Received: 19 April 2023, Accepted: 11 May 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 6, 1037-1043

Evaluating the Efficacy of Commercial *Escherichia coli* Killed Vaccine in Broiler Chickens

Omar Amen[1], Abeer H.M. El Hendy[2], Wael K. Elfeil[3], Abeer G. Hussein[4*]

[1]Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, 71511, Egypt.

[2]Pathology and Clinical Pathology, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt.

[3]Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt.

[4]Poultry Diseases Department, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt.

***Correspondence**
Corresponding author: Abeer G. Hussein
E-mail address: Abeergamal@ahri.gov.eg

Abstract

Escherichia coli infections cause significant financial losses for the worldwide chicken economy, so this research is being conducted to evaluate the efficacy of the inactivated multivalent *Escherichia coli* vaccine. Six groups were created from a total of 120 broiler chicks; the groups (1 and 3) were received *E. coli* inactivated vaccine at day 7 and challenged with O78 and O128 respectively, groups 2 and 4 were referred to as the control of challenge for the *Escherichia coli* strains O78 and O128. Groups 5 and 6 were maintained as the non-vaccinated and non-challenged control negative group and vaccinated non challenged group respectively. Challenged with *E. coli* strains of heterologous O128 and homologous O78 were given at 28 days old broilers by injection into the muscle of the thigh with 0.2 ml contained 10^7 CFU/bird. Moreover, the performance measurements of body weight per gram, feed intake and feed conversion ratio were measured; histopathological findings, clinical symptoms, rate of mortality and total intestinal counts of *Escherichia coli* were also examined. The study revealed that the vaccination of COLI-VAC™ *E. coli* provided a significant defense against the challenge between homologous O78 strain and heterologous O128 strain. Just used the aforementioned parameters, Groups 1 and 3 that vaccinated at seven days old and challenged at 28 days old chicks with homologous and heterologous (O78 and O128) serotypes of *E. coli* provided impressive protection against all those non-vaccinated chicks (Groups 2 and 4) that were challenged with O78 and O128 serotypes of *E. coli*.

KEYWORDS
Inactive vaccine, Body weight gain, Serotypes, COLI-VAC™.

INTRODUCTION

Avian pathogenic *Escherichia coli* (APEC) caused poultry colibacillosis often appears as just a secondary infection to the primary homeostatic abnormalities that might arise because of respiratory dysfunction, infected or non-infected immunosuppressive, or even other widespread infectious disorders (Dho-Mulin and Fairbrother, 1999).

One of the most prevalent bacterial infections in chicken production is colibacillosis. Although the infection on farms might be localized, it is typically systemic and causes a high mortality rate (Nolan *et al*., 2020; Mageiros *et al*., 2021).

Antimicrobials appear to be the fastest and most effective treatment, but they act universally, affecting the entire microbiota (Kairmi *et al*., 2022). Wide-spectrum antimicrobials are typically used as part of the treatment, frequently without any susceptibility testing first. Because of the excessive and ineffective application, extremely resistant and virulent strains of *Escherichia coli* are gradually identified, which results in an increase in resistance levels (Ievy *et al*., 2020; Christensen *et al*., 2021). Which increases the risk of resistance genes transmitting to people who eat various poultry products (Johnson *et al*., 2008; Popy *et al*., 2011).

Strict hygiene measures might be used to regulate APEC, or if infection develops, treatment will involve using the antibiotic of choice after performing a sensitivity test. However, many *E. coli* isolates that are multi-drug resistant in poultry have made bacterial resistance a large problem in many broiler farms (Ogunleye *et al*., 2008). This makes a new method of avoiding *E. coli* infection necessary. Nowadays, either inactivated vaccinations or live vaccines are effective at controlling this bacterial disease (La Ragione *et al*., 2013; Sadeyen *et al*., 2015). The most intriguing method for colibacillosis control outside of antibiotics is colibacillosis vaccination. *E. coli* infection in chicken has been evaluated using live, subunit, and inactivated vaccinations (Ghunaim *et al*., 2014). Since they contain multiple strains of *E. coli*, inactivated vaccines that are administered via injection also have strict homologous protection (Ghunaim *et al*., 2014). When challenged with *E. coli*, vaccines primarily induce cell-mediated immunity because, in comparison to humoral immunity and antibodies, this type of immunity is most crucial for the protection of birds (Sadeyen *et al*., 2015). Experimental studies have already indicated that inactivated *E. coli* vaccinations are effective. Inactivated vaccinations with various adjuvants were examined for effectiveness in a Canadian study, and the results showed that the challenged *E. coli* recovered less from vaccinated than from uninfected chicks (Gomis *et al*., 2007).

Yaguchi *et al*. (2009) found that when *E. coli* exposure of certain specific pathogen free chicks, clinical signs and counts of bacteria in chicken blood were successfully reduced by the administration of an inactivated liposomal *E. coli* vaccination. An-

ISSN: 2090-6277/2090-6269/

other study (Shehata *et al*., 2019) reported that after challenging specified pathogen free chicks, it was determined whether the formalin-inactivated *E. coli* vaccination, which contained seven different APEC serotypes, was effective. The effectiveness was evaluated using mortality, clinical symptoms, and seroconversion. The vaccinated birds were seen to be completely resistant when exposed to an O157:H7 challenge and when just partially protected following an O125 challenge. On other hand, researchers suggested that autogenous vaccines are used to prevent *E. coli* infections having insufficient protective effectiveness (Li *et al*., 2016). According to the researchers, this could be due to the use of a high infective dose or an insufficient amount of humoral stimulation to give protection against an *E. coli* challenge.

Protection developed from inactivated vaccine of *E. coli* need additional research because several aspects, such as the vaccine strain serotype, the type of adjuvant utilized, the technique utilized to inactivate *E. coli*, the administration and frequency route, and the chicks' age at the day of vaccination, which is crucial for vaccine protection (Sadeyen *et al*., 2015). After 21 days of immunization should offer sufficient protection, because the peak of *E. coli* infections at this age old (Dho-Mulin and Fairbrother, 1999). The changing of microbiota in broiler intestines may thus indirectly impact the effects of the chicken *E. coli* vaccinations. Immunization did consistently alter the microbiota of intestine, which may be accounted for the enhanced health of the intestine observed after vaccination in many fields. (Beirao *et al*., 2021).

The present study's objective was to show benefit of applying an inactivated vaccine against pathogenic *Escherichia coli* infection in broilers and to protect the broilers against colibacillosis due to the variety serotypes of *E. coli* in farms. In addition to its potential effects on the reduction of antibiotic use, it also effectively resolves the use of inactive vaccination as a different approach to antimicrobial therapy.

