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EFFECT OF EXPERIMENTAL YOLK SAC INFECTION WITH ESCHERICHIA COLI ON IMMUNE STATUS OF BROILER CHICKS

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ABSTRACT

In this study, 100 day-old broiler chicks were divided into two groups, A and B containing 50 birds each. Experimental infection with E. coli was given intra yolk to group A on day-1 of experiment, while group B was kept as control. Parameters studied were examination of yolk sac, yolk sac:body weight ratio, antibody titer against Newcastle disease virus (NDV) and analysis of fractional serum proteins. Results showed that yolk sac infection with E. coli led to gross pathological changes of yolk sac (enlarged, discoloured, changed consistency and congested blood vessels). Yolk sac:body weight ratio was increased and geometric mean titer against NDV was decreased in serum and yolk of treatment group. Analysis of fractional serum proteins revealed that percentage area covered by most of serum protein fractions was decreased in treatment group as compared to control group. It was concluded that intra yolk infection with E. coli resulted in pathological changes of yolk sac, increased yolk sac:body weight ratio, decreased immunity and altered serum proteins profile of chicks.

Key words: Yolk sac infection, Escherichia coli, maternal immunity, broiler chicks.

INTRODUCTION

The first two weeks of age are very important in the life of a broiler chick. Out of total mortality, 30-50% occurs in this period. Omphalitis, avian encephalomyelitis, brooder pneumonia, spiking mortality, dehydration, ammonia burns and pullorum disease are major problems of the early life (Charlton, 1996). Amongst these problems, omphalitis is the major cause of early chick mortality in Pakistan (Anjum, 1997). Major cause of omphalitis is E. coli but other bacteria may be found in single or mixed infections (Deeming, 1995, Rehman et al., 1996, Anjum, 1997).

Newly hatched birds emerge from the sterile environment of the egg and require temporary immunological assistance. Serum immunoglobulins are readily transferred from hen serum to the yolk of egg. IgA and IgM are found in albumen while IgG is found in yolk of egg. As the chick embryo develops, it absorbs some of the yolk IgG, which appears in its circulation. The maternal IgM and IgA from albumen diffuse into the amniotic fluid, are swallowed by embryo and present in its intestine on hatching. These maternal antibodies effectively protect chicks from diseases until they disappear between 10 and 20 days after hatching. Alteration in structure of these proteins accompanies with microbial infection, results in immunosuppression (Sander et al., 1998).

The present study was undertaken to evaluate the effect of experimental yolk sac infection with E. coli on maternal immunity and serum proteins profile of broiler chicks with the objective to understand the pathogenesis of yolk retention and thus contribute to devise measures for controlling early chick mortality in broiler flocks.

MATERIALS AND METHODS

Preparation of inoculum

Pathogenic strain of E. coli was isolated from the birds suspected for colibacillosis. Identification of the organism was done by studying their morphological, cultural and staining characteristics, sugar fermentation and biochemical reactions (Jalil and Das, 2001; Khan et al., 2002). Pathogenicity was determined by inoculating over night cultured broth of E. coli to day-old broiler chicks (Lee and Arp, 1998). Total viable count was done by plate count method (Collins et al., 1995).

Experimental birds

One hundred day-old chicks were reared under optimal managemental conditions in Experimental Room of Pathology Department, University of Veterinary and Animal Sciences, Lahore. Feed and water were provided ad libitum.

Experimental design

Chicks were distributed into two groups, A and B, each containing 50 birds on day-1. In group A, experimental yolk sac infection with E. coli was induced on day-1 of experiment. The inoculum of pathogenic isolate of E. coli (10^9 c.f.u/0.1 ml) was
inoculated into the yolk sac of each chick using sterilized insulin syringe (Kloryga, 1986). Chicks of group B acted as control. Sterile nutrient broth (0.1ml/chick) was injected into yolk sac on day-1 in this group.

Collection of samples
Ten chicks were slaughtered from each group at interval of 48 hours, i.e., on 3rd, 5th, 7th and 9th day post inoculation (PI). Blood and yolk of each chick were collected to study the experimental parameters.

Experimental parameters

Examination of yolk sac
The yolk sac of each chick was thoroughly examined to record gross pathological lesions.

