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IMMUNOLOGICAL STUDIES ON AVIAN E.COLI VACCINE WITH SPECIAL REFERENCE TO PREPARATION AND EVALUATION

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SUMMARY

Local prepared *E.coli* F11 fimbriae vaccine was prepared and compared with an imported one against chicken *E.coli* infection. The antibody response in sera of chickens vaccinated with the prepared E.coli F11 fimbriae vaccine (group 1) and the antibody response in sera of chickens vaccinated with oil imported *E.coli* vaccine (group 2) as determined by ELISA were similar. The antibody response in sera of chickens of groups 1&2 appeared from the first week post vaccination and reached the maximum at the third week post second vaccination. Challenge of vaccinated and control chickens were done at the third week post second vaccination. Chickens of groups 1&2 have decreased number of lesion scores than the control one. Also, chickens of groups 1 and 2 and challenged with heterologous *E.coli* strains (08, 114, and 119) have increased number of lesion scores than that challenged with homologous *E.coli* strains (01, 02, and 078). In addition, chickens of groups 1 and 2 have higher percent of protection (PIS: had 84, 70, 80, and 67 % respectively) than the control one (PIS: 34 %). In conclusion, the prepared F11 fimbriae vaccine from a combination of 01, 02 and 078 *E.coli* strains was seemed to cover good range of protection and has been elicited a protective immune response against virulent *E.coli* challenge with homologous and hetorologous strains. Over all, strong correlation was found between antibody response in vaccinated groups and low lesion score that indicated a good protection.

INTRODUCTION

Escherichia coli septicemia or colibacillosis is a common disease in poultry; include egg peritoni-

tis, omphalitis, coligranuloma, cellulites, and colisepticaemia, of which the latter is the most severe form. In domestic poultry, avian colibacillosis is frequently affects chickens, turkeys and ducks of 3-12 weeks of old broiler Gross,(1994). *E.coli* infection may be due to egg transmission from infected parent stock Gross, (1994) . Colibacillosis is usually a secondary infection, with *E. coli* entering via the respiratory tract after damage caused by Mycoplasma sp. or viral e.g. New Castle Disease virus, Infectious Bronchitis Virus infection that lead to invasion of the blood and internal organs Environmental stresses such as overcrowding, and poor ventilation predispose birds to *E. coli* infections. A wide variety of *E. coli* serotypes are involved, but in most studies more than half of the infecting strains belong to one of the serotypes O1:K1, 02:K1, 078:K80, and 035 Gomis et al (2001) , kariyawasam et al (2004). With regard to the pathogenesis of avian colibacillosis, a correlation between virulence and adherence to tracheal or pharyngeal epithelial cells was suggested Dho-Moulin and Fairbrother (1999). Adherence to epithelial cells is likely to be a fundamental requirement for colonization of the respiratory tract by *E. coli* Laragione and Woodward (2002).

Avian colisepticaemia is a multi-factorial disease and that to date only a limited number of vjrulence factors of Avian Pathogenic *Escherichia coli* APEC have been thoroughly elucidated LaRagione and Woodward (2002). Several potential virulence factors have been associated with APEC strains, including type 1 (F1A) and P (F11) fimbriae, curli, the aerobactin iron-sequestering system, outer membrane proteins, K1 capsular antigen, temperature-sensitive hemagglutinin (Tsh), FimH and resistance to the bactericidal effect of serum Dho-Moulin and Fairbrother.(1999). Fimbriae are thought to be involved in infection and colonization. Pourbakhsh et al (1997). *Escherichia coli* strains that cause septicemia of poultry often possess F1 (type 1) fimbriae and/or P fimbriae Dozois et al (1995). Colibacillosis is one of most economically world wide lead to death of poultry, carcass condemnation and the cost of treatment resulting in millions of dollars lost each year Gross, (1994). The Coasts associated with using antibacterial agents have led to increase the trials to have alternative methods for protecting flocks against *E.coli* infection Dhillon and jack (1996); Killed, subunit and live vaccines all have been evaluated to develop an effective vaccine against colibacillosis in poultry. Zigterman et al. (1993). Vaccines containing killed or attenuated virulent bacteria protect against infection with the homologous strain but are less efficient against heterologous strains. Hence, vaccination against colibacillosis is not widely practiced because of the large variety of serogroups involved in field outbreaks Deb and Harry (1978). Pillus vaccines have protected chickens against challenge with homologous E.coli strain Gyimah et al (1986).

