
Mansoura Veterinary Medical Journal

IMMUNOLOGICAL STUDIES ON E.COLI VACCINE IN CHICKEN

*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,Cairo.

ABSTRACT

The present study was carried out on One hundred and fifty , 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 O₁, O₂ and O₇₈*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 a variety 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 fifty, 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 ID₅₀ of *E. coli* strains as well as chicks were used for evaluation of safety of prepared vaccine used in this study. *E. coli* serotypes O₁, O₂, O₇₈ 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 according 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* experimental 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°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*) while the end 50% end point was calculated according to *Reed and Muench (1938)*.

Challenged procedure after vaccination of chickens.

Mortality rates, lesion scores and *E. coli* re-isolation according to *Gyimah et al., (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 O₁ plate antigen increase from 0.05 prevaccination level to reach 1.2 and 1.8 at 3rd and 4th weeks of age. After boosting at 4th 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 O₂ plate antigen were increased from 0.07 prevaccination level to reach 1.6, 2.01, 2.2 and 2.4 at the 3rd, 4th, 5th and 6th week of age respectively.

The mean ELISA values (OD) against O₇₈ plate antigen were increased from 0.05 prevaccination level to reach 1.4, 1.9, 2.01 and 2.1 at the 3rd, 4th, 5th and 6th week of age respectively.

The control unvaccinated chicken showed ELISA antibodies against O₁, O₂ and O₇₈ plate antigen that ranged between 0.07 - 0.05 at various age intervals.

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 O₁, O₂ and O₇₈ 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 O₁, O₂ and O₇₈ *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.coli* pili vaccine at 14 & 28 days of age, after challenge with *E.coli* O₁ showed lesion scores of 0.01 & 0.3 for airsacculitis and perihepatitis, regarding birds challenged with *E.coli* O₂ serotype the lesion scores were 0.2, 0.2 & 0.4 for airsacculitis, pericarditis & perihepatitis, concerning vaccinated birds challenged with *E.coli* O₇₈ serotype the lesion scores were 0.03 & 0.1 for

airsacculitis, pericarditis & perihepatitis respectively.

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

Protective index (PI) assessment in chicken vaccinated with the multivalent *E.coli* pili 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.coli* pili 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 lesions in vaccinated chickens with the inactivated trivalent *E.coli* vaccine 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 F₁₁ 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

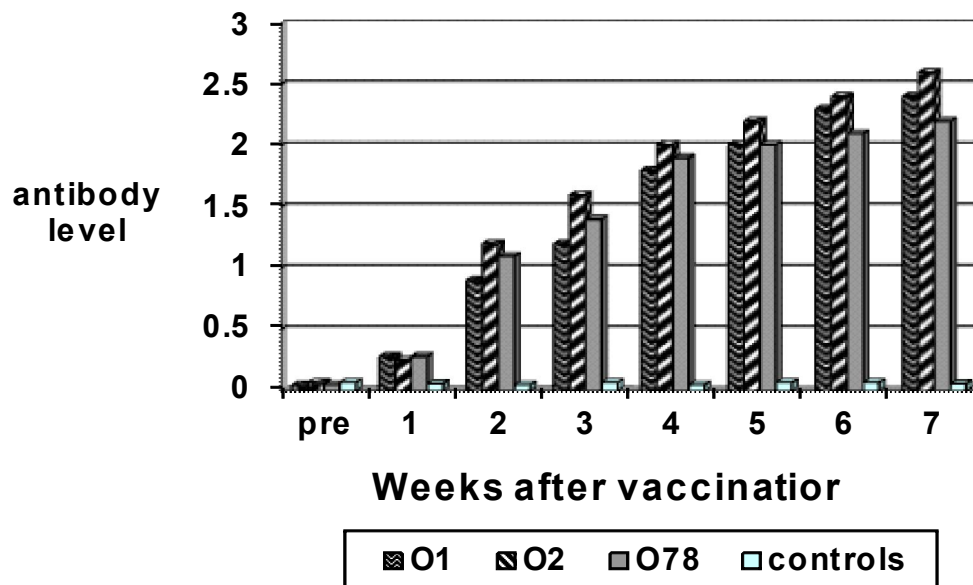


Figure (1) : Antibody levels against F₁₁ insera of chicken postvaccination with multivalent E.colipili vaccine at 14 & 28 days of age as measured by enzyme linked immune – sorbent assay (ELISA)

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 <i>E.coli</i> (%)
	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.

