Field Application For Inactivated Multivalent E.coli Vaccine In Chickens

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ABSTRACT

A field trails was conducted to evaluate the immunogenicity of an inactivated *Escherichia coli* vaccine composed of serotypes O_1, O_2 and O_{78}. Two Hubbard broiler chick flocks (1000 each) were used through the experimental work of this study. In trial No1, chickens were vaccinated twice at 10 and 20 days of age, while those in trial No2 were vaccinated once at 10 days of age. Another 100 chickens of same age were served as controls . Humoral immune responses as assessedd by Enzyme Immuno Sorbant Assay (ELISA) documented an increase in serotype –specific antibodies IGy in vaccinated birds in the two trials. Mortality and gross lesions scores of both vaccinated flocks were compared with those of unvaccinated flocks, on the other hand chicken in trial No1 had significantly lower mortality and lower score lesions than those in flock No2 indicating that two doses of vaccine gave long -last immunity than one dose. A protective indices (PIs) system which provide a very sensitive measurement of effective vaccination was conducted. Vaccination trials showed that active immunization with the prepared vaccine twice at 10 and 20 days of age provided better protection than one dose. These results suggest that the locally prepared *E.coli* vaccine elicits a non- specific health improvement of the vaccinated chickens in addition to the specific protection. The vaccine could be used either a single vaccinal dose in broilers prepared for marketing from beginning 35 days of age or double doses in breeder used for long period of production.

INTRODUCTION

Colibacillosis is a common systemic disease of economic importance in poultry and occurs worldwide. *Escherichia coli* (*E. coli*) infection occurs as an acute fatal septicemia or subacute pericarditis and airsacculitis, as well as perihepatitis, arthritis, and also cellulites *(1)*. Among bacterial infections, colibacillosis is very often the first cause of morbidity and mortality in poultry. Large numbers of *E. coli* are maintained in the poultry house environment through fecal contamination. Systemic infection occurs when large numbers of pathogenic *E. coli* gain access to the blood stream via the respiratory tract or intestine. Bacteremia progresses to septicemia and death, or the infection extends to serosal surfaces, pericardium, joints and other organs.

The literature suggests that serotypes O1, O2 and O 78 of *E. coli* associated with colibacillosis are the most common serotypes found in chickens and turkeys *(2,3)*. Many isolated strains are also untypeable and are considered especially virulent.

Chemotherapeutics and antibiotics have been hitherto used therefore. However, the value of these medicines is now decreasing because of the appearance of resistant strains. Therefore, in place of these medicines, a useful vaccine for the protection of poultry from *E. coli* infections has been developed *(4)*.The present investigation was undertaken to examine the protection of formalin – inactivated aluminum hydroxide gel *E. coli* vaccine.

MATERIAL AND METHODS

1-Experimental birds

Two broilers Hubbard flocks (1000 each) of one –day old were used through out the experimental work of this study. The two flocks were fed on a balanced diet free from antibiotics allover the period of the experiment. Random serum samples were screened for detection of *E. coli* antibodies using ELISA test

2- E.coli strains

*E.co*li serotypes O_1; K1, O_2; K1, were isolated and identified *(5)* and O_{78}; K_{80} was kindly supplied as identified strain by ***Animal Health Research Institute-Dokki.*** The relative pathogenicity of these strains was re-evaluated in day- old susceptible chicks before vaccine preparation and challenge procedure. ID_{50}For each strain was estimated *(6)*.

3-Preparation of an inactivated E.coli vaccine

Each serovar E.coli (O_1, O2 and O_{78}) was separately seeded into tryptic soya broth medium containing o.05% yeast extract and incubated for 24 hours at $37C^0$. The culture of each strain was adjusted at a concentration $3.8x10^9$ colony forming unit (CFU)/0.05/ml *(7)*. The broth cultures were taken to check purity, before the inactivation by 0.5% formalin at $37C^0$ for 48 hours. After completion of inactivation, the three inactivated cultures were mixed together in equal volumes and Adjuvavated with aluminum hydroxide gel 2% (Honel,UK) in a final concentration of 20%.

