

## **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 heterologous strains. Over all, strong correlation was found between antibody response in vaccinated groups and low lesion score that indicated a good protection.

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### **INTRODUCTION**

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

titis, 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, O2:K1, O78:K80, and O35 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 virulence 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 fimbriae or f 11 are expressed in air sacs of chickens, it might be involved in colonization of systemic organs and subsequent septicemia Laragione 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, O2:K1 and O78:K80 that isolated from septicemic chicken were used for preparation and evaluation of the vaccine. *E.coli* serotypes O8, O114, O119 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 manufacture in struction of spectrium diagnosis kit. The fimbria was emulsified in oil adjuvants consists of whiterex 09 oil, 9 parts, span (one part) which represents oil phase. Tween was added to fimbriae antigen in percentage of 2% aqueous phase the ratio of aqueous phase to oil phase was 1: 2. All these substance were mixed together with an emulsifier until obtaining a stable emulsion.

Fimbriae were purified from *Escherichia coli* strains (O1:K1, O2:K1, and O78: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, Netherlands).

##### **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 antichickens horse peroxidase 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  $1.0 \times 10^5$  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 Kariyawasam 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 following formula described by Timms and Marshall (1989) protective indices (PIS) were assessed according to mortality (M) and post-mortum (PM) lesions (PML).

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

## RESULTS AND DISCUSSION

Colibacillosis caused 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 mycoplasma). 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 should involve a 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) revealed 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 heterologous 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<br>30<br>chicken | E.coli F11<br>locally prepared<br>vaccine | Two doses of<br>0.5ml S/C three<br>weeks a part at<br>the base of neck,<br>the first dose at<br>two weeks of age. | Ten random<br>blood samples<br>were<br>aseptically<br>collected from<br>each group of<br>chickens just<br>before<br>vaccination<br>then after 1, 2,<br>3, and 4 weeks<br>post<br>vaccination. | Mixed with 0.1ml s/c of E.Coli 01, 02, 078<br>each containing $1.0 \times 10^5$ (CFU)    |
|              | B<br>30<br>chicken |   |   |   | Mixed with 0.1ml s/c of E.Coli 08, 0114,<br>0119 each containing $1.0 \times 10^5$ (CFU) |
| Second group | A<br>30<br>chicken | Oil imported<br>E.coli vaccine            | Two doses of<br>0.5ml S/C three<br>weeks a part at<br>the base of neck,<br>the first dose at<br>two weeks of age. |   | Mixed with 0.1ml s/c of E.Coli 01, 02, 078<br>each containing $1.0 \times 10^5$ (CFU)    |
|              | B<br>30<br>chicken |   |   |   | Mixed with 0.1ml s/c of E.Coli 08, 0114,<br>0119 each containing $1.0 \times 10^5$ (CFU) |
| Third group  | 30<br>chicken      | Control                                   | Control   |   | Mixed with 0.1ml s/c of E.Coli 01, 02, 078<br>each containing $1.0 \times 10^5$ (CFU)    |
|              | 30<br>chicken      |   |   |   | Mixed with 0.1ml s/c of E.Coli 08, 0114,<br>0119 each containing $1.0 \times 10^5$ (CFU) |
| fourth group | 20<br>chicken      | Control<br>Negative blank                 | Control<br>Negative blank   |   | Control<br>Negative blank  |

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

| Chicken groups       |           | Antigen used |                           | Weeks Post first dose of Vaccination |                   |                            | Weeks Post Second dose of Vaccination |                   |                   |
|----------------------|-----------|--------------|---------------------------|--------------------------------------|-------------------|----------------------------|---------------------------------------|-------------------|-------------------|
|                      |           |              |                           | 1 <sup>st</sup> w                    | 2 <sup>nd</sup> w |                            | 1 <sup>st</sup> w                     | 2 <sup>nd</sup> w | 3 <sup>rd</sup> w |
| Vaccinated groups    | *Group 1  | 01           | First dose of vaccination | 1.7                                  | 1.9               | Second dose of vaccination | 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<br>(prepared vaccine) | A          | 25                          | 2 | 2 | - | - | 1  |
|                                       | B          | 21                          | 4 | 3 | 1 | - | 1  |
| Group 2 imported<br>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.