MATERIALS AND METHODS

Bacterial isolation

Two strains of *E. coli* ATCC 25922 serotype O78 and *E. coli* ATCC BAA-1704 seotype O128 *E. coli* serotypes were generously received from the Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Dokki, Giza, Egypt and were inoculated onto a medium of MacConkey agar, which was kept at 37°C for 24 hours. Using oxidase urea, sulfide-indole-motility (SIM), triple sugar iron (TSI), and citrate usage to identify the isolates, each strain was biochemically identified (Quinn et. al., 2002).

Inactivated Vaccine

COLI-VAC™ inactivated multivalent vaccine for immunization against *E. coli* 500MI (1000 Dose). Inactivated *E. coli* bacteria ≥ 3x 10^7 CFU/ each of O166, O126, O157-H7, O18, O78, O127 and hemolytic un-typed strains.

COLI-VAC™ manufactured by Middle East for Vaccines (ME-VAC™). Second Industrial Zone, Extension Part No. 21, 22, 24, 25. El Salhya El Gdeda, El Sharkya Governorate, Egypt.

Experimental designs and Vaccination

One hundred twenty day old chicks were separated into six groups, each group with 20 chicks, and housed in pens at the Faculty of Veterinary Medicine, Assiut University, for seven days prior to vaccination. Each group had two replicates, each with 10 chicks. A commercially available balanced food without any antibacterial or anticoccidial ingredients was fed to chicks.

At the 7th days of age chicks of groups 1, 3 and 6 were vaccinated by COLI-VAC™ inactivated multivalent vaccine 0.5 ml IM dose in the thigh muscle and groups 2 and 4 were kept as non – vaccinated while group 5 was kept as an unvaccinated negative control group. After 3 weeks post vaccination, groups 1 and 2 were challenged with strain O78, while groups 3 and 4 were challenged with strain O128, by injection into the thigh region of 0.2 ml containing 10^7 CFU of each (APEC) serotype according to Chaffer *et al*. (1997). Chicken group 6 was maintained as control group for vaccinations. All the challenged groups were maintained under supervision for a week. Clinical signs, mortality, and PM lesions were noted.

The total number of birds that died after being exposed to *E. coli* for seven days was recorded, and all birds that survived were necropsied and checked for the presence of clear Colibacillosis lesions.

Experimental parameters

Morbidity and mortality rates were calculated

Mortality rates were calculated at 7 days after the challenge, along with clinical colibacillosis symptoms and morbidity (ruffled feathers, nasal secretions, gasping and diarrhoea), as well as peritonitis, clouded air sacs whether their caseous exudate, pericarditis and perihepatitis were scored according to Charleston *et al*. (1996).

Feed conversion rate (FCR)

Feed conversion rate was calculated for each group by taking the total of weight per gram of food consumption per chickens over total weight gain per gram (including the increase in weight gain of birds that died throughout the time period) based to Sainsbury (1984).

Lymphoid organs and average body weight

Liver, bursa and spleen weights of the broilers at each group were calculated at 35 days old. Organs were measured and the ratios of their weights were determined (Verma *et al*., 2004).

Total E. coli count CFU

From five chicks in each group were collected one gram of cecum content and ten-fold multiple dilutions in phosphate-buffered dilution were made and amounts of 0.1 ml each of the dilution were dispersed on newly created media of EMB agar. The experiment was carried out at 37°C for 24 hours under aerobic conditions. The findings were calculated in CFU/ml and examination of bacteria (Pineda *et al*., 2012).

Histopathological examination

Towards the conclusion of the experiment, 5 birds from each group were sacrificed and samples were obtained from heart, lung, liver, spleen, and bursa of Fabricius and taken immediately for fixation in neutral buffered formalin. The fixed tissues were dehydrated in an ascending series of ethyl alcohol, cleared in methyl benzoate, and embedded in paraffin wax, sectioned at 4-5 µm thickness then stained with Haematoxylin and Eosin stain for light microscopic analysis in accordance with Bancroft and

Layton (2013).

This study was approved by ethical committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, according to the OIE standards for use of animals in research under the number 06/2023/0083.

Statistical analysis

Analysis of differences between groups was done using Duncan's multiple comparison Post Hoc tests and One Way ANOVA (Duncan, 1955). Analysis of statistical was carried out using the statistical software package SPSS for Windows (version 2016; SPSS Inc., Chicago, IL, USA). Between mean values statistical significance was proved at P≤ 0.05.

RESULTS

Clinical findings and lesions

Clinical findings for groups 2 and 4, showed clinical signs 3 days post challenge, which included decreased feed intake, weakened, ruffled feathers, depression, and brownish diarrhea. Other groups didn't show any clinical signs. PM lesions for chicks of groups 2 and 4 have lesions of pericarditis, fibrinopurulent airsacculitis and perihepatitis.

Mortality rate

Mortalities in groups 2 and 4; in Group 2, there were 4/20 and 2/20 dead chicks in the 2nd and 4th day post challenge respectively, while in Group 4 there were 5/20, 1/20 and 1/20 dead chicks in the 3rd, 4th and 5th day post challenge respectively and the other groups showed 0% mortality (Table 1).

Table 1. Results of the challenge test performed on groups of vaccinated and unvaccinated chicks.

Groups		Dead birds' number/ total number	Rate of protection
Group (1)	Vacc. / chall. O78	0/20	100%
Group (2)	O78 +ve control	20-Jun	70%
Group (3)	Vacc. / chall. O128	0/20	100%
Group (4)	O128 +ve control	20-Jul	65%
Group (5)	Control -ve	0/20	100%
Group (6)	Vaccinated	0/20	100%

Total E. coli count

There was a significant reduction of *E. coli* count between all vaccinated broilers compared to groups of challenged non-vaccinated. While all vaccinated groups had significant variation in total count of the *E. coli* compared with challenged control group (Table 2).

Lymphoid organs and average body weight

Broilers in G1, G3, G5 and G6 seemed to have significant increases in body weight (P≤ 0.05) compared with other groups. However, G2 and G4 had significant decrease in body weight (P≤ 0.05) compared with other groups, Broilers in G2 had a significant increase in liver weight, while G5 and G6 had significant decreases in liver weight (P≤ 0.05) compared with other groups (Table 3).

Table 2. Mean count of *Escherichia coli* ± SD separated from cecal (CFU/gram) in broilers exposed to O78 homologous and O 128 heterologous *E. coli* serotypes (7 days old after challenged).