4-Quality control of prepared vaccine

The vaccine was tested for purity, sterility and biological safety tests following standard international protocols *(8, 9)*.

5-Vaccination

Vaccination was carried out twice using 0.5ml dose / bird , inoculated s/c into the Back of the neck. Chicken were divided into two groups and managed as shown in the following table:

Trial No	Chicken groups	No. Of Birds	age at vaccination	age at challenge
No1	Vaccinated	1000	10,20 days	35 days
No2	Vaccinated	1000	10 days	35 days
Controls		100		

N.B ; From each of vaccinated and control groups 90birds were selected and divided into three subgroups at challenge

Blood samples were collected directly before vaccination at 0 hours and every week til the end of –the experiment. Sera were separated and stored at $-20C^0$till used.

6-Evaluation of humeral immune response

Antibody response of vaccinated chickens was evaluated in their sera by Enzyme Linked Immunosorbnt Assay (ELISA) *(10)* .

7-Challenge procedure

Two weeks after the second dose of vaccination, all birds were challenged intrathoracic with 0.1ml of 24houers brain heart infusion culture containing 100ID50 of E.coli serotypes O1, O2 O78.

a- Mortality, lesions, and *E.coli* recovery

Following challenge, all birds were kept under observation daily for 7 days and the mortality rate was recorded. All dead birds and some survivors were subjected to post mortem examination, particular attention given to air sacs, liver, and heart. Lesions were scored from 0 to 4 according to severity (0 = no lesion, 1 = cloudy air sacs, pericarditis, or hepatitis; 2 = moderate air sacculitis, , pericarditis or hepatitis ; 3 = bilateral air sacculitis , pericarditis, or hepatitis , 4 = sever and extensive fibrinous air sacculitis , pericarditis, or hepatitis). The heart blood, pericardium, air sacs and livers were cultured onto MacConkey media for E.coi recovery

b- Protective indices (PI)

Using the following formula *(11)* ,protective indices were assessed according to mortality (M), and PM lesions(PML).

$$PI = \frac{\%\ (M\&PML)\ controls - \%\ vaccinated}{\%\ controls} \times 100$$

8-The weight gain and feed conversion rate (FCR)

were calculated using the following equation *(12)*

$$FCR = \frac{Mass\ of\ food\ eaten}{The\ body\ mass\ gain}$$

RESULTS AND DISCUSSION

The use of antibiotics drugs for controlling of *E. coli* infection was thought to be possible , transferable resistance to this products and their prohibitive costs have shown a deficient of this type of control *(13)*. Vaccination has therefore a rational proposed as a n alternative method of control *(4)*.

As shown in Tables 1, 2 the immunogenicity of formalinized inactivated *E.coli* trivalent vaccine was evaluated by the detection of antibody titer in the sera of vaccinated and control chickens as measured by ELISA test .It is evident that the vaccine used elicited high level antibody titer and that ELISA is a highly efficient and sensitive method for assessment *E.coli* antibodies . These results coincide with the previous finding *(10)* which reported that ELISA served to be as sensitive test and it detect specific antibody against *E.coli* in sera of vaccinated chickens.

The results of ELISA test in chicken vaccinated twice at 10 & 20 days of age (trial No1) showed detectable antibodies at second week following initial vaccination, then it increase gradually and reached to 0.96-1.10 at time of challenge. These levels tend to increase and persist all over the period of the experiment. On the other hand antibody titers in sera of chicken vaccinated with single dose at 10 days of age (trial No 2) reached to 0.74-0.76 at time of challenge and then it decreased gradually. These results cleared that chicken vaccinated twice has a higher antibody production. These findings supported *(14,15)* which observed that the antibody response to *E.coli* immunization is greater if chicken are at least two weeks of age at vaccination. Meanwhile, chicken immunized with E.coli vaccine at earlier ages produced lower antibody titers *(16)* . Vaccination at one day of age were poorly protected as compared to those vaccinated at 14 days *(4)*. The lack of full protection could be due to the immune response of the chick at this age being immature and unable to responsed effectively. The results of these experiment showed that vaccination at 10 days is acceptable because the aluminum adjuvanated vaccine tend to become encapsulated by a granulomatious inflammatory reaction *(17)*, a granulomatious inflammatory reaction response is constant with vaccine deposits at necropsy.

