

قسم الميكروبيولوجيا  
كلية الطب - جامعة أسيوط  
رئيس القسم : أ. د / ماهر مختار زكى

دراسات عن الميكروب القولونى فى الدجاج  
٢ - تحضير وتقييم اللقاحات المختلفة

عماد نافع ، مصطفى الرهيوى ، عوض عبد الحافظ\* ، صلاح موسى\* ، سامية كامل\*\*  
باهى الجمال\*\*

تم تحضير لقاح متعدد من أكثر العترات انتشارا وتم تقسيمه الى جزئين عومل  
الاول بالحرارة والثانى بالفورمالين •  
كانت الجرعة المستخدمة كالحقن فى ١ سم<sup>٣</sup> سواء عن طريق الفم أو الحقن فى  
العضل •

تم عمل اختبار تحدى المناعه فى الطيور المحصنه بعد أسبوعين من آخر جرعة  
بكلا من العترات المشابهة أو المخالفة •

تم قياس الاجسام المضادة للتلازن فى سيرم الدم للطيور التى تم تحصينها بكلا  
اللقاحين •

هذا وقد ارتفع معدل الاجسام المضادة فى الدم بالنسبة للطيور التى تم حقنها  
بعترات مشابهة لتلك المستخدمة فى اللقاح ولم يحدث هذا الارتفاع بالنسبة للطيور التى  
حقنت بعترات مختلفة عن المستخدمة فى اللقاح •

---

\* : قسم أمراض الدواجن - كلية الطب البيطري - جامعة أسيوط •

\*\* : معهد بحوث صحة الحيوان - أسيوط •

Dept. of Microbiology,  
Fac. of Med., Assiut University,  
Head of Dept. Prof. Dr. M. Zaki.

**STUDIES ON *E. coli* IN POULTRY**  
**II- PREPARATION AND EVALUATION OF DIFFERENT**  
**TYPES OF VACCINES**

(With 3 Tables)

By

**E.K. NAFIE; M. EL-REHAWY; A.A. IBRAHIM\*; S. MOUSA\*;**  
**S. KAMEL\*\* and BAHY EL-GAMAL\*\***

(Received at 29/3/1988)

**SUMMARY**

A polyvalent vaccine was prepared from 078: K 80 (B-), 0125: K 70 (B15) and 080: K 61 (B7) and divided into two parts, the first was heat-inactivated and the second was a formalinized 2<sup>ed</sup> vaccine. The vaccinal dose was one ml. administered either by oral or intramuscular route for both types of vaccine.

The birds challenged, 15-days after the administration of the second vaccinal dose, either by homologous or heterologous strains.

*E. coli* agglutinating antibodies were detected in the serum of birds with a titres up to 480 following heat inactivated vaccine and up to 320 following formalinized one.

The serum of test group showed a higher titres of antibodies up to 800 following challenge, while no change occurred after homologous challenge.

**INTRODUCTION**

GROSS (1956) studied the efficacy of *E. coli* vaccines prepared from O2 serogroup and he concluded that neither live nor killed vaccines were effective.

YADAV and MALIK (1971) reported that phenolized polyvalent *E. coli* vaccine produced a higher antibody response in hens than formalin killed or heat-killed polyvalent vaccines. They found that the peak of "O" titers was attained earlier than that of "K" titres.

DEB and HARRY (1976) prepared an effective vaccine for colisepticaemia in chickens from formaline inactivated alum-precipitated *E. coli* 078 bacterium. Most immunized chickens were protected against challenge with homologous *E. coli*, but had only low or undetectable "O" and "K" serum agglutinins.

DEB and HARRY (1978) prepared another vaccine made from *E. coli* O2: K 1 and they mentioned that it produced high titres of "O" and "K" agglutinins in chickens, but minimal protection to challenge with homologous *E. coli*. They concluded that "O" and "K" agglutinins did not correlate with immunity to Colisepticaemia in chickens.

CESSI (1979) recorded 82-94% resistance to challenge among chickens immunized at the age of 20 days with bivalent (078 and O2) *E. coli* emulsified vaccine.

HASSANAIN (1983) tried a formal and heat inactivated vaccines, prepared from O1,

\*: Dept. of Poultry Diseases, Fac. Vet. Med., Assiut Univ.

\*\* : Animal Health Research Lab, Assiut.