Groups		CFU / Gram	
		Mean	SD
Group (1)	Vacc. / chall. O78	4.57E+06^{b}	6.24E+6
Group (2)	O78 +ve control	1.04E+08^{a}	1.37E+8
Group (3)	Vacc./ chall. O128	5.66E+06^{b}	9.01E+6
Group (4)	O128 +ve control	1.00E+08^{a}	1.33E+8
Group (5)	Control -ve	1.85E+05^{b}	3.01E+5
Group (6)	Vaccinated	8.67E+05^{b}	9.45E+5

$^{a-b}$Mean values followed with different superscripts within one column have statistical significance (P≤0.05).

Table 3. Body and relative organs weights at the end of experimental.

Group	Body weight	Liver	Spleen	Bursa
Group (1)	1964±54.3^{ab}	2.79±0.07^{ab}	0.11±0.00^{cd}	0.182±0.01^{a}
Group (2)	1806±110.8^{b}	2.97±0.1^{a}	0.19±0.00^{a}	0.187±0.01^{a}
Group (3)	1941±62.1^{ab}	2.76±0.05^{ab}	0.11±0.01^{cd}	0.180±0.01^{a}
Group (4)	1907±51.2^{b}	2.62±0.06^{bc}	0.13±0.01^{bc}	0.184±0.01^{a}
Group (5)	2003±39.1^{ab}	2.45±0.08^{c}	0.09±0.00^{d}	0.180±0.01^{a}
Group (6)	2171±102.4^{a}	2.46±0.09^{c}	0.15±0.01^{b}	0.195±0.00^{a}

$^{a-b}$Mean values followed with different superscripts within one column have statistical significance (P≤0.05).

Body weight gain, feed conversion rate and feed intake

Concerning final weight of body gain and feed conversion rate at the end of the 3rd week in gram after vaccination (1week after challenge) results indicated that control vaccinated (G6) showed the highest average body weight gain in gram (2139±44) and best FCR followed by control non-vaccinated (G5) which was (1964±7), then G1 which was (1925±25), then G3 which was (1901±9), while G2 and G4 were the lowest which were (1769.5±19.5) and (1869±15) respectively (Table 4).

Table 4. Performance of BWG, FI and FCR in all chicken groups.

Group	BWG	FI	FCR
Group (1)	1925±25^{bc}	2951±26.9^{a}	1.5403±0.0004^{b}
Group (2)	1769.5±19.5^{d}	2789±83.4^{b}	1.6017±0.0023^{a}
Group (3)	1901±9^{bc}	2926±48.1^{a}	1.5595±0.0134^{ab}
Group (4)	1869±15^{c}	2917±39.6^{a}	1.5624±0.0472^{ab}
Group (5)	1964±7^{b}	2984±33.9^{a}	1.5202±0.0140^{b}
Group (6)	2139±44^{a}	3004±19.8^{a}	1.4097±0.0207^{c}

$^{a-d}$Mean values followed with different superscripts within one column have statistical significance (P≤ 0.05); BWG= body weight gain; FI= feed intake; FCR= feed conversion rate.

Histopathological findings

The severity of histopathological lesions of the lung, liver, spleen, heart, and bursa are illustrated in Table 5. While the histopathological images of these organs in all groups are indicated in Figures 1-5. As indicated in Figure 1A, liver tissue of healthy chicks (control negative, G6) revealed that healthy hepatic architecture and cellular parenchyma. Also, liver of (non-challenged and vaccinated G5) showed normal hepatocytes with minimal leukocytes infiltration within sinusoids (Figure 1B). In contrast, the liver of *E. coli* O78 challenged bird (G2) revealed massive aggregation of mononuclear and heterophiles inflammatory cells, focal necrosis, congested blood vessels and dilated sinusoids (Figure 1C). While liver of challenged O78 and vaccinated G1 restored their normal hepatic architecture with mild perivascular leukocytes infiltration

and kupffer cells in dilated sinusoids (Figure 1D). In case of liver of *E. coli* O129 challenged G4, chickens revealed prominent peri-portal inflammatory cells (lymphocytes and heterophiles) infiltration, focal coagulative necrosis, dilated blood sinusoids and hemorrhage (Figure 1E). An improvement was observed in challenged O129 and vaccinated G3 showed mild focal periportal leukocytes aggregation and between hepatic parenchyma and vascular congestion (Figure 1F).

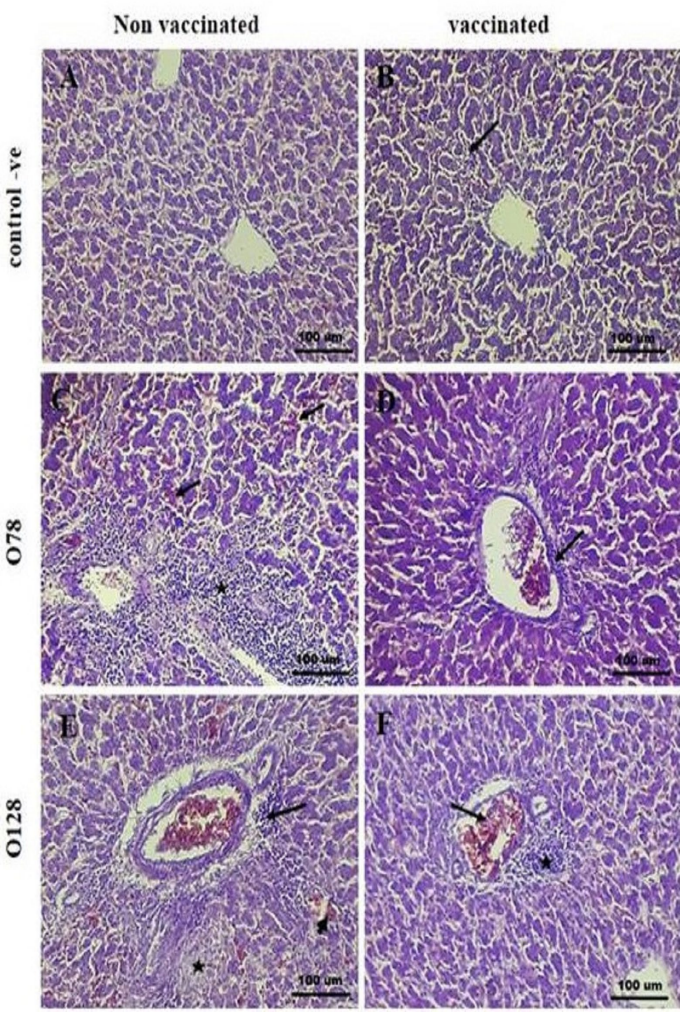

Fig. 1. Liver photomicrographs of chicken stained with hematoxylin and eosin stains. (A) G6 showing normal hepatic parenchymal cells and blood vessels. (B) G5 showing minimal inflammatory cells infiltration within blood sinusoid (arrow) (C) G2 showing severe mononuclear cells and heterophiles inflammatory cells infiltration (asterisk) dilated blood sinusoids and hemorrhage (arrows). (D) G1 showing mild periportal inflammatory cells infiltration (arrow) and mild congestion. (E) G4 showing degeneration and necrosis of hepatocytes (asterisk), edema and mononuclear cells and heterophiles inflammatory cells infiltration in periportal area (thin arrow), dilated blood sinusoids and hemorrhage (thick arrow). (F) G3 showing congestion (arrow) and inflammatory cells infiltration (asterisk). Scale bars = 200 µm & 100 µm.

