Original Article

Evaluation of the Efficacy of Humoral Immunity Response of Killed Oil Adjuvant *Escherichia coli* Vaccine in Layer Chicken against Avian *E. coli* Serotype O_{78} Infection

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ABSTRACT

Colibacillosis, a major bacterial disease affecting chickens and turkeys, is caused by avian pathogenic Escherichia coli (APEC). The disease has significant economic implications for poultry farms, resulting in increased mortality, reduced body weight, and higher feed conversion ratios (FCRs). These factors can lead to higher carcass condemnation at slaughterhouses.In recent years, significant progress has been made in the development of vaccines against APEC, including both homologous and heterologous vaccines. The present study employed mineral oil as an adjuvant for inactivated E. coli, which was inoculated via injection route to layer chickens.At 28 days of age, 60 birds were subsequently divided into six experimental groups of 10 chickens per group. The control group did not receive the E. coli vaccine, whereas the five treatment groups were vaccinated subcutaneously with a formalin-inactivated, mineral-oil adjuvant E. coli vaccine containing an isolate of E. coli serotype O78. The T1, T2, T3, T4, and T5 groups were vaccinated at The T1, T2, T3, T4 and T5 groups were inoculated at 28 days of age with 0.2 ml (8 x106, 16 x106, 33 x106, 66 x106 and 133 x106 cfu/ml) of E. coli O78, respectively.Ten weeks after inoculation, the levels of IgG antibody titres against E. coli were evaluated using an ELISA method. The results demonstrated a significant increase in IgG antibody titres in the immunised birds compared to the unimmunised control group ($P < 0.05$). Anti-IgG antibodies increased on a weekly basis after injection in most vaccinated groups up to four weeks.In conclusion, the prepared E. coli vaccine at the Razi Institute, Shiraz branch, induced high levels of immune responses in the vaccinated group, as revealed by ELISA. In order to elicit a substantial immunological stimulus, it is recommended that all chickens in the experimental group receive a booster dose four weeks after the initial immunization.

Keywords: APEC, Colibacillosis, Immunization, Vaccine.

1. Introduction

Colibacillosis is a disease caused by infection with a strain of Escherichia coli (1). Escherichia coli is a gram-negative, rod-shaped bacterium that is normally found in the intestine of poultry and other vertebrates. Though many E. coli are not pathogenic, some have acquired virulence factors, greatly increasing their capacity to cause disease (2). Colibacillosis results in a localised or systemic infection caused by avian pathogenic Escherichia coli (APEC) (3). Syndromes associated with colibacillosis can vary, and include acute fatal septicemia, airsacculitis, pericarditis, perihepatitis, peritonitis, and lymphocytic depletion of the bursa and thymus. (4); salpingitis and cellulitis (5).In laying hens, peritonitis and salpingitis are prevalent, whereas disease in young chicks may include omphalitis (yolk sac infection) or swollen head syndrome (4).Previously, most APEC isolates were assigned to three main serogroups: O1, O2, and O78; however, it has been demonstrated that there is a significant diversity in serogroups of APEC causing colibacillosis (6).Colibacillosis is one of the most prevalent and economically detrimental bacterial diseases of poultry worldwide (7), resulting in multimillion-dollar losses annually that affect numerous aspects of poultry production. The challenge of controlling colibacillosis is compounded by the pervasive antimicrobial resistance among APEC isolates (8), limitations on the use of antimicrobial agents in poultry, and the absence of vaccines that offer comprehensive protection against all types of APEC isolates associated with colibacillosis. There are reports on Escherichia coli autogenous vaccines, predominantly utilized in breeder flocks; however, evidence on the efficacy of such vaccines in terms of mitigating E. coli infections is scarce. The present study was therefore undertaken to evaluate the efficacy of humoral immunity response in layer chickens vaccinated with an Escherichia coli vaccine developed at the Shiraz Razi Institute.

2. Materials and Methods

2.1. Animals

A total of sixty unvaccinated layer chickens (Hy-line) of mixed sex were obtained on the day of hatching from a commercial hatchery of the Razi Vaccine and Serum Research Institute (Shiraz branch). These birds were then kept in a controlled area with free access to food and water at the poultry department of the Razi Vaccine and Serum Research Institute (Shiraz branch).