Yolk sac:body weight ratio
Yolk sac weight and body weight were determined and then yolk sac:body weight ratio for each chick was calculated by following formula:

\[
\text{Yolk sac:body wt. ratio} = \frac{\text{Yolk sac weight}}{\text{Body weight}} \times 100
\]

Determination of antibody titer against Newcastle disease virus
Antibody titer against Newcastle disease virus (NDV) in serum and yolk was determined by haemagglutination inhibition (HI) test (Silim and Venne, 1989). Geometric mean titer was calculated as described by Villegas (1998).

Analysis of fractional serum proteins
Determination of fractional serum proteins was done by using sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) method, as described by Laemmli (1970).

Statistical analysis
Data thus collected were statistically (P<0.05) analyzed by applying unpaired t-test (Steel and Torrie, 1982).

RESULTS AND DISCUSSION
Results of the present study are tabulated in Tables 1-4. Body weight of infected chicks was lower than that of control chicks. Reduced weight gain in yolk sac infection was also observed by Khan et al (2002). This might be due to refusal of feed by chicks. Yolk sac:body weight ratio in infected chicks was higher than in control group Deeming (1995) also reported that yolk sacs of infected chicks were bigger than the uninfected yolk sacs from poults of same age. Sander et al. (1998) and Khan et al. (2002) also reported similar findings. Higher yolk sac:body weight ratio indicates decreased yolk absorption in infected chicks probably due to protein alteration, binding or decreased permeability of the yolk sac membrane. Reduced weight gain and high yolk sac weight resulted in higher yolk sac:body weight ratio in E. coli infected group as compared with control group. Similar observations in E. coli infection were also reported by Khan et al. (2002).

Examination of yolks revealed that the yolks of infected chicks were discoloured, having abnormal consistency (watery in initial stage and hard in latter stage) and congested yolk sac blood vessels. Similar findings were also reported by Jordan (1990), Sainsbury (1992), Anjum (1997) and Khan et al. (2002).

Geometric mean haemagglutination inhibition (HI) titers of serum and yolk against NDV were highest at day 3 and lowest at day 9. These results are in congruent with findings of Saeed et al. (1988), Mitra et al. (1998) and Sander et al. (1998). Geometric mean titer against NDV in serum and yolk were higher in control than infected chicks. Sander et al. (1998) also reported similar results. It was probably due to alteration of protein structure of globulin caused by microbial infection and decreased antibody absorption from yolk.

Analysis of fractional serum proteins revealed that percentage area covered by most of the serum proteins was decreased in treatment group as compared to control. On day 3, protein fractions of molecular weight 141.3 kDa, 100 kDa (hepatoglobulin), 50.1 kDa (pre-albumen), 35.5 kDa (α acid glycoprotein), 25.1 kDa and 22.4 kDa protein decreased, while 31.6 kDa increased in treatment group as compared with control group. On day 5, 141.3 kDa, 50.1 kDa (pre-albumen), 25.1 kDa and 19.9 kDa decreased, while 100 kDa (hepatoglobulin) and 22.4 kDa increased in treatment group as compared with control group. On day 7, 141.3 kDa, 100 kDa (hepatoglobulin), 50.1 kDa (pre-albumen) 28.2 kDa, 25.1 kDa, 22.4 kDa and 19.9 kDa decreased, while 44.6 kDa increased in treatment group as compared with control group. On day 9, 141.3 kDa, 70.8 kDa, 35.5 kDa (α acid glycoprotein) 25.1 kDa and 19.9 kDa decreased, while 56.2 kDa (hemopein) and 22.4 kDa increased in treatment group as compared with control group. These results are partially similar to the findings of Ching and Yeh (1992), who reported that the serum proteins of 30, 40, 83 and 94 kDa increased but 24, 70...
### Table 1: Gross pathological changes of yolk sac in control and infected groups

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Discoloration</th>
<th>Offensive odour</th>
<th>Engorged blood vessels</th>
<th>Watery Consistency</th>
<th>Caseous Consistency</th>
<th>Hard Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>A</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>++</td>
<td>++++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>A</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>_</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Absorbed</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>_</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Absorbed</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

### Table 2: Mean yolk sac/body weight ratio in control and infected groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sampling days</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>4.86</td>
<td>1.792</td>
<td>2.0668*</td>
<td>2.4443*</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>3.45</td>
<td>1.373</td>
<td>0.2773</td>
<td>0.20331</td>
</tr>
</tbody>
</table>

A = Treatment  
B = Control  
* = Significant difference (P<0.05).