As illustrated in Figures 2A-E, the lung of negative control G6 recorded that squamous epithelium lined healthy hexagonal parabronchial extending to healthy respiratory atria, divided by blood capillaries and fibrous septa (Figure 2A). In addition, the lung of G5 showed normal parabronchi and atria with minimal edema of septa (Figure 2B). In contrast, the lung of G2 showed heterophiles infiltration in parabronchial respiratory atria, congestion, and diffuse edema of inter alveolar septa (Figure 2C). However, the lung of G1 revealed normal parabronchi without exudation and intact respiratory atria, prominent interalveolar septa (Figure 2D). While the lung of G4 revealed swelling of parabronchial lumen beside heterophiles infiltration in respiratory atria, distended septa, and congested capillaries (Figure 2E). Also, the lung of G3 showed improvement of respiratory atria and parabronchi, edema in the septa between parabronchi with distended capillaries blood (Figure 2F).

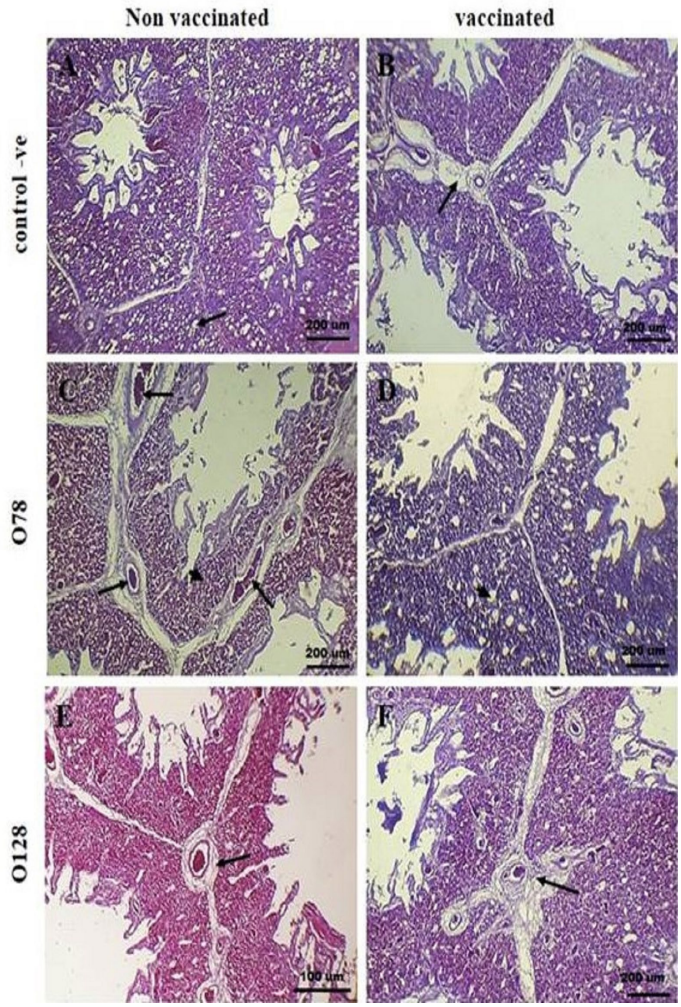

Fig. 2. Lung photomicrograph of chicken stained with H&E (A) G6 showing normal secondary bronchi and peribronchial air capillaries (arrow). (B) G5 showing mild edema within interstitial tissue (arrow). (C) G2 showing marked congestion of peribronchial blood vessels (arrows) edema and inflammatory cells infiltration within the parabronchus wall (arrowhead). (D) G1 showing normal peribronchial air capillaries (arrowhead). (E) G4 showing congested peribronchial blood vessel associated with marked interstitial tissue exudation (arrow). (F) G3 showing congested blood vessel surrounded by edema (arrow). Scale bars = 200 µm & 100 µm.

Regarding (Figures 3A-F), the heart of normal healthy chicks (G6) revealed normal myocardial fiber striations (Figure 3A). Also, heart of G5 showed normal cardiac muscle fiber striations (Figure 3B). On the other hand, the heart of G2 revealed severe necrotic degeneration of myocardium, loss of striations and leukocytic infiltration figure (3C). A noticeable improvement was observed in the heart of G3 which showed mild degeneration of myocardium and edema (Figure 3D). While the heart of G4 showed myocardial degeneration associated with inflammatory cell infiltration and hemorrhage (Figure 3E). However, the heart of G3 showed improvement of lesions associated with mild edema and congestion (Figure 3F).

As illustrated in Figures 4A-E, the spleen of negative control (G6) exhibited normal splenic architectures of white and red pulps with normal resting lymphoid follicles appearance (Figure 4A). Additionally, the spleen of G5 showed normal lymphoid follicle of white pulp with mild congestion of red pulp sinusoid (Figure 4B). In contrast, the spleen of G2 revealed prominent depletion of white pulp lymphoid follicles and most of the splenocytes showed deeply hematoxylin stained nuclei especially those in

the red pulp and vacuolar degenerative change of splenic blood vessels (Figure 4C). Interestingly, the spleen of G1 restores normal lymphoid follicle appearance with mild lymphoid depletion (Figure 4D). While the spleen of G4 showed noticeable depletion in lymphocyte population of white pulp and hemorrhage in the red pulp.