P fimbrai or f 11 are expressed in air sacs of chickens, it might be involved in colonization of systemic organs and subsequent septicemia Lara-grone and Woodward (2002).

The purpose of this work was to develop F11 fimbriae vaccine that would protect poultry against heterologous strains of *E.coli*, detection of *E.coli* antibody titers in sera of vaccinated chicken, evaluation of the efficacy of this vaccine to heterologous challenge with *E.coli* strains.

3. Material & Methods:

3.1. Bacterial strains:

E.coli serotypes O1:K1, 02:K1 and 078:K80 that isolated form septicemic chicken were used for preparation and evaluation of the vaccine. *E.coli* serotypes 08, 114, 119 were used for challenge of vaccinated chickens only. All *E.coli* strains were kindly supplied by Professor Dr. Zakria, Animal Health Research Institute, Dokki, Cairo. The relative pathogenicity of these strains were re-evaluated in day old susceptible chickens before vaccine preparation and pre-challenged. The E.Coli strains were confirmed by biochemical tests on API 20E strips (biomeriax) according to Edward and Ewing (1972).

3.2. Vaccines used:

3.2.1. Preparation of *E.coli* F11 vaccine:

According to Johannes et al (1993) the vaccine of E. Coli F11 was prepared and F11 fimbria was measured at 546nm by spectrium diagnosis kit according to manfucture in struction of spectrium diagnosis kit. The fimbria was emilsifect in oil adjuvants consists of whiterex 09 oil, 9 parts, spain (one part) which represents oil phase. Tween was added to fimbriae antigen in percentage of 2% aquase phase the ratio of aqueous phase to oil phase was 1: 2. All these substance were mixed together will in an emulsifier until obtaing a stable emulsion.

Fimbriae were purified from Escherichia coli strains (Ol:K1, O2:K1, and 078:K80), according to Van Den Bosch et al., (1993).

3.2.2. Oil imported *E.coli* vaccine:

An imported inactivated *E.coli* vaccine containing fimbrial antigen (F11) and flagellar toxin (FT) (intervet, Nethelands).

3.2.3. - Quality control of prepared vaccine:

The prepared F11 fimbriae vaccine and the Oil imported E.coli vaccine were subjected to a number of quality control tests based on sterility, safety and potency criteria following standard international protocols of British pharmacopoeia veterinary (2005). And code of American federal regulation (1985).

3.3. Experimental design:

Table 1 illustrate the design of the experiment·

3.4. Serological testing:

Ten random blood samples were aseptically collected from each group of chickens just before vaccination then after 1, 2, 3, and 4 weeks post vaccination. Sera were obtained and stored at -20°C. Sera were tested for estimation of humoral immune response to *E.coli* vaccines by using Enzyme Linked Immunosorbant Assay (ELISA).

3.4.1. Enzyme-linked immunosorbent assay (ELISA):

Indirect enzyme linked immunosorbent assay E;L.I. S.A :

ELISA were performed essentially as described by Johannes et al (1993). The antigens (of 01, 02, and 078) were diluted to concentration of 2.2ug/ml. ELISA 96 immunoplates were coated with 100ul/ml of 01, 02 078 separately the plates were blocked for 20 mite at 37°C with 200 ug of P.B.S Per well with 5% bovine serum albumin. Serum samples were diluted to 1 : 10 and added as 100ul/well induplicates for each sampl. Each Microtiter plates contained positive and negative sera as well as a blank as controls.

