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EFFICACY OF AN EXPERIMENTAL E.COLI INACTIVATED VACCINE IN TURKEY POULTS

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ABSTRACT

E. coli infections are responsible for great economic losses in the poultry industry worldwide, bringing serious threat to the turkey industry. The present investigation aimed to prepare a potent vaccine from *E. coli* serogroups O1 and O78 to aid in control of Colibacillosis in turkey. One hundred commercial 14 days-old turkey poults were used and divided into three groups; first group was vaccinated twice with 3 weeks interval by prepared inactivated E.coli vaccine adjuvanted with aluminum hydroxide gel, second group was vaccinated twice with 3 weeks interval by prepared inactivated E.coli vaccine adjuvanted with montanide ISA70, third group was left as unvaccinated control one. The immune response was measured by MAT (Microagglutination test), ELISA and challenge test. It was found that the protection rate for inactivated vaccine with aluminum hydroxide gel and *E.coli* inactivated vaccine with montanide ISA70 were 80% and 85% respectively although it was 30% among the unvaccinated group.

Keywords: Inactivated vaccine, E. coli, ELISA, MAT

INTRODUCTION

Colisepticemia is the most important disease in poultry caused by Avian Pathogenic E.coli (APEC) strains resulted in high mortality lead to high economic loses. The infection is also referred to as aero sac disease and usually occurs among birds with 2 to 12 weeks of age, with the majority of the cases occurring among birds with 4 to 9 weeks of age with mortality reaching rates as high as 20% **(Dho-Moulin and Fairbrother, 1999)**. Colibacillosis in turkeys exists in two forms: an egg-transmitted form found in poults which are less than 6 weeks old, and that found in poults over 6 weeks of age. The latter form is often exacerbated by other infections such as haemorrhagic enteritis virus or

turkey rhinotracheitis virus or by unfavorable environmental factors (**Sponenberg *et al.*, 1985 and Van den Hurk *et al.*, 1994)**.

Fifty-five clinical isolates of avian pathogenic Escherichia coli (APEC) obtained from seven outbreaks of acute haemorrhagic septicaemia in turkeys **(Olsen *et al.*, 2011)** APEC strains are predominantly belonging to serogroups O1, O2, O5, O8, O18 and O78 **(Blanco *et al.,* 1997).** Biotyping and serotyping are often undertaken on isolates recovered from cases of colibacillosis. In most countries, O1, O2 and O78 represent major *E.coli* serogroups isolated from diseased birds **(Dozois *et al.,* 1992; Ewers *et al*., 2004).**

Consequently, representative strains from these serotypes provide the focus for unraveling APEC virulence mechanisms and for the development and evaluation of vaccine candidates. Losses were occur through premature deaths, condemnation of carcasses at slaughter, reduced productivity and recurring costs associated with antibiotic prophylaxis and therapy **(Kemmett *et al*., 2014)**. A recent longitudinal survey of broiler flocks in the United Kingdom found evidence of colibacillosis in 39% of dead birds, colibacillosis in 70% of deaths of broiler chicks 2–3 days after placement **(Kemmett *et al*., 2013)**. Control of colibacillosis is problematic, due to restricted availability of relevant antimicrobial agents and frequent failure of vaccines to protect against the diverse range of ExPEC (external pathogenic E.coli) causing disease in birds **(Nolan and Jason, 2007)**.

The presence of several serotypes of *E. coli*, the lack of cross-protection between these serotypes, and the fact that field outbreaks are caused by different serotypes make it very difficult to prepare a vaccine to protect poultry flocks from field infections with this organism **(Abdul-Aziz and EL-Sukhon, 1998).** The control of APEC has been largely depending upon vaccination with autologous bacterins (**Trampel and Griffith, 1997**), but these confer short-lived serotype-specific protection and their effectiveness is blunted by the diversity of E. coli capable of infecting poultry. Several vaccines based on killed or

attenuated give sufficient protection against infection with homologous strains, but protection against heterologous strains is less efficient (**Dho-Moulin and Fairbrother, 1999**). Several experiments have been performed to prevent colibacillosis in turkeys by vaccination **(Bolin and Jensen, 1987, Kwaga *et al.,* 1994 and Sadeyen *et al.*, 2015)**.

So the aim of this study was planned to compare the immune response of two different prepared E.coli inactivated vaccines containing O1 and O78 serotypes. One of them was adjuvanted with aluminium hydroxide gel and the other was adjuvanted with montanide ISA70 in turkey poults.

MATERIAL AND METHODS

1- Bacterial Strains

Two *E.coli* strains serotypes O1 and O78 were kindly obtained from Central Laboratory for Evaluation Veterinary Biologics (CLEVB), Abbasia, Cairo and were inoculated onto MacConkey agar medium at 37°C for 24 hr. each strain was identified biochemically by using **API 20E** identification system following the procedures of kit manual.

