Mansoura Veterinary Medical Journal

Volume 18 | Issue 1 Article 28

12-12-2017

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Amany Abd.ELEmoaty
Dep. of Bacteriology, Mycology and Immunology, Fac. Vet.Med, Mans. Univ.

Ahmad Ammar
Dep. of Microbiology, Fac. Vet. Med, Zagazig Univ.

AlaaEldin Moustafa
Dep. of Bacteriology, Mycology and Immunology, Fac. of Vet. Med. KafrElsheikh Univ.

Ibrahim Ibrahim
Vaccines Research Institute,Abbasia,Cario

How to Cite This Article

Abd.ELEmoaty, Amany; Ammar, Ahmad; Moustafa, AlaaEldin; and Ibrahim, Ibrahim (2017) "IMMUNOLOGICAL STUDIES ON E.COLI VACCINE IN CHICKEN," *Mansoura Veterinary Medical Journal*: Vol. 18: Iss. 1, Article 28.
DOI: https://doi.org/10.21608/mvmj.2017.127395

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***Amany,M,Abd.ELEmoaty, **Ahmad,M,A,Ammar, ***AlaaEldin,H,Moustafa, ****Ibrahim,S,Ibrahim**

**Dep. of Bacteriology, Mycology and Immunology, Fac. Vet.Med, Mans. Univ.*
***Dep. of Microbiology, Fac. Vet. Med, Zagazig Univ.*
****Dep. of Bacteriology, Mycology and Immunology, Fac. of Vet. Med. KafrElsheikh Univ.*
*****Vaccines Research Institute,Abbasia,Cario.*

ABSTRACT

The present study was carried out on One hundred and fifity , one day old chicks to evaluate the immunogenicity of multivalent pilus vaccine containing pili from the three common pathogenic serotypes of E. coli (O1, O2 and O78). Nienty vaccinated birds at 14 days & 28 days of age by 0.5ml s/c of pilus vaccine at base of neck and 60 unvaccinated were divided to 4 subgroups (30 vaccinated & 20 unvaccinated) were used for challenge two weeks after second dose . Each group was challenged each with one type of E.coli strains O1 , O2 and O78. Blood samples were collected during the experiment from the flocks one week before vaccination and weekly for 7th week after the first dose of vaccination for evaluation of immune response by using ELISA test. The prepared multivalent E.coil pilivaccine from combination of $O_{1,}$ O_2 and O_{78}E.coli strains were seemed to cover good range of protection and has been elicited a protective immune response against virulent E.coli challenge with homologous and heterologous strains. strong correlation was found between antibody response vaccinated groups and low lesions score that indicated a good protection

Keywords, *enzyme linked immune sorbent assay (ELISA).Esherichia coli (E.coli).*

INTRODUCTION

Escherichia Coli is a major pathogen of worldwide importance in commercially produced poultry contributing significantly to economic losses in avian species E.coli has been associated with avariety of disease conditions in avian species including enteritis, arthritis , omphalitis , coligranulon, slapingitis, septicemia and complicated air sacculitis. The coli infection has become in the last decade a major problem in chickens all over the world *(Truscott, 1973; Awaad1975; Rosen Bergen et al., 1984 and Gross, 1991).* Several potential virulence factors are important in the virulence of avian pathogenic *E.coli*. These include pilli, resistance to complement (serum resistance), The aerobactin in scavenging system and the production of the K1 capsule (*Dozois et al., 1995 and Mellata et al., 2003*). Pili are commonly associated with adherence in *E.coli* and there have been several studies to identify pilli which might bear adhesions that are important in the virulence of avian pathogenic *E.coli*. *(Raveh et al., 1984)* demonstrated that three strains of *E.coli* (O1, O2 and O78) from colisepticaemia in poultry produced pilli.

MATERIAL AND METHODS

Samples One hundred and fifity , one day old chicks of specific pathogenic free (SPF) breed were obtained from El Fayoum and reared in battery brooders at faculty of veterinary Medicine, El Mansoura University for the experimental work of this study .Out of these flocks , chicks were used for the measurement of ID_{50} of *E. coli* strains as well as chicks were used for evaluation of safety of prepared vaccine used in this study. *E.coli* serotypes O_1, O_2, O_{78} were kindly obtained from Animal Health Research Institute - Dokki: as identified strains. These strains were the predominant common cause E.Colisepticaemia in chickens in Egypt. The pathogenicity of these strains was evaluated by Animal Health Research Institute Dokki before vaccine preparation and pre- challenge , confirmation of *E.coli* strains morphological examination,colonial appearance onto MacConkey bile salt lactose agar media, EMB media and finally by Biochemical identification accordimg to *krieg et al., (1994) and Collier et al., (1998).*

Serological typing of *E.coli* using slide agglutination test *(Sojka, 1965; Edwards and Ewing, 1972*) and preparation of *E.coli* experimenta vaccine.