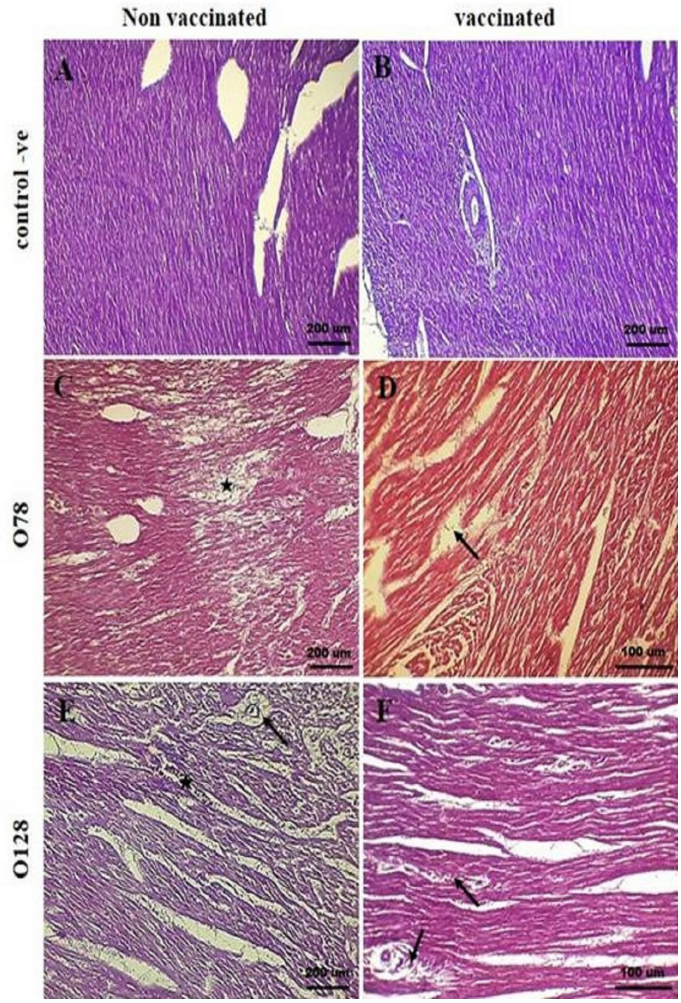

Fig. 3. Heart photomicrographs of chicken stained with H&E stain (A) G6 showing normal myocardial fibers. (B) G5 showing normal cardiac muscle fiber. (C) G2 showing lytic degeneration of muscle fiber and edema (asterisk). (D) G1 showing edema between myocardium muscle fiber (arrow). (E) G4 showing intermuscler hemorrhage (asterisk) and edema (arrow). (F) G3 showing mild intermuscler edema (arrows) scale bars = 200 µm &100 µm.

substantially lower average of *Escherichia coli* count (CFU/gram) when compared to unvaccinated groups. No mortality was observed in any of the injected birds using inactivated vaccine, which matched with Amer *et al*. (2015) who verified similar results that there no mortalities in vaccinated broilers experimental challenged with *E. coli* heterologous and homologous strains.

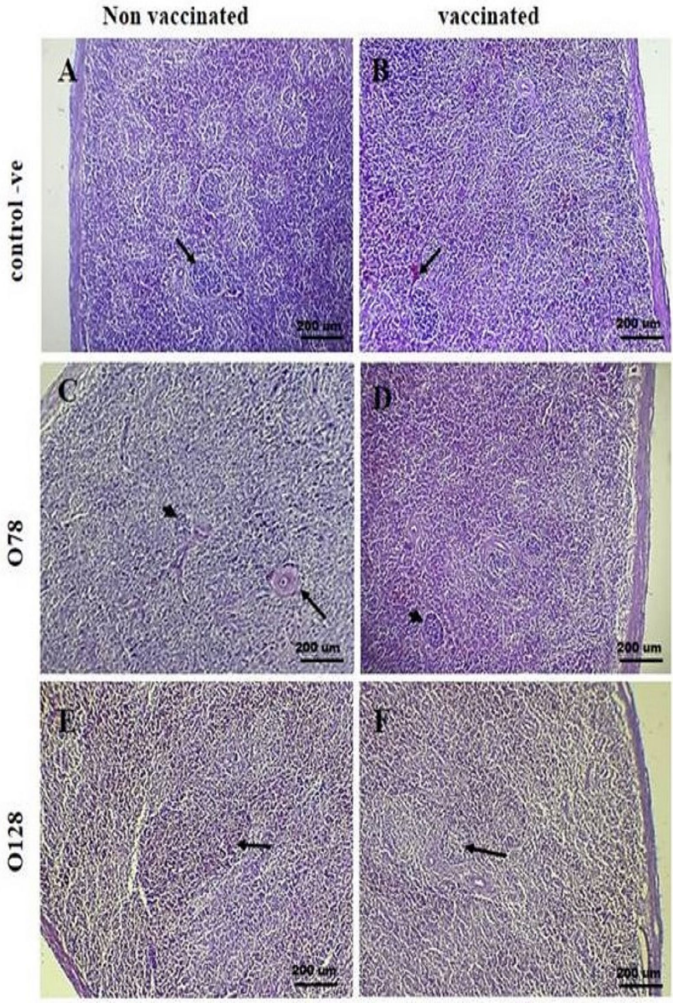

Fig. 4. Spleen photomicrograph of chicken stained with H&E stain (A) G6 showing normal resting lymphoid follicle (arrow). (B) G5 showing mild congested blood sinusoid (arrow). (C) G2 showing depletion of splenic lymphoid follicle (arrowhead) vacuolar degeneration in the wall of splenic blood vessel (arrow). (D) G1 showing small normal lymphoid follicle (arrowhead). (E) G4 showing depletion of lymphoid follicle (arrow) and congested blood sinusoids. (F) G3 showing mild depletion of splenic lymphoid follicle (arrow) and congested blood sinusoids. Scale bars = 200 µm &100 µm.

DISCUSSION

Pathogenic avian *Escherichia coli* is regarded as a major poultry pathogen. The widespread use of antimicrobial drugs for disease prevention and management are associated with an unprecedented rise in antibiotic-resistant organisms (Shrestha *et al*., 2017; Davis *et al*., 2018; Ibrahim *et al*., 2019). Antimicrobial medications can be used in this way to get eliminate of susceptible strains of bacteria that have genetic characteristics which make them resistant to them, which creates favorable conditions for the chosen strain's permanence and transmission in the farms (Castanon, 2007). In addition to, antibiotics used in animals can be harmful to humans in the form of resistance to antibiotics, necessitating the creation of new disease control strategies, including vaccination with an inactivated live vaccine (Anonymous, 2000).

In this study, the COLI-VAC™ inactivated multivalent vaccine is effective in using the intramuscular method to reduce the lesion against both homologous and heterologous infection may result in lower rates of morbidity and mortality as a result of a

So, the percentage of protection in chicks after being challenged with O78 and O128 strains of *E. coli* were showed in Table 1. The rate of protection in G2 was 70% while in G4 was 65% where it was less than those noted in control and vaccinated groups were 100% and these findings were consistent with El Jakee *et al.* (2016).

