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EVALUATION OF COMMERCIAL *ESCHERICHIA COLI* VACCINE IN BROILER CHICKENS

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ABSTRACT

This study is conducted to evaluate the protective effectiveness live-attenuated *E.Coli* vaccine. A total of 140 broiler chicks were classified into seven groups; the first two groups (1 & 2) were labeled as challenge control for *E.Coli* O78 and O91 strains, the second two groups (4 & 5) were vaccinated with the *E.Coli* live-attenuated vaccine at day 1, the third two groups (3 & 6) treated with *Difloxacin* "second generation fluoro-quinolone" 1ml/L drinking water for first three days of life then vaccinated at day 7 with the *E.Coli* live-attenuated vaccine. The last group (7) was kept as a control negative group.
The challenge was directed to the 21-day-old chicks with either homologous O78 or heterologous O91 *E.Coli* strains 1.2x10^9 Colony Forming Unit/mL injected intratracheally using 0.5 ml/ bird of each avian pathogenic *E.Coli* (APEC) strain. Clinical signs, gross and histo-pathological lesions, the intestinal total count of *E.Coli* (CFU), the chick body weight and the weight of lymphoid organs were calculated at 28 days of age. The results postulated that Poulvac® *E.Coli* vaccine gives a significant protective effect against the challenge with homologous O78 and the most common heterologous serotype O91. Based on the for-mentioned parameters, vaccinated birds at 7 days of age (Groups 3&6) which were treated with *Difloxacin* and challenged with both homologous and heterologous *E.Coli* serotypes (O78 & O91) showed superior protection over those vaccinated at day one of age (Groups 4&5), challenged with O78 & O91 *E.Coli* serotypes and did not receive any antibiotic.

Key words: *E.Coli*, live vaccine, serotypes, growth performance, Poulvac® *E.Coli*

INTRODUCTION

Escherichia coli is a gram negative, non-spore forming, facultative anaerobic bacilli, a widespread gut normal flora of vertebrates and a versatile pathogen (Tenaillon *et al.,* 2010). *Escherichia coli* is defined as the most common bacterial pathogen that causes deleterious health effects in humans and many production problems in the field of poultry (Landman and van Eck, 2015). Enterohemorrhagic *E.Coli* (EHEC) serotype O157:H7 is the cause of Enterohemorrhagic gastroenteritis that causes panic among fast food fans worldwide. (Saeedia *et al.,* 2017). APEC strains have been concerned with a diversity

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of disease conditions including perihepatitis, coli granuloma, omphalitis, cellulitis, salpingitis, air-sacculitis, pericarditis, egg peritonitis, osteomyelitis and arthritis (Nolan *et al.,* 2013).

Live vaccines are among the best implements available to combat *E.Coli* disease. These vaccines are safe and show efficacious outcomes under field conditions (Mombarg *et al.,* 2014). *E.Coli* vaccines are used to prevent and control *E.Coli* infections because of the high incidence of antibiotic resistance used to treat *E.Coli* on farms. In addition, Poulvac® *E.Coli* provides cross-protection against most heterologous APEC serotypes (Cookson *et al.,* 2009). The antigenicity of this pathogen is complicated, and the serogroups are diverse (Ewers *et al.,* 2004).

The *E.Coli* serotypes that are usually isolated from infected birds can be taken into consideration to be specifically pathogenic are O1, O2, O78, O8, and O35. (Dho-Moulin and Fairbrother, 1999). Antimicrobial resistance of *E.Coli* carries a huge burden to the general public fitness due to the fact that these "nasty bugs" will be handed to people through meals or direct touch with inflamed birds. Moreover, resistant E. coli strains can also act as a good vehicle for the transmission of antimicrobial-resistance genes to different pathogens (Akond, 2009). Avoidance and eradication of *E.Coli* are primarily dependent on reducing elements of its growth and flourishment in a herd (vaccinations against immune-compromising diseases, preserving hygienic requirements at hatcheries, right feeding approaches) (Dho-Moulin and Fairbrother,. 1999). Vaccination is one of the numerous methods that have been used to combat *E.Coli* (Saif, 2008). Live vaccines are considered to be the most suitable method for immunization against *E.Coli* (Peighambari *et al.,* 2002). Live *E.Coli* vaccines can target the intestine and interact with its symbiotic microbiota, and vaccine protection has been postulated to be at least partially due to intestinal immune components (Desselberger, 2018). This could be proven when gut-associated lymphoid tissue was not the predominant immune site to show signs of reaction following vaccination; instead, the respiratory-associated lymphoid sites and other secondary lymphoid tissues, such as the spleen did. Moreover, Vaccination did induce consistent changes to the intestinal microbiota, which may really account for the improved intestinal health reported in the field following immunization; the altered intestinal microbiota may then indirectly affect the outcomes of E. coli vaccination in poultry (Beirão *et al.,* 2021).

Owing to the variety of APEC serotypes in the field, this study is intended to assess the efficacy of live-attenuated *E.Coli* vaccine (Poulvac® *E.Coli*) and their proficiency to cross-defend against other heterologous serogroups (O91). The APEC serotypes were isolated from broiler chickens common in the practical field.

MATERIALS AND METHODS

A:Collection of Samples and Transportation:

One hundred and ten samples (liver, heart, lung and yolk sac) were collected from diseased freshly dead broiler chicken from 50 different poultry farms located in upper Egypt (Minya, Assiut, Sohag, Qena and Luxor Governorates) in separate zipper-locked bags, preserved in ice box and directly conveyed to the laboratory. Freshly dead chickens showed: omphalitis, chronic respiratory disease (CRD) or colibacillosis septicemia.

B: Bacteriological examination:

Loopfuls from each organ were inoculated onto nutrient broth for incubation at 37^{o}C for 12 hours. After that, loopfuls were streaked onto Eosin-methylene blue (EMB) agar plates and incubated for 24 hours at 37^{o}C to detect lactose fermenter colonies, suspected colony was streaked onto MacConkey’s agar plates and then

incubated at 37ºC for 24-48 hours. The lactose-fermented colonies were held in Semi-solid agar for more identification including morphology and biochemical reactions (Quinn *et al.*, 2002).