E.K. NAFIE *et al.*

02 and 078 *E. coli* strains for vaccination of chickens and he reported that formalized vaccine gave better protection than heat-inactivated, regardless of the route of administration. He detected a range of agglutinin titres between 1/20 and 1/40 following the intramuscular administration of the vaccines.

The present work was planned to evaluate the vaccines prepared from previously isolated *E. coli* serotypes and its protectivity to chickens against *E. coli* infection.

## MATERIAL and METHODS

### I. Experimental birds :

Thirty-five one week-old Hubbard chicks were obtained from the G.P.C. and used for testing the efficacy of the prepared vaccines.

### II. Preparation of *E. coli* vaccines :

This was carried out following the technique described by ARP (1980) and HASSANAIN (1983). Vaccines were prepared from pure smooth colonies of *E. coli* serogroups 078 : K80 (B-) 086 : K61 (B7) and 0125 : K70 (B15) which were the most frequent isolates.

### III. Preparation of *E. coli* antigen :

*E. coli* antigen was prepared following the technique described by STIPKOVITS (1964) and AWAAD (1975).

### IV. Design of the experiment :

Thirty five, one week-old Hubbard chicks were divided into 5 groups, each of 7- chicks. Groups 1 & 2 were vaccinated with heat-inactivated vaccine in a dose of one ml. administered orally in the first group and intramuscularly in the second group.

The birds of the third and fourth groups were given one ml. formalized vaccine orally in the third group and intramuscularly in the fourth group.

Group 5 were kept as non vaccinated control. The second dose of vaccines were given in the same sequence mentioned before.

### V. Challenge :

The birds of the test groups were subdivided to group of four birds which was given one ml. from the homologous viable bacterial suspension, and the remaining three chicks were given 1 ml. from the heterologous viable bacterial suspension as challenge "0114 : K-(B-) and 0124 : K72 (B17)" following the same sequence and routes of administration of both vaccines.

Two birds were taken a part from the control non vaccinated group and one of them was given one ml homologous viable bacterial suspension and the other was given one ml. heterologous viable bacterial suspension by the intramuscular route, the remaining five chicks kept as non-vaccinated non-challenged control.

All birds were kept under close observation for 20 days after challenged, symptoms and post-mortem lesions were recorded.

All birds were bled 15-days after challenge and serum was separated for tube agglutination test.

### VI. Tube agglutination test :

The test was performed according to the technique described by STIPKOVITS (1964) and AWAAD (1975).

E. coli IN POULTRY**RESULTS**

Non clinical symptoms were observed on birds vaccinated orally by either heat-inactivated or formalinized E. coli vaccines, while the heterologous challenge resulted in appearance of clinical symptoms on birds vaccinated with heat-inactivated vaccine but no symptoms were expressed by the birds given the formalinized vaccine.

The intramuscularly vaccinated birds by either formalinized or heat inactivated vaccine, showed no clinical symptoms on challenge by the homologous strains although, marked symptoms were reflected on the birds challenged by the intramuscular injection of the heterologous strains. Non-vaccinated birds died after challenge either by homologous or heterologous strains.

Results of tube agglutination test of the birds vaccinated by heat inactivated and formalinized vaccines and of non-vaccinated, non-challenged control birds are summarized in Tables (1, 2 and 3).

**DISCUSSION**

The clinical symptoms observed on non-protected vaccinated birds appeared in the form of weakness, loss of appetite, ruffled feathers, drowsiness and loss of weight, while the post-mortem examination of the birds died after challenge revealed the presence of lesions of septicaemia including congestion of the internal organs and petechial haemorrhages on coronary fat, liver and spleen. These symptoms and post-mortem lesions were similar to those recorded by SOJKA and CARNAGHAN (1961), AWAAD (1972), AWAD et al. (1973), HASSANAIN (1977) and ARP (1980).

The results of the tube agglutination test of birds vaccinated with formalinized E. coli vaccine intramuscularly revealed that the agglutination titre ranged between 160 and 320, which increased up to 800 when these birds challenged with homologous strains, while no change in the titre after heterologous challenge. These results are in accordance with that reported by YADAV and MALIK (1971) and AWAAD (1975).

The results of the tube agglutination test of the birds vaccinated with heat-inactivated E. coli vaccine by intramuscular route revealed that antibody titers were up to 480, those titres are nearly similar to the titres detected by YADAV and MALIK (1971), but they are higher than those recorded by DEB and HARRY (1976) and HASSANAIN (1983).

