

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* (10^4 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 poult 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 yolk sac 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 titers 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. 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

Day	Group	Discoloration	Offensive odour	Engorged blood vessels	Consistency		
					Watery	Caseous	Hard
3	A	+	+++	+++	+++	-	-
	B	-	-	+	+	-	-
5	A	++	++++	++++	++	++	-
	B	-	-	-	+	-	-
7	A	+++	++	++	-	+	++
	B	Absorbed	-	-	-	-	-
9	A	++++	++	++	-	+	++
	B	Absorbed	-	-	-	-	-

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

Groups	Sampling days			
	Day 3	Day 5	Day 7	Day 9
A	4.86	1.792	2.0668*	2.4443*
B	3.45	1.373	0.2773	0.20331

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

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

Day	GMT in serum		GMT in yolk	
	Group A	Group B	Group A	Group B
3	Log ₂ ^{6.5}	Log ₂ ^{8.7}	Log ₂ ^{8.6}	Log ₂ ^{8.8}
5	Log ₂ ^{6.7}	Log ₂ ^{8.2}	Log ₂ ^{8.5}	Log ₂ ^{8.8}
7	Log ₂ ^{5.7}	Log ₂ ^{6.1}	Log ₂ ^{6.7}	Absorbed
9	Log ₂ ^{5.6}	Log ₂ ^{5.7}	Log ₂ ^{6.4}	Absorbed

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

Fraction No.													
Mol. Wt. (KDa)													
Days	Group	1	2	3	4	5	6	7	8	9	10	11	12
	s	141.3	100	70.8	56.2	50.1	44.6	35.5	31.6	28.2	25.1	22.4	19.9
3	A	3.65	2.95	-	-	2.67	-	3.23	4.56	-	4.42	4.28	-
	B	4.14	3.02	-	-	2.95	-	4.07	4.14	-	5.05	4.77	-
5	A	3.18	3.32	-	-	2.61	-	-	-	-	2.68	4.16	4.17
	B	3.6	2.97	-	-	2.9	-	-	-	-	2.97	4.01	4.81
7	A	3.06	1.97	-	-	3.29	3.21	-	-	1.82	3.72	4.53	3.58
	B	3.21	2.48	-	-	3.65	3.07	-	-	2.26	4.09	4.6	4.09
9	A	2.34	-	3.19	3.05	-	-	2.34	-	-	2.98	4.61	3.41
	B	2.62	-	3.41	2.98	-	-	2.76	-	-	3.48	4.4	3.83

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 transferrin 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.

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