

Studies on Vaccination of Turkey Against *Escherichia coli* Infection

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Abstract: Evaluation of the immune response of turkeys to avian colibacillosis vaccine was studied, where sixty 3-weeks old turkeys were vaccinated twice subcutaneously (S/C) with a dose of 0.5 ml with 3 weeks intervals. Other forty turkeys were kept as non-vaccinated control. Birds were challenged S/C against O2 and O78 pathogenic strains of *Escherichia coli* (*E. coli*) using 10⁹ CFU/0.2 ml/ turkey. Clinical and necropsy examinations revealed that vaccinated birds showed 80-85% protection while non-vaccinated birds were unable to withstand the challenge. Vaccinated birds had reduced mortality compared to non-vaccinated controls. *E. coli* was recovered from vaccinated challenged turkeys at ratios ranged from 15-30% from the heart blood, liver, spleen and bone marrow on the 8th day post challenge while these ratios were ranged from 46.6-86.6% from non-vaccinated challenged birds. So, the vaccination studies performed here showed that turkeys immunized with two doses of inactivated *E. coli* vaccine were protected to a high degree to challenge with the same pathogenic *E. coli* strains.

Key words: Turkeys • Avian Colibacillosis Vaccine • *Escherichia coli*

INTRODUCTION

Both the incidence and severity of colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), have increased rapidly and current trends indicate that it is likely to continue and become one of the most important causes of economic losses in commercial chickens and turkeys [1]. The losses are caused by morbidity, lack of uniformity in a flock, lowered production, increased condemnation at the slaughter plant and mortality [2]. The most common lesions associated with colibacillosis are perihepatitis, airsacculitis and pericarditis, although other syndromes such as egg peritonitis, salpingitis, coligranuloma, omphlitis, cellulitis and osteomyelitis/ arthritis may be encountered [3]. In turkeys, APEC also causes osteomyelitis complex characterized by lesions including green discolouration of the liver, arthritis/synovitis, soft tissue abscesses and osteomyelitis of the proximal tibia in an otherwise normal-appearing processed turkey carcass [4]. Many *E. coli* isolates commonly associated with colibacillosis in poultry belong to serogroups O1, O2 and O78 [5-7].

Treatment strategies include the control of predisposing infections or environmental factors and the early use of antibiotics, unfortunately, a high frequency of resistance to tetracycline, oxytetracycline, chlortetracycline, doxycycline and erythromycin has occurred [8]. Furthermore, using of antibiotic drugs in the future will tend to be reduced and restricted in commercial farms so *E. coli* vaccines are an alternative way to prevent and control of *E. coli* infection [9]. Several vaccines based on killed or attenuated strains have been tested experimentally. In general, they give sufficient protection against infection with homologous strains, but protection against heterologous strains is less efficient [10]. Nevertheless, Melamed *et al.* [11] reported a certain degree of heterologous protection obtained with an inactivated vaccine. In turkeys, several experiments have been performed to prevent colibacillosis by vaccination [12-14].

The objective of the present work was to evaluate the efficacy of the commercial inactivated *E. coli* vaccine in immunizing and protecting turkeys against experimental colibacillosis. It also described the immune response of turkeys evoked by the vaccine.

MATERIALS AND METHODS

Avian Colibacillosis Vaccine: Oil adjuvanted vaccine (Nobilis® E. COLI inac) prepared from O2 and O78 strains of *E. coli* for poultry use was supplied by Intervet International B.V. Company.

***E. coli* Strains:** Two local strains of pathogenic *E. coli* serotype O2 and O78 isolated from infected turkeys were kindly supplied by Animal Health Research Institute, Dokki, Giza, Egypt. These strains were used for challenging of vaccinated turkeys.

Turkeys: One hundred of one-day old turkeys were obtained from farm of Faculty of Agriculture, Egypt. These turkeys were used to evaluate the potency of inactivated *E. coli* vaccine. These birds were tested and found to be free from *E. coli* infection and antibodies as determined serologically.

Mice: A total of 250 weaned Swiss albino mice of about 25 gm body weights supplied by Veterinary Serum and Vaccine Research Institute, Cairo, Egypt were used for passage and detection of the LD₅₀ of *E. coli* strains.

Potency Test: One hundred turkeys at 3 weeks old were divided as follow: 60 turkeys were vaccinated with 2 doses of the vaccine each of 0.5 ml inoculated subcutaneously (S/C) in the dorsal aspect of the neck with 3 weeks intervals depending on the recommended dose according to Chaffer *et al.* [15]. 40 turkeys were kept without vaccination as control. All birds were housed in separate isolates under hygienic measures receiving adequate ration and water. Serum samples were obtained regularly on week intervals to follow up the induced antibody levels up to 4 months post the first vaccination.

Challenge Test: The challenge was performed on 40 vaccinated and 30 non-vaccinated control birds by S/C injection in the neck at 5th weeks post vaccination. The number of organisms was adjusted to approximately 10⁹ CFU/0.2 ml/ turkey according to Chaffer *et al.* [15]. Mortality was recorded for 7 days after challenge and on the 8th day, the number of dead birds was noted and all the survivors were euthanized, necropsied and examined for the presence of grossly visible lesions of colibacillosis.

Serological Evaluation of Humeral Immune Response of the Vaccinated Turkeys by Micro Agglutination Test (MAT):

Antibody response in vaccinated and non-vaccinated turkeys was followed up on regular intervals post vaccination determined by micro agglutination test (MAT), according to the method described by Erganis and Hadimli [16]. The geometric mean of *E. coli* antibodies titers was calculated according to Brugh [17].