Table 1. Mean ELISA (OD) values in chicken vaccinated with trivalent inactivated E.coli vaccine at 10 AND 20 days of age(Trial No1)

Group of chicken	Plate Ag	Pre-vaccination	Time post primary vaccination						
			1week	2week	3Week	4week	5week	6week	7week
Vaccinated	O_1,	0.06	0.23	0.66	0.84	1.20	1.40	1.60	1.90
	O_2	0.06	0.26	0.56	0.89	1.10	1.40	1.80	2.10
	O_{78}	0.05	0.36	0.73	0.90	1.10	1.30	1.70	1.8•
Controls		0.07	0.06	0.05	0.06	0.07	0.05	0.05	0.07

Table 2. Mean ELISA (OD) values in chicken vaccinated with trivalent inactivated E.coli vaccine at 10 days of age(Trial No2)

Group of chicken	Plate Ag	Pre-vaccination	Time post vaccination						
			1week	2week	3week	4week	5week	6week	7week
Vaccinated	O_1	0.05	0.26	0.58	0.76	1.02	0.97	0.91	0.8
	O_2	0.07	0.23	0.63	0.80	1.04	0.84	0.82	0.74
	O_{78}	0.05	0.24	0.66	0.74	1.10	0.97	0.71	0.69
Controls		0,07	0.06	0.05	0.07	0.05	0.07	0.05	0.06

The results of challenge is depicted in Tables 3, 4 with virulent E.coli strains indicated that antibody has been show to play a central role in development of immunity against *E.coli (18)* . Chickens in trial No1 showed a sticking reduction in mortality and high protection level (72%) compared with this those in group of trial No2 (63%) . The gross lesions in group of No1 were so mild and recovery of *E.coli* was 8.8 % . Unvaccinated controls demonstrated that the mortality and lesions were varied according to the virulence of challenge strain, the birds showed extensive and sever lesions in the form of pericarditis , peri hepatitis and air saculitis. These results indicated that score lesions could be considered as a parameter for evaluation of protective efficacy of *E.coli* vaccines *(7)*.Vaccinated chickens with one or two doses of vaccine had a tendency to have heavier body weight gain than those of unvaccinated controls. These results suggest that the locally prepared *E.coli* vaccine elicits a non- specific health improvement of the vaccinated chickens in addition to specific protection

Finally it can be concluded that aluminum adjuvanated formalinized inactivated *E.coli* trivalent vaccine is potent immunogen and gave acceptable protection level against *E.coli* infection. The vaccine could be used either a single dose in broilers prepared for marketing from beginning at 35 days of age or double doses in breeder used for long period of production. Approches to control of *E.coli* infection with vaccination reduced the excessive use of antibiotics and will be met with favor from consumers and regulators, as shown in Table 7.

Table 3. Post challenge immunostatus assessment in chicken vaccinated with two doses of Trivalent E.coli vaccine (Trial No. 1)

Challenge Strain	Challenged birds with lesions					
	Vaccinated group			Controls		
	Dead/total	Survived/ Total	% of birds With lesion	Dead/total	Survived/ Total	% of birds With lesion
O_1	2/30	3/30	16.6	5/30	12/30	56.6
O_2	1/30	1/30	6.6	2/30	14/30	53.3
O_{78}	2/30	2/30	13.3	4/30	11/30	50

Table 4. Post challenge immunostatus assessment in chicken vaccinated with one dose of trivalent E.coli vaccine (Trial No. 2)

Challenge strain	Challenged birds with lesions					
	Vaccinated group			Controls		
	Dead/total	Survived/ Total	% of birds With lesion	Dead/Total	Survived/ Total	% of birds With lesion
O_1	4/30	5/30	30	6/30	13/30	63.3
O_2	3/30	8/30	36.6	11/30	15/30	86.8
O_{78}	3/30	3/30	20	6/30	10/30	33.3

Table 5. Lesions score in vaccinated and control chickens and challenged with Virulent E.coli strains