C: Serological identification of the isolates:

Forty *E.Coli* isolates that showed biochemically positive results were conducted for serological identification (Edwards & Ewing, 1986). Serological identification was carried out by Prof. Dr. Mohamed Ahmed Hassan (Food Analysis Center, Faculty of Veterinary Medicine, Benha University) according to (Kok *et al*., 1996) by using slide agglutination test and definite mono-valent and poly-valent sera. Group O somatic anti-sera.

D: In Vitro Pathogenicity Testing:

According to Berkhoff and Vinal (1986), Congo red dye binding test. Serotypes were tested for pathogenicity. Each isolate was inoculated on trypticase-soy-agar plates supplemented with 0.15% bile salts and 0.03% Congo red dye and incubated at 37ºC for 24 hrs. Positive results were indicated by the growth of black colonies with a dry crystalline consistency.

E: Bio-film formation ability:

All the tested *E.Coli* serotypes (n.=20) were confirmed to be bio-film producers by Congo red agar method (Berkhoff and Vinal, 1986).

Experimental design:

A: Grouping: one hundred and forty broiler chicks were separated into seven groups (20 each). The first two groups (1&2) were labeled as challenge control by either *E.Coli* O78 or O91 strains. The second two groups (4&5) were vaccinated with the live attenuated vaccine of *E.Coli* (Poulvac® *E.Coli*) on day 1, the third two groups (3&6) were treated with *Difloxacin* (1mL/L drinking water) for first three days of life then vaccinated at day 7 with Poulvac® *E.Coli* and the last group was kept to be the negative control group. the challenge was directed to the 21-day-old chicks with either homologous O78 or heterologous O91 *E.Coli* strains 1.2x10^9 Colony Forming Unit /mL injected intratracheally using 0.5 ml/ bird of each avian pathogenic *E.Coli* (APEC) strain according to McFarland standard reactions (Rawiwet and Chansiripornchai 2009). Pooled samples from all groups were collected to determine the best antibiotic by antibiotic sensitivity test.

***E.Coli* strains:** two local strains of pathogenic *E.Coli* serotype O78 and O91 were isolated from infected broilers. These strains were used for homologous and heterologous challenging of vaccinated broilers. Commercially available live attenuated *E.Coli* vaccine (Poulvac® *E.Coli*) prepared from *E.Coli* O91 strain. The lyophilized vaccine was reconstituted to contain 10^9 CFU/mL before use.

Evaluation parameters:

A- clinical signs and Mortality: at 7 days post-challenge, clinical signs and morbidity of colibacillosis (ruffled feathers, gasping, nasal discharge, and diarrhea), mortality rates were calculated including cloudy air sacs with or without caseous exudate, peritonitis, perihepatitis, and pericarditis.

B- Count of CFU of *E.Coli*: 1 gram cecal content and bacteriological examination

C: The average body weight and lymphoid organs: spleen, bursa and thymus of the birds in each group in addition to liver weight were measured at 28 days of age.

D: Histopathology: Tissue sections of liver, spleen, thymus and intestine were collected post necropsy in 10% neutral-buffered formalin for fixation and processed using paraffin embedding section then sectioned at 4 μm thickness and stained with Haematoxylin and Eosin (H&E) stain for histo-pathological examination (Bancroft and Cook. 1994).

E: Statistical analysis: All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by

using (Graph pad prism 9.3.1) software and (medcalc 20.0.0) software. One-way analysis of variance (ANOVA) followed by Tukey multiple comparisons for post hoc was used to demonstrate the significant differences between groups, values less than 0.05 were considered significant. Levene test was used to judge the homogeneity of variances, while the Shapiro-Wilk normality test was used to assess whether the data met the assumptions of the statistical approach, if the assumptions were not met, Kruskal-Wallis test ANOVA and Conover Multiple Comparison Test for Post hoc were used to demonstrate the significant differences between groups, P-values less than 0.05 were considered significant. (conover, 1999), (Lantz *et al.*, 2016).

RESULTS

A: Occurrence of *E.Coli* in broiler chicken:

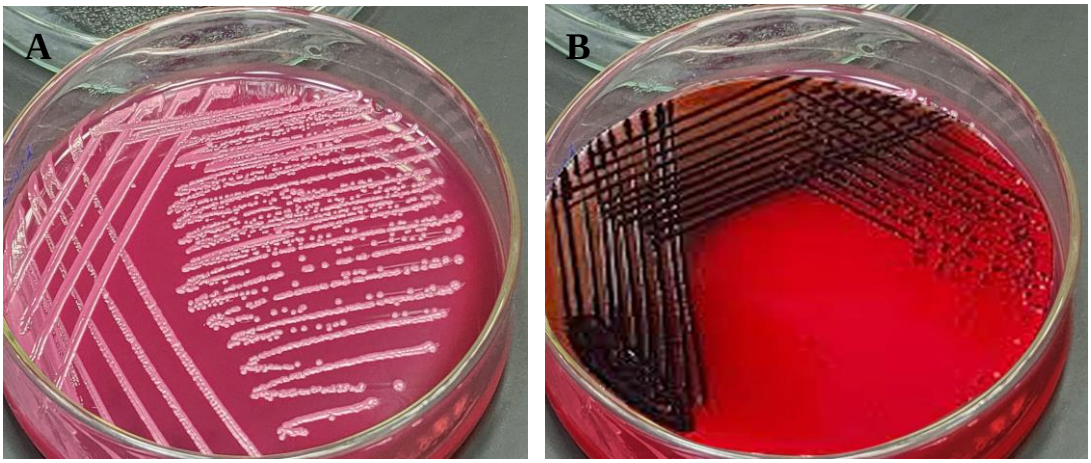
According to morphology and biochemical characteristics:

Our results disclosed that 40 suspected *E.Coli* isolates out of 110 examined samples were detected with a percentage 36.3 %

According to serological identification: The overall incidence of E. coli isolates in the total examined 110 chicks samples were 20 isolates (18.1 %). With regard to serotyping of *E.Coli* isolates, the most isolated serotypes were O78 & O91:H21 (4 isolates each), followed by O2:H6 (3 isolates), O1:H7& O128:H2 (2 isolates), O26 (2 isolates), O142, O86 and O111 (one isolate each) as shown in tables (1)

Pathogenicity Testing: CR-binding assay indicated that 32 isolates of 40 (80%) give black colonies with a dry crystalline consistency and 8 isolates (20%) were considered negative (pink color).

