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Some Studies of *E. coli* 0H157 on Ducks and some clinicopathological Changes

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ABSTRACT

Forty ducks 6 weeks old and 700 - 900 g average body weight were used. They kept in a balanced diet to study some performance, and clinicopathological changes under *E. coli* 0157:H7 infection. Ten ducks kept as control and 30 were infected with *E. coli* 0157:H7 by dose 0.05ml ($\times 10^7$ CFU) inoculated intramuscularly. Body weight were recorded, blood samples were collected at 7, 15, 30 days post infection, serum was separated for determination of AST, ALT, Total protein. Albumin, urea, creatinine, calcium phosphorous, sodium, potassium and cortisol hormone. The biochemical analysis showed increase in AST and ALT and a significant change in protein. Hypoalbuminemia, was observed, increase of serum urea, creatinine, hypocalcaemia, hyperphosphatemia, and decrease in level of potassium, sodium and cortisol hormone in areas. Blood examination revealed pancytopenia. This indicates that *E. coli* 0157:H7 causes deleterious effect on the Hematopoietic system.

Key words: *E. coli*; ducks; AST, ALT; Biochemical analysis.

INTRODUCTION

Infection with *E. coli* 0157:H7 presents with a wide spectrum of clinical manifestations, including severe abdominal cramps with little or no fever and watery diarrhea that often progresses to grossly bloody diarrhea [1]. Infection can be asymptomatic or can present with only nonbloody diarrhea [2]. Extra intestinal involvement, including cardiac and neurologic manifestations, has been reported, and infection can be associated with the hemolytic-uremic syndrom and thrombotic thrombocytopenic purpura. The disease can be fatal [3].

Escherichia coli comprises a group of bacteria found in the intestines of humans, animals and birds, *E. coli* 0157:H7 strain produces potent toxins and can cause food born pisones to person transmitted disease after ingestion of very low numbers of microorganism *E. coli* 0157:H7 was first identified as a human pathogen in 1982 [4].

Griffin and Tauxe, [5], a recorded reported that strain of *E. coli* infection is more often reported in the young, illness signs are bloody diarrhae, severe abdominal pain, low grade of fever and vomiting. The major source of food born *E. coli* 0157:H7 cited disease is undercooked grand beef. Roast beef, roast ducks, raw milk and water an out break of the disease in persons who had eaten fast food in these restaurant chain. Marks and Robert, [6], reported that the cytotoxins of *E. coli* 0157:H7 production seems to be important factors in the pathogenesis of disease. These cytotoxins are among the most potent bacterial toxins. These toxins in active host cell ribosomes disrupting protein synthesis and causing cell death [7].

Prevention of illness is especially critical in addition to strategies designed to prevent food born illness. Controlled production of live animal, meat processing relatively little information is available on clinicopathological changes in experimental animals with this disease. The present work designed to study some serum biochemical changes after experimental infections of ducks with *E. coli* 0157:H7.

MATERIALS AND METHODS

E. coli strain:

E. coli 0157: H7 strain was used for the experimental infection for 40 ducks 6 weeks old as well as 700-900 gm average body weight. Bird proved to be free from pathogenic bacteria and parasitic infection. I/M with 0.05×10^7 cfu colony forming unit ml of viable organisms of *E. coli* 0157:H7.

Blood samples:

Two blood samples were collected from wing vein the first blood sample was collected into dry clean tube containing dipotassium. Ethylene diamine tetracetate (EDTA) as anticoagulant to covering out the hematological studies including red blood cells counts (RBCs) hemoglobin, (H) packed cell volume (PCV), mean corpuscular. Hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from these hematological parameters according to Jain, [8].

Second blood samples were collected from wing vein and serum was separated and used for the determination the activity of aspartate aminotransferase. (AST) and alanine aminotransferase (ALT) according to Reitman and Frankel [9]. Total protein and albumin according to Doumas and Biggs [10]. Urea according to Sanders [11] creatinine was estimated according to Bartel [12], calcium was determined according to Sarkar and Chankan [13], phosphorous was measured according to Goodwin [14], Na, K were determined by atomic absorption. Serum cortisol was analyzed by means of a gammacoat 125 I cortisol radioimmunoassay kit according to the method described by Campbell and Coles [15]. Also at the time of scarification feces from these birds are cultivated on sorbitol Ma Conkey agar medium for bacteriological examination according to Ratnam and March [16].