Purified pili were prepared according to the method of *salit and Gotschlich (1977),*The prepared vaccines in the present study were tested for purity, sterility, completion of inactivation and safety tests, according to the standard international protocols as described by *the British veterinary codex (1970) and code of American Federal Regulation (1985)From each bird, blood samples* were collected .from the wing vein.Sera were separated and collected separately in sterile tubes and kept at - 20^{o}c for testing by using enzyme linked immune sorbent assay (ELISA) according to *leitner (1999).* Measurement of ID50 of *E.coli* strains according to *(Mackie and MacCartney,1989*) whil the end 50% end poit was caculate according to *Reed and Muench (1938).*

Challenged procedure after vaccination of chickens.

Mortolity rates, lesion scores and *E.coli* reisolation according to *Gyimah etal., (1985, 1986).*

Protective indices (PIS) was done according to *Timms and Marshall (1989).*

RESULTS

As could be seen from table (1) and figure (1) the mean ELISA values (OD) against O_1 plate antigen incrase from 0.05 prevaccination level to reach 1.2 and 1.8 at 3^{rd} and 4^{th} weeks of age . After boostering at 4^{th} week of age the values reach 2.01 and 2.3 at the 5th and 6th week of age . The mean ELISA values (OD) against O_2 plate antigen were increased from 0.07 prevaccination level to reach 1.6 , 2.01 , 2.2 and 2.4 at the 3^{rd},4^{th} , 5^{th} and 6^{th} week of age respectively.

The mean ELISA values (OD) against O_{78} plate antigen were increased from 0.05 prevaccination level to reach 1.4 , 1.9 , 2.01 and 2.1 at the 3^{rd} . 4^{th} , 5^{th} and 6^{th} week of age respectively .

The control unvaccinated chicken showed ELISA antibodies against O_1,O_2 and O_{78} plate antigen that ranged between 0.07 - 0.05 at various age interva.

Results of bioassay for evaluation the immunizing efficacy of multivalent *E.coli* pili vaccine:

From data available in table (2) it is evident on challenging vaccinates with *E.coli* serotypes O_1, O_2 and O_{78} the percent of chicken with lesions were 6.6%,10% and 3.3% respectively . While in the control unvaccinated chicken , the percent of birds with lesions were 60% , 80% and 50% after challenge with O_1 , O_2 and O_{78}E.coli serotype respectively .

Challenged with virulent E.coli serotypes :-

The gross lesion on post mortem examination of dead and sick vaccinated and control chicken after challenge were examined including the air sacs , pericardial sacs and liver of chicken . Results of lesion scores scaled from 0.4 are tabulated in table (3) describe the gross lesions on post mortem examination of dead and sick birds vaccinated with multivalent E,colipili vaccine at 14 & 28 days of age , after challenge with E.coli O_1 showed lesions score of 0.01 & 0.3 for air saculitis and perihepatitis , regarding birds challenged with E.coli O_2 serotype the lesion score were 0.2 , 0.2 & 0.4 for air saculitis , pericarditis & perihepatitis , concerning vaccinated birds challenged with E.coli O_{78} serotype the lesion score were 0.03 & 0.1 for air soculitis , pericarolitis & perihepatitis respectively .

E.coli was recovered from vaccinated chicken after challenge at apercentage of 4% . While the recovery rate from control unvaccinated chicken was 79.3%.

Protective index (PI) assessment in chicken vaccinated with the multivalent E.colipili vaccine:-

The data given in table (4) and figure (2) indicated that the total percent of birds with lesions in vaccinates were 6.7 % while it was 63.3 % in the control unvaccinated chicken . It was estimated that the protective index afforded by the multivalent E.colipili vaccine was 89.4 %.

Protective indices (PIs) assessment in vaccinated chicken with inactivated Trivalent E.coli vaccine at 14 and 28 days of age

The data given in table (5) and figure (3) indicated that the total percent of birds with lesion in vaccinated chickens with the inactivated trivalent E.coli vaccines was 40 % while it was 55 % in the control unvaccinated chicken . It was estimated that the protective index afforded by the inactivated vaccine at 14& 28 days of age was 27.2 % .