Figure (1) Showing the results of *E.Coli* on Congo red agar:



A: Negative results with pink colonies. B: Positive results with black colonies with a dry crystalline consistency.

Results of Experimental infection of broiler chicks with APEC serotypes O78 & O91:

A: Clinical signs, lesions:

Diseased birds especially G1 and G2 presented clinical signs 2 days post-challenge including low feed intake, whitish-brownish diarrhea, coughing and sneezing. PM lesions include fibrinous pericarditis and perihepatitis. Sever PM lesions noticed in G1&G2 also presence of fibrinous whitish yellow exudates in the air sacks (cloudy air sacs with or without caseous exudates) and fibrinous pneumonia. There were very mild clinical signs and PM finding in all vaccinated groups challenged by serotype O78 or O91. The score lesion is shown in figure 2:

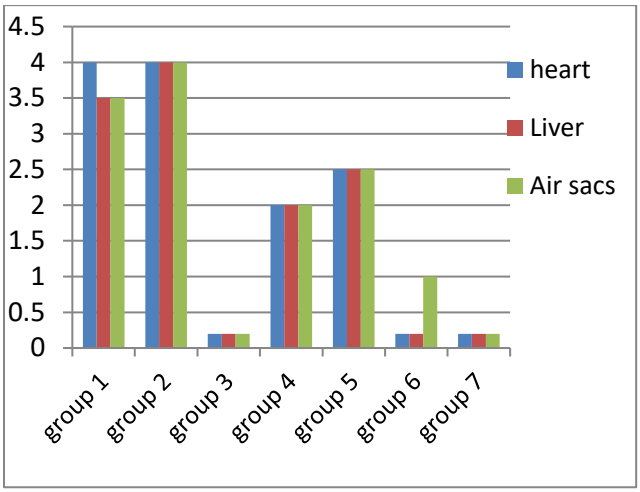
Table (1): Incidence of *E.Coli* serotype isolated from different organs of diseased chicks.

E. coli Serotypes	Numbers	%
O78	4/20	20
O91	4\20	20
O2	3/20	15
O1	2/20	10
O128	2/20	10
O26	2/20	10
O142	1/20	5
O86	1/20	5
0111	1/20	5

B: Mortality rate: was 0% in G3,G4,G6&G7, 1/20 in G5, 5/20 mortality was reported in chicken G1 and 4/20 in G2.(Table 2)

C: Total *E.Coli* count: All vaccinated chickens had a significant reduction of *E.Coli* count compared to the challenged non-vaccinated groups. While, there is no significant difference in the *E.Coli* count between all vaccinated groups compared with non vaccinated non-infected group (Table 3).

Figure (2): heart, liver and air sacs score lesion



D: Body weight and lymphoid organs: Final weight of body, liver, spleen, bursa and thymus (figure 3): Chicken in G3 &G6 had significantly higher body weight and liver ($p < 0.05$) compared to the other groups, while G1&G2 had significantly lower B.WT ($p< 0.05$) compared to the other groups, Chickens in G1, G3 &G4 had a significantly higher weight of bursa, while G2 had a significantly lower bursal weight ($p< 0.05$) compared to the other groups.

Table 2: The protection rate of broilers after challenge with homologous (O78) or heterologous (O91) *E.Coli* by the intra-tracheal route

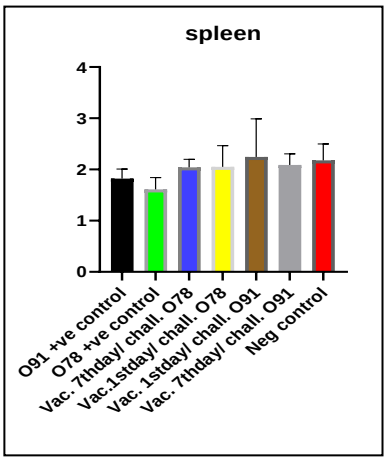
	Groups	Mortality	Protection rate
1	*O91* +ve control	5/20	75%
2	*O78* +ve control	4/20	80%
3	Vac. 7thday/ chall. O78	0/20	100%
4	Vac.1stday/ chall. O78	0/20	100%
5	Vac. 1stday/ chall. O91	1/20	95%
6	Vac. 7thday/ chall. O91	0/20	100%
7	Negative control	0/20	100%

Table 3: Mean *E.Coli* count ±SD isolated from cecum (CFU/Gram) in chicken challenged with *E.Coli* serotype homologous O78 & heterologous O91 at 28 days old (7 days post challenge).

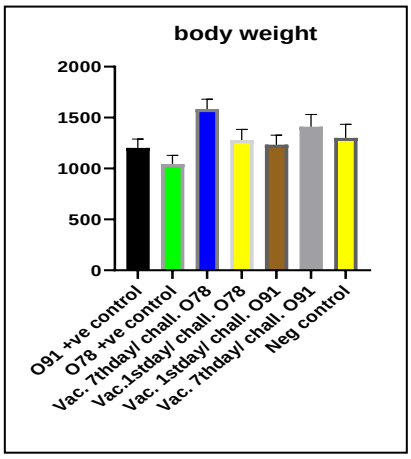
Groups		CFU/Gram	
		M	SD
[G1]	O91 +ve control	5.97E+08 [a]	5.86E+08
[G2]	O78+ve control	1.82E+09[a]	4.01E+09
[G3]	Vac. 7thday/ chall. O78	7.02E+04[b]	8.14E+04
[G4]	Vac.1stday/ chall. O78	4.67E+06[b]	8.58E+05
[G5]	Vac. 1stday/ chall. O91	4.09E+06[b]	8.89E+06
[G6]	Vac. 7thday/ chall. O91	2.29E+05[b]	3.43E+05
[G7]	Neg. control	1.30E+06[b]	2.10E+06

a,b Mean in the same column with the different superscript are statistically significant