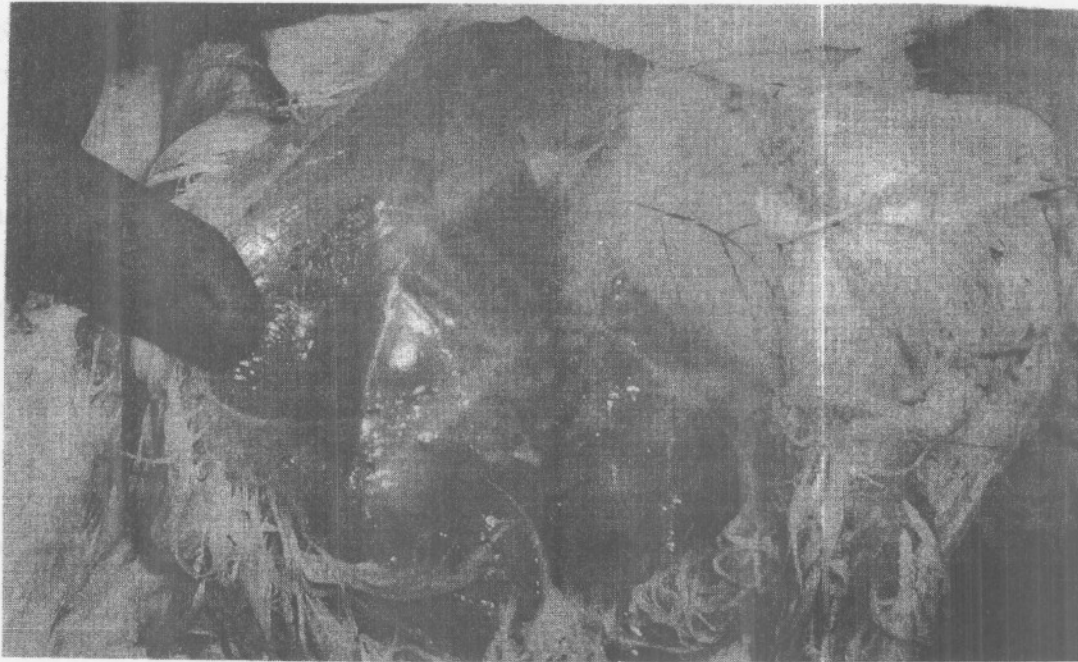
Subgroup A: Challenged with homologous E.Coli strains (01, 02, and 078).

Subgroup B: Challenged with heterologous E.Coli strains (08, 0114, and 0119).

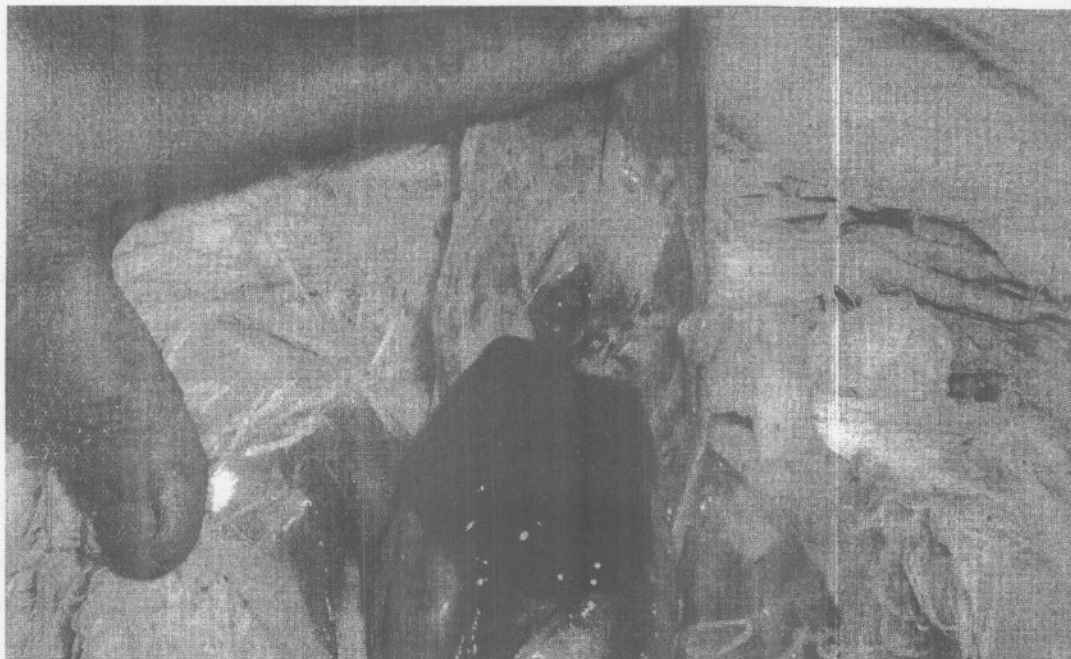
**Table (4): Protective indexes PIS assessment in chickens vaccinated with locally prepared F11 vaccine, imported vaccine and non vaccinated control groups.**