Data analysis reveals a significant difference in the experimentally vaccinated broilers have 7 days old, which challenged with O78 homologous serotype (G1) while getting greater body weight, best FCR and least gross lesions than that of other challenged groups. The greater protection specifically against challenged with O78 serotype of *E. coli* could be explained by similarities in antigen of serotypes between the strains of O78 in COLI-VAC™. The same findings were achieved by Gina *et al.* (2016) who reported that inactivated polyvalent vaccine of *E. coli* was valuable and effective as a vaccine for broiler colibacillosis caused by both heterologous and homologues strains.

About the final body weight gain in grams that appeared in Table 4, results revealed that vaccinated G6 showed the highest average body weight gain followed by control non-vaccinated

(G5), followed by G1 and G3, while G2 and G4 were had the lowest gain, this finding was explained by Amer *et al.* (2015) who stated that inactivated vaccine has an exceptional benefit as it substantially enhanced the body weight of the vaccinated chickens.

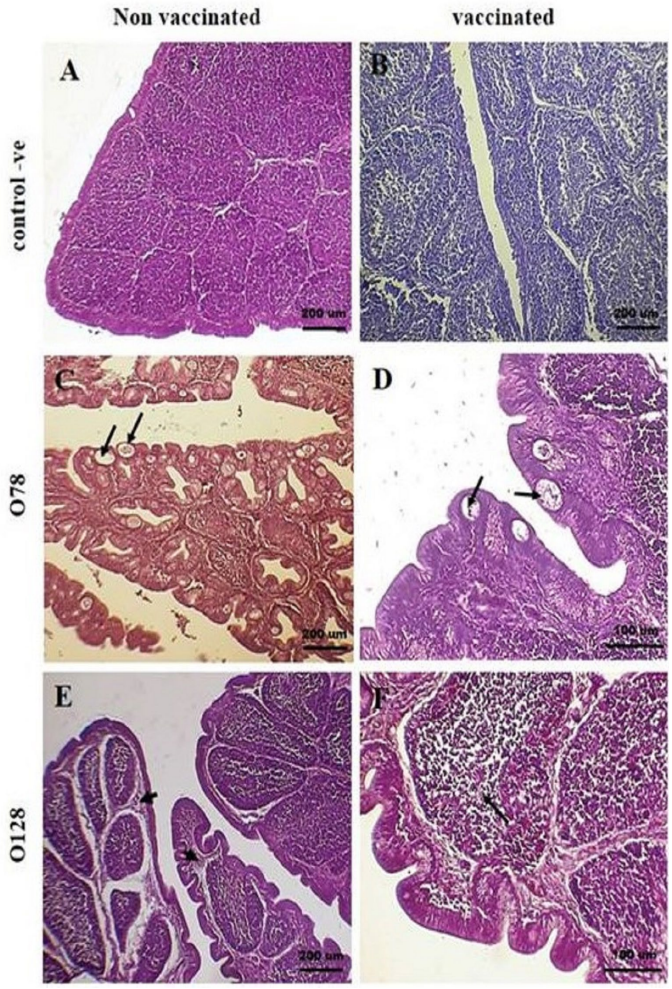

Fig. 5. Bursa of Fabricius photomicrograph of chicken stained with H&E (A) G6 showing normal epithelial lining cells and developed bursal follicles. (B) G5 showing minimal depletion of bursal follicles. (C) G2 showing hyperplasia of bursal epithelium associated with cysts formation (arrows). (D) G1 showing reduction in hyperplasia of bursal epithelium and few cysts formation (arrows). (E) G4 showing mild depletion bursal follicle and thickening of connective tissue stroma (arrows). (F) G3 showing mild hyperplasia of bursal epithelium and depletion bursal follicle (arrow). Scale bars = 200 µm &100 µm.

Feed conversion rate results which showed in Table 4 was better in G1 which received polyvalent inactivated vaccine and challenged with O78 strain followed by group vaccinated and challenged with O128 strain than groups non vaccinated and challenged with O78 and O128 strains this were parallel with results found by Melamed *et al.* (1991) who stated that preferable to use multiple circulating strains of the polyvalent inactivated vaccination to maximize protection against homologous and heterologous strain challenges., The results matched what Gina *et al.* (2016) had previously found.

Additionally, broilers vaccinated with COLI-VAC™ inactivated multivalent vaccine non-challenged G6 has been demonstrated high weight gain, protection, and best result of FCR than control group (G5) which is capable of improving weight gain and FCR in cases of non-infection. The obtained findings agreed with Amer *et al.* (2015) who reported that as compared to monovalent vaccination, groups receiving polyvalent inactivated vaccination had higher feed conversion rates and Gina *et al.* (2016) who stated that the use of inactivated *E. coli* vaccination resulted in a significant immune reaction and protection that can induce a long-lasting immunity.

Vaccinated and challenged chickens' lesions rates were reported in Table 5, post-challenge lesion scores, that were discovered that least postmortem effects were given in groups obtaining and challenged with inactivated polyvalent vaccine of *E. coli* (G1 and G3), while the highest lesion score were recorded in groups challenged with O78 and O128 *E. coli* strain (G2 and G4). Both control vaccinated groups of chicken and non-vaccinated non-challenged control group were found to be free of lesion score or PM lesions and these findings were comparable to Gina *et al.* (2016).

Table 5. Severity of the histopathological lesions in selected organs of all groups.

Organs / Groups	Liver	Lung	Heart	Spleen	Bursa of fabricius
G1 (vacc + O78)	+	+	+	+	+
G2 (O78)	+++	+++	+++	++	+++
G3 (vacc + O128)	+	+	+	+	+
G4 (O128)	++	++	++	++	++
G5 (vaccinated)	-	-	-	-	-
G6 (control –ve)	-	-	-	-	-

Liver: -: normal hepatic parenchyma, blood vessels and sinusoids; +: mild hepatocytes degeneration, inflammatory cells infiltration and congested B.V.; ++: moderate degree of hepatocytes degeneration, periportal inflammatory cells infiltration and sinusoidal congetion; +++: severe hepatocytes degeneration, necrosis and leukocytic aggregation (Coligranuloma). Lung: -: normal bronchi and peribronchial air capillaries; +: mild congestion, perivascular and interlobular edema; ++: marked serofibrinous exudates; heterophils infiltration and thicken the interlabour septum; +++: severe congestion, thickening with serofibfinous exudates, collapse of air capillaries and leukocytes infiltration. Heart: -: normal myocardial fibers; +: mild myocytes degeneration and edema; ++: moderate degree of myocytes degeneration and leukocytic infiltration; +++: marked myocardial fibers degeneration, interstitial edema and leukocytic infiltration. Spleen: -: normal splenic resting follicles; +: Mild lymphoid follicles depletion and congested blood vessels; ++: marked depletion of lymphoid follicles and vacuoler degeneration in the wall of splenic BV. Bursa of Fabricius: -: normal bursal follicle and epithelium lining; +: mild degree of depletion and thickening of stroma; ++: marked hyperplasia of lining epithelium and depletion; +++: marked increase epithelium hyperplasia with small cysts formation and depletion of lymphoid follicle.