2.2. Isolation, Identification, and Serotyping

Poultry pathogenic *Escherichia coli* serotype O_{78} was obtained from the microbiology department of the Razi Vaccine and Serum Research Institute (Shiraz branch), this bacterium selected for this study was isolated in the laboratory from the hearts and livers of 4–8-day-old broiler chickens suffering from colibacillosis infection with perihepatitis and pericarditis. For isolation of *E. coli*, tryptic soy broth (Merck, Germany), MacConkey agar (MCA) and Eosin-methylene blue (EMB) agar were used as enrichment, differential and selective medium respectively. The enrichment, the MCA, and EMB agar were incubated at 37°C for 24 hr. The smooth, moist colonies having metallic sheen on EMB agar were randomly sub-cultured. The isolates were identified on the basis of their cultural, morphological and biochemical characteristics (9). Identification of *Escherichia coli* O_{78} was done by polymerase chain reaction (PCR) in experimentally infected specimens (10). The PCR amplifications were conducted in a 25 µL reaction volume containing 12.5 µL of Master Mix 2X AMPLIQON (Denmark), 1.5 U Taq polymerase, 1.5 Mm $MgCl_2$,1 µL of each primer, PCR buffer, and RNase-free water. To confirm *Escherichia coli* strain O_{78} from PCR test using forward and reverse primers OG_{78} related to wzx gene was used based on the presence of 992 bp fragment (Table 1; Figure 1). Polymerase chain reaction was done in an Eppendorf thermocycler (Germany) during 30 cycles with denaturing temperature of 94°C for one minute, annealing temperature of 55°C for 40 seconds, and an extension temperature of 72°C for 1 minute was done. The polymerase chain reaction product was electrophoresed on a 1% agarose gel (Figure 1). Poultry pathogenic Escherichia coli serotype O_{78} was obtained from the microbiology department of the Razi Vaccine and Serum Research Institute (Shiraz branch). The bacterium was isolated in the laboratory from the hearts and livers of 4–8-day-old broiler chickens suffering from colibacillosis infection with perihepatitis and pericarditis. The isolation of E. coli was performed using tryptic soy broth (Merck, Germany), MacConkey agar (MCA) and eosin-methylene blue (EMB) agar as enrichment, differential and selective media, respectively. These were incubated at 37°C for 24 hours. Smooth, moist colonies with a metallic sheen on EMB agar were then randomly sub-cultured. The isolates were identified on the basis of their cultural, morphological and biochemical characteristics (9). The identification of Escherichia coli O78 was done by polymerase chain reaction (PCR) in experimentally infected specimens (10). The PCR amplifications were conducted in a 25 µL reaction volume containing 12.5 µL of Master Mix 2X AMPLIQON (Denmark), 1.5 U Taq polymerase, 1.5 Mm MgCl2, 1 µL of each primer, PCR buffer, and RNase-free water. The confirmation of the Escherichia coli strain O78 from the PCR test using forward and reverse primers OG78 related to the wzx gene was based on the presence of a 992 bp fragment (Table 1 & Figure 1). The polymerase chain reaction (PCR) was performed in an Eppendorf thermocycler (Germany) for 30 cycles, with a denaturing temperature of 94°C for one minute, an annealing temperature of 55°C for 40 seconds, and an extension temperature of 72°C for one minute.The polymerase chain reaction product was then subjected to electrophoresis on a 1% agarose gel (Figure 1).

2.3. Antigens

The PCR confirmed isolate was proliferated in tryptic soy broth (Merck, Germany) (24 h/37°C). After incubation, cell suspension was inactivated with 0.3% formaldehyde at

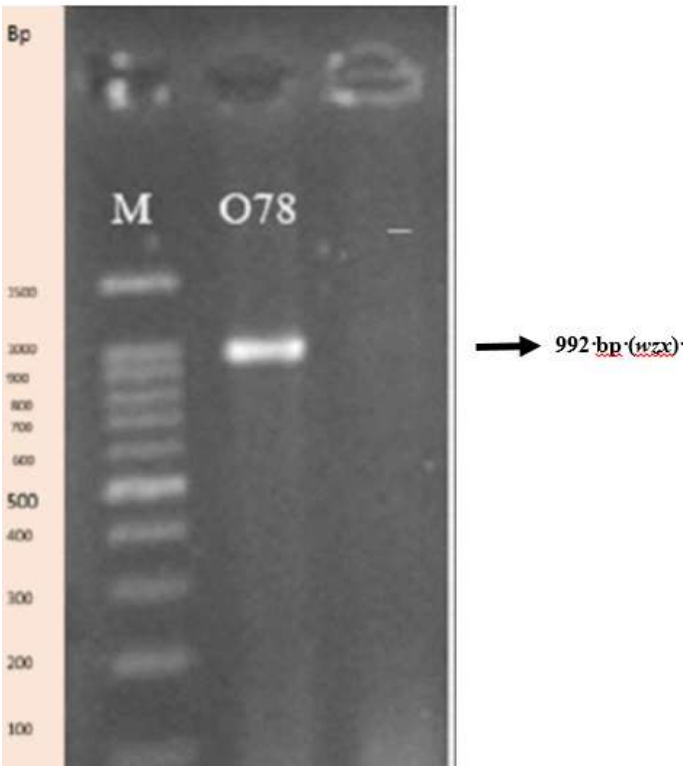

Figure 1. Molecular PCR test to confirm *Escherichia coli* O_{78}.