2-Vaccine preparation: (Chaffer *et al,* 1997)

E.coli strains serotypes O1 and O78 were grown separately onto brain heart agar in Roux bottles and incubated at 37ºC for 24 hr. The colonies were collected using normal saline then mix together and bacterial suspension was adjusted to be $1x10^9$ CFU/0.5 ml (vaccinal dose). The bacteria were then inactivated by adding 0.5% formalin with agitation then montanide ISA70 (SEPPIC®, France) was mixed with one part of bacterial suspension in a ratio of 70 adjuvent: 30 antigen to one vaccine and for the other vaccine the aluminium hydroxide gel 20% was added in a ratio (1:1).

3-Turkey poults

One hundred and ten commercial, 1 day-old turkey poults vaccinated against Newcastle, Mycoplasma and Marek's disease were obtained from Faculty of Agriculture, Cairo University and maintained in pens at

the animal husbandry facilities of Central Laboratory for Evaluation Veterinary Biologics (CLEVB). These birds were ascertained first to be free from *E. coli* infection and antibodies. The turkey poults were fed *ad libitum* with no antibacterial or anticoccidial components in their feed.

4- Experimental design

One hundred commercial 14 day-old turkey poults were divided into three groups.

Group (1): Fourty turkey poults were vaccinated by E.coli inactivated vaccine adjuvanted by aluminium hydroxide gel in a dose of 0.5 ml S/C ($1x10^9$ CFU/dose).

Group (2): Fourty turkey poults were vaccinated by E.coli inactivated vaccine adjuvanted by mentonide ISA70 using with the same dose as group (1).

Group (3): Twenty turkey poults injected 0.5 ml S/C with normal saline, left as control group.

Birds in group (1) and group (2) boostered with the same vaccine with the same route and dose 3 weeks after first immunization. Serum samples were obtained regularly before immunization, weekly after each vaccination and post challenge for two weeks (once/week), then pooled and stored at -20 °C till used for following up the induced antibodies.

5- Quality control testing of the prepared experimental vaccines

5. 1.Sterility test

The prepared vaccines were tested to be free from any contaminant (aerobic and anaerobic bacteria, fungus and mycoplasma according to **OIE (2013).**

5.2. Safety test

Safety of the prepared vaccine was tested according to **OIE (2013);** in which 10 turkey poults 14 days of age were injected subcutaneously with double field dose of the prepared vaccines. The inoculated poults were observed for 14 successive days to detect any signs of local reaction attributed to E.coli clinical symptoms or death.

5.3. Determination of immune response to the prepared experimental vaccines

5.3.1. Serological evaluation of humeral immune response of the vaccinated turkeys

5.3.1.1. Micro-agglutination test (MAT)

Antibody response in vaccinated and unvaccinated turkeys was followed up on regular intervals post vaccination determined by Micro-agglutination test (MAT) using sonicated antigen, according to the method described by **Thaxton *et al.* 1970 and Brown *et al.* 1981**

5.3.1.2. ELISA

ELISA was performed on the same serum sample, according to the method described by **(Voller, 1976 and Briggs and Skeels (1984)**. The results were calculated according to the following formula:

$$S/p \text{ (sample/positive)} = \frac{sample\ mean - negative\ control}{positive\ control - negative\ control}$$

Log_{10} titre= $1.09(\log_{10} S/P) + 3.63$

Titre = $Antilog_{10}$

5. 3.2.Challenge test

Three weeks post-boostering, each group was divided into two subgroups; one subgroup were injected into the thigh region with 0.2ml containing 10^7 CFU of E.coli serotype O1 and the other subgroup injected with E.coli serotype O78 and monitored for clinical signs, Mortality was recorded for 7 days after challenge according to the method described by **Chaffer *et al.* (1997)**.

6. Statistical analysis

Results of MAT in tables (1 and 2) and ELISA test in tables (3 and 4) were analyzed and compared with parametrical correlation using Student's T test **(Snedecor and Cochran, 1980).**

RESULTS

The two vaccines were found to be safe and sterile during all period of observation.The humeral immune response against E.coli serotypes O1 and O78 was measured by MAT were illustrated in tables1,2 and figure 1,2, it was clear that for both antigens the titer start with 40 at 3rd week post first vaccination then increased to 80 and reached peak to 160 at 3rd week post boostering then decreased to 40 after first week post challenge then increased to 80 and 160 at 2nd week post challenge, also it was clear that MAT titer for inactivated vaccine adjuvanted with montanide ISA 70 was higher or double the titer of inactivated vaccine adjuvanted with aluminium hydroxide gel for both antigens. The obtained results in table (2) were analyzed statistically using Student's T test and it was found that there is a significant difference at P $\geq$ 0.05 between group 2 (vaccinated with *E.coli* inactivated vaccine adjuvanted by montanide ISA70) and group 1(vaccinated with E.coli inactivated vaccine adjuvanted by aluminium hydroxide gel).