The rabbit antichicken horse peroxidose conjugate antibody was diluted as (1: 10.000) and 100 ul was added to each well. The cut off means absorbance value of 01, 02 and 078 were 0.6. The above these cut off values a serum samples were regarded as positive.

3.5. Challenge of chickens:

Table (1) illustrated the chickens in which all vaccinated and control chickens were challenged with 0.1ml s/c of 24 hrs *E.coli* old broth culture Each strain were challenged with 0.1 ml of a 24 hour brain heart infusion broth culture containing 1.0×10^5 colony forming units (CFU) of one strain of *E.coli* broth culture, (01, 02, 078, 08, 114, 119) serotypes Gyimah and Panigrahy, 1985).

After 3, 7, and 10 days of challenge, dead and survivors birds of post challenge were examined euthanatized and necropsied. Samples were taken for bacteriological examination and data obtained from all groups were used to evaluate the effects of vaccine according to British pharmacopoeia veterinary (2005).

3.5.1. Gross lesions in the air sacs, pericardial sac and livers were scored as follow):

Using the flowing formula described by Kanyawasam et al (2004)

O = no lesions

1 = cloudy air sac or pericardial sacs or hepatitis.

2 = Moderate air sacculitis or pericarditis or hepatitis.

3 = bilateral airsacculitis or pericarditis or hepatitis.

4 = severe and extensive fibrinous air sacculitis or pericarditis.

3.5.2. Protective indices (PIS):

Using the flowing formula described by Timms and marshal (1989) protective indices (PIS) were assessed according to mortality (M) and post-mortum (PM) lesions (PML).

$$PIS = \frac{\% (M + PML) \text{ controls} - \% \text{ vaccinated} \times 100}{\% \text{ control}}$$

RESULTS AND DISCUSSION

Colibacillosis cause by Infections with avian pathogenic *Escherichia coli* (APEC) organism. Experimental studies have shown that the respiratory tract, principally the gas-exchange region of the lung and the interstitial of the air sacs are the most important sites of entry for APEC. Resulting in massive lesions in multiple internal organs and in sudden death of the birds Ewers et al., 2003). Vaccines provide limited homologous protection against the pathogen and they suggest that research is needed to develop a good, broad-spectrum vaccine. Vandemaele et al.,(2002). APEC strains being resistant to a wide range of antibiotics due to wide use of antibiotherapy. However residues in eggs may occur in antibiotics chosen on the basis of sensitivity testing. Vaccines containing killed or attenuated virulent bacteria protect for infection with the homologous strain but are less efficient against heterologous strains Dho-Moulin and Fairbrother (1999).the results of sterility test of the prepared vaccine indicated that inactivated *E.coli* vaccine is free from contamination (aerobic ,anaerobic bacteria ,fungus and mycoplasm).Concerning safety of the prepared vaccine ,it was found that chickens vaccinated with double vaccine doses did not show any abnormalities or adverse reaction. Hence, vaccination for colibacillosis is should involve large number of (APEC) strains because of the large variety of serogroups involved in field outbreaks.

Avian colibacillosis generally affects broilers between 3 and 10 weeks of age Marc et al., (1998). Vaccination of chickens at 2 weeks of age with two doses.

From the results of table 2 that the antibody response in sera of chickens vaccinated with the prepared *E.coli* F11 fimbriae vaccine (group 1) and the antibody response in sera of chickens vaccinated with oil imported *E.coli* vaccine (group 2) were similar. The antibody response in sera of chickens vaccinated with the prepared E.coli F11 fimbriae vaccine (group 1) appeared from the first week post vaccination with titer of 1.7, 1.9, and 1.6 for 01, 02, and 078, respectively and reached the maximum at the third week post second vaccination with Titer of 2.6, 2.8 and 2.5 for 01, 02, and 078, respectively. The antibody response in sera of chickens vaccinated with oil imported E.coli vaccine (group 2) appeared from the first week post vaccination with titer of 1.6, 1.8, and 1.5 for 01, 02, and 078, respectively and reached the maximum at the third week post second vaccination the end of experiment with titer

2.5, 2.7 and 2.4 for 01, 02, and 078 respectively. High titer post challenge in dictating further maturation of humeral immune response generated by vaccine. These results were agree with Noha et al., (2007).