Table 1. The exclusive primer sequences used for molecular confirmation of Escherichia coli bacteria by PCR.

Gene	Primer sequence (5′-3′)	Size (bp)	Reference
wzx	Forward: GGTATGGGTTTGGTGGTA	992	
	Reverse: AGAATCACAACTCTCGGCA		Liu B. et al. Vet Microbiol.2010

37°C for 24 h. The cells were separated through centrifuging (5,000 g/20 min), and the pellet was resuspended in phosphate-buffered saline (PBS, pH 7.2). A suspension was adjusted to the turbidity standard 2 $x10^9$ bacteria / ml. The PCR-confirmed isolate was then propagated in tryptic soy broth (Merck, Germany) at 37°C for a duration of 24 hours. Following this incubation period, the cell suspension was inactivated using 0.3% formaldehyde at 37°C for a further 24 hours. Subsequently, the cells were separated through a process of centrifugation (5,000 g/20 minutes), after which the pellet was resuspended in phosphate-buffered saline (PBS, pH 7.2). The suspension was then adjusted to a turbidity standard of 2 x109 bacteria/ml.

2.4. Adjuvants and Vaccines

The production of oil-adjuvanted vaccines involved the preparation of standard W/O emulsions, with Montanide™ ISA 70 being utilized as an example. These emulsions were subjected to gentle agitation in a mixer at room temperature. The subsequent step involved the combination of the aqueous phase, with a ratio of 70:30 (w/w, adjuvant: antigen or PBS), as recommended by the adjuvant manufacturer, Seppic (France).

2.5. Immunization of Chickens

A total of 60 chickens were randomly allocated to six treatment groups (ten birds each), namely the control group, and the groups designated T1, T2, T3, T4, and T5. The birds in the control group were not given E. coli vaccines; those in the groups T1, T2, T3, T4, and T5 were vaccinated at 28 days of age with 0.2 ml of the vaccine (8 x106, 16 x106, 33 x106, respectively). 06, 66 x106, 133 x106 cfu/ml per dose of E. coli O78, respectively. The vaccine was formulated with formalin-inactivated, mineral-oil-adjuvanted E. coli (serotype O78) vaccine subcutaneously.

2.6. Serum Titer of Anti- *E. coli* O_{78}Antibody

In this study, a total of 60 chickens were randomly selected for blood sample collection from the brachial vein prior to immunization. Subsequent to this, additional blood samples were collected from 10 chickens per group on a weekly basis, continuing for a period of 10 weeks following immunization. The separated sera were then stored at -20°C until analysis of the antibody responses against E. coli O78

using an indirect enzyme-linked immunosorbent assay (ELISA).

2.7. Serology

The antigen response following challenge with E. coli O78 was defined by Enzyme-Linked Immunosorbent Assay (ELISA). As previously mentioned, the samples were subjected to centrifugation at 3500×g for 10 minutes, after which the serum fraction was transferred to separate tubes and stored at -20 °C until ELISA evaluation of antibody content. The ELISA assay was performed on 96-well plates, which were coated overnight at 4 °C with 0.5 µg of whole cell sonicates of E. coli O78. The sonicates were then diluted with carbonate-bicarbonate buffer (pH 9.6). Subsequent to this, each well was washed by adding 300 µL of washing buffer (PBS + 0.05% Tween 20). Following this, 200 µL of bovine serum albumin (BSA) was added as a blocking solution and the plates were left to stand at room temperature for 2 hours. After this time, the blocking solution was removed and the plates were washed. Following this, 100 µL of serum samples were added to each well. After incubation and washing, 100 µL polyclonal goat anti-chicken IgG diluted 1:4000 in diluting buffer were added to each well and the plates incubated for 1 h at 37 °C. Then, the plates washed. To ascertain the binding, 100 µL of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was added to each well and incubated for 15 minutes. The reaction was then halted by the addition of 100 µL 1 M H2SO4, and the optical density was measured at 450 nm using a spectrophotometer (BioTek Instruments).

3. Results

The results of the serological tests are displayed in Table 2. The data demonstrated a significant increase in IgG antibody titers in the immunized birds in comparison to the unimmunized control group ($P < 0.05$; Figure 2). The E. coli vaccine, which was developed in Shiraz at the Razi Institute, elicited higher IgG titers in most weeks (Figures 3 and 4). No differences in antibody titers against Escherichia coli were found between the experimental and control groups at weeks 5 to 10 (Figures 4 and 5). The mean OD titer of sera from the vaccinated groups was generally higher than that of the control groups (Figure 2). However, no differences were found between the T5 and T1 groups, but significant differences were found in the T5 group compared to the corresponding values for the T2, T3, and T4 groups.