Recovery of *E. coli* from Experimental Turkeys: On 8th day post challenge, samples were collected from the heart blood, liver, spleen and bone marrow from vaccinated and non-vaccinated challenged turkeys for recovery of the *E. coli* organism. Bacterial determination was carried out through cultivation of prepared samples on MacConkey medium according to Barnes and Gross [3].

RESULTS AND DISCUSSION

Colibacillosis is one of the most frequent diseases in turkeys and chickens resulting in mortality losses at all stages of life and decreased production efficiency in older birds [18, 19]. Although previous attempts to develop a vaccine have not been very successful, vaccination is still considered the most effective way of controlling the disease. Therefore, a number of experimental vaccines have been developed for the prevention of colibacillosis in poultry. Various strains of *E. coli* inactivated either by heat, formalin, or attenuation have been used for immunization of chickens to prevent colibacillus infections. However due to repeated outbreaks of this organism in the field, there is a need for an effective vaccine [20]. The results of this study have direct implication in considering an inactivated oil emulsion *E. coli* vaccine prepared from O2 and O78 strains for chickens as a vaccine option to protect turkeys against *E. coli* infections.

The humeral immune response of inactivated *E. coli* vaccine was evaluated in vaccinated turkeys by micro agglutination test (MAT) as shown in Table (1). The geometric mean of *E. coli* antibody titers against *E. coli* strains, O2 and O78 in sera of vaccinated birds increased from (6) and (7) pre-vaccination to reach (453) and (394) at 6th weeks post vaccination, such rising in antibody titer was continued to reach (610) and (557) by the 4th month post vaccination with inactivated *E. coli* vaccine, respectively, while the control un-vaccinated birds showed steady levels (6-10). These results agree

Table 1: Means of *E. coli* antibody titers in turkeys' sera as measured by micro agglutination test

Turkey Groups	Strain used	Geometric mean of <i>E. coli</i> antibody titers in pre and post vaccination periods									
		Pre-V	1WPV	2WPV	3WPV	4WPV	5WPV	6WPV	2MPV	3MPV	4MPV
Vaccinated	O2	6	49	70	98	226	343	453	520	597	610
	O78	7	28	57	75	171	206	394	485	520	557
Control	O2	8	7	8	7	8	9	8	10	10	9
	O78	6	8	9	10	9	8	8	9	8	9

Pre-V= pre-vaccination

WPV= week post vaccination

MPV= month post vaccination

Table 2: Protective efficacy of the vaccines against challenge with O2 and O78 strains of *E. coli*

Turkey Groups	Type of challenge strain	No. of challenged turkeys	No. of survived turkeys	Mortality Rate	Protection Rate
Vaccinated	O2	20	17	15%	85%
	O78	20	16	20%	80%
Control	O2	15	8	46.7%	53.3%
	O78	15	7	53.3%	46.6%

Table 3: Recovery of *E. coli* from challenged turkeys

Turkeys Groups	Type of challenge strain	Number of positive samples for <i>E. coli</i> recovery			
		Heart blood	Liver	Spleen	Bone marrow
Vaccinated	O2	6/20 (30%)	4/20 (20%)	5/20 (25%)	3/20 (15%)
	O78	6/20 (30%)	6/20 (30%)	6/20 (30%)	4/20 (20%)
Control	O2	11/15 (73.3%)	13/15 (86.6%)	12/15 (80%)	9/15 (60%)
	O78	12/15 (80%)	13/15 (86.6%)	11/15 (73.3%)	7/15 (46.6%)

with Erganis and Hadimli [16] who used the micro agglutination test for estimation of *E. coli* antibody titers. The antibody titers in pullets vaccinated once or twice with trivalent *E. coli* vaccine (TECA) prepared from serotypes O1, O2 and O78 were found to be ~0.5-3.6 times higher than controls, respectively. In breeder hens, the antibody titer figures were observed ~1.5-4.8 times higher than those from non-vaccinated hens.

The protection percentage of turkeys after challenge with the virulent strains O2 and O78 were summarized in Table (2), protection rates in the vaccinated groups were (80 and 85%) both significantly higher than those recorded in the control groups (46.6 and 53.3%) against challenge with $10^9/0.2$ ml *E. coli* O2 and O78 respectively. Thus, the turkeys vaccinated with *E. coli* vaccine could have a good protection rate confirming that the vaccine is a potent vaccine able to protect turkeys against infection through the induction of sufficient antibody titers. These results all agree with Rosenberger *et al.* [21] who found that the mortality increased to 60% in control birds when challenged with a dose of 10^7 CFU of *E. coli*.

These findings come in agreement with Chaffer *et al.* [15] and also, Erganis and Hadimli [16] who recommended the use of trivalent *E. coli* vaccine (TECA) for protection of both chicks and layers against infection of *E. coli*.

The tabulated results in Table (3) revealed that *E. coli* could be recovered from vaccinated challenged turkeys with ratios ranged from 15-30% from the heart blood, liver, spleen and bone marrow on the 8th day post challenge while these ratios were ranged to 46.6-86.6% from same organs of non-vaccinated challenged turkeys. These results came confirming by Abdul-Aziz and EL-Sukhon [22] who found that chickens hyperimmunized with the *E. coli* J5 strain are protected against experimental challenge with *E. coli* O78 serotype. *E. coli* O78 was re-isolated only from the heart blood or heart surface of all the chickens in control challenged group.

So, the vaccination studies performed here showed that turkeys immunized with two doses of inactivated *E. coli* vaccine are protected to a high degree from challenge with the same pathogenic *E. coli* strains.

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