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STUDIES ON THE IMMUNOPATHOLOGY AND HAEMA TOLOGY OF BROILERS EXPERIMENTALLY INFECTED WITH *ESCHERICHIA COLI*

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Abstract: This project was carried out to study immunopathological and haematological effects of experimental *E. coli* infection in broiler chicks. For this purpose one hundred (day-old) broiler chicks were randomly divided in two equal groups A and B on day-21 of the experiment. At the same day chicks of group A were inoculated with confirmed pathogenic isolate ($3x10^7$ bacilli/0.1 ml) of *E. coli* intraperitoneally while chicks of group B received sterile nutrient broth and acted as control. Result showed that haemoglobin (Hb), packed cell volume (PCV), Total erythrocyte count (TEC), antibody titre against Newcastle disease virus (NDV) and feed conversion ratio (FCR) were significantly lower while total leukocyte count (TLC) was significantly higher in treatment than control group on all the three sampling days. Moderate to marked gross and microscopic pathological changes in heart, liver and spleen were also observed in treatment group as compared to control one. Mean lymphoid organs weight/body weight ratio and post-infection mortality were higher in treatment group as compared to control one. From the foregoing study it was concluded that infection with *E. coli* resulted not only in haematological and pathological alterations but it also impaired the immune system along with high mortality in infected birds.

Key words: *Escherichia coli*, histopathology, immunopathology, haematology, broilers.

INTRODUCTION

Escherichia coli (*E. coli*) has been associated with many disease conditions of poultry. Various *E. coli* infections include colibacillosis, colisepticaemia, Hjarre's disease, coligranuloma, peritonitis, salpingitis, omphalitis and air sac disease (Gross, 1991). *Escherichia coli* infection and its association with various viral and bacterial diseases in poultry is not uncommon. Moreover, the poultry industry is constrained by high incidence of disease, high morbidity and mortality, poor quality of day-old chicks and high cost of health care (Anonymous, 1996). Colibacillosis

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is characterized by pansystemic involvement and great economic losses. It is responsible for high mortality during rearing, reduced weight gain and poor feed conversion (Anjum, 1997).

Collectively, infections caused by *E. coli* are responsible for significant economic losses to the poultry industry (Barnes *et al.* 2003). The multiplicity of disease occurring in broilers in which *E. coli* infection has been implicated represents one of the largest problems in poultry industry. Much work appears to have been done on various aspects *E. coli* infection including epidemiology, isolation and characterization, serology, drug resistance and chemotherapy. The present project was planned to study immunopathological and haematological effects of experimental infection with *E. coli* in broilers.

MATERIALS AND METHODS

Preparation of inoculum

E. coli was isolated from the birds suspected for colibacillosis. Identification and confirmation of isolated organisms was done on the basis of cultural, morphological and staining characteristics, sugar fennentation and biochemical reactions as described by Khan (2002). Pathogenicity of *E. coli* isolates was determined (Lee and Arp, 1998) and total viable count was made (Collins *et al.*, 1995).

Experimental design

A total of one hundred (day-old) broiler chicks were procured from local commercial hatchery. The birds were kept under standard managemental conditions for first 21 days of the experiment. On day-21 the chicks were randomly divided into two groups A and B having fifty chicks each. All the birds were vaccinated against Newcastle disease virus (NDV).

Group A

With the help of insulin syringe broth culture (0.5 ml/chick) of pathogenic *E. coli* ($3XI0^7$ bacilli/0.1 ml) was inoculated intraperitoneally in birds of group A on day-2l.

Group B

Chicks in this group acted as control. Sterile nutrient broth (0.5ml/chcik) was injected intraperitoneally on day-2l.

Collection of sample

The blood samples were taken from five randomly selected chicks of each group at 48, 72 and 96 hours post-infection. The blood was taken in two clean test tubes, one containing anticoagulant Ethylene diamine tetra acetate (EDTA) for hematological studies and other without anticoagulant, to separate serum. Serum samples were kept at -20°C till used for HA and HI tests. At the end of I experiment five randomly selected birds were

slaughtered and liver, heart and spleen were collected for pathological studies while bursa of Fabricius, spleen and thymus were taken and weighed.