Table (1):Microagglutination titer in sera of vaccinated turkey poults using O1 antigen and challenged with E.coli serotype O1

Groups	Microagglutination Titre										
	Prevac	Weeks post vaccination			Booster	Weeks post boostering			Challenge #	Weeks post challenge	
		1	2	3		1	2	3		1	2
Group (1)	0	0	0	40		40	40	80		40	80
Group (2)*	0	0	0	40		80	160	160		80	160
Control	0	0	0	0		0	0	0		0	0

Group (1): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with aluminum hydroxide gel.

Group (2): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with montanide ISA70. # Challenge with virulent *E. coli* (O1) strain

Control: Unvaccinated group * Significant at P $\geq$ 0.05

Table (2) Microagglutination titer in sera of vaccinated turkey poults using O78 antigen and challenged with E.coli serotype O78:

Groups	Microagglutination Titre										
	Prevac c.	Weeks post vaccination			Booster	Weeks post boostering			Challenge #	Weeks post challenge	
		1	2	3		1	2	3		1	2
Group (1)	0	0	0	40		40	80	160		40	40
Group (2)*	0	0	0	40		80	160	160		80	80
Control	0	0	0	0		0	0	0		0	0

Group (1): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with aluminium hydroxide gel.
Group (2): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with montanide ISA70. # Challenge with virulent *E. coli* (O78) strain.
Control: Unvaccinated group. * Significant at P $\geq$ 0.05

B-ELISA

Concerning ELISA titers for both antigens in both vaccine as shown in tables 3, 4 and figures 3 and 4 it was clear that results was paralleled with that of MAT, also there was a significant difference in ELISA titer between both vaccines.

Table (3) ELISA titer in sera of vaccinated turkey poults using O1 antigen and challenged with E.coli serotype O1:

Groups	ELISA antibody Titre										
	Prevac	Weeks post vaccination			Booster	Weeks post boostering			Challenge #	Weeks post challenge	
		1	2	3		1	2	3		1	2
Group (1)	96	430	460	1412		1779	2818	3550		1949.5	2575
Group (2)*	117	740	870	2755		3550	3890	4217		2399	2650
Control	100	193	120	240		100	157	166		120	106

Group (1): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with aluminium hydroxide gel.
Group (2): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with montanide ISA70. # Challenge with virulent *E. coli* (O1) strain.
Control: Unvaccinated group * Significant at P $\geq$ 0.05

Table (4) ELISA titer in sera of vaccinated turkey poults using O78 antigen and challenged with E.coli serotype O78

Groups	ELISA antibody Titre										
	Prevac	Weeks post vaccination			Booster	Weeks post boostering			Challenge #	Weeks post challenge	
		1	2	3		1	2	3		1	2
Group (1)	93	410	735	1318		2089	2344	2691		1465	2450
Group (2)*	110	520	890	1524		2317	2582	2884		1655	2645
Control	100	193	120	240		157	166	244		120	106

Group (1): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with aluminium hydroxide gel
Group (2): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with montanide ISA70. # Challenge with virulent *E. coli* (O78) strain Control: Unvaccinated group * Significant at $P \geq 0.05$

3. Challenge test

The protection rates as measured by challenge test were 80% and 85% in turkey poults vaccinated with inactivated vaccine adjuvanted with aluminium hydroxide gel and inactivated vaccine adjuvanted with montanide ISA70 repectively, as shown in Table (5). Meanwhile, the protection rate was 30% among the unvaccinated chicken group.

Table (5): Results of challenge test among turkey poults vaccinated with inactivated *E.coli* adjuvanated with aluminiumhydroxide gel and inactivated E.coli adjuvanated with montanide ISA70 vaccines
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Groups	Total No. of challenged birds	No. of dead birds / Total No.	Protection rate
Group (1)	40	8/40	80 %
Group (2)	40	6/40	85 %
Control	20	14/20	30 %

Group (1): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with aluminium hydroxide gel.
Group (2): Turkey poults vaccinated with inactivated *E. coli* vaccine adjuvanted with montanide ISA70.
Control: Unvaccinated group.