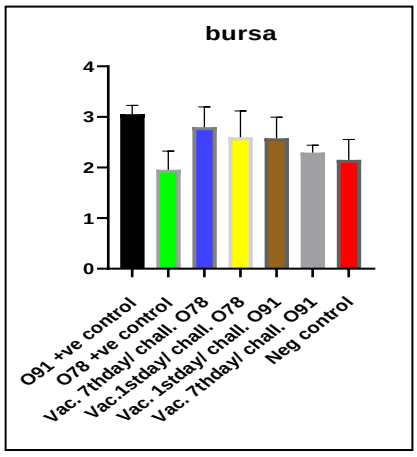
Figure 3: Average weight of: spleen (fig.3.1), body (fig.3.2), bursa (fig.3.3), thymus (fig.3.4) and liver (fig.3.5) in chicken challenged with E. coli serotype homologous O78 & heterologous O91 at 28 days old (7d.p.c):



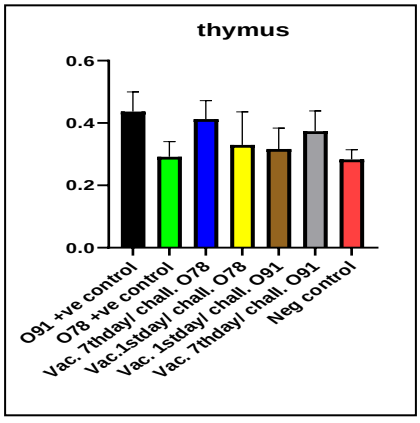
Average weight of spleen
Figure (3.1)



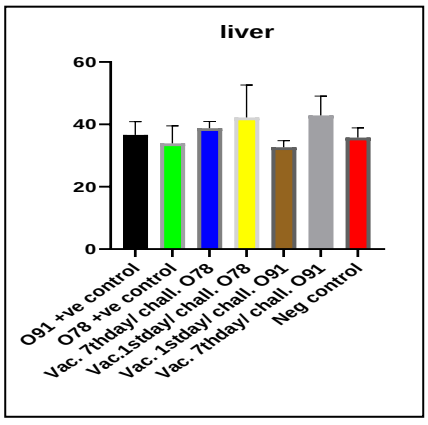
Average body weight
Figure (3.2)



Average weight of bursa
Figure (3.4)



Average weight of thymus
Figure (3.4)



Average weight of liver
Figure (3.5)

D: Histopathological findings:

The recorded histo-pathological findings in chicken liver of G1&G2 challenged by serotypes O91 & O78 respectively, showed congested central veins and dilated sinusoids, locally extensive leukocytes cellular infiltration around portal areas, degeneration of bile duct epithelial cells and multifocal coagulative necrosis of hepatocytes Fig.4 (A,B,C and D) compared to normal hepatic structure of negative control group (G7) Fig.4 (E,F). The spleen showed congested blood vessel and sinuses with light depletion of lymphocytes Fig.7 (A). Thymus showed necrosis and lysis of lymphoid elements Fig.8 (A, B). The intestine showed edematous wide villi with mild inflammatory cells infiltration in lamina propria Fig.9 (A). While the microscopic findings of two groups (4&5) that vaccinated at day 1 with the live vaccine of *E.Coli* revealed vacuolar hepatocytes degeneration, heterophils as well as mononuclear inflammatory cells infiltration, congested vessels and sinusoids Fig.5 (A,B). Less pathological changes in G4 appeared in sub-scapular inflammatory cell infiltration and dilated sinusoid with leukocytes Fig.5 (C, D). The spleen of G5 showed depletion of lymphoid follicle Fig.7 (B) while, spleen of group 4 showed less depletion than group 5 Fig.7 (C). Thymus showed increase thickness of septa, mild congestion and less necrosis of lymphoid elements Fig.8 (C). The intestine of group 5 showed inflammatory cell infiltration in villi associated with mild edema Fig.9 (B), while, intestine of group 4 showed mild inflammatory cells infiltration with minimal shortening of villi and increase intestinal glands Fig.9 (C). The last two groups (3&6) that vaccinated at day 7 with the live vaccine of *E.Coli*, and treated with *Difloxacin* showed nearly normal hepatic parenchyma with minimal pathological changes like dilated sinusoids Fig.6 (A, B, C, D). The spleen showed more or less normal splenic architecture Fig.7 (D). The thymus showed no histopathological changes Fig.8 (D). Concerning the intestine showed normal intestinal villi with intact enterocytes mucosa Fig.9 (D).

Lesion score for the selected organs was recorded. Table (4).

Table (4): Histopathological score lesion for the selected organs in all 7 groups:

Groups / Lesion	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Liver:							
*Congestion	+ + +	+ + +	-	+	+ +	-	-
*Infiltration of inflam. Cells	+ + +	+ + +	-	+	+ +	-	-
*Degeneration of hepatocytes	+ + +	+ + +	-	+	+ +	-	-
*Necrosis of hepatocytes	+ + +	+ + +	-	+	+ +	-	-
Spleen:							
*Congestion	+ + +	+ + +	-	+	+ +	-	-
*Depletion of lymphocytes	+ + +	+ + +	-	+	+ +	-	-
Thymus:							
*Necrosis of lymphoid tissue	+ + +	+ + +	-	+	+ +	-	-
*Depletion of lymphocytes	+ + +	+ + +	-	+	+ +	-	-
Intestine:							
***Edema**	**+ + +**	**+ + +**	**-**	**+**	**+ +**	**-**	**-**
***Infiltration of inflam. cells**	**+ + +**	**+ + +**	**-**	**+**	**+ +**	**-**	**-**

N.B: (-) means 1- 4 slides showing lesion, (+) means 5-9 slides showing lesion, (++) means 10-14 slides showing lesion and (+++) means 15-20 slides showing lesion.