Table 2. LS mean±SE of antibody titer against *Escherichia coli* (SP %) obtained in Hyline selected laying chickens at different weeks post-immunization.

Traits	Treatment						*P*-value		
	Control	T_1	T_2	T_3	T_4	T_5	Treatment	Time	Treatment × time
	1.0499±0.043^{c}	1.5800±0.046^{ab}	1.6226±0.043^{b}	1.6189±0.044^{b}	1.5900± 0.043^{b}	1.7488±0.045^{a}	<.0001	<.0001	0.0293

T_1, T_2, T_3, T_4, and T_5 groups were vaccinated at 28 days of age with 0.2 ml (8 x10^6, 16 x10^6, 33 x10^6, 66 x10^6, and 133 x10^6 cfu/ml) per dose of *E. coli* O_{78} respectively. Means with different letters differ significantly ($P \leq 0.05$).

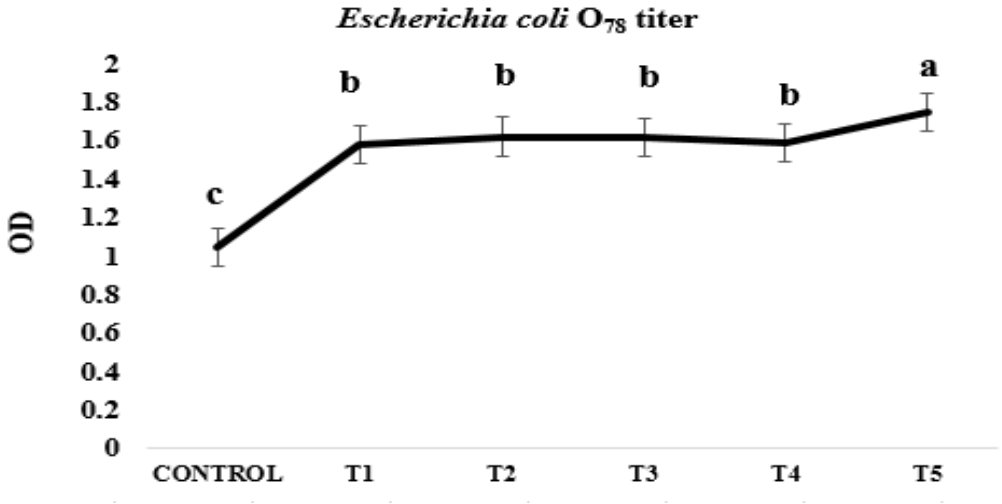

Figure 2. Mean titre of OD of sera of laying chickens following vaccination with *Escherichia coli* O_{78} which produced in Shiraz, Razi Institute. T_1, T_2, T_3, T_4, and T_5 groups were vaccinated at 28 days of age with 0.2 ml (8 x10^6, 16 x10^6, 33 x10^6, 66 x10^6, and 133 x10^6 cfu/ml) per dose of *E. coli* O_{78} respectively. Means with different letters differ significantly ($P \leq 0.05$).

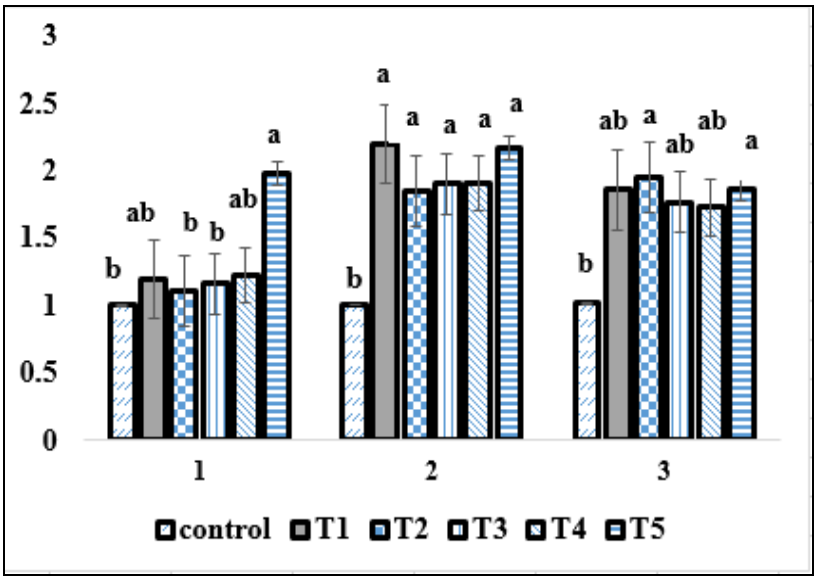

Figure 3. Effect of treat × time (wk) interaction on antibody titers against *E. Coli* (SP%) in Hyline selected laying chickens at different weeks post-immunization. T_1, T_2, T_3, T_4, and T_5 groups were vaccinated at 28 days of age with 0.2 ml (8 x10^6, 16 x10^6, 33 x10^6, 66 x10^6, and 133 x10^6 cfu/ml) per dose of *E. coli* O_{78} respectively. [a,b]Within each week, least squares means with different letters differ significantly ($P \leq 0.05$).