DISCUSSION

Avian colibacillosis is a costly disease to the poultry industry, accounting for multimillion-dollar losses annually. There is a need for improved control of avian colibacillosis because current control strategies, which focus on reducing predisposing conditions among production, have proven largely ineffective (**Barnes *et al*., 2008**). In addition, strategies that have controlled colibacillosis in the past may not be as effective in the future, as recent studies show that *E. coli* infections are responsible for great economic losses in the poultry industry worldwide, bringing serious threat to the poultry industry.

The presence of several serotypes of *E. coli*, the lack of cross-protection between these serotypes, and the fact that field outbreaks are caused by different serotypes make it very difficult to prepare a vaccine to protect poultry flocks from field infections with this organism **(Abdul-Aziz and EL-Sukhon, 1998).** The control of APEC has been largely reliant upon vaccination with autologous bacterins (**Trampel and Griffith, 1997**), but these confer short-lived serotype-specific protection and their effectiveness is blunted by the diversity of E. coli capable of infecting poultry. They give sufficient protection against infection with homologous strains, but protection against heterologous strains is less efficient (**Dho-Moulin and Fairbrother, 1999**).

Inactivated vaccines based on formalin or heat inactivated E. coli are generally believed to confer protection against avian colibacillosis in an antibody-dependent manner **(Arp, 1980 and Leitner *et al*., 1990).** Because cross-protection is usually not observed with E. coli serotypes from poultry **(Arp *et al*., 1980)**, a suitable vaccine would have to contain the most common serotypes.

So, in this study two different E.coli formalin inactivated vaccines containing serotypes O1 and O78 were prepared, one of them was adjuvanted with aluminium hydroxide gel and the other was adjuvanted with montanide ISA70. The immune response in turkey poults was

monitored using MAT, ELISA and challenge test, it was clear that antibody titers in sera of turkey poults for both tests were paralleled to each other in starting and increasing titer and also after challenge as illustrated in tables and figures1, 2, 3 and 4 for both antigens which agree with that obtained by **El Jakee *et al***. (**2016).**

The protection percentages in turkeys after challenge with (O1 and O78) *E. coli* were summarized in Table (5). Protection rate in group (1) was 80% while in group (2) was 85% which was higher than those recorded in the control groups and these results agreed with **El Jakee *et al***. (**2016)**. So, the turkey poults vaccinated with *E. coli* vaccine adjuvanted with montanide ISA70 gave high immune response and protection which is capable of improving vaccine efficacy via the induction of a strong and long lasting immunity. Also it has been demonstrated an excellent adjuvant to stimulate humoral and cellular responses. This product is recommended for bacterial, mycoplasma, viral or parasite antigens for producing a potent vaccine able to protect turkey poults against colibacillosis. The obtained results agreed with **Chaffer *et al.,*1997** and **Sadeyen *et al.,* 2015** who found that using inactivated E.coli vaccine adjuvanted with montanide ISA70 was more potant than inactivated vaccine with Th2-biasing (Titermax® Gold or aluminium hydroxide).

CONCLUSION

From the above results it could be concluded that producing a vaccine from locally isolated E.coli strains adjuvanted with montanide ISA70 is recommended to aid in controlling colibacillosis.

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كفاءة لقاح الاشيريشيا كولى الميت التجريبى فى كتاكيت الرومى

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الملخص العربى

الاصابة بميكروب الاشيريشيا كولى يؤدى الى مرض القولون المعوى الذى يتسبب فى خسائر اقتصادية كبيرة تجلب تهديدا خطيرا لصناعة الدواجن في جميع أنحاء العالم وتهدف هذه الدراسة الى تحضير لقاح فعال من عترات الاشيريشيا كولاي (O1, O78) للسيطرة على مرض القولون المعوى بالدواجن. تم استخدام مائة كتكوت عمر يوم وتقسيمهم الى ثلاث مجموعات , تم تحصين المجموعة الاولى باللقاح المثبط محمل على الومنيوم هيدرواكسيد وتم تحصين المجموعة الثانية باللقاح الزيتى باستخدام زيت المونتانيدISA70 و وتركت المجموعة الثالثة مجموعة ضابطة دون حقن. تم قياس الاستجابة المناعية باستخدام اختبار التحدى وقياس عيارية الاجسام المناعية النوعية المكتسبة بالتحصين باستخدام اختبار التلازن الدقيق (MAT) و اختبارالاليزا(ELISA). وكان معدل الحماية للقاح المثبط على الومنيوم هيدرواكسيد 80% بينما كان معدل الحماية للقاح الزيتى باستخدام زيت المونتانيدISA70 85% وكانت نسبة الحماية 30% فى المجموعة الضابطة الغير محصنة وبعد الانتهاء من هذه الدراسة ينصح باستخدام اللقاح المثبط المحمل على زيت المونتانيد ISA70.