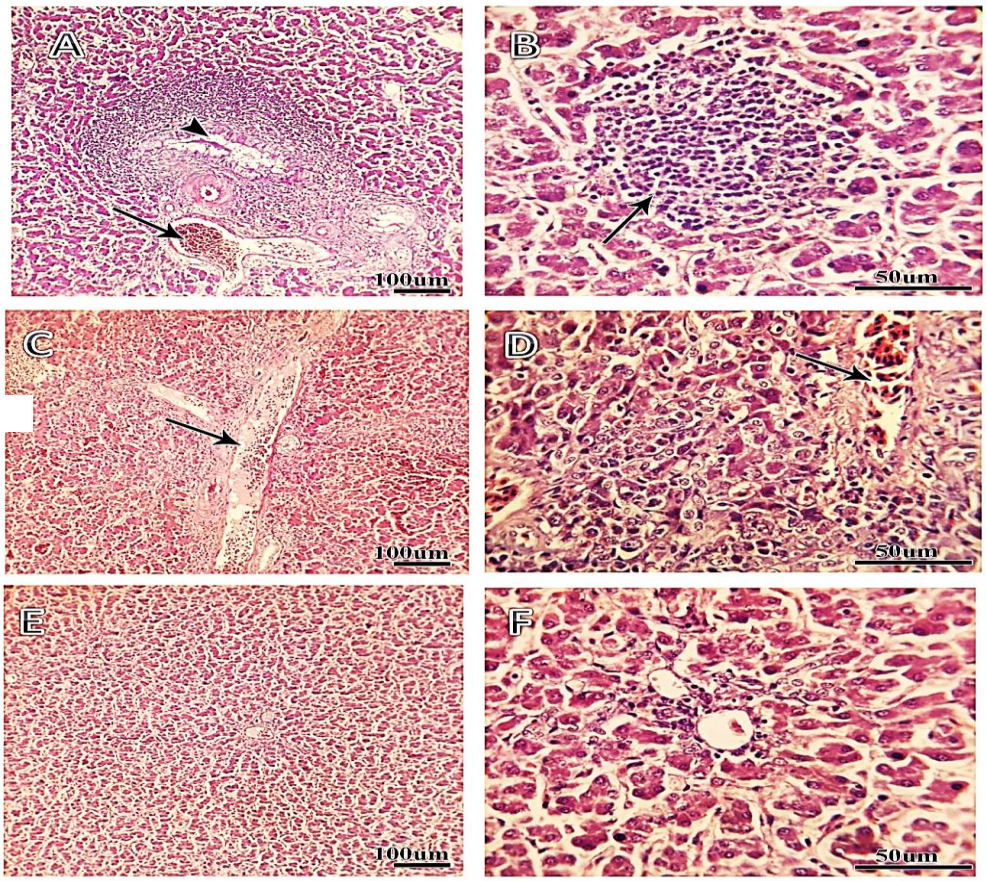


Fig. (4): photomicrograph of chicken liver showing:
*****control +ve O78 challenged group showing: congested central vein (arrow) and degeneration of bile duct epithelial cells (arrow head) (A). *focal leukocytes cellular aggregation (arrow) (B).
*Control +ve O91 challenged group showing congested blood vessel (arrow) (C, D).
*Control –ve group 7 showing: normal hepatic structure (E, F) (H&E x100 and 400**)**

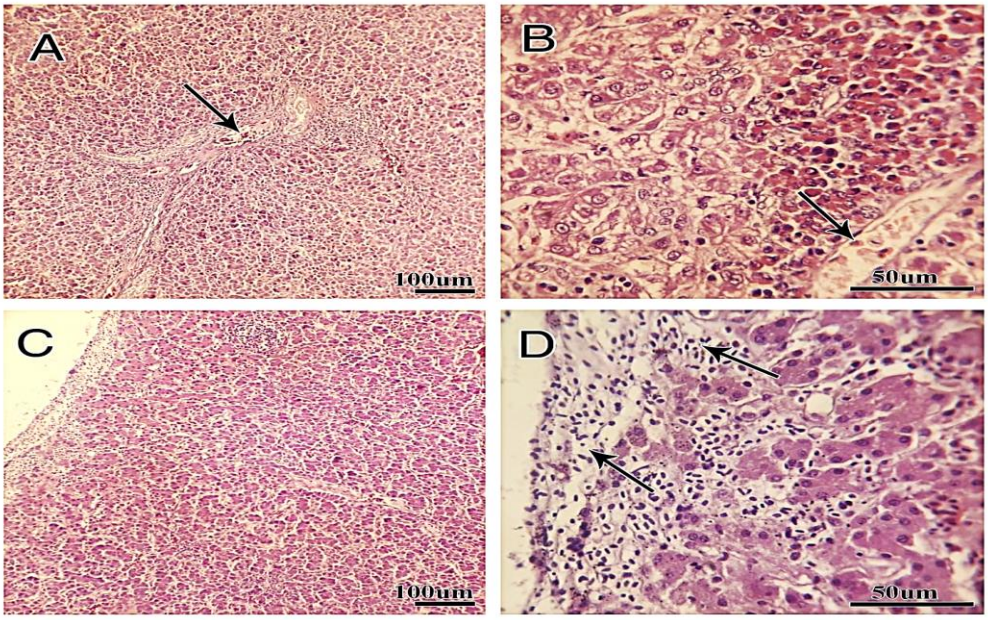


Fig. (5): pho tomicrograph of chicken liver showing:
*1 day vaccinated and homologues challenged O78 group showed congested blood vessel (Arrow) (A, B).
*****1 day vaccinated and heterologous challenged O91 group showing sub-scapular inflammatory cell infiltration and dilated sinusoid with leukocytes (Arrows) (C, D). (H&E x100 and 400).