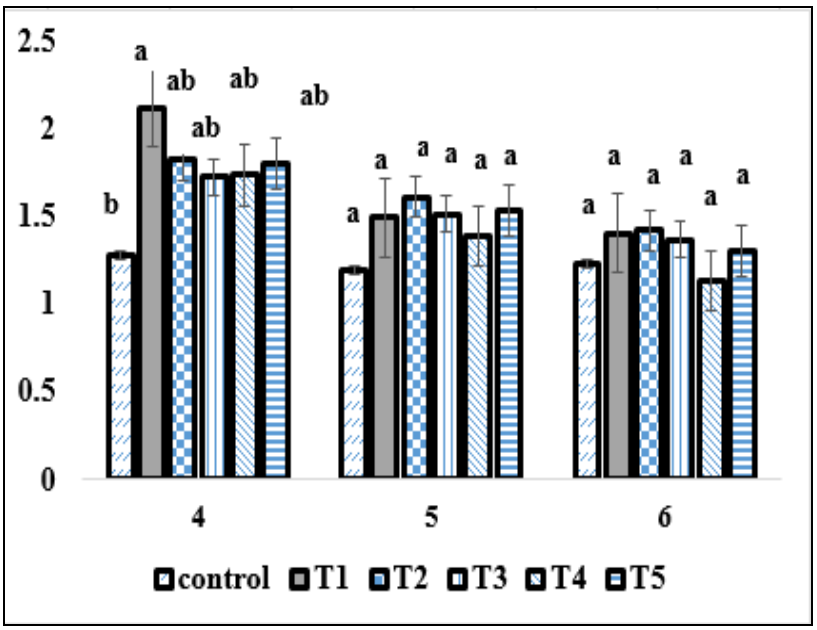

Figure 4. Effect of treat × time (wk) interaction on antibody titers against *E. Coli* (SP%) in Hyline selected laying chickens at different weeks post-immunization. T_1, T_2, T_3, T_4, and T_5 groups were vaccinated at 28 days of age with 0.2 ml (8 x10^6, 16 x10^6, 33 x10^6, 66 x10^6, and 133 x10^6 cfu/ml) per dose of *E. coli* O_{78} respectively. [a,b]Within each week, least squares means with different letters differ significantly ($P \leq 0.05$). No differences were found at wk 5 and 6.

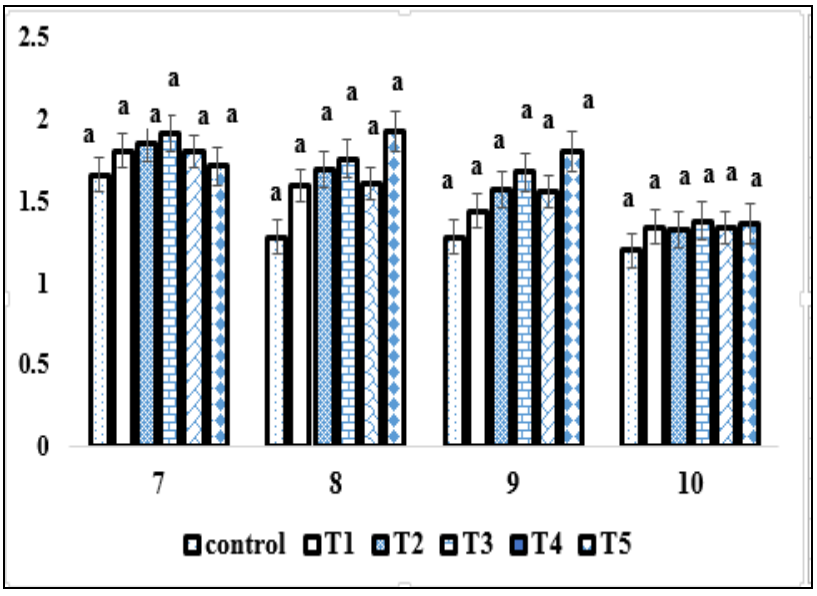

Figure 5. Effect of treat × time (wk) interaction on antibody titers against *E. Coli* (SP%) in Hyline selected laying chickens at different weeks post-immunization. T_1, T_2, T_3, T_4, and T_5 groups were vaccinated at 28 days of age with 0.2 ml (8 x10^6, 16 x10^6, 33 x10^6, 66 x10^6, and 133 x10^6 cfu/ml) per dose of *E. coli* O_{78} respectively. No differences were found at wk 7, 8, 9, and 10.