Samples were taken to re-isolate the challenged strain from all dead birds. Mortality and Macroscopic lesion scores are shown in table (3 & 4) and photo (1& 2).

Regarding challenge test table 3 chickens vaccinated with the prepared *E.coli* F11 fimbriae vaccine (group 1) and chickens vaccinated with oil imported *E.coli* vaccine (group 2) reveled that the number of lesion scores was decreased than the control one. Also, chickens of groups 1 and 2 and challenged with heterologous *E.coli* strains (08, 0114, and 0119) recorded that the number of lesion scores was increased than that challenged with homologous *E.coli* strains (01, 02, and 078). Vaccination trials showed that active immunization with the prepared vaccine twice at 14 and 28 days of age provided the best protection against challenge. These results were agreed with Hassan et al., (1999).

It could be seen also from the results of table 4 that chickens vaccinated with the prepared F11 fimbriae vaccine (group 1) and chickens vaccinated with oil imported vaccine (group 2) showed higher acceptable percent of protection (PIS: 84, 70, 80, and 67 % respectively) than the control one (PIS: 34 %), which indication of acceptable levels for the locally prepared F11 vaccine and the imported vaccine. Also, chickens of groups 1 and challenged with homologous *E.coli* strains (01, 02, and 078) showed percent of protection PIS: 84% than that challenged with heterologous E.coli strains (08, 114, and 119) which showed PIS: 70%. Chickens of groups 2 and challenged with homologous *E.coli* strains (01, 02, and 078) showed percent of protection (PIS: 80%) than that challenged with heterologous *E.coli* strains (08, 114, and 119) that showed (PIS: 67%). These results were agreed with British Pharmacopia vet. (2005).

In conclusion, the prepared F11 fimbriae vaccine from a combination of 01, 02 and 078 *E.coli* strains seem to cover good range of protection and has been elicited a protective immune response against challenged with virulent *E.coli* either homologous or hetorologous strains. Over all, strong correlation was found between antibody response in vaccinated groups and low lesion score which , indicating good protection.

Table 1: Vaccination and challenge schedule of chicken groups:

Groups	No. of chick.	Vaccine	Dose of vaccine	bleeding	Challenge
First group	A 30 chicken	E.coli F11 locally prepared vaccine	Two doses of 0.5ml S/C three weeks a part at the base of neck, the first dose at two weeks of age.	Ten random blood samples were aseptically collected from each group of chickens just before vaccination then after 1, 2, 3, and 4 weeks post vaccination.	Mixed with 0.1ml s/c of E.Coli 01, 02, 078 each containing 1.0 x 10^4 (CFU)
	B 30 chicken				Mixed with 0.1ml s/c of E.Coli 08, 0114, 0119 each containing 1.0 x 10^4 (CFU)
Second group	A 30 chicken	Oil imported E.coli vaccine	Two doses of 0.5ml S/C three weeks a part at the base of neck, the first dose at two weeks of age.		Mixed with 0.1ml s/c of E.Coli 01, 02, 078 each containing 1.0 x 10^5 (CFU)
	B 30 chicken				Mixed with 0.1ml s/c of E.Coli 08, 0114, 0119 each containing 1.0 x 10^5 (CFU)
Third group	30 chicken	Control	Control		Mixed with 0.1ml s/c of E.Coli 01, 02, 078 each containing 1.0 x 10^5 (CFU)
	30 chicken				Mixed with 0.1ml s/c of E.Coli 08, 0114, 0119 each containing 1.0 x 10^5 (CFU)
fourth group	20 chicken	Control Negative blank	Control Negative blank		Control Negative blank

Table (2): ELISA mean absorbance value of chicken sera using the E.coli serotypes 01, 02, 078.

