lymphocytes, HCT and WBC in both treatment groups (8h and 24 hours) but decreased the percentage of Heterophil significantly in flor24h compared with flor8h (p<0.05).Results obtained from MIC showed that florfenicol 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).

Keywords: Broiler Chickens, Florfenicol, Colibacillosis, Mic, Hematological and Biochemical Parameters

INTRODUCTION

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 extra intestinal infections. Avian pathogenic *E.coli* (APEC) strains are commonly of the O1, O2, and O78 sero groups, 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 untype able. 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 fibrinopurulentairsacculitis, 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)**.

Florfenicol is a synthetic, fluorinated analogue of chloramphenicol which lacks chloramphenicol's associated human health risk. It has been used in Asia for aquaculture since the 1980's **(Fukui *et al*., 1987)**. In early 1996, an injectable formulation of florfenicol was approved for the treatment of bovine

respiratory disease in the United States. It has not yet been approved for poultry, and, in fact, an animal feed formulation is not available. Florfenicol is bacteriostatic, and its mechanism of action is similar to that of chloramphenicol **(Cannon, 1990)**. The mechanism of resistance to florfenicol is unknown but is associated with theflo determinant, a highly conserved gene sequence detected in Salmonella entericaserovar Typhimurium DT104 **(Bolton *et al*., 1999; Briggs and Fratamico, 1999)** and in the fish pathogen Pasteurellapiscicida (Photobacteriumdamsela) **(Kim *et al*., 1993)**. The flogene confers resistance to both chloramphenicol and florfenicol **(Kim *et al*., 1993)**. The aim of present study was to clinical investigation and some biochemical indices in broiler chickens with colibacillosis following treatment with florfenicol.

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, AST, ALT, Creatinine, RBS, heterophils and hematocrit were measured. In one salon florfenicol 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 florfenicol. 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 florfenicol 8h with control group

Data showed that administration of florfenicol in broiler chickens with colibacillosis increases the serum values of ALP significantly ($p=0.042$). As well as, florfenicol caused no changes in serum values of AST, ALT, protein and creatinine in compared control group ($p>0.05$).

Comparison of florfenicol 24h with control group

Data showed that administration of florfenicol in broiler chickens with colibacillosis decreases the serum values of ALP significantly (p=0.032). Also, it decreases the level of serum protein non-significantly (p=0.068). As well as, florfenicol increases serum values of AST, ALT (p=0.040) and creatinine non-significantly (p=0.073) in compared control group.

Comparison of florfenicol8h and 24h

Our results showed that there is no significant changes in measured parameters in florfenicol 8h and 24h (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 flor 8h before	55	46	130	3	0.25
2 flor8h after	85	39	160	2.1	0.23
3 flor8h after	95	39	150	3.5	0.42
4 flor8h after	65	45	130	2.5	0.42
5 flor8h after	85	39	132	4.1	0.52
6 flor8h after	82	42	143	2.8	0.38
7 flor8h after	85	39	140	3.3	0.40
8 flor8h after	79	41	146	2.7	0.38
9 flor8h after	82	38	141	3.2	0.40
10 flor 8h after	82	40	145	3	0.39
11flor24h before	69	56	160	4.9	0.32
12flor24h after	52	60	170	4.2	0.46
13flor24h after	59	73	180	4.6	0.42
14flor24h after	53	70	190	4.2	0.32
15flor24h after	53	55	170	4.4	0.45
16flor24h after	55	65	175	4.3	0.39
17flor24h after	52	63	180	4.1	0.40
18flor24h after	58	63	175	4.4	0.42
19flor24h after	57	66	180	4.4	0.41
20flor24h after	55	65	177	4.3	0.42
21flor24h after	59	67	176	4.4	0.47

Table 2: comparison data obtained from hematological factors

Group	HCT%	Eosinophil%	Monocytes %	Lymphocytes %	Heterophil %	WBC/mm3
florfenicol 8h	**33**	**3**	**5**	**60**	**32**	**17000**
florfenicol 24h	**33**	**3**	**3**	**80**	**14**	**16000**
Control	**37**	**4**	**3**	**71**	**22**	**21000**

Results of hematological parameters

It showed that administration of florfenicol make no changes in percentage of Eosinophil, Monocytes, Lymphocytes, HCT and WBC in both treatment groups (8h and 24 hours) but decreased the percentage of Heterophil significantly in flor24h compared with flor8h (p<0.05).

Results of MIC

Results obtained from MIC showed that florfenicol 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).

DISCUSSION AND CONCLUSION

Florfenicol is a broad-spectrum bacteriostatic antibacterial that belongs to amphenicol family, with a wide range of activity against

different types of Gram-negative and Gram-positive organisms including: *Mannheimiahaemolytica, Pasteurellamultocida, Haemophilus somnus, Enterobacter cloacae, Escherichia coli, Klebsiellapneumoniae, Salmonella typhi, Shigella dysenteriae and Staphylococcus aureus* **(Graham *et al.*, 1988)**. In addition, florfenicol is active at lower concentrations than its structural analogs, thiamphenicol and chloramphenicol, against a number of bacterial pathogens and against many chloramphenicol or thiamphenicol-resistant strains **(Lobell *et al.*, 1994)**. Florfenicol is approved in the European Union for use in cattle, sheep, pigs and chickens.