Experimental parameters

The following experimental parameters were studied:

1. **Blood analysis**
   a) Haemoglobin determination: Haemoglobin was determined in blood by Sahli's method (Khan and Aslam, 2001).
   b) Packed cell volume (PCV): PCV percentage was estimated by using microhaematocrit method (Khan and Aslam, 2001).
   c) Erythrocyte and leukocyte count: Erythrocyte and leukocyte counts were determined by the methods described by (Khan and Aslam, 2001).
2. **Determination of antibody titre against NDV**
   Antibody titre against NDV in serum was determined by haemagglutination inhibition (HI) test as described by Thayer and Beard (1998).
3. **Pathological studies of different organs**
   a) Gross pathological examination: Liver, heart and spleen were collected at the end of experiment and were examined to record any gross pathological change.
   b) Histopathological examination: Liver, heart and spleen were processed for histopathological studies (Drury and Wallington, 1980).
4. **Lymphoid organ/body weight ratio**
   Lymphoid organs were weighed separately to determine the lymphoid organs weight/body weight ration by using following fonnula (Giambome and Closser, 1990):
   Organ-body weight ratio = weight of the organ/body weight x 1000
5. **Post-infection mortality**
   Post-infection mortality (%) in chicks of both groups was recorded.
6. **Estimation of feed conversion ratio (FCR)**
   At the end of experiment, FCR was calculated using the following formula (Singh and Panda, 1992):
   Feed Conversion Ratio = Total feed consumed/Total weight gained

Statistical analysis

Data collected were analyzed by applying unpaired t-test (Steel and Torrie, 1982).

RESULTS AND DISCUSSION

Escherichia coli is a large group of ecologically advantageous organisms and is able to grow aerobically and anaerobically. Coliform infection in birds is indicated by

depression, yellowish or greenish watery dropping, hyperthermia and gross lesions including mild to acute enteritis, perihepatitis, pericarditis, salpingitis and septicaemia.

The chicks of group A inoculated with *E. coli* became dull, depressed and took food and water very often. The chicks became dejected, had reduced weight gain and were pot bellied, The vent of these chicks was pasty with greenish feces.

Haematological alterations studies included haemoglobin (Hb) estimation, packed cell volume (PCV) and total erythrocyte (TEC) and leukocyte (TLC) counts (Table 1). There was a significant difference between these blood parameters in both treatment and control groups. Hb, PCV and TEC values were significantly lower while TLC values were significantly higher in treatment than control group on all the three sampling days. These findings are in line with the findings of Qazi (1989) who reported lowered Hb, PCV and TEC values and higher TLC values in chicks inoculated with *E. coli*.

Table 1: Mean (±S.E.) of different blood parameters of chicks of group A and B

Blood parameters	48 hrs post-infection		72 hrs post-infection		96 hrs post-infection	
	A	B	A	B	A	B
Hb estimation (gm/dl)	9.3±1.03^{a}	9.8±1.02^{b}	8.5±1.05^{c}	10.1±0.98^{b}	7.7±1.12^{d}	10.0±1.06^{b}
PCV (%)	24.7±1.07^{a}	26.4±1.10^{b}	22.7±1.05^{c}	26.9±1.10^{b}	20.3±0.92^{d}	28.4±1.09^{b}
Erythrocyte count (10^{6}/µl)	2.11±1.12^{a}	2.48±0.98^{b}	1.88±1.22^{c}	2.51±1.08^{b}	1.62±1.01^{d}	2.50±1.12^{b}
Leukocyte count (10^{3}/µl)	40.0±0.90^{a}	26.0±1.12^{b}	46.0±1.13^{c}	27.0±1.03^{b}	51.0±1.21^{d}	28.0±1.09^{b}

Mean with different superscripts across the rows are significantly different ($P<0.05$).
S.E = Standard error.