Chicken groups		Antigen used	First dose of vaccination	Weeks Post first dose of Vaccination		Second dose of vaccination	Weeks Post Second dose of Vaccination		
				1st w	2nd w		1st w	2nd w	3rd w
Vaccinated groups	*Group 1	01		1.7	1.9		2.2	2.4	2.6
		02		1.9	2.2		2.3	2.5	2.8
		078		1.6	1.8		2.1	2.3	2.5
	**Group 2	01		1.6	1.8		2.1	2.3	2.5
		02		1.8	2.1		2.2	2.4	2.7
		078		1.5	1.8		2	2.2	2.4
Non vaccinated group	Group 3	01		0.6	0.5		0.6	0.4	0.6
		02		0.5	0.5		0.6	0.5	0.6
		078		0.7	0.5		0.6	0.6	0.5

* group 1 : vaccinated with prepared vaccine

** group 2 : vaccinated with imported vaccine

Table (3): lesion scores of chickens vaccinated with locally prepared F11 vaccine, imported and non vaccinated control groups, after challenge.

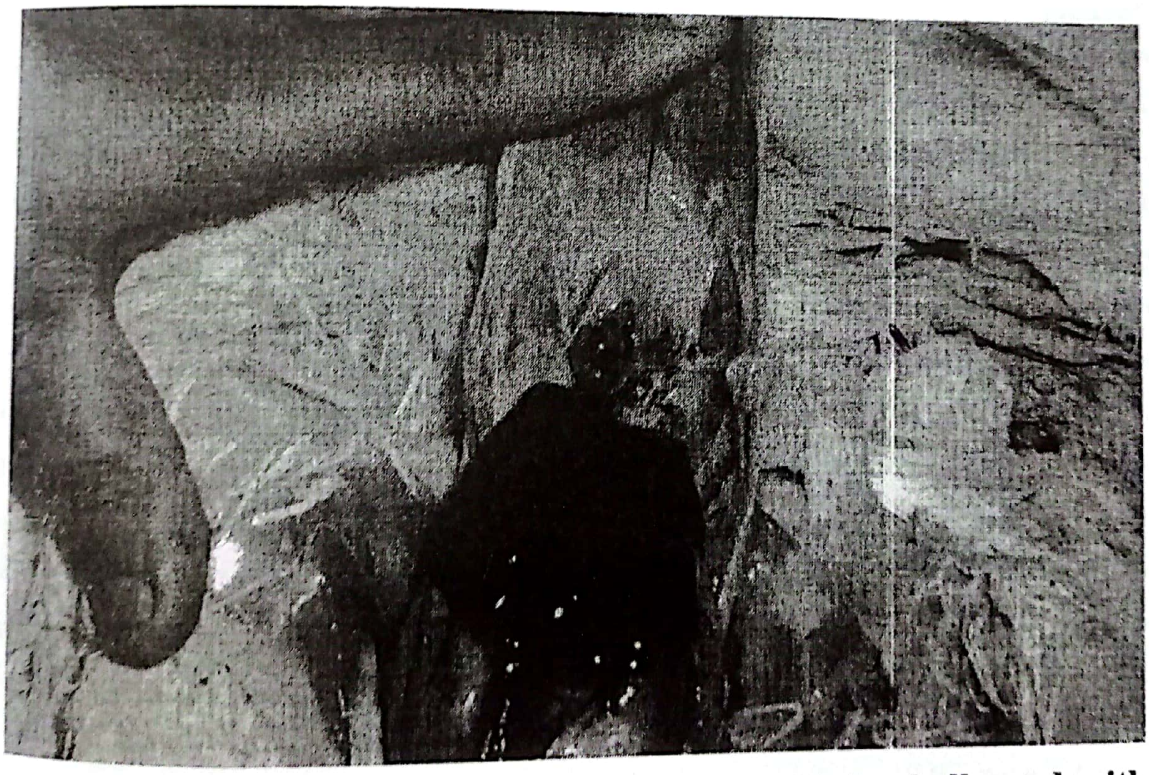
Chicken groups	**Subgroups*	*No of birds with each group*					
		0	1	2	3	4	5
Group 1 locally (prepared F11 vaccine)	A	25	2	2	-	-	1
	B	21	4	3	1	-	1
Group 2 imported vaccine	A	24	2	2	1	-	1
	B	20	2	3	2	2	1
Group 3 (Control)	A	-	-	3	6	8	13
	B	-	-	4	7	7	12

