Efficacy of a live *Escherichia coli* vaccine for protection of turkeys against homologous and heterologous field strains infection

Hanan A. Ahmed, Abdel Hakim M. Ali and Mansour H. Abdel Baky

Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo Egypt, P.O. Box131

A B S T R A C T

The immune response of turkeys to live attenuated vaccine for avian pathogenic Escherichia coli (APEC) constructed from O78 strain was evaluated, where ninety-one-day old turkeys were vaccinated twice with 3 weeks intervals. Other thirty turkeys were kept as non-vaccinated control. Birds were challenged against homologous O78 and heterologous O1 and O2 pathogenic strains of *E. coli* using 10^7 CFU/0.2 ml/ turkey. Clinical and necropsy examinations revealed that vaccinated birds showed 96.7% protection after homologous challenge while vaccinated birds were unable to withstand the heterologous challenge. *E. coli* was recovered from vaccinated challenged turkeys at ratio ranged from13.3%-20% from the heart blood, liver, spleen and bone marrow on the 8th day post homologous challenge while these ratios were ranged from 53.3%-66.7% from vaccinated birds post heterologous challenge. So, the vaccination studies performed here showed that live attenuated *E. coli* vaccine was protective against homologous challenge with O78 strain but con not protect turkeys against heterologous challenge.

Key Words: APEC, *E. coli*, Vaccine.

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1. INTRODUCTION

Avian pathogenic Escherichia coli (APEC) infections are a serious impediment to sustainable poultry production worldwide. Colibacillosis causes a complex of respiratory and systemic diseases that exert substantial welfare and economic costs on poultry producers (Russell, 2003). Losses are incurred through premature deaths, condemnation of carcasses at slaughter, reduced productivity and recurring costs associated with antibiotic prophylaxis and therapy (Kemmett et al., 2013). A longitudinal survey of broiler flocks found evidence of colibacillosis in 39% of dead birds, and the same authors implicated colibacillosis in 70% of deaths of broiler chicks 2–3 days after placement (Kemmett et al., 2013). A number of risk factors are known, including prior or concurrent infection with respiratory viruses or Mycoplasma, stress and injury associated with formation of a social hierarchy, onset of sexual maturity and intense laying, and poor biosecurity, hygiene and ventilation. 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 (Barnes and Gross, 1997). In turkeys, APEC also causes osteomyelitis complex characterized by lesions including green discoloration of the liver, arthritis/ synovitis, soft tissue abscesses, and osteomyelitis of the proximal tibia in an otherwise normal-appearing processed turkey carcass (Huff et al., 2000). Many *E. coli* isolates commonly associated with colibacillosis in poultry belong to sero-groups O1, O2 and O78 (Cloud et al., 1985 and Whiteman and Bickford, 1989).

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 (Cortes et al., 2010). 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 (Wang et al., 2010). The control of colibacillosis has been largely reliant upon vaccination with autologous bacterins (Trampel and Griffith, 1997) but these confer short-lived serotype-specific protection and their effectiveness is blunted by the diversity of *E. coli* capable of infecting poultry. Licensed vaccines are available, but the immunological basis of protection is ill-defined and a need exists to extend cross-serotype efficacy. 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 (Dho-Moulin and Fairbrother, 1999). Live-attenuated vaccines are preferable owing to ease of administration and improved cross-serotype protection and hence are entering the market (Kwaga et al, 1994). In turkeys, several experiments have been performed to prevent colibacillosis by vaccination (Arp, 1980; Bolin and Jensen, 1987) Vaccination of turkeys with a low dose of a live virulent strain appeared to give better protection compared to a heat- or formalin inactivated non-adjuvanted vaccine (La Ragione, et al 2013).

The objective of the present work is to evaluate the efficacy of the commercial live attenuated *E. coli* vaccine in immunizing and protecting turkeys against experimental homologous and heterologous colibacillosis infection. It also describes the immune response of turkeys evoked by the vaccine.

2. MATERIAL AND METHODS

2.1. Avian Colibacillosis Vaccine:

Commercial live attenuated Escherichia coli vaccine (Poulvac® *E. COLI*) prepared from O78 strain of *E. coli* used for vaccination of turkey poults. The lyophilized vaccine was reconstituted to contain 10^9 colony forming units (CFU) per ml prior to use.

2.2. E. coli strains:

Three local strains of pathogenic *E. coli* serotype O78, O1 and O2 were isolated from infected turkeys were kindly supplied by Animal Health Research Institute, Dokki, Giza, Egypt. These strains were used for homologous and heterologous challenging of vaccinated turkeys.

2.3. Turkeys:

Ninety, one-day old turkeys were obtained from farm of Faculty of Agriculture, Egypt. These turkeys were used to evaluate the potency of live attenuated *E. coli* vaccine. These birds were tested and found to be free from *E. coli* infection and antibodies as determined serologically. All birds were housed under hygienic measures in separate isolates receiving balanced ration and adequate water.

2.4. 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 LD_{50} of *E. coli* strains.

2.5. Potency test:

Ninety, turkeys were divided at 1-days old as follow: 60 turkeys were vaccinated by aerosol spray with 2 doses with 3 weeks' intervals depending on the recommended dose according to manufacturer instructions. 30 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.

2.6. Challenge test:

The homologous and heterologous challenge was performed on the vaccinated and the non-vaccinated control birds. All birds were inoculated into the air-sac with 10^7 CFU/0.2 ml/ turkey of the virulent strains (O78, O1 and O2) at 5th weeks post vaccination according to Chaffer *et al.* (1997). 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.