Statistical analysis according to Snedecor and Cochran, [17].

RESULTS

Bacteriological results recorded fail to ferment sorbitol can be recognized as colorless colonies. Further confirmation we made by agglutination test with anti-serum against the flagella antigen H7 it give positive results.

In Table (1) there is a significant decrease on body weight at 7, 15, 30 days and mortality rate is increase in the 1st week after infection, signs of infection appears in the form of depression, loss of body weight, bloody diarrhea and ascites.

Table (3) revealed a significant decrease in RBCs count Hb concentrations PCV, MCV, MCH, MCHc also TLC such decrease was very highly significant on days 30 of infection ($P < 0.01$).

In Table (4) there is a significant increase in ALT and AST if compared with control group $P < 0.05$. Total protein and Albumin showed highly significant decrease if compared with control group. Concerning cortisol the result showed highly significant increase if compared with control group.

Table (5) revealed a significant increases in serum urea and creatinine and Hyperphosphatemia in d15 and d30 $p < 0.01$. also there is a significant decrease in serum level sodium, potassium and Hypocalcemia in d15 and d30 $p < 0.01$.

Table 1: Changes in body weight in infected ducks with E coli

	7 days	15 days	30 days
Control	750 ± 0.62	76 ± 0.13	90000 ± 0.40
Infected	800 ± 0.70	600 ± 0.75*	608 ± 0.56**

* $P < 0.05$ ** $P < 0.01$

Table 2: Commercial of the basal diets (according to National Research Council)

Ingredients (%)	Started diet	Grower-Finisher diet
Yellow corn ground	67.40	72.90
Soyabean meal (44 %)	21.30	18.30
Fish meal (72 %)	4.00	3.00
Meat meal (60 %)	5.84	4.30
Bone meal	0.08	0.10
Dicalcium phosphate	0.15	0.10
Limestone ground	0.79	0.94
Salt	0.71	0.17
Methionine	0.12	0.04
Premix *	0.15	0.15
Calculated analysis		
Crude protein (%)	2150	19
ME (Kcal/kg)	2989	3040
C/P ratio	139.02	160

Birds premix furnishing the following ingredients per kg of feed vit. A 12000 IU, vit D₃ 2000 IU, Vit E 10 mg, folic acid 1 mg vit Niacin 20 mg, pantothenic acid 10 mg, vit K 2 mg, vit B₁ 1mg, vit B₂ 4 mg, vit B₆ 1.5 mg vit B₁₂ 10 µg, iron 30 mg, copper 10 mg, Zinc 55 mg, Mn 55 mg, Iodine 1 mg, Se 0.1 mg, choline chloride 500 mg.

Table 3: Hematological values (mean Values ± S.E of normal and affected duck by *E. coli*)

Groups	R.B.C.S 10 ⁶ µL	HB gm/dl	P.cv %	M.cv	MCH pg	M.CH.C% * 10 ³ µl	TL. C
Control	3.1 ± 0.23	7.4 ± 0.16	26.7 ± 0.43	115.5 ± 11.8	41.1 ± 1.72	36.3 ± 0.17	21.2 ± 0.21
7 days	2.6* ± 0.20	7.6 ± 0.32	24.8 ± 0.73	113 ± 6.2	34.4 ± 0.23	32.1 ± 0.16	22.2 ± 0.70
15 days	2.5 ± 0.84	6.60 ± 0.57*	23.8 ± 0.11*	104.5 ± 4.13*	32.6 ± 0.25*	21.4 ± 0.26*	21.2 ± 0.23*
30 days	1.34 ± 0.62*	7.80 ± 0.70*	22.0 ± 0.70*	99.0 ± 2.20*	22 ± 0.64*	21.4 ± 0.13**	21.2 ± 0.13**

Table 4: Changes Of Liver function test and cortisol hormone in duck infested with *E coli* 0157:H₇

