The Effect Of E. Coli Infection On Chickens Immune Response

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ABSTRACT

This study is to evaluate the effect of *E. coli* infection on humeral and cellular immune response post ND vaccination as well as evaluation of inactivated E. coli vaccine. Two vaccinations with inactivated *E. coli* vaccine at 14, & 28 day of age gave 100% protection, meanwhile single vaccination at 14 day of age gave 90% protection.

E. coli infection was carried before ND vaccination by 2-5 days has adverse effect on humeral and cellular immune response, the geometric mean titer (GMT) was 11.3, 22.6, and 18.4 in group infected with *E. coli* at 5day of age and vaccinated with HB1 at 7 day of age, while in vaccinated non infected was 22.6, 42.2 and 36.8 at 7, 14, 21 day postvaccination respectively. The protection rate was 12.5% in infected vaccinated group and 25% in vaccinated non infected. Phagocytic index was 1.76, 0.983, and 0.889 in infected vaccinated group and in vaccinated non infected group was 2.03, 1.43, and 1.09 at 7, 14, 21 day postvaccination respectively. The effect of *E. coli* infection on chickens lymphoid organs weight was ranged from 0.150±0.041 to 0.102±0.0079gm in bursa of fabricius while in spleen ranged from 0.101±0.038 to 0.184±0.038, gm .

INTRODUCTION

Colibacillosis and Newcastle disease are widespread diseases resulting in economic losses ***(1,2)***. Avian pathogenic *Escherichia coli* (APEC) strains cause extra intestinal diseases in chickens, and other avian species ***(3)***. Avian colibacillosis starts as a respiratory infection (airsacculitis) frequently followed by generalized infections which manifested by perihepatitis, pericarditis, and septicemia ***(4-6)***. Clinically apparent *E. coli* infection is generally indicative of immunosuppression in poultry ***(7)***. *E. coli* infection damaged the immune systems of the chickens including lymphocyte depletion in both bursa and thymus ***(8,9)***. Oil emulsion *E. coli* vaccine exhibited good immunogenicity, which protect chickens from respiratory tract infections caused by avian pathogenic *E. coli* ***(10,11)***.

NDV vaccination failure demonstrated by several authors due to certain predisposing factors or agent such as *E. coli* infection ***(12,13)***. The aims of our work was to study the effect of *E.coli* infection on the lymphoid organs and immune response to ND vaccination, as well as evaluation of inactivated *E. coli* vaccine.

MATERIAL AND METHODS

I. Material

I. 1. Experimental birds

Two hundred and thirty apparently healthy day old chicks, Cobb breed obtained from El Kahera Poultry Company were used in this study. All chicks were reared in floor pens under hygienic conditions.

I.2. Ration

Chicks fed on a starter ration (Elkahira) contain energy 3000 kilo-calories, not less than 21% protein, 3.6% fat.

I. 3. Vaccines

a. Hitchner Bl and La Sota ND vaccines were obtained from Vet. Serum& Vaccine Research Institute, Abbasssia, Cario, Egypt, with virus titer of $10^{9.5}$ EID_{50}/ vial 1000 dose.

b. ND inactivated vaccine and *E. coli* formalized vaccine were kindly supplied by Intervet company. Batch/ Lot: A427A05, and A373A05 respectively.

I. 4. Challenge agents

a. Very Virulent NDV with EID_{50} $10^{6.5}$ / 0.5ml

b. E. coli serotype used was O78: K80 (F103).

These strains were kindly supplied from the department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University.

I. 5. Media

MacConky agar, Nutrient agar, and Nutrient broth obtained from *Oxoid*

I.6. Antibiotic media

MacConky Novobiocine agar, and Nutrient Novobiocine broth

I. 7. Fertile chicken egg

Two hundred and Forty fertile chicken eggs were obtained from hatchery located at Sharkia Governorate.

I. 8. Washed chicken erythrocyte *(14).*

I. 9. Antibiotic: Penicillin 250 I.U/ml., Streptomycin 100 µg./ml.

I. 10. Materials used for assessment of phagocytic activity:

I. 10.1. Reagent

i-Hank`s solution *(15)*

ii- Anticoagulant

A. Heparin solution ampoules: (5000 i.u.)

B. Sodium Citrate 3.8% *(16).*

I. 10. 2. Buffer:

i- Phosphate buffered Saline (PBS) *(15)*

I. 10. 3. *Candida albicans (C. albicans)*

It was kindly supplied by Department of Bacteriology, Mycology and Immunology, Faculty of Vet. Medicine, Zagazig University

I. 10. 4. Media

i- Sabouraud`s dextrose broth *(15)*

ii- Roswell Park memorial institute medium (RPMI Medium 1640), *(17)*

I. 10. 5. Stain

i- Leishman stain *(15).*

I. 10. 6. Foetal calf serum

I.11. Material for ELISA, *(18).*

II. Methods

1.Preparation of bacterial cultures for experimental infection

E. coli O78 was reconstituted in 5ml nutrient broth and incubated at 37C/ 24hr, then sub-cultured on Mac Conkey agar and incubated for 24hr at 37°C.

II.2.Preparation of Novobiocin *E. coli* marked strains *(19).*

II.3. Titration

Bacterial titration ***(20)*** and viral titration ***(21).***

II. 5.1. Experimental design

It aimed to study the effect of *E. coli* O_{78} infection on ND immune response post ND vaccination, at different ages (5, 10, 20 days), and evaluation of the immune response post formalized inactivated *E. coli* vaccine. One hundred and sixty chicks one day old were divided into six groups (A, B, C, D, E, F). Weights of lymphoid organs (bursa of fabricius and spleen) were recorded. The experimental designs is shown in Table,1.