| 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.**

## REFERENCE

- British Pharma Copoeia Veterinary (2005): Published by the stationary office limited under the department of health of the agriculture ministers London, England.
- Code of American Federal Regulation (1985): Published by: The office of federal register national Archives record service General service Administration.
- Deb, J.R. and Harry, E.G (1978): Laboratory trails with inactivated vaccines against *Escherichia coli* O2: k1 infection in fowls. *Ret. Vet. Sci.*, 24: 308 - 313.
- Dhillon, A.S. and Jack, O.K. (1996): Two outbreaks of colibacillosis in commercial caged layers. *Avian Dis.*, 742 - 746
- Dho-Moulin, M. and Fairbrother, J.M. (1999): Avian pathogenic *Escherichia coli* (APEC). *Vet. Res.*, 30(2-3):299-316.
- Dozois CM, Pourbakhsh SA. and Fairbrother JM, (1995) Expression of P ant type 1 (F1) fimbriae in pathogenic *Escherichia coli* from poultry. *Vet. Microbial*; 45: 297 - 307.
- Edward, P.R. and Ewing, W.H. (1972): Identification of enterobacteriaceae Minneapolis, Burgess publishing Co, P.P. 709.
- Ewers, C.; Janssen, T.; and Wieler, L.H. (2003): Avian pathogenic *Escherichia coli*. *Berl Münch Tierärztl Wschr.* 116(9-10):381-95.
- Fiommer, A, Freidlin P.J, Bock RR, leitner G, Chaffer, M. and Heller E.D (1994): Experimental vaccination of young chickens with a live, non pathogenic strains of *E. Coli*. *Avian pathol.* 23 : 425 - 433.
- Gross W. B (1994): Disease due to *Escherichia coli* in poultry in domestic animals and humans C.L. Gyles. *CaB International*, walling ford, United Kingdom 237 - 259.
- Gomis, S.M, Riddell, C. potter, A.A. and Allan, B.J. (2001): Phenotypic and genotypic characterization of virulence factors of *Escherichia coli* isolated from boiler chicken with simultaneous occurrence of cellulitis and other colibacillosis lesions. *Can. J. vet. Res.* 65. 1-6.
- Gyimah J.E. and Panigrahy B. (1985): Immunogenicity of an *Escherichia coli* (serotype O1) pili vaccine in chickens. *Avian Dis*, 29, 4, 1078 - 1083.
- Gyimah, J.E. Panigrahy, B and William J.D. (1986): immunogenicity of an *Escherichia coli* multivalent pillus vaccine in chickens. *Avian Dis.*, 30: 687 - 689.
- Hassan, H, A, Waffa A. Goniemy, Halla. A. Fadle, Hussein, A.Z. and Ibrahim I.S. (1999): Immunogenicity of oil - Emulsified *Escherichia coli* vaccine in chickens. *Beni-Suef Vet. J.*, g, 3, -149 - 164.
- Noha, A. Helmy.; El Kholy.A.A.; Amal M. El Sawah; Aboul Saoud, S.M.; and Abdel 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, --
- Johannes F. van Den Bosch, Jos H.I.M. Hendriks, Irene Gladigau, Hemmie. M.C. Willems, Storm, P. K. and Graaf., F. K. (1993). Identification of F11 fimbriae on chicken *Escherichia coli* strains. *Infection and immunity*, 61, 3, 800 - 806.
- Kariyawasam S, Wilkie and B.N., Gyles, CL. (2004): Resistance of broiler chickens to *Escheria coli* respiratory tract infection induced by passively transferred egg-yolk antibodies. *Vet. Microbiology* 98, 273-84.
- Laragione. R. M and Woodward M.J. (2002): Virulence

- factors of *Escherichia coli* serotypes associated with avian colistpticaemia Res. Vet. Sco 73, 27-35.
- Marc,D.; Arne, P.; Bree, A.; and Dho-Moulin, M.(1998): Colonization ability and pathogenic properties of a fun- mutant of an avian strain of *Escherichia coli*. Res. Mi- crobiol., 149, 473-485.
- Pourbankhsh S.A. Dho-Moulin MB. Bree A, Desaultells C, Doize B. M. and Fairbrother JM. (1997): Localization of in vivo expression of P and F1 fimbriae in chickens experimentally inoculated with pathogenic *Escherichia coli* Microbial patho. 22 : 331 - 41.
- Timms, L.M. and Marshall, N (1989): laboratory assess- ment of protection given by experimental Pasteurella antipestifer vaccine: Br. Vet. J 145, 483.
- Vandemaele,F.; Assadzadeh,A.; Derijcke,J.; Vereecken,M.; and Goddeeris,B.M. (2002): Avian pathogenic *Escheri- chia coli* (APEC). Tijdschr Diergeneesked, 127 (19):582.
- Van Den Bosch, J.F.; Hendriks,J.H.; Gladigau,I; Willems, H.M.C.; Storm,P.K.; and Graaf,F.K. (1993): Identifica- tion of FII Fimbriae on Chicken *Escherichia coli* Strains. Infection and Immunity, 61, 3, 800.
- Zigterman GJ, van de Ven W., van Geffen, C., Loeffen, A.H., Pan huijen J, Rihke E.O and Vermeulen A.N. (1993): Detection of mucosal immune responses in vac- cines after immunization or infection. Vet. Immunol. Immunopathol., 36 : 281 - 291.

## دراسات لتحضير وتقييم لقاح الأيشريشيا كولاي فى الطيور

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فى هذه الدراسة تم تحضير لقاح الفميريا ف11 المحلى متعدد العترات من عترات القولون المعوى ومقارنته باللقاح الزيتى المستورد.

تم اختيار كفاءة لقاح الفميريا 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.