

Evaluation Of Sonicated *E. coli* Vaccine In Chickens

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ABSTRACT

Laying hens were vaccinated with sonicated *E. coli* O78 vaccine at 20 and 25 week old, the immune response was estimated by ELISA and IHT test in hens, eggs and hatched chicks after 1st, 2nd, 3rd and 4th weeks post vaccination and hatching.

The titer was measured by ELISA in hens ranged from 8.86-9.84. While GMT titer ranged from 1.99-2.71, while in eggs was ranging from 8.1-8.86 and from 1.32-2.43. In chicks ranged from 8.81-5.79 and 1.32 -2.1 respectively. The protection by sonicated vaccine with homologous strain at 14,21 and 28 day post hatching was 80, 60 and 50% while it was 70, 50 and 50% when challenge with heterologous strain respectively. On the other hand body weight gain and feed conversion rate significantly improved than that of control non vaccinated groups.

INTRODUCTION

Colibacillosis, is an acute and mostly systemic disease resulting in significant economic losses in poultry industry worldwide. The disease is characterized by multiple organ lesions with airsacculitis and associated pericarditis, perihepatitis and peritonitis. Several challenge experiments in chickens proofed the role of virulent avian pathogenic *Escherichia coli* (APEC) strains as the single aetiological agent. Currently serotypes O₁:K₁, O₂:K₁ and O₇₈:K₈₀ are recognized as the most prevalent, however, the number of published serotypes is increasing. The respiratory tract, principally the gas exchange region of the lung and the interstitium of the air sacs are the most important sites of entry for APEC strains adhere to the epithelial cells of air sacs presumably through F1-fimbriae. After colonization and multiplication the bacteria enter the bloodstream, and the temperature-sensitive hemagglutinin (tsh) seems to be important in this step. Septicemia resulting in massive lesions in multiple internal organs and in sudden death of the birds (1).

Broiler hens are vaccinated once at 20 weeks or twice at 20 and 25 weeks of age with a formalin-inactivated oil-emulsion *Escherichia coli* bacterin composed of serogroups 02, 078, and 035. Serological responses as assessed by

micro-agglutination documented an increase in serotype-specific antibody in vaccinated birds. Challenge of progeny from vaccinates and non vaccinates with homologous *E. coli* demonstrated that maternally derived antibodies could protect against mortality and/or lesions for as long as 2 weeks post-hatching (2).

Progeny of hens with high antibody titer challenged with the homologous bacteria at 7 and 14 days of age exhibited total resistance, while those challenged at 21, 34 and 45 days of age, when antibody titers were undetectable still showed 30 to 40% higher resistance than the control group. The results indicated a correlation between the hen's antibody titer and percentage of her progeny survival. Challenged progeny with a heterologous strain exhibited no protection. Hens which were vaccinated with a bivalent vaccine gave rise to chicks which were protected against both serotypes (3).

Ultrasonic inactivation of *Escherichia coli* followed by irradiation was the most efficient method for preparation of an effective vaccine against colibacillosis. Challenge experiments revealed that this vaccine provided the best protection compared with other methods of inactivation: heat, formaldehyde, and irradiation. Preparing the ultra-sonicated vaccine from O₂:K₁ strain increased its range and also supported

adequate protection against heterologous strain *O*₇₈:K₈₀ (4).

Aim of the work: Evaluation of sonicated *E. coli* vaccine by Elisa, IHT and challenge test to Detect severity of *E. coli* infection in broiler chicks from vaccinated and non vaccinated hens

MATERIAL AND METHODS

Experimental breeder hens: Thirty apparently healthy H&N breeder hens was divided into two equal groups, each of 15 were used in this study. The hens were reared in a separate hygienic pen.

Eggs: Fertile eggs were collected daily according to the schedule of the experiment for measuring immunoglobulin and chick hatching. Hatched chicks were housed in separate pens.

Ration: All groups were fed on commercial balanced ration obtained from El-Kahera Co.

***E. coli* vaccines:** Prepared sonicated *E. coli* *O*₇₈ vaccine (3,5).

***E. coli* strain:** *E. coli* *O*₇₈ was isolated and identified by (6) *E. coli* *O*₅₅ was isolated and identified by (7).

Serum samples: Blood samples were collected under aseptic condition according to the schedule of the experiment. Sera were separated and stored at -20°C until use.

Enzyme linked immunosorbent assay (ELISA): for detection of antibodies against *E. coli* (3,8).

Indirect hemagglutination test (Passive hemagglutination): for detection of antibodies against *E. coli* (9,10).

Body Weight and Feed Conversion Rate were calculated.

Statistical Analysis: All available data were analyzed using SPSS statistical analysis system package (11). Individual titers were transformed to log and log geometric mean (log GM) was calculated (12).