4. Discussion

As before mentioned, colibacillosis is an economically important for the avian industry, which causes multimillion-dollar losses annually (11). Preparing effectual colibacillosis control measures highly favorable. Colibacillosis control mostly concentrates on management methods made biosecurity plan for reduce preparing conditions among production birds, such as mycoplasma or viral infections (11). Although, management methods that have reduced colibacillosis in the past may not be as efficient in the future. Moreover, use of antimicrobial factors in animal production is being given close investigation at this time with restriction being placed on the use of certain therapeutic factors in avian production (12). Finally, control of avian colibacillosis using vaccines in specified conditions may demonstrate favorable. Up to the present time, vaccines formulated to impede avian colibacillosis have been faced with mixed results. Vaccines against APEC of different serogroups have been generated. Killed bacterial vaccines, including autogenous vaccines, sub-unit vaccines, and live-attenuated vaccines are in use for prevention of APEC (13, 14, and 15). As previously stated, colibacillosis represents a significant economic burden on the avian industry, causing losses amounting to millions of dollars on an annual basis (11).The development of effective control measures for colibacillosis is of paramount importance. These measures primarily focus on the implementation of biosecurity plans aimed at mitigating risk factors such as mycoplasma or viral infections in production birds (11). However, it should be noted that the efficacy of management methods employed in the past to control colibacillosis may not be guaranteed in the future. Furthermore, the use of antimicrobial factors in animal production is currently under close investigation, with restrictions being placed on the use of certain therapeutic factors in avian production (12). Finally, the use of vaccines to control avian colibacillosis in specified conditions may prove to be advantageous. To date, vaccines formulated to impede avian colibacillosis have yielded equivocal outcomes. Vaccines against APEC of different serogroups have been generated, including killed bacterial vaccines, such as autogenous vaccines, sub-unit vaccines, and live-attenuated vaccines, which are in use for the prevention of APEC (13, 14, and 15). A great number of these vaccines have only been effective against homologous challenge. The present report is to address the efficacy of humoral immunity response of killed oil adjuvant *Escherichia coli* vaccine in layer chicken against avian *E. coli* infection. The titer in IgG antibodies in the experimental groups were higher compared to the control group. Increased titer in IgG antibodies was more pronounced in T_5 birds which receiving the highest number of bacteria per mL. Śmiałek et al. (16) showed that the use of live, attenuated, aroA gene-deleted vaccine against colibacillosis cause a reduction in the amount of *E. coli* in the population of avian. These results are in agreement with findings of El-Mawgoud et al. (17), who showed that live *E. coli* spray vaccination of broiler chickens decreased the APEC colonization in the liver and heart of the birds after *E. coli* infection. Also Roland et al. (18) reported the use of live, attenuated, *E. coli* vaccine derived O_{78} LPS, protected the white leghorn chicks against avian pathogenic *E coli* O_{78} strain. In the present study killed *Escherichia coli* vaccine was used, because the production process of live vaccine requires a lyophilizer and the facility of live vaccine preparation was not available in Shiraz, Razi Institute. Killed *E. coli* vaccines, including autogenous vaccines, protect only against homologous challenge (5, 19). A significant proportion of these vaccines have only demonstrated efficacy against homologous challenge (Smith et al., 2022).The present report aims to address the efficacy of humoral immunity response of killed oil adjuvant Escherichia coli vaccine in layer chicken against avian E. coli infection.The experimental groups exhibited higher titres of IgG antibodies in comparison to the control group, with the increase being more pronounced in T5 birds, which received the highest number of bacteria per mL. In a related study, Śmiałek et al. (16) demonstrated that the utilisation of a live, attenuated, aroA gene-deleted vaccine against colibacillosis resulted in a decline in the population of E. coli. These results align with the findings of El-Mawgoud et al. (17), who demonstrated that live E. coli spray vaccination of broiler chickens led to a reduction in APEC colonization in the liver and heart of the birds following E. coli infection.Additionally, Roland et al. (18) reported that the use of a live, attenuated E. coli vaccine derived from O78 LPS provided protection to white leghorn chicks against the avian pathogenic E. coli O78 strain. In the present study, a killed Escherichia coli vaccine was utilised due to the requirement of a lyophilizer for the production of a live vaccine, which was not available at the Shiraz Razi Institute.It is noteworthy that killed E. coli vaccines, including autogenous vaccines, offer protection exclusively against homologous challenges (5, 19). Finally, the use of killed vaccines requires knowledge of the serotype(s) of *E. coli*, which are included in the substantial outbreaks. Unfortunately, vaccination with killed vaccines may stress the birds, and the adjuvants may induce local reactions (20). Sub-unit vaccines may provide an extensive protection against more serotypes of APEC. However, the disadvantage of stress to birds during vaccination, and side effects of adjuvants have also been recorded for the sub-unit vaccine (20). Vaccination of broiler parents by the inactivated subunit Nobilis® *E. coli* was found to reduce the number of sequence types of *E. coli* isolated from diseased broiler parents in the vaccinated flock compared to the control group, which shows a potential for sub-unit vaccine to make less the outbreak of specific clones of APEC (21). The live Poulvac® *E. coli* vaccine includes an aroA mutant of a strain of serotype O78:K80 and ST23, but protection is not limited to this specific serotype and sequence type (22). Recent experimental studies have combined vaccination with live attenuated *E. coli* vaccine with autogenous vaccines and it seems possible to take a