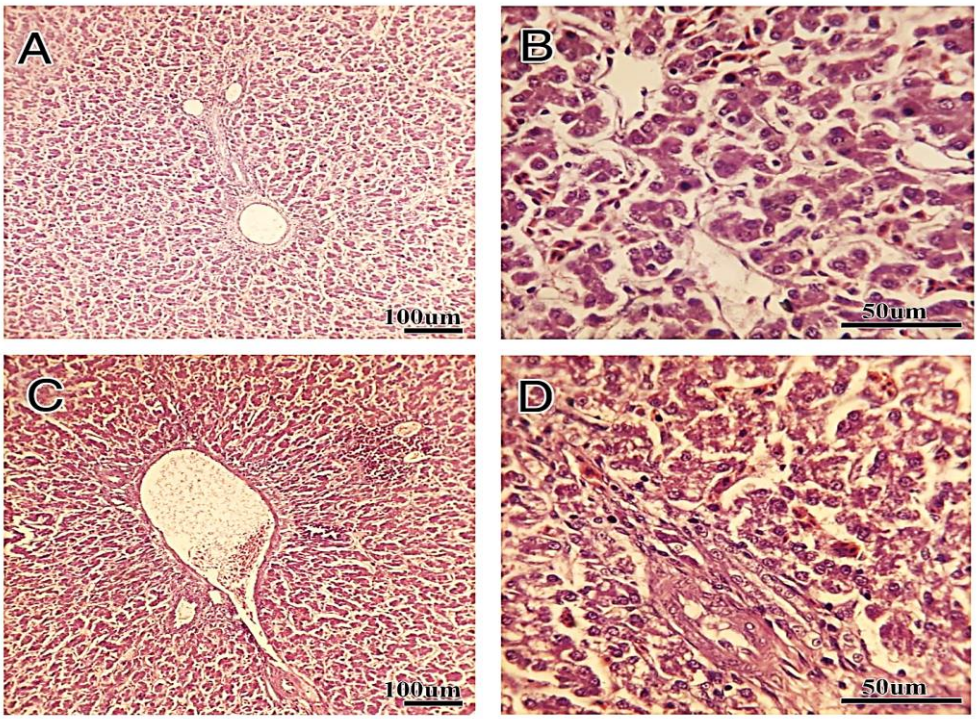


Fig. (6): photomicrograph of chicken liver showing:
* 7 days vaccinated and homologues challenged O78 group showing normal hepatic structure (A, B).
* 7 day vaccinated O78 and heterologous challenged O91 group showing minimal pathological changes like dilated sinusoids (C, D). (H&E x100 and 400)

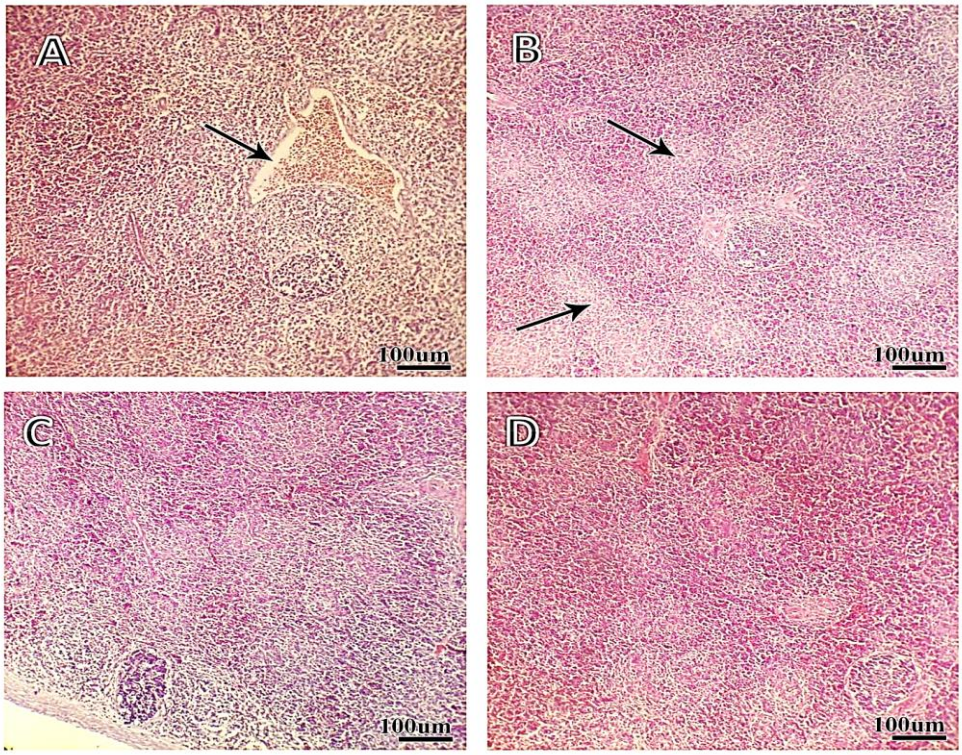


Fig. (7): photomicrograph of chicken spleen showing:
* Control +ve O78 group and Control +ve O91 group showing severe congested blood vessel (Arrow**)** (A).
***** 1 day vaccinated and homologues challenged O78 group showing moderate depletion of lymphocytic follicles. (Arrows) (B).
***** 1 day vaccinated O78 and heterologous challenged O91 group showing mild depletion of lymphoid follicles (C).
* 7 days vaccinated and homologues challenged O78 group 3 and heterologous challenged O91 group showing more or less normal splenic architecture (D). (H&E x100)

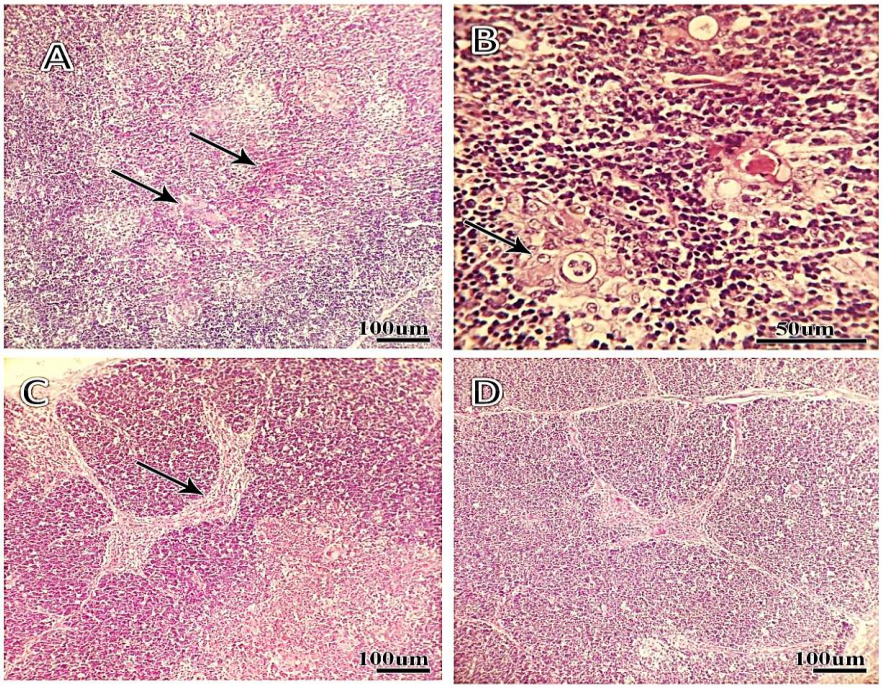


Fig. (8): photomicrograph of thymus showing:
*Control +ve O91 group and Control +ve O78 group showing necrosis and lysis of lymphoid elements (arrows) (A).
*****Higher magnification of previous photo necrosis and lysis of lymphoid elements (arrow)**.** (B).
*1 day vaccinated and homologues challenged O78 group and heterologous challenged O91 group showing thickening of septa (Arrow) and mild congestion and less necrosis of lymphoid elements (C).
***** 7 days vaccinated and homologues challenged O78 group and heterologous challenged O91 group showing no histopathological changes (D). (H&E x100, x400)