RESULTS AND DISCUSSION

In this study efficacy of sonicated *E. coli* vaccine was tested by serological evaluation,

protection and performance parameters. ELISA titer and GMT of IHT test of vaccinated breeder hens and their egg yolk showed significant increase than that of non vaccinated groups at 1st, 2nd, 3rd and 4th weeks post vaccination.

ELISA and GMT of IHT test of hatched chicks from vaccinated hens show significant increase than that from non vaccinated hens at 1st, 7th, 14th, 21st and 28th days post hatching (Table 2).

The obtained results revealed an increase in antibody titer in both breeder hens and eggs. There is a high correlation between ELISA and IHT where the titer were parallel in both tests (10). Moreover, the increase of antibodies activity occurred firstly in hens serum and then in egg yolk (13) and the increase in yolk antibodies titer were similar to those of breeder hens receiving *E. coli* vaccine (14).

While the antibody titer in hatched chicks from vaccinated dams decreased weekly up to the fourth week Table (2). On contrast it has been found that at hatching the level of maternal antibody to *E. coli* in chickens was 55-60% of their hens post-vaccination then declined to an undetectable level at 21 day old (3).

The protection percent of hatched chicks from vaccinated hens with homologous strain of *E. coli* *O*₇₈ at 14, 21 and 28 days old was 80, 60 and 50% post challenge while it was 70, 50 and 50% when chicks were challenged with *E. coli* *O*₅₅. On the other hand chicks from non vaccinated hens showed highest degree of mortalities 60, 60 and 50% post challenged with *E. coli* *O*₇₈ while it was 50, 50 and 50% post challenge with *E. coli* *O*₅₅ at 14, 21 and 28 day old, respectively Table (2).

Severe mortalities occurred in challenged chicks from unvaccinated breeder hens and the maternally derived protection in first 21 day old can protect progeny during the growing age immediately after hatching when chicks are most susceptible to *E. coli* infection (14).

Homologous challenge revealed protection % ranging from 73.3 – 86.7%, while after heterologous challenge was 60.7%. Non

vaccinated birds showed protection percent ranged from 20-40% (15).

At 6 weeks old, the recorded body weight for chicks with maternal antibody were 380gm, 375gm, 390 post challenge with homologous strain of *E. coli* O₇₈ while it were 360gm, 365gm and 385gm post challenge with heterologous strain of *E. coli* O₅₅ at 14, 21, 28 days respectively. On the other hand control groups recorded body weight 354gm, 353gm and 370gm and 359gm, 360gm and 370gm post challenge with *E. coli* O₇₈ and *E. coli* O₅₅ at age of 14, 21 and 28 day old respectively.

Challenged chicks with homologous strain of *E. coli* O₇₈ showed lower FCR (3.2, 4.2, 3.6) than groups challenged with heterologous strains of *E. coli* O₅₅ (3.38, 4.5, 4.1) at 14, 21 and 28

days old respectively. The control group showed higher FCR (Table 3).

Vaccinated chickens with *E. coli* vaccine were protected against active respiratory infection in that they gained body weight comparable to those unvaccinated (16).

Clinical signs of birds exposed to dust and *E. coli* infection had lower feed intake and body weight gain than the control group (17). The body weight of birds infected with *E. coli* were significantly lower than the control group (18).

It is concluded that vaccination of breeder hens with sonicated *E. coli* vaccine protected their offspring with maternal antibodies from *E. coli* infection up to the 4th week of age.

Table1. Experimental design

Group ⁽¹⁾	No. of hens ⁽²⁾	E. coli vaccination ⁽⁴⁾				Challenge tests ⁽³⁾					
		Type	Age	Dose	Rout	⁽⁵⁾ E. coli O78			⁽⁵⁾ E. coli O55		
						2 nd week	3 rd week	4 th week	2 nd week	3 rd week	4 th week
1	15	Sonicated vaccine	20 and 25 week of age	0.5ml	I/M	+	+	+	+	+	+
2	15	Non vaccinated	20 and 25 week of age	0.5ml	I/M	+	+	+	+	+	+

(1) Detection of immune response post vaccination by ELISA and IHT.

(2) Serum samples from breeders and eggs collected after 1, 2, 3, and 4 weeks post vaccination

(3) Serum samples from newly hatched chicks collected at 1, 7, 14, 21 and 28 day old

(4) Body weight, BWG, FI and FCR were estimated

(5) Hatched chicks from vaccinated and non vaccinated hens were challenged by E. coli O78 and O55, 1×10^8 I/M at 14, 21 and 28 days post hatching

Table 2. Antibody titer in breeders, eggs and hatched chicks (20-25 week age), dosed with 0.5ml I/M.