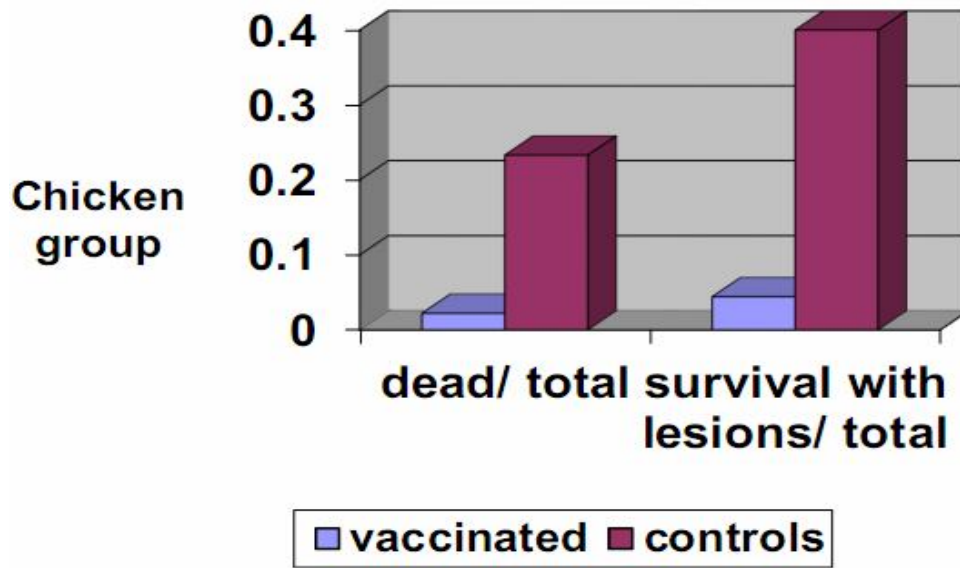


Figure (2): Protective indices (PIs) assessment in vaccinated chicken with multivalent E.colipili vaccine

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.

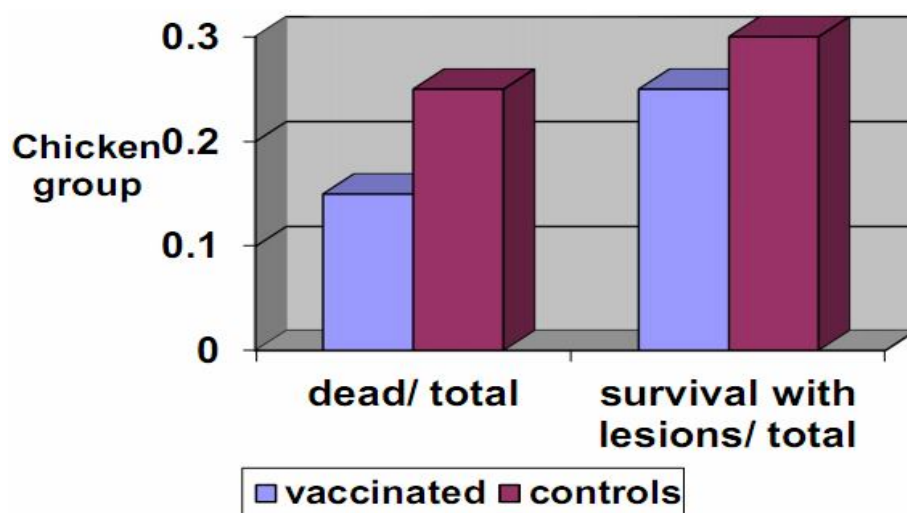


Figure (3): Protective indices (PIs) assessment in vaccinated chicken with inactivated Trivalent E.coli vaccine at 14 and 28

DISCUSSION

Recently, surface fimbriae (pili) of *E. coli* have been implicated as mediators of adhesion to the tracheal epithelium of chicks and there is some evidence that the bacteria invade the tracheal mucosa after limited colonization (Dho *et al.*, 1990 and Yerushalmi *et al.*, 1990) while Naveh *et al.*, (1984) demonstrated that three strains of *E. Coli* (O₁, O₂ & O₇₈) from colisepticaemia in poultry produced pili.