Table (1): Antibody levels against F_{11}insera of chicken postvaccination with multivalent E.colipili vaccine at 14 & 28 days of age measured by enzyme linked immune – sorbent assay (ELISA)

Group of chicken	Plate AG	Pre-vaccination	Weeks post vaccination						
			1week	2week	3week	4week	5week	6week	7week
Vaccinated	O_1	0.05±0.042	0.28±0.071	0.9±0.072	1.2±0.067	1.8±0.066	2.01±0.053	2.3±0.069	2.4±0.062
	O_2	0.07±0.012	0.25±0.074	1.2±0.067	1.6±0.043	2.01±0.050	2.2±0.091	2.4±0.062	2.6±0.058
	O_{78}	0.05±0.042	0.28±0.071	1.1±0.057	1.4±0.056	1.9±0.067	2.01±0.055	2.1±0.048	2.2±0.082
Controls	O_1, O_2 O_{78}	0,07±0.012	0.06±0.032	0.05±0.042	0.07±0.012	0.05±0.042	0.07±0.012	0.05±0.042	0.06±0.032

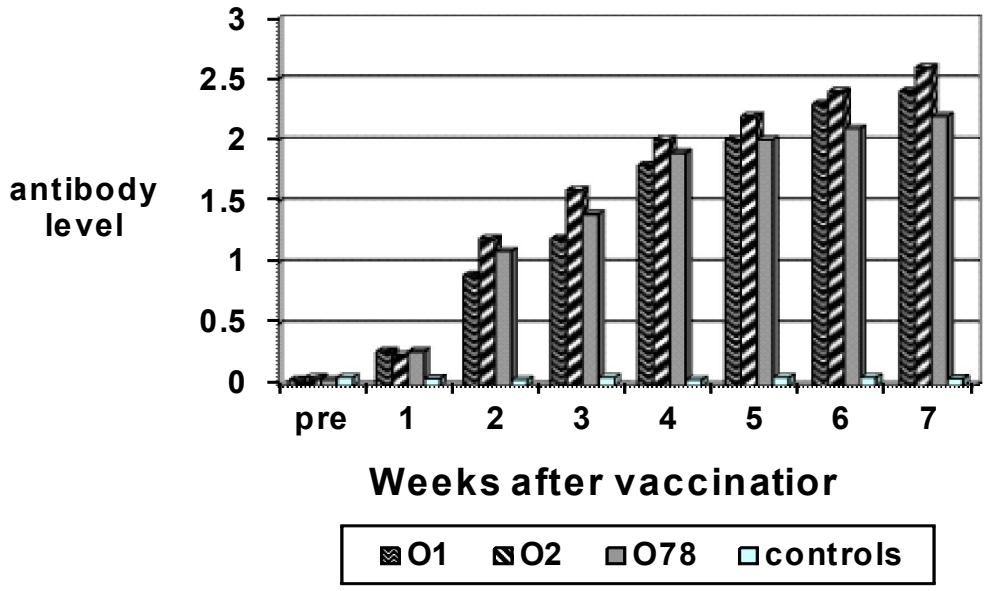

Figure (1) : Antibody levels against F_{11}insera of chicken postvaccination with multivalent E.colipili vaccine at 14 & 28 days of age as measured by enzyme linked immune – sorbent assay (ELISA)

Table (2): Post challenge immunostatus assessment in chicken vaccinated with multivalent E.colipili vaccine :-

Challenge Strain	Challenged birds with lesions					
	Vaccinated group			Controls		
	Dead/total	Survived/ Total*	% of birds With lesions**	Dead/Total	Survived/ Total	% of birds With lesion
O_1	1/30	1/30	6.6%	4/20	8/20	60%
O_2	1/30	2/30	10%	6/20	10/20	80%
O_{78}	0/30	1/30	3.3%	4/20	6/20	50%

* Surviving chicken that had symptoms typical of colibacillosis.

** Chicken with lesion/ total x 100.

Table(3): Lesions score in vaccinated and control chickens and challenged with virulent *E.coli* strains

Groups	Challenge strain									Recovery of E.coli (%)
	O_1			O_2			O_{78}			
	AS	PE	PH	AS	PE	PH	AS	PE	PH	
Vaccinated	0	0.1	0.3	0.2	0.2	0.4	0	0.3	0.1	4%
Controls	1.2	1.4	0.8	1.5	2.2	1. 0	1.2	1.2	0.3	79.3%

Table (4): Protective indices (PIs) assessment in vaccinated chicken with multivalent E.colipili vaccine

Group	Dead / Total	Survival with lesions/total*	%of birds With lesions**	PIs
Vaccinated	2/90	4/90	6.7%	89.4%
Unvaccinated Controls	14/60	24/60	63.3%	

* Surviving chicken that had symptoms typical of colibacillosis.