0 = No lesions
1 = Cloudy air sacs or pericardium or hepatitis.
2 = Moderate air sacculitis or pericarditis or hepatitis.
3 = bilateral air sacculitis or pericarditis or hepatitis.
4 = Sever bilateral fibrinous air sacculitis or pericarditis or hepatitis.
5 = Dead birds.
*Each group divided into 2 subgroups A and B.
Subgroup A: Challenged with homologous E.Coli strains (01, 02, and 078).
Subgroup B: Challenged with heterologous E.Coli strains (08, 0114, and 0119).

Table (4): Protective indexes PIS assessment in chickens vaccinated with locally prepared F11 vaccine, imported vaccine and non vaccinated control groups.

Chicken groups	*Subgroups*	Dead/total	Survival with lesion/total	% of birds with lesions	PIS
Group 1	A	2/30	5/30	16	84%
	B	5/30	9/30	30	70%
Group 2	A	3/30	6/30	20	80%
	B	6/30	10/30	33	67%
Group 3	A	10/30	20/30	66	34%
	B	10/30	20/30	66	34%

Photoo (1): Post mortem lesions of non vaccinated birds challenged with virulent *E.coli* strain of 01.02 and O78.



Photoo (2): Post mortem lesions of vaccinated birds challenged with virulent *E.coli* strain of 01.02 and O78.

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دراسات لتحضير وتقييم لقاح الأيشريشيا كولاى فى الطيور

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**المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية - العباسية - القاهرة

***كلية الطب البيطرى - جامعة بنها

- فى هذه الدراسة تم تحضير لقاح الفمبريا ف11 المحلى متعدد العترات من عترات القولون المعوى ومقارنته باللقاح الزيتى المستورد.
- تم اختيار كفاءة لقاح الفمبريا المحلى بتحصين مجموعتين من الكتاكيت أ،ب بجرعتين من اللقاح المحضر فى الأسبوع الثانى والأسبوع الرابع وكذلك اللقاح الزيتى المستورد.
- تم تقييم الإستجابة المناعية باستخدام اختبار الإليزا حيث أظهرت النتائج إرتفاع معدل الأجسام المناعية فى الكتاكيت المحصنة ابتداء من الأسبوع الثانى وحتى الأسبوع الرابع حيث وصلت إلى أعلى مستوى فى الأسبوع الثالث من التحصين وهى النتائج كانت مشابهة لنتائج السيرولوجية للقاح الزيتى المستورد.
- تم إجراء اختبار التحدى باستخدام العترات الضارية المحضر من اللقاح فى المجموعة (أ) وعترات الـ 8 والـ 114 ، 119 من عترات القولون المعوى فى المجموعة (ب)
- وذلك عن طريق حساب نسبة النفوق ونسبة التأثير المرضى فى الكتاكيت المحصنة ومقارنتها بالضوابط
- وقد أظهرت النتائج أن الكتاكيت المحصنة نسبة حماية مقارها 84٪ للقاح المحلى ، 80٪ للقاح المستورد.
- مما يثبت أن اللقاح الفميريا المحلى يعطى حماية جيدة يمكن استخدامه فى التحصين فى المزارع وتطبيقه فى المزارع.