2.7. 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 (2002). The geometric mean *E. coli* antibodies titer was calculated according to Brugh (1977)

2.8. 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 Whiteman and Bickforf (1989).

3. RESULTS

The humeral immune response of live attenuated *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* strain O78 in sera of vaccinated birds increased from (6) pre-vaccination to reach (394) at 5th weeks post vaccination, while the control un-vaccinated birds showed steady levels (7-9). The protection percentage of turkeys after challenge with homologous (O78) or heterologous (O1 or O2) *E. coli* by the intra-air sac route were summarized in Table (2), protection rate in the vaccinated groups was (96.7%) in case of homologous challenge with O78 that was significantly higher than those recorded in the vaccinated groups (53.3% and 46.6%) against heterologous challenge with *E. coli* O1 and O2 respectively. Mortality was recorded and surviving turkeys were euthanized after the challenge and examined for macroscopic lesions as shown in table (3) which revealed that *E. coli* could be recovered from vaccinated challenged turkeys with the ratio ranged from 13.3%-20% from the bone marrow, spleen, liver and heart blood on the 8th day post homologous challenge while these ratios were ranged in case of heterologous challenge from 53.3%- 66.7 % from same organs of vaccinated challenged turkeys.

Table (1): E. coli mean antibody titers in turkeys' sera as measured by microagglutination test

Turkey Groups	Strain used	Geometric mean of *E. coli* antibody titers on periods post vaccination					
		Pre-V	1WPV	2WPV	3WPV	4WPV	5WPV
Vaccinated	O78	6	49	70	98	226	343
Control	O78	8	7	8	7	8	9

Pre-V= pre-vaccination WPV= week post vaccination

Table (2): Protective efficacy of the vaccines against challenge with homologous O78 and heterologous O1 and O2 strains of *E. coli*

Turkey Groups	Type of challenge strain	No. of challenged turkeys	No. of survived turkeys	Protection Rate
Vaccinated	O78	30	29	96.7%
	O1	30	16	53.3%
	O2	30	14	46.6%
Control	O78	10	4	40%
	O1	10	4	40%
	O2	10	3	30%

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

Turkeys Groups	Type of challenge strain	Number of positive samples for *E. coli* recovery			
		Heart blood	Liver	Spleen	Bone marrow
Vaccinated	O78	6/30 (20%)	5/30 (16.7%)	5/30 (16.7%)	4/30 (13.3%)
	O1	18/30 (60%)	18/30 (60%)	17/30 (56.7%)	16/30 (53.3%)
	O2	20/30 (66.7%)	20/30 (66.7%)	19/30 (63.3%)	18/30 (60%)
Control	O78	8/10 (80%)	7/10 (70%)	8/10 (80%)	6/10 (60%)
	O1	8/10 (80%)	8/10 (80%)	7/10 (70%)	6/10 (60%)
	O2	8/10 (80%)	7/10 (70%)	7/10 (70%)	5/10 (50%)

4. 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 (Barnes et al., 2008 and Russell, 2003). Although previous attempts to develop a vaccine have not been very successful, vaccination is still considered the most effective way of controlling the disease (Altekruse et al., 2002; Blanco, 1997). Therefore, a number of experimental vaccines have been developed for the prevention of colibacillosis in poultry. However due to repeated outbreaks of this organism in the field, there is a need for an effective vaccine (Vaez Zadeh et al., 2004). The results of this study have direct implication in considering live attenuated *E. coli* vaccine prepared from O78 strain for chickens as a vaccine option to protect turkeys against *E. coli* infections.

The humeral immune response of live attenuated *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* strain O78 in sera of vaccinated birds increased from (6) pre-vaccination to reach (343) at 5th weeks post vaccination, while the control un-

vaccinated birds showed steady levels (7-9). These results agreed with Erganis and Hadimli (2002) who used the micro-agglutination test for estimation of *E. coli* antibody titers. The antibody titers in pullets vaccinated with *E. coli* vaccine was found to be ~0.5-3.6 times higher than non-vaccinated controls. The protection percentage of turkeys after challenge with homologous (O78) or heterologous (O1 or O2) *E. coli* by the intra-air sac route were summarized in Table (2), protection rate in the vaccinated groups was (96.7) in case of homologous challenge with O78 that was significantly higher than those recorded in the vaccinated groups (53.3% and 46.6%) against heterologous challenge with *E. coli* O1 and O2 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 homologous infection through the induction of sufficient antibody titers. These results all agree with La Ragione et.al (2013) who found that chickens and turkeys vaccinated with an O78 live attenuated vaccine were protected against a challenge at 6 wk of age by homologous virulent APEC strain. Also (Dho-Moulin and Fairbrother, 1999) proved that live attenuated *E. coli* vaccine give sufficient protection against infection with homologous strains, but protection against heterologous strains is less efficient.

Mortality was recorded and surviving turkeys were euthanized after the challenge and examined for macroscopic lesions as shown in table (3) which revealed that *E. coli* could be recovered from vaccinated challenged turkeys with the ratio ranged from 13.3%-20% from the bone marrow, spleen, liver and heart blood on the 8th day post homologous challenge while these ratios were ranged in case of heterologous challenge from 53.3%-66.7 % from same organs of vaccinated challenged turkeys. These results came confirming by Abdul-Aziz and EL-Sukhon (1998) 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.

Therefore, after the analysis of the immune responses induced by commercial APEC O78-based live-attenuated vaccine and its association with protection. We verified that the vaccine was protective against homologous intra-air sac challenge with O78 strain but con not protect turkeys against heterologous challenge.

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