synergy of protection (23). The investigation of Kariyawasam et al. (24) revealed that collected IgY from eggs took from hens under different vaccination programs could cause passive maternal protection of day-old chicks when *E. coli* was used for challenge compared to the control groups, documenting that vaccination of parents may transfer the immunity to the chicks, under experimental conditions. Based on the results of this study the application of killed oil adjuvant *Escherichia coli* vaccine which produced in Shiraz, Razi Institute had greater efficacy in rising IgG titers in layer hens in comparison with unvaccinated group; however, to evoke immunological response, the second immunization is suggested four weeks after the first immunization. Finally, it is imperative to acknowledge the necessity of understanding the serotype(s) of E. coli implicated in substantial outbreaks, particularly in the context of the utilization of killed vaccines. It is noteworthy that vaccination with killed vaccines has the potential to exert stress on birds, and the adjuvants employed may elicit local reactions (20). Conversely, sub-unit vaccines offer the advantage of providing extensive protection against a broader spectrum of APEC serotypes. However, it should be noted that stress to birds during vaccination and side effects of adjuvants have also been recorded for the sub-unit vaccine (20). The vaccination of broiler parents with the inactivated subunit Nobilis® E. coli has been demonstrated to reduce the number of sequence types of E. coli isolated from diseased broiler parents in the vaccinated flock when compared to the control group. This finding suggests a potential for the sub-unit vaccine to mitigate the outbreak of specific clones of APEC (21). The live Poulvac® E. coli vaccine incorporates an aroA mutant of a strain of serotype O78:K80 and ST23, but protection is not limited to these specific serotypes and sequence types (22).Recent experimental studies have combined vaccination with live attenuated E. coli vaccines with autogenous vaccines, and it seems possible to achieve a synergistic effect in terms of protection (23). The investigation by Kariyawasam et al. (24) revealed that IgY collected from eggs obtained from hens under different vaccination programs could elicit passive maternal protection in day-old chicks when E. coli was used for challenge, in comparison to the control groups. This suggests that vaccination of parents may transfer immunity to chicks under experimental conditions. The findings of this study indicate that the employment of a killed oil adjuvant Escherichia coli vaccine, which was produced in Shiraz at the Razi Institute, resulted in a higher level of IgG titers in layer hens when compared to the unvaccinated group. However, to elicit an immunological response, it is recommended that a second immunization be administered four weeks after the initial immunization.

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Authors' Contribution
Study concept and design: A.S; M.H.H; F.S.
Acquisition of data: F.S; R.R; F.D; M.H; S.A; A.R.
Analysis and interpretation of data: F.S; M.H.
Drafting of the manuscript: F.S
Critical revision of the manuscript for important intellectual content: A.S; M.H; F.S.

Ethics
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Data Availability
The data that underpin the findings of this study are available upon request from the corresponding author.

References

1.Rawiwet V, Chansiripornchai N. The efficacy of Escherichia coli aroA-live vaccine in broilers against avian E. coli serotype O78 infection. The Thai Journal of Veterinary Medicine. 2009; 39(4):337-42.
2.Ghunaim H, Abu-Madi MA, Kariyawasam S. Advances in vaccination against avian pathogenic Escherichia coli respiratory disease: potentials and limitations. Veterinary microbiology. 2014 Aug 6; 172(1-2):13-22.
3.Lozica L, Morteza Gholi CS, Kela A, Lošić I, Horvatek Tomić D, Gottstein Ž. Autogenous Escherichia coli vaccine application as an innovative antimicrobial therapy in poultry farming—A case report. Vaccines. 2022 Sep 19; 10(9):1567.
4.Alber A, Stevens MP, Vervelde L. The bird's immune response to avian pathogenic Escherichia coli. Avian Pathology. 2021 Sep 3;50(5):382-91.
5.Li L, Thøfner I, Christensen JP, Ronco T, Pedersen K, Olsen RH. Evaluation of the efficacy of an autogenous Escherichia coli vaccine in broiler breeders. Avian Pathology. 2017 May 4; 46(3):300-8.
6.Ali A, I Abd El-Mawgoud A, M Dahshan AH, A EL-Sawah A, A Nasef S. Escherichia coli in broiler chickens in Egypt, its virulence traits and vaccination as an intervention strategy. Novel Research in Microbiology Journal. 2019 Aug 1; 3(4):415-27.
7.Filho TF, Fávaro Jr C, Ingberman M, Beirão BC, Inoue A, Gomes L, Caron LF. Effect of spray Escherichia coli vaccine on the immunity of poultry. Avian Diseases. 2013 Sep 1; 57(3):671-6.