It was concluded that the immune response and the degree of protection of birds were higher in case of formalinized vaccine than in case of heat-inactivated one. This supports the use of formalinized vaccine which is better than heat-inactivated vaccine in protection of chickens against E. coli infection.

**REFERENCES**

- Arp, L.H. (1980): Consequences of active or passive immunization of turkeys against *Escherichia Coli* 078. *Avian Dis.* 24, 808-815.
- Awaad, M. (1972): Studies on colisepticaemia in chickens. M.V.Sc. Thesis, Faculty of Vet. Med., Cairo Univ.
- Awaad, M. (1975): Studies on E. coli infection in chickens. Ph.D. Thesis, Faculty of Vet. Med., Cairo Univ.
- Awad, F.I.; Bassicuni, A.A.; El-Sisi, M.A. and Awaad, W.H. (1973): Studies on colisepticaemia in chickens. *Egyptian J. Vet. Sci.* 10, 85.

- Cassi, D. (1979): Prophylaxis of *E. coli* infection in fowls with emulsified vaccine. *Clinical Vet.* 102, 270.
- Deb, J.R. and Harry, E.G. (1976): Laboratory trials with inactivated vaccines against *E. coli* (O78 : K80) infection in fowls. *Res. Vet. Sci.* 20, 131-138.
- Deb, J.R. and Harry, E.G. (1976): Laboratory trials with inactivated vaccines against *Escherichia Coli* (O2 : K1) infection in fowls. *Res. Vet. Sci.* 24, 308-313.
- Gross, W.B. (1956): *Escherichia Coli* as a Complicating Factor in chronic respiratory disease of chickens and infectious sinusitis of turkeys. *Poult. Sci.* 35, 765-771.
- Hassanain, Z.A. (1977): Studies on Celi Septicaemia in chickens with particular reference to the role of K-antigen. M.V.Sc. Thesis. Faculty of Vet. Med., Cairo Univ.
- Hassanain, Z. (1983): Further studies on coli infection in poultry, Ph.D. Thesis, Faculty of Vet. Med., Cairo Univ.
- Safka, W.J. and Carnaghan, R.B.A. (1961): *Escherichia coli* infection in poultry. *Res. Vet. Sci.* 2(4): 340-352.
- Stipkovits, L. (1964): Indirect H.A. test for chickens infected artificially with *E. coli*. *Avian Dis.* 8: 637-648.
- Yada, M.P. and Malik, B.S. (1971): Isolation and serotyping of *Escherichia coli* from chickens and their eggs in India. *Ind. Vet. J.* 48: 879-884.

**Table (1):** Results of the tube agglutination test of the birds vaccinated with the heat-inactivated vaccine.

Serum samples	Reactions against "O" antigens				
	O <sub>78</sub>	O <sub>86</sub>	O <sub>125</sub>	O <sub>114</sub>	O <sub>124</sub>
<b>I. Post vaccination</b>					
1 I.M.	120	480	120	480	120
2 Ora U	120	240	120	240	120
<b>II. Post challenge</b>					
A- I/M					
1(Homo.)	80	160	80	800	800
2 (Homo.)	160	40	80	800	400
3 (Het.)	240	240	240	240	120
B- Oral					
1 (Homo.)	240	240	480	240	480
2 (Homo.)	800	800	800	800	800
3 (Het.)	80	160	80	160	160

I/M = Intramuscular.

Homo. = Homologous

Het. = Heterologous.

E. coli IN POULTRY**Table (2):** Results of the tube agglutination test of the birds vaccinated with the formalized vaccine.

Serum samples	Reactions against "O" antigens				
	O <sub>78</sub>	O <sub>86</sub>	O <sub>125</sub>	O <sub>114</sub>	O <sub>124</sub>
<b>I. Post vaccination</b>					
1. I.M.	160	160	160	320	320
<b>II. Post challenge</b>					
A- I/M					
1 (Homo.)	400	800	800	400	400
2 (Homo.)	240	120	480	480	480
3 (Het.)	320	160	160	320	160
B- Oral					
1 (Homo.)	80	320	160	320	80
2 (Homo.)	160	40	40	800	400
3 (Het.)	480	480	240	480	240

**Table (3):** Results of the tube agglutination test of the non-vaccinated, non-challenged control birds.

Serum samples	Reactions against "O" antigens				
	O <sub>78</sub>	O <sub>86</sub>	O <sub>125</sub>	O <sub>114</sub>	O <sub>124</sub>
1	30	-	30	30	30
2	20	20	10	20	10
3	10	10	10	20	10