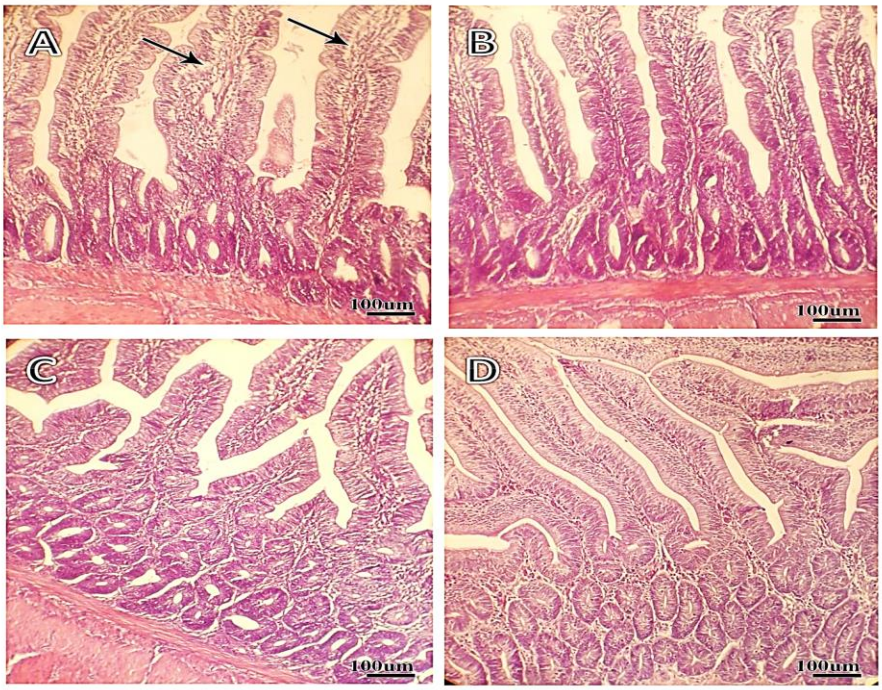


Fig. (9): photomicrograph of chicken intestine showing:
* Control +ve O78 group and Control +ve O91 group showing edematous wide villi (Arrows), with mild inflammatory cells infiltration in lamina propria (A).
*1 day vaccinated and homologues challenged O78 group showing inflammatory cells infiltration in villi associated with mild edema (B).
*****1 day vaccinated and heterologous challenged O91 group showing mild inflammatory cell infiltration with minimal shortening of villi and increase intestinal glands (C).
* 7 days vaccinated and homologues challenged O78 group and heterologous challenged O91 group showing normal intestinal villi with intact enterocytes mucosa (D). (H&Ex100)

DISCUSSION

In the current study, the global incidence of *E.Coli* in chicken was 36.3 % similar isolation rate was detected by Amer *et al.* (2018) who isolated *E.Coli* from 35% of 160 broilers showing septicemia.

The results of CR-binding assay revealed that 80% of *E.Coli* isolates were positive. The result of *in vitro* pathogenicity testing was in agreement with Berkhoof and Vinal (1986), who reported a strong correlation between the expression of CR phenotype and virulence in avian *E.Coli*. Pathogenic *E.Coli* can be identified by their ability to bind CR and produce brick red colonies. (Abhilasha and Gupta2001).

The most predominant serotypes were as follows: 4(O78& O91), 3(O2), 2 (O1, O26 & O128) and 1(O111, O86 & O142). The serotypes isolated in this study were in accordance with Amer *et al.* (2018**)** who reported that O78 serotype was the most predominant isolate from broilers in Egypt.

In the present study, the aro-A gene deleted live-attenuated O78 *E.Coli* vaccine is effective in diminishing the lesion versus both homologous & heterologous infection by using the intratracheal route that may lead to the reduction of morbidity and mortality rates as a consequence to significantly reduced total count of *E.Coli* (CFU/g) compared to the non-vaccinated group. Frommer *et al.* (1994) verified similar results 15, 55 and 60% mortalities in chicken experimentally challenged with homologous and heterologous *E.Coli* serotypes; however, these mortality percentages decreased down to 3–10% with the use of the vaccine.

Fibrinous pericarditis, perihepatitis, enteritis and airsaculitis were more severe in G1 and G2 and confirmed histo-pathologically with the presence of moderate to severe tracheitis pulmonary lesions with severe enteritis. While the examined chickens in Groups 3,4,5&6 presented the least degree of tracheal, pulmonary, hepatic and enteric lesions. This may be an indication for systemic infection and septicemia associated with both O78 and O78 serotypes. Dho-Moulin and Fairbrother (1999) documented the matching finding in experimentally and naturally infected broiler chickens with different *E.Coli* serotypes.

Histopathology findings revealed a lower level of lesions in the 7-day vaccinated group compared to several lesions observed in one day-vaccinated group. This could be attributed to the vaccination at the age of one day is exposed to the existing maternal antibodies. Additionally, one vaccination dose to immunologically immature one-day-old chicks may be un sufficient to produce wanted protection against pathogenic *E.Coli* infection (Mohamed *et al.*, 2016). Also, Spray droplet size might be an effective factor in vaccine efficacy (La Ragione *et al.*, 2013).

This study proved that Poulvac® *E.Coli* given at seven days of age, combined with *Difloxacin* treated during the first 3 days, and challenged with both homologous and heterologous field *E.Coli* serotypes (G3&G6) provided the superlative results on the premises of protection. Moreover, a significant reduction in *E.Coli* count during the challenge with avian pathogenic *E.Coli* field isolates. 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 challenge at 6 weeks of age by homologous virulent APEC strain.