They also showed that piliated strains adhered both *in vitro* and *in vivo* to ciliated tracheal epithelial cells and inoculation of chicken with piliated strains resulted in a significantly higher occurrence of disease compared with birds inoculated with these non-piliated strains.

level and that ELISA is a highly efficient and sensitive method for assessment of *E. coli* antibodies. This result coincides with the previous findings of Leither *et al.*, (1990), Hassan *et al.*, (1999), Melamed *et al.*, (1991) and Noha *et al.*, (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 present work 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 broiler chick with two doses of 0.5 ml dose/bird, inoculated S/C or I/M at 2 and 4 weeks of age gave good protection than that vaccinated at 1 & 14 days of age. From the results in tables (3,4), it is noticed that multivalent pilus vaccine had a satisfactory prophylactic effect against challenge with virulent *E. coli* serotypes. The data given in table (5) and figure (5)

indicated that after challenging vaccinates the percent of birds with lesions was 6.7%. Meanwhile it is 63.3% in control, the protection indices was 89.4 respectively. The above mentioned results are in agreement with those of Gyimah and Panigraphy (1985) and Gyimah *et al.*, (1986) who vaccinated chick twice s/c at 2 and 4 weeks of age with an oil emulsified *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 Fairbrother (1999).

REFERENCE

- Awaad M.H. (1975)** : Studies on colisepticaemia in chicken M.V. Sc. Thesis (poultry), fac. Vet. Med., Cairo Univ.
- Azzam, A. H. (1998)**: Studies on colisepticaemia in poultry in Dakhliya province. M. V. Sc. Thesis (poultry Diseases), Fac, Vet. Med., Cairo University.
- British veterinary codex (1970)**: The pharmaceutical press, London.
- Code of American federal regulation (1985)**: Published by : the office of the federal register national archives records service. General services administration.
- Colier, I.; Balows. A; and Sussman. M. (1998)** : Topley and Wilson's Microbiology and Microbial infections. 9th edition volume 2 systematic bacteriology.
- Dho – Moulin, M. and Fairbrother, J.M. (1999)** : Avian pathogenic coli (APEC). Vet. Res., 30 (2-3):299-316.

- Dho , M.; Van den Bosch , J.P.; Girardeau , A.; Bree , A.; Barat, T. and Lafont , J. (1990):** Surface antigens from Escherichia coli O2 and O78 strains of avian origin . *Infect . Immun.*, 58:740-745.
- Dozois, C. M. ; Pourbakhsh, S. A. and Fairbrother, J. M. (1995):** Expression of pandemic type 1 (f1) fimbriae in pathogenic Escherichia coli from poultry . *vet. Microbial.*, 45: 297-309.
- Ewing, W.H. (1972):** Identification of Enterobacteriaceae 3rd. Ed., Burgess Publishing co. Minneapolis
- Gross, W. B. (1991):** Colibacillosis in: diseases of poultry . 9th Ed. Editors calnek, B. W. et al ., Iowa State Univ. press, Ames Iowa, USA. Pp. 138-144.
- Gyimah , J. E .; Panigraphy . B.; Hall , C.F. and William , J.D. (1985) :** Immunogenicity of an oil emulsified Escherichia coli bacterin against heterologous challenge . *Avian Dis . , 29 (2) : 540 – 545 .*
- Gyimah, J. E.; Panigraphy, B. and Williams, J. D. (1986):** Immunogenicity of an Escherichia coli multivalent pilus vaccine in chickens. *Avian Dis.*, 30(4): 687-689.
- Hassan ,H.A.;waffa, A.Goniemy; Halla, A.Fadlei; Hussein, A.Z.and Ibrahim , I.S.(1999):** Immunogenicity of an oil emulsified Escherichia coli vaccine in chickens .*Beni – suef Vet .Med .J.*,9 (3A) : 149 – 164 .
- Krieg, N.R.; Holt, J.C.; Murray, R.G.E; Brenner, D.J.; Bruant, M.P.; Moulder, J.W.; and staley, J.T. (1994):** Bergey's Manual of determinative Bacteriology Ninth edition REFQR81.B47.
- Leitner, G.D.; Melamed, N. Drabkin and Heller, E. D. (1990):** An enzyme linked immunosorbent assay for detection of antibodies against Escherichia coli association with indirect haemagglutination test and survival. *Avian Dis.*, 34: 58-62.
- Mackie, T. J. and MacCartney, J. E. (1989):** Biochemical tests for identification of medical bacteriology . 2nd. Wilkins company , Baltimore, USA.
- Melamed , D.; Leitner , G. and Heller , E.D. (1991):** A vaccine against avian colibacillosis based on ultra sonic inactivation of Escherichia coli .
- Mellata, M.; Dho-Moulin, M.; Dozoiz, C. M. Curtiss, R.; Brown, P. K.; Arne, P.; Bree, A.; Desautels, C. and Fairbrother, J. M. (2003):** "Role of avian pathogenic Escherichia coli to serum and in pathogenicity " *Infect. Immun*, 71 (1): 536 - 40.
- Naveh , M.W.; Zusman , T.; Shutelsky , E. and Ron , E. (1984) :** Adherence pili in avian strains of Escherichia coli : Effect on pathogenicity . *Avian Dis . , 28 : 651 – 661 . .*
- Noha, A. Helmy.; El Kholy. A.A.; Amal M.EL Sawah; AboulSaoud, S.M.; and Adel Rahman, A.O. (2007):** Preliminary preparation and evaluation of local lysate vaccine for protection of chicken against E.coli infection . *Vet.Med.j.giza*,55,1,--.
- Raveh, M.W.; Zusman, T.; shutelsky, E. and Ron, E.(1984):** Adherence pilli in avian strains of Escherichia coli : Effect on pathogenicity. *Avian Dis.*, 28 : 651 – 661 .
- Read, L. J. and Muench, H.(1938):** A simple method of estimating fifty percent end points. *Am. J. Hyg.*, 27(3): 493-497..