### Table 3: Haemagglutination inhibition titer against NDV in serum and yolk

<table>
<thead>
<tr>
<th>Day</th>
<th>GMT in serum</th>
<th>GMT in yolk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>3</td>
<td>Log₂6.5</td>
<td>Log₂6.7</td>
</tr>
<tr>
<td>5</td>
<td>Log₂5.7</td>
<td>Log₂6.1</td>
</tr>
<tr>
<td>7</td>
<td>Log₂5.6</td>
<td>Log₂5.7</td>
</tr>
<tr>
<td>9</td>
<td>Log₂5.6</td>
<td>Log₂5.7</td>
</tr>
</tbody>
</table>

### Table 4: Mean % area covered by different fractions of serum proteins in control and infected groups

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol. Wt. (KDa)</td>
<td>141.3</td>
<td>100</td>
<td>70.8</td>
<td>56.2</td>
<td>50.1</td>
<td>44.6</td>
<td>35.5</td>
<td>31.6</td>
<td>28.2</td>
<td>25.1</td>
<td>22.4</td>
<td>19.9</td>
</tr>
<tr>
<td>Days Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 A</td>
<td>3.65</td>
<td>2.95</td>
<td>-</td>
<td>-</td>
<td>2.67</td>
<td>-</td>
<td>3.23</td>
<td>4.56</td>
<td>-</td>
<td>4.42</td>
<td>4.28</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>4.14</td>
<td>3.02</td>
<td>-</td>
<td>-</td>
<td>2.95</td>
<td>-</td>
<td>4.07</td>
<td>4.14</td>
<td>-</td>
<td>5.05</td>
<td>4.77</td>
<td>-</td>
</tr>
<tr>
<td>5 A</td>
<td>3.18</td>
<td>3.32</td>
<td>-</td>
<td>-</td>
<td>2.61</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.68</td>
<td>4.16</td>
<td>4.17</td>
</tr>
<tr>
<td>B</td>
<td>3.6</td>
<td>2.97</td>
<td>-</td>
<td>-</td>
<td>2.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.97</td>
<td>4.01</td>
<td>4.81</td>
</tr>
<tr>
<td>7 A</td>
<td>3.06</td>
<td>1.97</td>
<td>-</td>
<td>-</td>
<td>3.29</td>
<td>3.21</td>
<td>-</td>
<td>-</td>
<td>1.82</td>
<td>3.72</td>
<td>4.53</td>
<td>3.58</td>
</tr>
<tr>
<td>B</td>
<td>3.21</td>
<td>2.48</td>
<td>-</td>
<td>3.65</td>
<td>3.07</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.26</td>
<td>4.09</td>
<td>4.6</td>
<td>4.09</td>
</tr>
<tr>
<td>9 A</td>
<td>2.23</td>
<td>3.19</td>
<td>3.05</td>
<td>-</td>
<td>2.34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.98</td>
<td>4.61</td>
<td>3.41</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>2.62</td>
<td>3.41</td>
<td>2.98</td>
<td>-</td>
<td>2.76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.48</td>
<td>4.4</td>
<td>3.83</td>
<td>-</td>
</tr>
</tbody>
</table>
and 90 KDa proteins remained unchanged due to acute stress of heat in chickens. Youssaf et al. (1988) reported increase in serum total protein, pre-albumen, post albumen, gamma globulin and transferring values, while decrease in albumen value, after subcutaneous infection with *Salmonella arizonae*. Sefer et al. (1999) reported that alpha and gamma globulin concentrations increased, albumen concentration decreased and albumen-globulin ratio narrowed in T-2 toxin treated chicks.

Based on the results of the present study, it can be concluded that intra yolk infection with *E. coli* resulted in pathological changes of yolk sac, increased yolk sac: body weight ratio and decreased immunity.

REFERENCES