Group	Vaccine	ELISA titer					GMT titer					*Challenge test (protection %)					
		0 day	1 st w	2 nd w	3 rd w	4 th w	0 day	1 st w	2 nd w	3 rd w	4 th w	E. coli O78			E. coli O55		
												14	21	28	14	21	28
Breeders	Sonicated	-	8.86 ^a ±2.33	9.50 ^a ±4.02	9.79 ^a ±4.3	9.84 ^a ±1.2	-	1.99 ^a ±7.37	2.35 ^a ±6.02	2.59 ^a ±6.02	2.71 ^a ±2.86	-	-	-	-	-	-
	Control	-	5.79 ^b ±1.58	5.79 ^c ±0.44	5.79 ^c ±0.48	5.79 ^c ±0.49	-	1.14 ^b ±6.02	1.14 ^c ±6.02	1.14 ^c ±6.02	1.14 ^c ±6.02	-	-	-	-	-	-
Eggs	Sonicated	-	8.1 ^a ±4.62	8.4 ^a ±6.8	8.7 ^a ±4.04	8.86 ^a ±2.9	-	1.32 ^a ±7.73	1.62 ^a ±7.73	1.98 ^a ±6.02	2.43 ^a ±6.02	-	-	-	-	-	-
	Control	-	5.79 ^c ±4.6	5.79 ^c ±1.03	5.97 ^c ±1.37	5.79 ^c ±4.6	-	1.08 ^b ±7.73	1.08 ^b ±7.73	1.08 ^b ±7.73	1.08 ^b ±7.73	-	-	-	-	-	-
Hatched Chicks	Sonicated	8.81 ^a ±4.1	7.29 ^a ±4.02	6.8 ^a ±4.02	6.8 ^a ±4.01	5.79 ^a ±9.03	2.1 ^a ±6.02	1.92 ^a ±7.73	1.62 ^a ±7.73	1.32 ^a ±7.97	1.20 ^a ±1.34	80	60	50	70	50	50
	Control	5.79 ^c ±5.01	5.79 ^c ±5.01	5.79 ^c ±5.01	5.79 ^c ±5.01	5.79 ^a ±5.01	1.08 ^a ±7.37	1.08 ^c ±7.37	1.08 ^c ±7.37	1.08 ^c ±7.37	1.08 ^b ±7.37	40	40	50	50	50	50

Means of different variable within the same column having different superscripts are significantly different (P<0.05)

*All birds challenged with 1×10^8 cfu IM

Table 3. Performance parameters of newly hatched chicks .

●Group		Chicks from hens vaccinated with sonicated vaccine							Chicks from non vaccinated hens						
Time of challenge		14 day old		21 day old		28 day old		A	14 day old		21 day old		28 day old		B
Challenge Strain	Performance Parameters	O78	O55	O78	O55	O78	O55		O78	O55	O78	O55	O78	O55	
		B.W.	*2 weeks	117 ^b ±.81	117 ^b ±.81	120 ^{ab} ±.81	120 ^{ab} ±.81	120 ^{ab} ±.81	120 ^{ab} ±.81	120 ^{ab} ±.81	120 ^a ±.95	120 ^a ±.95	120 ^a ±.95	120 ^a ±.95	120 ^a ±.95
**6 weeks	380 ^d ±.81		360 ^h ±.70	375 ^e ±.81	365 ^g ±.91	390 ^b ±.81	385 ^c ±.91	400 ^a ±.81	354 ⁱ ±1.06	359 ^h ±.76	353 ⁱ ±.99	360 ^h ±.81	370 ⁱ ±.81	370 ⁱ ±.81	390 ^b ±.81
FI	2 weeks	119.5 ^a ±0.95	119.5 ^a ±0.95	119.5 ^a ±0.95	119.5 ^a ±0.95	119.5 ^a ±0.81	119.5 ^a ±0.81	119.5 ^a ±0.95	119.5 ^a ±0.95	119.5 ^a ±0.95	119.5 ^a ±0.95	119.5 ^a ±0.95	119.5 ^a ±0.95	119.5 ^a ±0.95	
	6 weeks	263 ^{ef} ±17.5	253.2 ^g ±0.99	252.1 ⁱ ±1.10	270 ^{cde} ±0.91	252.5 ^f ±0.95	268.7 ^{cde} ±1.23	210.5 ^h ±0.95	273 ^h ±0.76	266.5 ^h ±0.64	283.8 ^{abc} ±1.74	293.7 ^a ±0.68	274 ^{bcde} ±0.81	294 ^a ±0.81	209.7 ^h ±1.24
B.W.G	2 weeks	51 ^b ±0.8	51 ^b ±0.8	53.7 ^{ab} ±0.3	53.7 ^{ab} ±0.3	54.4 ^{ab} ±1.3	54.4 ^{ab} ±1.3	53.4 ^{ab} ±1.4	54.6 ^{ab} ±0.9	54.6 ^{ab} ±0.9	54.6 ^{ab} ±0.9	54.6 ^{ab} ±0.9	54.6 ^{ab} ±0.9	54.6 ^{ab} ±0.9	
	6 weeks	80 ^c ±0.81	75 ^d ±1.19	60.1 ^g ±1.00	60 ^g ±0.00	70 ^e ±0.42	65 ^f ±0.89	75 ^d ±0.21	63.5 ⁱ ±0.76	65 ⁱ ±0.44	54 ^h ±1.15	60 ^g ±1.15	64.4 ^f ±1.15	70.2 ^e ±1.79	69.9 ^e ±0.31
FCR	2 weeks	2.34 ^a ±3.9	2.34 ^a ±3.9	2.22 ^{ab} ±2.5	2.22 ^{ab} ±2.5	2.2 ^{ab} ±5.07	2.2 ^{ab} ±5.07	2.24 ^{ab} ±4.8	2.18 ^{ab} ±4.6	2.19 ^{ab} ±4.6	2.19 ^{ab} ±4.6	2.19 ^{ab} ±4.6	2.19 ^{ab} ±4.6	2.19 ^{ab} ±4.6	
	6 weeks	3.2 ⁱ ±0.22	3.38 ⁱ ±4.07	4.2 ^{bc} ±2.1	4.5 ^b ±1.5	3.6 ^g ±1.5	4.1 ^{fg} ±6.44	2.8 ^j ±1.65	4.3 ^h ±4.16	4.1 ^h ±2.45	5.2 ^a ±0.13	4.9 ^{ab} ±0.12	4.2 ^{de} ±0.11	4.2 ^e ±0.10	3 ^j ±2.35