Data analysis points out significant differences in the experimental vaccinated birds at 7 days of age, challenged by homologous *E.Coli* serotype O78 throughout having better weight gain and lowest gross lesions rather than other groups. This higher protection especially against the challenge with *E.Coli* serotype O78 may be attributed to the similarity of serogroup

antigens between the aro-A mutant O78 strains in Poulvac® *E.Coli*. Similar results have been obtained by La Ragione *et al.* (2013) who studied an aro-A construct live-attenuated *E.Coli* (RML17 vaccine) and was shown to be valuable as a vaccine against colibacillosis in chicken and turkeys caused by a homologous strain. Also, they studied an un-type-able APEC strain in chicken which directed a favorable solution for broader cross-protection than other vaccines. Also (Dho-Moulin and Fairbrother, 1999) proved that the live-attenuated *E.Coli* vaccine provides satisfactory protection against infection with homologous strains; on the other hand, protection against heterologous strains is less effective.

Additionally, the immature immune system of a day-old chick may not react to a single vaccine dose (Sadeyen *et al.,* 2015). Maternally imitative antibodies even in little amounts may hinder vaccine effectiveness (Elazab *et al.,* 2009).

Poulvac® *E.Coli* vaccine needs to last for an adequate time to express a sufficient amount of increased serum survival (Iss) in order to reach efficacy (Lynne *et al.,* 2006). Achieved gross lesions were well-matched with some studies as they demonstrated a decrease in the gross visible lesion of colibacillosis in vaccinated birds and challenged with homologous *E.Coli* serotype as a consequence of a significant reduction of the total *E.Coli* count as reported by (Mombarg *et al.,* 2014**)**. Vaccination reduced the count of *E.Coli* isolates and these isolates were further sensitive to antimicrobial agents, Broiler vaccination against *E.Coli* should be deliberated as part of a regular immune prophylaxis regime (Śmiałek, 2020). Alternatively, Mohamed *et al.* (2016) reported a reduction in necropsy lesions in the challenge with homologous *E.Coli* strain but not in heterologous one. *E.Coli*. Vaccinated broilers with Poulvac® *E.Coli* vaccine contributed significant protection against the challenge with homologous O78 and the most prevalent heterologous APEC serotype in Upper Egypt, O91.

Figures suggested significant elevated body weight, liver, thymus, spleen and bursa weight ($p \leq 0.05$) compared to the negative control group in all 7-day vaccinated groups, which was treated with *Difloxacin* and challenged with both homologous and heterologous *E.Coli* serotypes O78 & O91.Moreover, much more than 1-day-old vaccinated groups which not treated with *Difloxacin* and challenged with both O78 & O91 *E.Coli* serotypes. The main immune organs in broilers (Thymus, bursa of Fabricius, and spleen) are involved in humeral and cell-mediated immunities. Bursa of Fabricius and thymus are central lymphoid organs, which are not only important in improving the ontogenetic development of adaptive immunity, but are also the generative organs for T and B lymphocytes. The spleen, as the peripheral lymphoid organ, acting an important key role in the generation of the immune response. Fox and Grasman (1999) also realized that lymphoid cell numbers in both the thymus and bursa of Fabricius were correlated with the mass of the organs. Therefore, the increased index of immune organs suggested enhanced immune function and the ability to fight different infections and stress. (Zheng *et al.,* 2013).

Based on final body weight, lymphoid organs weight, clinical signs, PM lesions, total *E.coli* count and histopathological lesions, The *E.Coli* vaccine gave a significant protection against the challenge with different *E.Coli* serotypes. (Elbestawy *et al.,* 2021).

CONCLUSION

The results postulated that the vaccine tends to prevent *E.Coli* infection and the vaccinated chickens in all groups tended to show low morbidity and pathological findings including fewer air-sacculitis, pericarditis, perihepatitis and peritonitis than the non-vaccinated chickens.

The liver, bursa, spleen, thymus and body weight of the chicks were evaluated in the

concept of vaccination with E. coli aro-a live vaccine

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تقييم اللقاح التجاري للميكروب القولوني في بدارى التسمين

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تهدف هذه الرسالة الي تقييم مدي كفاءة لقاح الإي كولاي الحي المثبط المحضر من عترة . O78 تم تقسيم عدد 140 كتكوت عمر يوم إلي سبعة مجموعات متساوية (20 طائر / مجموعة). أول مجموعتين رقم (1 و 2) تم استخدامهم كمجموعات إيجابية ضابطة, حيث لم تحصن هاتين المجموعتين وتمت عليهم العدوى فقط بعترتين مختلفتين O78 و .O91 ثاني مجموعتين رقم (4 و 5) تم استخدام اللقاح فيهم عند عمر يوم وتمت العدوى بعترتين مختلفتين O78 و .O91 ثالث مجموعتين تم تحصينهم باللقاح عمر 7 أيام بعد أن تم معالجتهم بمضاد حيوي (داي فلوكساسين) خلال الثلاث أيام الأولي بجرعة 1 مل / لتر , ثم تمت العدوى بعترتين مختلفين O78 و .O91 المجموعة الأخيرة رقم (7) تم استخدامها كمجموعة ضابطة سالبة بدون تحصين أو عدوى. تمت العدوى في جميع المجموعات عند عمر 21 يوم باستخدام العترة المشابهة O78 أو العترة المختلفة O91 بجرعة 0.5 مل / طائر بتركيز قدره 1.2 x 10^9 (C.F.U) من بكتيريا الإي كولاي الممرضة الخاصة بالطيور. تم حساب النتائج عند عمر 28 يوم اعتمادا علي النقاط الاتية: الأعراض العامة, العلامات النسيجية والجسمية, العدد الكلي لميكروب الإي كولاي داخل الأمعاء, وزن الطائر بالإضافة الي وزن الأعضاء الليمفاوية. النتائج توضح أن لقاح بولفاك® إي كولاي يعطي حماية واضحة ضد العدوى بالعترتين المشابهة O78 أو المختلفة . O91 بناءً علي الحيثيات سابقة الذكر تبين أن المجموعات التي تم تحصينها عند عمر 7 أيام (3 و 6) والتي تم معالجتها بالمضاد الحيوي (داي فلوكساسين) والعدوى بالعترتين سواء المشابهة O78 أو المختلفة O91 تعطي نتائج أعلي من المجموعات التي تم تحصينها عمر يوم (4 و5) وتمت العدوى فيهم بالعترتين O78 و O91 ولم يتم معالجتهم باستخدام أي مضاد حيوي.