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 fibrinous exudative airsacculitis, 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 the flo determinant, a highly conserved gene sequence detected in Salmonella enterica serovar Typhimurium DT104 (Bolton et al., 1999; Briggs and Fratamico, 1999) and in the fish pathogen Pasteurella piscicida (Photobacterium damselae) (Kim et al., 1993). The flo gene confers resistance to both chloramphenicol and florfenicol (Kim et al., 1993). The aim of present study was to conduct a 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 salons with 10,000 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 florfenicol 8h 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

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>ALP (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>Protein (g/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 flor 8h before</td>
<td>55</td>
<td>46</td>
<td>130</td>
<td>3</td>
<td>0.25</td>
</tr>
<tr>
<td>2 flor 8h after</td>
<td>85</td>
<td>39</td>
<td>160</td>
<td>2.1</td>
<td>0.23</td>
</tr>
<tr>
<td>3 flor 8h after</td>
<td>95</td>
<td>39</td>
<td>150</td>
<td>3.5</td>
<td>0.42</td>
</tr>
<tr>
<td>4 flor 8h after</td>
<td>65</td>
<td>45</td>
<td>130</td>
<td>2.5</td>
<td>0.42</td>
</tr>
<tr>
<td>5 flor 8h after</td>
<td>85</td>
<td>39</td>
<td>132</td>
<td>4.1</td>
<td>0.52</td>
</tr>
<tr>
<td>6 flor 8h after</td>
<td>82</td>
<td>42</td>
<td>143</td>
<td>2.8</td>
<td>0.38</td>
</tr>
<tr>
<td>7 flor 8h after</td>
<td>85</td>
<td>39</td>
<td>140</td>
<td>3.3</td>
<td>0.40</td>
</tr>
<tr>
<td>8 flor 8h after</td>
<td>79</td>
<td>41</td>
<td>146</td>
<td>2.7</td>
<td>0.38</td>
</tr>
<tr>
<td>9 flor 8h after</td>
<td>82</td>
<td>38</td>
<td>141</td>
<td>3.2</td>
<td>0.40</td>
</tr>
<tr>
<td>10 flor 8h after</td>
<td>82</td>
<td>40</td>
<td>145</td>
<td>3</td>
<td>0.39</td>
</tr>
<tr>
<td>11 flor 24h before</td>
<td>69</td>
<td>56</td>
<td>160</td>
<td>4.9</td>
<td>0.32</td>
</tr>
<tr>
<td>12 flor 24h after</td>
<td>52</td>
<td>60</td>
<td>170</td>
<td>4.2</td>
<td>0.46</td>
</tr>
<tr>
<td>13 flor 24h after</td>
<td>59</td>
<td>73</td>
<td>180</td>
<td>4.6</td>
<td>0.42</td>
</tr>
<tr>
<td>14 flor 24h after</td>
<td>53</td>
<td>70</td>
<td>190</td>
<td>4.2</td>
<td>0.32</td>
</tr>
<tr>
<td>15 flor 24h after</td>
<td>53</td>
<td>55</td>
<td>170</td>
<td>4.4</td>
<td>0.45</td>
</tr>
<tr>
<td>16 flor 24h after</td>
<td>55</td>
<td>65</td>
<td>175</td>
<td>4.3</td>
<td>0.39</td>
</tr>
<tr>
<td>17 flor 24h after</td>
<td>52</td>
<td>63</td>
<td>180</td>
<td>4.1</td>
<td>0.40</td>
</tr>
<tr>
<td>18 flor 24h after</td>
<td>58</td>
<td>63</td>
<td>175</td>
<td>4.4</td>
<td>0.42</td>
</tr>
<tr>
<td>19 flor 24h after</td>
<td>57</td>
<td>66</td>
<td>180</td>
<td>4.4</td>
<td>0.41</td>
</tr>
<tr>
<td>20 flor 24h after</td>
<td>55</td>
<td>65</td>
<td>177</td>
<td>4.3</td>
<td>0.42</td>
</tr>
<tr>
<td>21 flor 24h after</td>
<td>59</td>
<td>67</td>
<td>176</td>
<td>4.4</td>
<td>0.47</td>
</tr>
</tbody>
</table>

### Table 2: comparison data obtained from hematological factors

<table>
<thead>
<tr>
<th>Group</th>
<th>HCT%</th>
<th>Eosinophil%</th>
<th>Monocytes%</th>
<th>Lymphocytes%</th>
<th>Heterophil%</th>
<th>WBC/mm3</th>
</tr>
</thead>
<tbody>
<tr>
<td>florfenicol 8h</td>
<td>33</td>
<td>3</td>
<td>5</td>
<td>60</td>
<td>32</td>
<td>17000</td>
</tr>
<tr>
<td>florfenicol 24h</td>
<td>33</td>
<td>3</td>
<td>3</td>
<td>80</td>
<td>14</td>
<td>16000</td>
</tr>
<tr>
<td>Control</td>
<td>37</td>
<td>4</td>
<td>3</td>
<td>71</td>
<td>22</td>
<td>21000</td>
</tr>
</tbody>
</table>

### 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...
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different types of Gram-negative and Gram-positive organisms including: *Mannheimia haemolytica*, *Pasteurella multocida*, *Haemophilus somnus*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *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 (Switalsa 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 (Switalsa 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 (Switalsa 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 microorganisms (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


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