The histopathological examination of the liver, lung, spleen, heart, and bursa of Fabricius confirmed the results obtained previously. In this study, there was severe alterations in hepatic, cardiac, bursa and pulmonary tissues of G2 challenged with O78 higher than G4 challenged with O128, this could be attributed to the potency of *E. coli* O78 pathogenicity reflecting the septicemia and infection system with strain O78 rather than O128. Similar results were reported by Zahid *et al.* (2016) who revealed that similar lesions in natural and experimental infected broilers with various serotypes of *E. coli*. While the examined bird of G1 (O78 challenged and vaccinated) showed minimal pathological lesions in different organs rather than birds of G3 (O128 challenged and vaccinated), this may be attributed to the fact that the inactivated vaccine gives sufficient protection mediated by high titre of circulating specific antibodies against homologous strains more efficient than the defense against strains of heterologous (Dho-Moulin and Fairbrother, 1999).

Findings in Table 4 indicated significantly increased body gain, FI and FCR ($p \leq 0.05$) comparable to control negative group and vaccinated groups in 7 days old groups of challenged and vaccinated, while was challenged with both homologous and heterologous serotypes of *E. coli* O78 & O128. The bursa and spleen, which are the primary immunological organs in broiler chicks, play a role in both cell-mediated and humeral immunity. The Bursa is a major lymphoid organ that effect essential function in both generations of B and T lymphocytes and the enhancement of the ontogenetic development of adaptive immunity. The spleen plays a crucial function in the development of the immune response as the peripheral lymphoid organ. Also, Fox and Grasman (1999) reported that the organ mass was inversely linked with the lymphoid cell count in the bursa and thymus. Therefore, Zheng *et al.* (2013) studied that the raised immune organ index indicated improved immune system and ability to fight off many stresses and diseases (Zheng *et al.*, 2013). The vaccine of *Escherichia coli* provided a considerable level of defense against

several challenged serotypes of *Escherichia coli*, according to final weight gain, clinical symptoms, postmortem signs, weight of lymphoid organs, histological lesions, and total count of *E. coli* (Elbestawy *et al*., 2021).

CONCLUSION

From the above results it could be concluded that the vaccination typically protects against infection of *E. coli*, among all groups, the vaccinated broilers seemed to have lower rates of pathologic results and morbidity than the non-vaccinated chickens. COLI-VAC™ inactivated polyvalent vaccine has better protection when compared with unvaccinated groups, while the vaccine working to improve weight gain and FCR in cases of non-infection. In order to prevent the mortality of the vaccine strain, numerous factors, including the usage of antibiotic drugs and their residues, are taken into consideration.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Ogunleye, A.O., Oyekunle, M.A., Sonibare, A.O., 2008. Multidrug resistant *Escherichia coli* isolates of poultry origin in Abeokuta, South Western Nigeria. Veterinarski Arhiv 78, 501-509.

Amer, M.M., Bastame, M., Elbayoumi Kh.M., Bosila, M.A., Mervat Salem, 2015. Evaluation of Prepared Inactivated Polyvalent *E. coli* Vaccine as Compared with Commercial Available Live Attenuated Vaccine. Advances in Environmental Biology 9, 299-306.

Anonymous, 2000. WHO global principles for containments of antimicrobial resistance in animals intended for food. Geneva: WHO/CDS/CSR/APH/2001.4.

Bancroft, J.D., Layton, C., 2013. The hematoxylin and eosin. In: Suvarna, S.K., Layton, C., Bancroft, J.D. (Eds.), Theory Practice of Histological Techniques. Churchill Livingstone of El Sevier, Philadelphia.

Beirao, B.C., Ingberman, B., M., Mesa D., Salles G.B.C., Muniz E.C., Caron L.F., 2021. Effects of aro-A deleted *E. coli* vaccine on intestinal microbiota and mucosal immunity. Comparative Immunology, Microbiology and Infectious Diseases 75, 101612.

Castanon, J.I.R., 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. 86, 2466–2471.

Chaffer, M., Schwartsburd, B., Heller, E.D., 1997. Vaccination of turkey poults against pathogenic *Escherichia coli*. Avian Pathol. 26, 377–390.

Charleston, B., Aitken, I.A., Reeve-Johnson, L., 1996. Efficacy oftilmicosin in the control of experimental *Mycoplasma gallisepticum* infection in chickens. Proceedings of the XX World Poultry Con-gress, New Dehli.

Christensen, H., Bachmeier, J., Bisgaard, M., 2021. New strategies to prevent and control avian pathogenic *Escherichia coli* (APEC). Avian Pathol. 50, 370–381.

Davis, G.S., Waits, K., Nordstrom, L., Grande, H., Weaver, B., Papp, K., Horwinski, J., Koch, B., Hungate, B.A., Liu, C.M., Price, L.P., 2018. Antibiotic-resistant *Escherichia coli* from retail poultry meat with different antibiotic use claims. BMC Microbiol. 18, 174.

Dho-Moulin, M., Fairbrother, J.M., 1999. Avian pathogenic *Escherichia coli* (APEC). J. Vet. Res. 30, 299–316.

Duncan, D.B., 1955. Multiple Range and multiple F tests. Biometrics 11, 1-42.

EL Jakee, J.K., EL Amry, G.M., Hessain, A.M., Hemeg, H.A., Shafei, S.M., Moussa, I.M., 2016. Production and evaluation of autogenous vaccine against avian colibacillosis. J. Anim. And Plant Scien. 26, 79-87.

Elbestawy, A.R., Ellakany, H. F., Abd El-Hamid, H. S., Ibrahim, M.S., Gado, A.R., Mustafa, N.S., Moussa, I.M., Al-Maary, K.S., Al-Sarar, D. S., Alshammari, H.O., Dawoud, T.M., Hemeg, H.A., Galal, H.M., 2021. Comparative evaluation of a live *E. coli* vaccine and cefotaxime treatment against three *E. coli* serotypes in broilers. Journal of King Saud University – Science 33, 1013532

Fox, L.L., Garsman, K.A., 1999. Effects of PCB 126 on primary immune organ development in chicken embryos. J. Toxicol.Env. Heal. A 58, 233–244.