- Rosen Berger, J. K; Fries, P. A. and Cloud, S. S. (1984):** in vitro and in vivo characterization of avian Escherichia coli. 111- Immunization. Avian Dis., 29:1108-117
- Saitanu, K. (1993):** Escherichia coli infection in the respiratory system of broilers: part IV Immune response to Escherichia coli bacterin. Thai. J. Vet. Med., 23(3): 717-235..
- Salit, I.E., and Gotschlich, E.C. (1977):**Hemagglutination by purified type 1 Escherichia coli. J. Exp. Med. 14b : 1169 – 1187 . Lowry ,O.H., Rosebrough, N.J , farr, A.L. and Randall. J. Biol. Chem. 193 : 265 – 275.
- Sojka, W. J. (1965):** Escherichia coli in domestic animals and poultry. 1st edition, Common Wealth Agricultural Bureaux, Farnham, Royal, Bucks. England. Pp. 221-231.
- Timms ,L.M and Marshall , N. (1989):** Laboratory assessment of protection given by experimental pasterurellaanatipestifer vaccine .Br .Vet .J.,154:483 – 487..
- Truscott,R.B. (1973):** Studies on the chick lothal Toxin of Escherichia coli. Comp. Med. 37 : 375-381 .
- Vandemeal,F., Vandekerchove , D.; Vereecken , Mm.; Derijcke , J.; Dho . MOulih , M . and Goddeeris , B .M. (2003) :** " sequence analysis demonstrates the conservation of fim H and variability of fim H throughout avain pathogenic Escherichia coli (PEC) " Vet . Res ., 34 (2) : 153 .

الملخص العربي

دراسات مناعية عن لقاحات الميكروب القولوني في الدواجن

أمانى محمد عبد المعطي .¹ أحمد محمد أحمد عمار.² علاء الدين حسين مصطفى³ 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

البيطري جامعة كفر الشيخ

**** رئيس بحوث بمعهد الأمصال واللقاحات البيطرية – العباسية – القاهرة

يعتبر مرض الكوليباسيلوزيس واحدا من أهم الأمراض التي تصيب الدجاج والتي تؤدي إلى خسائر اقتصادية كبيرة نتيجة لارتفاع نسبة الإصابة، والنفوق بالإضافة إلى زيادة تكاليف العلاج . وتصاحب الإصابة بالمرض أعراض تشريحية كثيرة منها التسمم الدموي الحاد ، التهاب 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 ، ٤٢ يوم من عمر الكتاكيت ثم تقييم الإستجابة المناعية باستخدام اختبار الإليزا حيث أظهرت النتائج ارتفاع معدل الأجسام المناعية في الكتاكيت المحصنة من ٠,٥ ، ٠,٧ ، ٠,٥ لكل من O_{1},O_{2},O_{78} قبل التحصين لتصل إلى 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