Parameter	A S T µ/l	A L T µ/l	Total protein gm/dl	Albumin gm/dl	Cortisol µg/dl
Control	25.0 ± 0.12	14 ± 0.24	4.0 ± 0.12	11.50 ± 0.04	0.07 ± 0.11
Infected 7 days	26 ± 0.12	16 ± 0.17	3.8 ± 0.80	1.56 ± 0.19	0.10 ± 0.64
Infected 15 days	36 ± 0.12	19 ± 0.20	2.8 ± 0.56*	0.93 ± 0.13*	0.8 ± 0.65*
Infected 30 days	45 ± 0.27*	27.8 ± 0.66**	2.6 ± 0.70	0.99 ± 0.70	0.16 ± 0.90**

* $P < 0.05$ ** $p < 0.01$

Table 5: Renal Function in ducks infested with *E coli* 0157:H₇

Parameter	Urea mg/dl	Creatinine mg/dl	Calcium mg/dl	Phosphorous mg/dl	Sodium Meq/l	Potassium Meq/l
Control	3.15 ± 0.72	1.43 ± 0.76	8.8 ± 0.12	6.15 ± 0.23	152 ± 0.62	7.98 ± 0.13*
Infected 7 days	3.98 ± 0.12*	1.9 ± 0.10*	7.50 ± 1.00*	6.09 ± 0.248*	138 ± 0.7*	6.96 ± 0.2*
Infected 15 dys	4.802 ± 0.21*	2.6 ± 0.30	7.00 ± 0.21*	6.98 ± 0.11	128 ± 0.20*	5.6 ± 0.9*
Infected 30 days	5.580 ± 0.72**	2.78 ± 0.90*	5.90 ± 0.12*	7.9 ± 0.80	120 ± 0.10*	4.97 ± 0.8

** $P < 0.01$ * $P < 0.05$

DISCUSSION

Hemorrhagic colitis caused by *E. coli* 0157:- H₇ is a clinical syndrome that consists of abdominal cramps; diarrhea that progresses to become bloody; radiologic or endoscopic evidence of clonic mucosal edema, erosion, or hemorrhage; and the absence of conventional enteric organisms in the stool.

The present study shows a significant decrease in RBcs count, Hb concentration and PCV in the affected birds indicate anemia of microcytic hypochromic as showed by the erythrocytic indices that were proportionally correlated with the severity of infection of *E. coli*. This result is in accordance with Jain [8].

The increase in serum AST levels in this work could be due to liver damage produces by the infected bacteria. Campell and Coles [15]; mentioned that the increased of the activity of.AST has been associated with hepatocellular damage in birds.

Concerning ALT in duck some studies reported elevation of ALT *it* birds infected with bacteria [18]. Our result

agrees with Omaima [19], who observed a significant increase in (AST & ALT; in duck infected with *E. coli*. The significant change in total protein and albumin in the present work could be due to liver and kidney damage which could be associated with bacterial infection.

Similar findings were previously mentioned by Riley *et al.* [4] Pai [20]; Campbell and Coles [15] and Ostroff *et al.* [21].

The increase in Urea and creatinine could be due to the effect of the micro-organisms and its Toxin on the kidneys. Our results completely agree with Pai *et al.* [22]; Tzipori *et al.* [23] and Ogrig *et al.* [24] who reported increased creatinine, urea level in case of renal disease.

Hypocalcaemia, and Hyperphosphatemia could be due to decreased calcium resorption by damaged renal tubules and associated with Hypoalbuminemia as reported by Campbell and Coles [15]; Beer *et al.* [25] and Marks and Robert [6]. The decrease of potassium and sodium level in serum could be due to renal disease as reported by Campbell and Coles [15]. Also the metabolism of Calcium and Phosphorus is closely linked in the body and hypocalcaemia always accompanied with hyperphosphatemia concerning serum cortisol level, the significant increase of serum cortisol level may be attributed to the activation of Hypothalamus pituitary axis due to stress. Our results agree with Ghanem [26] and Campbell and Coles [15].

In conclusion infection of duck with *E. coli* 0157: H7 injured liver, and Kidneys. The change in liver and kidney function were more severe in days of infection.

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