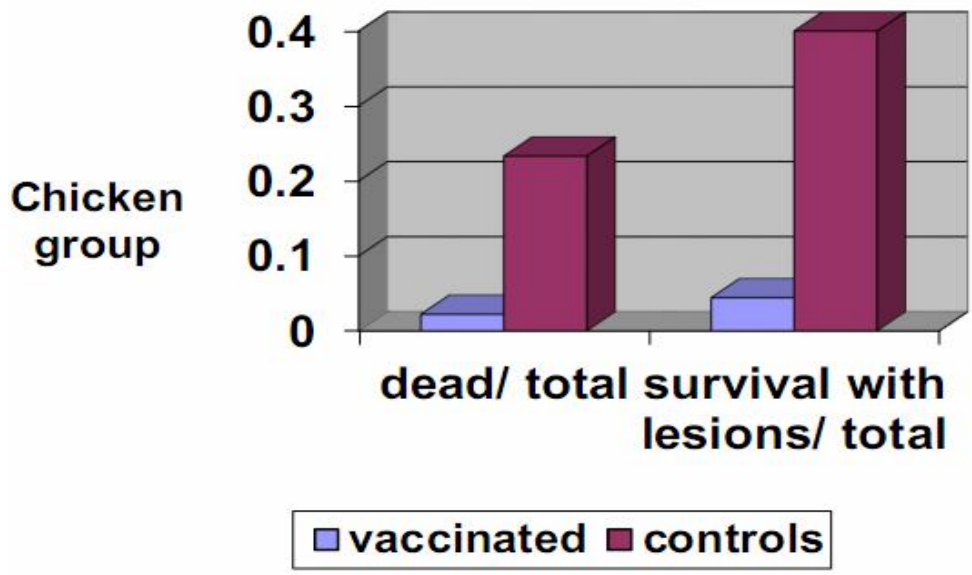

Figure (2): Protective indices (PIs) assessment in vaccinated chicken with multivalent E.colipili vaccine

Table (5): Protective indices (PIs) assessment in vaccinated chicken with inactivated Trivalent E.coli vaccine at 14 and 28 days of age

Group	Dead / Total	Survival with lesions/total*	% of birds With lesions**	PIs
Vaccinated	3/20	5/20	40%	27.2%
Controls	5/20	6/20	55%	

* Surviving chicken that had symptoms typical of colibacillosis.** Chicken with lesion/ total x 100.

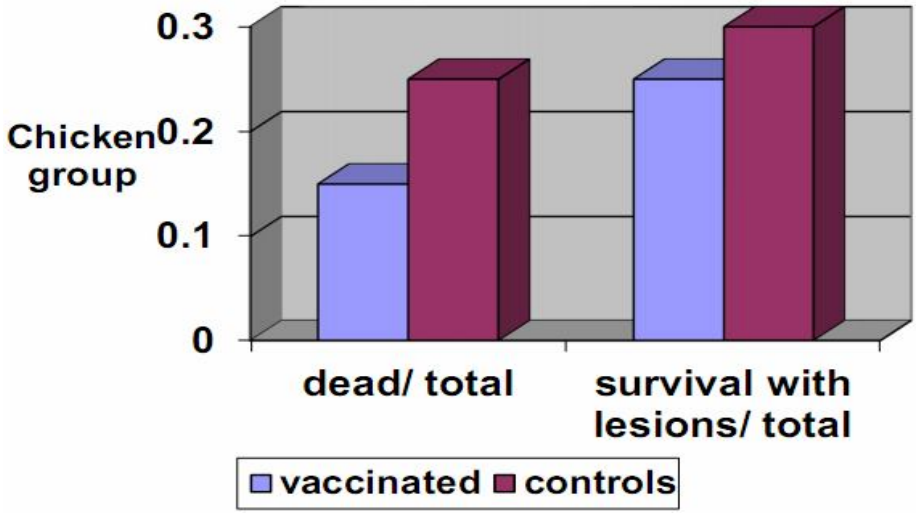

Figure (3): Protective indices (PIs) assessment in vaccinated chicken with inactivated Trivalent E.coli vaccine at 14 and 28