Geometric mean titres against NDV of group A and B on different sampling days are presented Table 2. On all sampling days antibody titres were significantly lower in treatment than control group. Our results are supported by the findings of Shah (2002) who reported a decreased antibody titre against NDV in chicks experimentally infected with *E. coli*.

Table 2: Geometric mean HI titre of chicks of group A and B

Group	48 hrs post-infection	72 hrs post-infection	96 hrs post-infection
A	226.0	189.3	174.6
B	368.2	352.4	371.8

The heart of treatment group was characterized by necrotic foci in cardiac muscles, thickened and inflamed pericardial sacs, discolouration of pericardial fluid and a fibrinous covering around it, There was mild to severe plasma cell infiltration. Epicardium

was edematous and epicardial sac was cloudy with light coloured exudates. The spleen of treatment group was enlarged markedly and congested. The liver from the infected birds was also enlarged and congested with green discolouration, There were multiple pale foci on liver, which were microscopically determined to be focal areas of early heterophilic, granulomatous hepatitis. Inflammatory cell infiltration, serous to fibrinous exudate, and cellular debris on serosal surfaces were present in the liver and spleen in treatment birds. Similar results in *E. coli* infection were also reported by Nakamura *et al.* (1985), Murakami *et al.* (1989), Fisher *et al.* (1998), Pourbakhsh *et al.* (1997) Anjum (1997) and Barnes *et al.* (2003).

The mean lymphoid organs (bursa of Fabricius, thymus and spleen) weights were higher while body weights were lower in treatment than control birds (Table 3). Thus mean lymphoid organs weight/body weight ratio was higher in treatment group as compared to control one, indicating a decreased confer of immunity in *E. coli* treated birds. Various workers have reported increased weight of bursa of Fabricius and thymus (Grizzle *et al.*, 1997) and spleen (Fisher *et al.*, 1998) in bacterial infections including *E. coli*. Reduced- body weight in *E. coli* infection is reported by Grizzle *et al.* (1997). Furthermore necrotic (Changlin *et al.*, 1996) and degenerative (Nakamura *et al.*, 1985) changes were also noted in bursa of Fabricius.

Table 3: Lymphoid organ weight/body weight ratio of chicks of group A and B

Group	Lymphoid organ weight/body weight ratio (Mean ± S.E)		
	Bursa of Fabricius	Spleen	Thymus
A	2.05 ± 0.04^{a}	2.29 ± 0.03^{c}	5.17 ± 0.07^{e}
B	1.83 ± 0.05^{b}	2.03 ± 0.02^{d}	3.47 ± 0.01^{f}

Means with different superscripts across the columns are significantly different ($P<0.05$).
S.E = Standard error.

Post-infection mortality percentage of group A and B is shown in Table 4. Up to day-21 no mortality was noted. In treatment group three birds died on day-2, four birds died on day-3 while five bird died on day-4 post-infection. In control group no bird died till the end of experiment. Thus total mortality in infected group was 24%. High mortality in *E. coli* infection is also reported by Leitner and Heller (1992) and Anjum (1997).

Table 4: Post-infection mortality percentage of organs of chicks of group A and B

Group	Total birds	Live birds	Dead birds	Mortality (%)
A	50	38	12	24.0
B	50	50	0	0.0

Feed conversion ratio was lower in treatment group than that of control group (Table 5). These findings are in line with the findings of Anjum (1997). It is tempting to speculate that poor FCR has resulted in reduced weight gain in bacterial infection.

Table 5: Feed conversion ratio of chicks of group A and B

Group	48 hrs post-infection	72 hrs post-infection	96 hrs post-infection
A	2.35	2.37	2.40
B	2.17	2.23	2.19

From the above discussion it can be concluded that infection with *E. coli* resulted not only in haematological and pathological alterations but it also impaired the immune system along with high mortality in infected birds.

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