The efficacy of florfenicol has been demonstrated against many diseases of domestic animals **(Nordmo *et al.*, 1994)**. However, to date, studies on the efficacy of florfenicol using pharmacokinetic/ pharmacodynamic (PK/PD) approaches have not been carried out in poultry. Nevertheless, the pharmacokinetics and bioavailability of florfenicol have been investigated in broiler chickens **(Shen *et al.*, 2002)**, turkeys **(Switała *et al.*, 2007)** and ducks **(El-Banna, 1998)**. Most of these studies used the same original preparation of florfenicol. There is therefore little information available regarding the differences between formulations of florfenicol used in poultry.

Florfenicol has been approved and become a valuable antibacterial in the treatment of serious bacterial infections in farm animals **(Shen *et al.*, 2003)**. In poultry, florfenicol is used extensively for the treatment of respiratory and gastrointestinal bacterial infections, administered via drinking water **(Switała *et al.*, 2007)**. It has been reported that florfenicol showed greater activity than chloramphenicol and thiamphenicol, especially against *Pasteurella, Salmonella, E. coli and Staphylococcus aureus* **(Soback *et al.*, 1995)**. Moreover, Florfenicol has superior pharmacological and pharmacokinetics features over some other antimicrobials used in chicken industry **(El-Banna, 1998)**. This drug is characterized by high bioavailability (F>80%), good tissue penetration and rapid elimination, which are important for the systemic treatment of domestic animals **(Switała *et al.*, 2007)**.

Several commercial local and international pharmaceutical preparations of florfenicol oral solution are currently available. In this respect, generic pharmaceutical preparations of florfenicol seeking approval to enter the market should demonstrate their ability to achieve Cmax and AUC values that are equivalent to that of the original preparation.

Inability to maintain high enough concentrations for sufficient periods of time may lead to therapeutic failure and may encourage the proliferation of resistant micro-organisms **(Toutain and Koritz, 1997)**. In conclusion it can be mentioned that florfenicol is one of the most important and chose drug in treatment of colibacillosis in poultry.

REFERENCES

[1] Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y (September 1997). "The complete genome sequence of Escherichia coli K-12". Science 277 (5331): 1453–62.

[2] Bolton, L. F., L. C. Kelley, M. D. Lee, P. J. Fedorka-Cray, and J. J. Maurer. 1999. Detection of multidrug-resistant Salmonella enterica serotype typhimurium DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J. Clin. Microbiol. 37:1348–1351.

[3] Briggs, C. E., and P. M. Fratamico. 1999. Molecular characterization of an antibiotic resistance gene cluster of Salmonella typhimurium DT104. Antimicrob.Agents Chemother. 43:846–849.

[4] Cannon, M. 1990. A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some fluorinated derivatives. J. Antimicrob. Chemother. 26:307–317.

[5] Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (June 2005). "Diversity of the human intestinal microbial flora". Science 308 (5728): 1635–8.

[6] El-Banna HA (1998) Pharmacokinetics of florfenicol in normal and Pasteurellainfected Muscovy ducks. Br PoultSci 39: 492–496.

[7] fish pathogens. Fish Pathol. 22:201–207.

[8] Fukui, H., Y. Fujihara, and T. Kano. 1987. In vitro and in vivo antibacterial activities of florfenicol, a new fluorinated analog of thiamphenicol, against

[9] Graham R, Palmer D, Pratt BC, Hart CA (1988) In vitro activity of florphenicol. European Eur J ClinMicrobiol Infect Dis 7: 691-694.

[10] Ishii S, Sadowsky MJ (2008). "Escherichia coli in the Environment:

Implications for Water Quality and Human Health". Microbes Environ. 23 (2): 101–8.

[11] Kim, E. H., T. Yoshida, and T. Aoki. 1993. Detection of R-plasmid-encoded with resistance to florfenicol in Pasteurellapiscicida. Fish Pathol. 28:165–170.

[12] Lobell RD, Varma KJ, Johnson JC, Sams RA, Gerken DF, et al. (1994) Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle. J Vet PharmacolTher 17: 253–258.

[13] Nordmo R., Varma KJ, Sutherland IH, Brokken ES (1994) Florfenicol in Atlantic salmon, Salmosalar L: field evaluation of efficacy furunculosis in Norway. J Fish Dis 17: 239–244.

[14] Shen J, Hu D, Wu X, Coats JR (2003) Bioavailability and pharmacokinetics of florfenicol in broiler chickens. J Vet PharmacolTher 26: 337-341.

[15] Shen J, Wu X, Hu D, Jiang H (2002) Pharmacokinetics of florfenicol in healthy and Escherichia coli-infected broiler chickens. Res Vet Sci 73: 137-40.

[16] Soback S, Paape MJ, Filep R, Varma KJ (1995) Florfenicol pharmacokinetics in lactating cows after intravenous, intramuscular and intramammary administration. J Vet PharmacolTher 18: 413–417.

[17] Switała M, Hrynyk R, Smutkiewicz A, Jaworski K, Pawlowski P, et al. (2007) Pharmacokinetics of florfenicol, thiamphenicol, and chloramphenicol in turkeys J Vet PharmacolTher 30: 145-150.

[18] Toutain PL, Koritz GD (1997) Veterinary drug bioequivalence determination. J Vet PharmacolTher 20: 79-90.