8.Gyles CL. Antimicrobial resistance in selected bacteria from poultry. Animal health research reviews. 2008 Dec;9 (2):149-58.
9.Cowan ST. Cowan and Steel's manual for the identification of medical bacteria.1974.
10. DebRoy C, Roberts E, Fratamico PM. Detection of O antigens in Escherichia coli. Animal health research reviews. 2011 Dec;12(2):169-85.
11. Barnes HJ. Colibacillosis. Diseases of poultry. 2003:631-52.
12. Lynne AM, Foley SL, Nolan LK. Immune response to recombinant Escherichia coli Iss protein in poultry. Avian Diseases. 2006 Jun 1;50(2):273-6.
13. Bolin CA, Jensen AE. Passive immunization with antibodies against iron-regulated outer membrane proteins protects turkeys from Escherichia coli septicemia. Infection and immunity. 1987 May; 55(5):1239-42.
14. Roland K, Karaca K, Sizemore D. Expression of Escherichia coli antigens in Salmonella typhimurium as a vaccine to prevent airsacculitis in chickens. Avian diseases. 2004 Sep 1; 48(3):595-605.
15. Kariyawasam S, Wilkie BN, Hunter DB, Gyles CL. Systemic and mucosal antibody responses to selected cell surface antigens of avian pathogenic Escherichia coli in experimentally infected chickens. Avian Diseases. 2002 Jul 1; 46(3):668-78.
16. Śmiałek M, Kowalczyk J, Koncicki A. Influence of vaccination of broiler chickens against Escherichia coli with live attenuated vaccine on general properties of E. coli population, IBV vaccination efficiency, and production parameters-A field experiment. Poultry science. 2020 Nov 1; 99(11):5452-60.
17. Abd El-Mawgoud AI, El-Nahass ES, Shany SA, El-Sawah AA, Dahshan AH, Nasef SA, Ali A. Efficacy of live attenuated vaccine and commercially available lectin against avian pathogenic E. coli infection in broiler chickens. Veterinary Sciences. 2020 May 13;7(2):65.
18. Roland K, Curtiss III R, Sizemore D. Construction and evaluation of a Δcya Δcrp Salmonella typhimurium strain expressing avian pathogenic Escherichia coli O78 LPS as a vaccine to prevent airsacculitis in chickens. Avian diseases. 1999 Jul 1:429-41.
19. Landman WJ, Van Eck JH. The efficacy of inactivated Escherichia coli autogenous vaccines against the E. coli peritonitis syndrome in layers. Avian Pathology. 2017 Nov 2;46(6):658-65.
20. Christensen H, Bachmeier J, Bisgaard M. New strategies to prevent and control avian pathogenic Escherichia coli (APEC). Avian Pathology. 2021 Sep 3;50(5):370-81.
21. Gregersen RH, Christensen H, Ewers C, Bisgaard M. Impact of Escherichia coli vaccine on parent stock mortality, first week mortality of broilers and population diversity of E. coli in vaccinated flocks. Avian Pathology. 2010 Aug 1;39(4):287-95.
22. La Ragione RM, Woodward MJ, Kumar M, Rodenberg J, Fan H, Wales AD, Karaca K. Efficacy of a live attenuated Escherichia coli O78: K80 vaccine in chickens and turkeys. Avian diseases. 2013 Jun 1;57(2):273-9.
23. Koutsianos D, Gantelet H, Franzo G, Lecoupeur M, Thibault E, Cecchinato M, Koutoulis KC. An assessment of the level of protection against colibacillosis conferred by several autogenous and/or commercial vaccination programs in conventional pullets upon experimental challenge. Veterinary Sciences. 2020 Jun 30;7(3):80.
24. Kariyawasam S, Wilkie BN, Gyles CL. Resistance of broiler chickens to Escherichia coli respiratory tract infection induced by passively transferred egg-yolk antibodies. Veterinary microbiology. 2004 Mar 5;98(3-4):273-84.