Trial No.	Groups	Challenge strain									Recovery of E.coli (%)
		O1			O2			O78			
		AS	PE	PH	AS	PE	PH	AS	PE	PH	
No1	Vaccinated*	0.2	0.2	0.3	0.8	0.5	0.8	0.4	0.6	0.2	8.8
	Controls	1.2	1.4	0.8	1.5	2.2	1. 0	1.2	1.2	0.3	79.3
No2	Vaccinated**	1.1	1.1	0.8	0.8	1.3	1.1	0.9	1.1	0.4	14.2
	Controls	1.6	1.6	1.2	2.1	1.5	2.2	1.2	1.4	0.3	62.4

AS :air saculitis
PH : perihepatitis
PE : peritonitis
* Birds vaccinated with two doses of vaccine
** Birds vaccinated with one dose of vaccine

Table 6. Protective indices (PIs) assessment in vaccinated chicken with inactivated Trivalent E.coli vaccine at different ages

Trial No	Group	Total/Dead	Survival with lesions/total	% of birds With lesions	Pis
No1	Vaccinated*	5/90	6/90	17%	72%
	Controls	18/90	37/90	61%	
No2	Vaccinated**	10/90	16/90	28.8%	63%
	Controls	23/90	38/90	78.8%	

* Birds vaccinated with two doses of vaccine
** Birds vaccinated with one dose of vaccine

Table 7. Effect of vaccination trials on weight gain and feed conversion of broilers

Group	Weight gain							Feed conversion ratio(FCR)						
	Days post vaccination							Days post vaccination						
	0 - 7	7-14	14-21	21-28	28-35	35-42	42-49	0 - 7	7-14	14-21	21-28	28-35	35-42	42-49
Trial No.1	83	199.2	400.89	654.93	1.22	1.50	1.65	1.6	1.8	2.48	1.2	1.3	2.2	2.2
Trial No2	101.25	188	392	669	1.05	1.42	1.52		2.5	2.49	1.7	1.45	1.2	1.2
Controls	91	176	362	590	800	1300	1400	1.9	2.7	2.8	1.9	1.7	1.6	British_P harmaco poeia

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الملخص العربى

تطبيق حقلى للقاح الإشيرشياكولاى المثبط والمتعدد العترات فى الدجاج

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تم القيام بتجربة حقلية لتقييم القوة المناعية للقاح الأشيرشياكولاى المثبط والمكون من عترات O_1 , O_2 , O_{78} فى قطعيين من بدارى التسمين الهبرد. فى التجربة الأولى تم تحصين الدجاج مرتين عند عمر ١٠ , ٢٠ يوم باللقاح محل الدراسة، أما فى التجربة الثانية تم تحصين الدجاج مرة واحدة عند عمر ١٠ أيام. وتم استخدام ١٠٠ دجاجة لمجموعة ضبط سالبة للتجربتين. وبقياس الإستجابة المناعية الغير خلوية باستخدام الإليزا تم تسجيل زيادة معنوية للأجسام المناعية المتخصصة (1gy) فى الدجاج المحصن فى المجموعتين، من ناحية أخرى تم مقارنة نسبة الوفيات وأيضاً وجودالتغييرات الباثولوجية فى الدجاج المحصن بالنسبة لمجموعة الضبط السالبة بعد إجراء اختبار التحدى، حيث وجد أن نسبة الوفيات والتغيرات الباثولوجية أقل فى الدجاج المحصن مرتين عنه فى الدجاج المحصن مرة واحدة. مما يؤكد أن التحصين مرتين باللقاح محل الدراسة يعطى الدجاج مناعة قوية وممتدة المفعول عنه فى الدجاج المحصن مرة واحدة.

وباستخدام دلالات الحماية- وهو نظام دقيق وحساس لقياس مدى كفاءة لقاح الأشيرشياكولاى- وجد أن التحصين مرتين عند عمر ١٠ ، ٢٠ يوم باللقاح محل الدراسة يعطى حماية للدجاج أفضل من التحصين مرة واحدة، وهذه النتائج تؤكد أن اللقاح محل الدراسة والمحضر محلياً يوفر تحسن فى الصحة العامة للدجاج المحصن بالإضافة للحماية من الإصابة بالأشيرشياكولاى .