DISCUSSION

Recently, surface fimbriae (pili) of *E.coli* have been implicated as mediators of adhesion to the trachealepithelium of chicks and thereissomeevidencethat the bacteriainvade the trachealmucosaafterlimitedcolonization (*Dho et al., 1990 and yerushalmi et al., 1990) whileNaveh et al., (1984*) demonstratedthatthreestrains of *E. Coli* (O_1, O_2& O_{78}) fromcolisepticaemia in poultryproducedpili. Theyalsoshowedthatpiliatedstrainsadheredboth in vitro and in vivo to ciliatedtrachealepithelialcells and inoculation of chickenwithpiliatedstrainsresulted in a significantlyhigher occurrence of diseasecomparedwithbirdsinoculatedwiththese non piliatedstrains.

level and that ElISA is a highly efficient and sensitive method for assessment of *E.coli* antibodies. This resultscoincidewith the previousfindingsof *Leither et al., (1990)* , *Hassan et al., (1999) , Melamed et al., (1991) and Nohaetal., (2007).*

Who stated that the average gross lesions were generally higher in the unvaccinated chicken and that they were significantly different from those of vaccinates. In the presentwork the protection rate 89.4% confirms the findings of *saitanu (1993), Azzam (1998).*

Furthermore, *Vandemaele et al., (2003)* in their study sequenced that vaccination of broilerchickentwo doses of 0.5ml dose/ bird, inoculated S/C or I/M at 2 and 4 week of age gave good protection than that vaccinatedat 1 & 14 days of age. From the results in tables (3,4), itisnoticedthat multivalent pilus vaccine had a satisfactory prophylactic effect against challenge with virulent *E. coli* serotypes .The data given in table (5) and figure (5) indicatedthatafterchallengingvaccinates the percent of birdswithlesionswas 6.7% . Meanwhileitis ,63.3% in control ,the protection indices was 89.4 respectively. The abovementionedresults are in agreement withthose of *Gyimah and panigraphy (1985) and Gyimah et al., (1986)* whovaccinatedchickentwicely s/c at 2 and 4 week of agewith an oilemulsified E. coli multivalent pilus vaccine .Meanwhile, the same findings were reported by *Suwanickul et al., (1987).* Vaccines containing killed or attenuated protect against infection with the homologous strain but less efficient against heterologous strains *Dho. Moulin and Fair brother (1999).*

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الملخص العربى

دراسات مناعية عن لقاحات الميكروب القولوني في الدواجن

أمانى محمد عبد المعطي .* أحمد محمد أحمد عمار.** علاءالدين حسين مصطفي *** إبراهيم سليمان إبراهيم

* كلية الطب البيطري جامعة المنصورة
** استاذ متفرغ الميكروبيولوجيا قسم الميكروبيولوجيا كلية الطب البيطري جامعة الزقازيق
***استاذ مساعد الميكروبيولوجيا قسم البكتريولوجيا والفطريات والمناعة قسم البكتريولوجيا والفطريات والمناعة كلية البيطرى جامعة كفر الشيخ
****رئيس بحوث بمعهد الأمصال واللقاحات البيطرية – العباسية – القاهرة

يعتبر مرض الكوليباسيلوزيس واحدا من أهم الأمراض التي تصيب الدجاج والتي تؤدي إلي خسائر اقتصادية كبيرة نتيجة لارتفاع نسبة الإصابة، والنفوق بالإضافة إلي زيادة تكاليف العلاج . وتصاحب الإصابة بالمرض أعراض تشريحية كثيرة منها التسمم الدموي الحاد ، إلتهاب الغشاء التاموري والغشاء المحيط بالكبد ، والتهاب الأكياس الهوائية لذلك تم تحضير لقاح الفمبريا متعدد العترات من عترات القولون المعوي الأكثر ضراوة (O_1,O_2,O_{78}) ثم اختبار كفاءة اللقاح بتحصين الكتاكيت عند عمر ١٤ ،٢٨ يوم من عمر الكتاكيت ثم تقييم الإستجابة المناعية باستخدام اختبار الإليزا حيث أظهرت النتائج ارتفاع معدل الأجسام المناعية في الكتاكيت المحصنة من ٠,٠٥ ، ٠,٠٧ ، ٠,٠٥ لكل منO_1,O_2,O_{78}قبل التحصين لتصل إلي ٢,٣ ، ٢,٤ ، ٢,١ في الأسبوع السادس من التحصين بينما كانت نسبة الطيور الإيجابية للآفات التشريحية ٤% ومعامل الحماية ٨٩,٤ %الإيجابية للآفات التشريحية ٧٩,٣ % في المجموعة الضابطة مما يثبت أن اللقاح الفمبريا المحلي يعطي حماية جيدة يمكن إستخدامه في التحصين في المزارع