Ghunaim, H., Abu-Madi, A.M., Kariyawasam, S., 2014. Advances in vaccination against avian pathogenic *Escherichia coli* respiratory disease: Potentials and limitations. Vet. Microbiol. 172, 13–22

Gina, M., Mohammed, Shell, W.S., Sayed, M.L., Ibrahim, H.M., Hanan, M.H., Ghada, M. El-Sadek, 2016. Efficacy of an experimental *E. coli* inactivated vaccine in turkey poults. Journal of Applied Veterinary Sciences 1, 07–14.

Gomis, S., Babiuk, L., Allan, B., Willson, P., Waters, E., Hecker, R., Potter, A., 2007. Protection of chickens against a lethal challenge of *Escherichia coli* by a vaccine containing cpg oligodeoxynucleotides (cpg-odn) as an adjuvant. Avian Dis 51, 78–83.

Ibrahim, R.A., Cryer, T.L,. Lafi, S.Q., Basha, E., Good, L., Tarazi, Y.H., 2019. Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. BMC Vet. Res. 15, 159.

Ievy, S., Islam, M.S., Sobur, M.A., Talukder, M.; Rahman, M.B., Khan, M.F.R., Rahman, M.T., 2020. Molecular detection of avian pathogenic *Escherichia coli* (APEC) for the first time in layer farms in Bangladesh and their antibiotic resistance patterns. Microorganisms 8, 1021.

Johnson, T.J., Y. Wannemuehler, S.J. Johnson, A.L. Stell, C. Doetkott, J.R. Johnson, K.S. Kim, Spanjaard, L., Nolan, L.K., 2008. Comparison of extraintestinal pathogenic*Escherichia coli* strains from human and avian sources reveals a mixedsubset representing potential zoonotic pathogens. Appl. Environ. Microbiol. 74, 7043–7050.

Kairmi, S.H., Taha-Abdelaziz, K., Yitbarek, A., Sargolzaei, M., Spahany, H., Astill, J., Shojadoost, B., Alizadeh, M., Kulkarni, R.R., Parkinson, J., 2022. Effects of therapeutic levels of dietary antibiotics on the cecal microbiome composition of broiler chickens. Poul. Sci. 101, 101864.

La Ragione, R.M., Woodward, M.J., Kumar, M., Rodenberg, J., Fan, H.A.D., Wales, Karaca K., 2013. Efficacy of a live attenuated *Escherichia coli* O78 : K80 vaccine in Chickens and Turkeys. Avian Dis. 57, 273-279.

Li L., Thofner I., Christensen J.P., Ronco T., Pedersen K., Olsen R.H., 2016. Evaluation of the efficacy of an autogenous *Escherichia coli* vaccine in broiler breeders. Avian Pathol. 46, 300-308.

Mageiros, L., Méric, G., Bayliss, S.C., Pensar, J., Pascoe, B., Mourkas, E., Calland, J.K., Yahara, K., Murray, S., Wilkinson, T.S., 2021. Genome evaluation and the emergence of pathogenicity in avian *Escherichia coli*. Nat. Commun. 12, 765–767.

Melamed, D., Leitner, G., Heller, E.D., 1991. A vaccine against avian colibacillosis based on ultrasonic inactivation of *Escherichia coli*. Avian Dis. 35, 17–22.

Nolan, L.K., Vaillancourt, J.P., Barbieri, N.L., Logue, C.M., 2020. Colibacillosis. In Diseases of Poultry, 14th ed.; Swayne, D.E., Ed.; Wiley-Blackwell: Hoboken, NJ, USA, pp. 770–860.

Pineda, L., Chwalibog, A., Sawosz, E., Lauridsen, C., Engberg, R., Elnif, J., Hotowy, A., Sawosz, F., Gao, Y., Ali, A., Moghaddam, H.S., 2012. Effect of silver nanoparticles on growth performance, metabolism and microbial profile of broiler chickens. Arch. Anim. Nutr. 66, 416-429.

Popy, N., Asaduzzaman, M., Miah, M.S., Siddika, A., Sufian, M.A., Hossain M.M., 2011. Pathological study on the upper respiratory tract infection of chickens and isolation, identification of causal bacteria. The Bangladesh Veterinarian 28, 60–69.

Quinn, p., Markey, B., Carter, M., Donelly, W., Leonard, F., 2002. Veterinary Microbiology and microbial disease. Black Well Science.

Sadeyen, J.R, Wu, Z., Davies, H., Diemen, P.M., Milicic, A., Ragione, M., Kaiser, Mark, P. Dziva, F., 2015. Immune responses associated with homologous protection conferred by commercial vaccines for control of avian pathogenic *Escherichia coli* in turkeys. J. Vet. Res. 46, 5.

Sainsbury, D., 1984. System of management in Poultry health and management. 2nd ED.Granda Publishing (TD), 8 Grafton st., London.WIX-3LA.

Shehata, A.A., Kilany W., Ali A., Radwan M., Radi M., Elfeil W. K., Wasfy M., 2019. Phenotypic, genotypic and pathogenic features of Avian Pathogenic *E. coli* in Egypt and the development of a multivalent vaccine against the predominant serotypes. In: Proceedings of World Vet. Poultry Association Congress, Thailand. pp. 360-361.

Shrestha, A., Bajracharya, A.M., Subedi, H., Turha, R.S., Kafle, S., Sharma, S., Neupane, S., Chaudhary, D.K., 2017. Multi-drug resistance and extended spectrum beta lactamase producing Gram negative bacteria from chicken meat in Bharatpur Metropolitan, Nepal. BMC Res. Notes 10, 574.

Verma, J., Johri, T.S., Swain, B.K., Ameena, S., 2004. Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers. British Poultry Science 45, 512-518.

Yaguchi, K., Ohgitani T., Noro, T., Kaneshig,e T., Shimizu, Y., 2009. Vaccination of chickens with liposomal inactivated avian pathogenic *Escherichia coli* (APEC) vaccine by eye drop or coarse spray administration. Avian Dis. 53, 245-249.

Zahid, A.H., AL-Mossawei, M.T.M., Mahmood, A.B., 2016. In vitro and In vivo Pathogenicity tests of Local Isolates APEC from Naturally Infected Broiler in Baghdad. Int. J. Adv. Res. Biol. Sci. 3, 89–100.

Zheng, L., Ma, Y.E., Gu, L.Y., Yuan, D., Shi, M.L., Guo, X.Y., Zhan, X. A., 2013. Growth performance anti-oxidant status and non-specific immunity in broilers under different lighting regimens, J. Appl. Poult. Res. 22, 798–807.