A= Control Positive (vaccinated non challenged groups)

**= Performance parameters at the end of experiment

B= Control negative (non vaccinated non challenged groups)

* = Performance parameters before challenge of all groups

● Means of different variable within the same raw having different superscripts are significantly different (P<0.05)

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الملخص العربى

تقييم لقاح الميكروب القولونى المعامل بالموجات فوق الصوتية فى الدجاج

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فى هذه الدراسة تم تحضير لقاح الميكروب القولونى (E. coli O78) باستخدام الموجات فوق الصوتية حيث تم تحصين الدجاج البياض عمر ٢٠ أسبوع ثم عمر ٢٥ أسبوع وتم قياس الأجسام المناعية بعد التحصين باستخدام اختبار الاليزا واختبار التلازن الدموى الغير مباشر وتبين من النتائج الآتى:

١- الأمهات :

باستخدام اختبار الاليزا كان مستوى الأجسام المناعية بعد أسبوع ، ٢ أسبوع ، ٣ أسبوع ، ٤ أسبوع ٦,٨٦ ، ٩,٥٠ ، ٩,٧٩ ، ٩,٨٤ على التوالي بينما باستخدام اختبار التلازن الدموى الغير مباشر كان المتوسط الهندسى للأجسام المناعية ١,٩٩ ، ٢,٣٥ ، ٢,٥٩ ، ٢,٧١ على التوالي .

٢- البيض:

باستخدام اختبار الاليزا كان مستوى الأجسام المناعية فى البيض المخصب من الأمهات المحصنة بعد أسبوع ، ٢ أسبوع ، ٣ أسبوع ، ٤ أسبوع ٨,٨٦ ، ٨,٧ ، ٨,٤ ، ٨,١ على التوالي بينما باستخدام اختبار التلازن الدموى الغير مباشر كان المتوسط الهندسى للأجسام المناعية ١,٣٢ ، ١,٦٢ ، ١,٩٨ ، ٢,٤٣ على التوالي .

٣- الكتاكيت:

نسبة الأجسام المناعية فى الكتاكيت من أمهات محصنة باستخدام اختبار الاليزا عمر يوم ، أسبوع ، ٢ أسبوع ، ٣ أسبوع ، ٤ أسبوع ٨,٨١ ، ٧,٢٩ ، ٦,٨ ، ٦,٨ ، ٥,٧٩ على التوالي بينما باستخدام اختبار التلازن الدموى الغير مباشر كان المتوسط الهندسى للأجسام المناعية ٢,١ ، ١,٩٢ ، ١,٦٢ ، ١,٣٢ ، ١,٢ على التوالي .

٤- اختبار التحدى :

نسبة الحماية فى الكتاكيت من أمهات محصنة والعدوى باستخدام E. coli O78 عمر ١٤ ، ٢١ ، ٢٨ يوم من الفقس ٨٠ ، ٦٠ ، ٥٠% بينما باستخدام E. coli O55 كانت نسبة الحماية ٧٠ ، ٥٠ ، ٥٠% على التوالي .

٥ - بقياس معدل نمو الدجاج تبين أن وزن الجسم ومعامل التحويل الغذائى قد تحسنا بصورة ملحوظة عن مجموعة الضوابط .