II. 6. a. Sera: blood samples were collected from wing vein (7,14,21 day postvaccination. Sera were stored at -20 °C until used.

II. 6. b. Heparinized blood: 2.5ml blood was collected using heparin 50 I.U./ml blood, at 7, 14, 21 day postvaccination.

II. 7. Evaluation of humeral immune response:

II. 7.1. Haemoagglutination inhibition test (H.I- test): was carried out according to the previously described technique ***(22).***

II. 7.2. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA has been modified to detect antibodies against *Escherichia coli* ***(18)***, and ELISA for NDV ***(23)***

II.8. Evaluation of innate or non specific cell mediated immune response- Detection of phagocytic activity (*24*).

II. 9. Statistical analysis:- The obtained data was statistically analyzed ***(25).***

Table 1. Experimental design: Effect of *E. coli* infection on ND immune response.

Group No	Sub group	No of birds	Infection			Vaccine			Serum samples time	Challenge test
			Type	Age	Route	Type	Age	Route		
A	A1	10	*E. coli* O_{78} 1×10^5 cfu/ ml	5d	I/M	Hitchner B1	7d	Eye drops	14, 21, 28d	29d (3 weeks post vaccination
	A2	10	—	—	—	Hitchner B1	7d	Eye drops	14, 21, 28d	
B	B1	10	*E. coli* O_{78} 1×10^5 cfu/ ml	10d	I/M	La Sota	18d	Eye drops	25, 32, 39d	40d
	B2	10	-	-	-	La Sota	18d	Eye drop	25, 32, 39d	40d
	B3	10	*E. coli* O_{78} 1×10^5cfu/ ml	10d	I/M	Hitchner B1	7d	Eye drops	25, 32, 39d	40d
						La Sota	18d			
	B4	10	—	—	—	Hitchner B1	7d	Eye drops	25, 32, 39d	40d
						La Sota	18d			
	B5	10	*E. coli* O_{78} 1×10^5cfu/ ml	10d	I/M	Hitchner B1	7d	Eye drops	21, 28, 35d	36d
						Killed ND v	14d	I/M		
	B6	10	-	-	-	Hitchner B1	7d	Eye drops	21, 28, 35d	36d
						Killed ND v	14d	I/M		
C	C1	10	*E. coli* O_{78} 1×10^5cfu/ ml	20d	I/M	Hitchner B1	7d	Eye drops	25, 32, 39d	40d
						La Sota	18d			
D	D	10	-	-	-	Killed *E. coli* V	14d	S/C	21, 28, 35d	36d
E	E	10	-	-	-		14d	S/C	35, 42, 49d	50d
							28d			
*F	F	35	*E. coli* O_{78} 1×10^5 cfu/ ml	5d	I/M					
G	G	85	*C-ve*							29,36,40,50d

* Bursa of fabricius and spleen were weighted 24, 48, 72hr, 5d, 7, d, 10d, and 14day post infection

RESULTS AND DISCUSSION

E. coli infection caused highly significant decrease in ND antibody titers when chickens were infected 2 days before ND vaccination (A1) the GMT was 18.4 much lower than vaccinated only (A2) which was 36.8 at 3 weeks PV (Table 2). ND vaccination after *E. coli* and IB virus infection(after three and seven days respectively) reduced anti- ND titer and depression of lymphopoietic organs ***(26)***.

Also ELISA showed low antibody titer expressed by positive OD value in infected group (A1) in double fold dilutions begins (1/100). The positive dilutions were 2, 3, and 2 dilutions, (Table 3), while in vaccinated only (A2) was 3, 4, and 3 dilutions at 1, 2, and 3 weeks PV respectively, (Table 3). Several previous studies recorded that *E. coli* infection has adverse effect on humoral immunity to ND vaccine as measured by ELISA test at 4, 7 and 10 days postvaccination ***(27,28)***.

The phagocytic indices were 1.76, 0.983, and 0.889 in infected vaccinated group while in vaccinated group only were 2.03, 1.43, and 1.09 and in negative control were 1.02, 0.94, 0.86 at 1, 2, and 3 weeks PV respectively, (Table 5). The increased Phagocytic indices in birds received lentogenic live vaccine strains agreed with study which recorded that the

phagocytic indices in negative control were 0.84, 1.27, 0.85 and 0.9 at 3, 7, 14 and 21 days and 1.5, 3, 1.43, and 1.09 at 3, 7, 14 and 21 days postvaccination with Hitchner B1 via eye drop respectively *(29)*. The decreased Phagocytic indices in birds received *E. coli* infection before vaccination similarly it has recorded that there was decrease in neutrophil phagocytes in *E. coli* infected broiler against *Staph aureus (30).*

The protection rate was 12.5% in infected vaccinated group (A1) and 25% in vaccinated non infected (A2) post challenge with VVNDV (Table 5). The commercial broilers exhibited 66% mortalities *(31),* and the protection % ranged from 20-80% *(29).*

No significant differences in the ND GMT, ELISA values and phagocytic index when *E. coli* infection was injected before ND vaccination by 8 days (Tables 2,3,5). *E. coli* infection may induce transient lymphocytic depletion of lymphoid tissues in the chicken for 5-7 days *(8).* The protection rate was 50% in both infected and vaccinated (B1) and in vaccinated birds only (B2). No significant effect on protection was recorded in *E.coli* infected chickens and VND virus challenged 10 days postvaccination *(28).*

Table 2. The effect of *E. coli* infection on immune response to ND vaccination

Group	Subgroup	No of samples	At 7th day post vaccination		At 14th day post vaccination		At 21st day post vaccination	
			Mean	G.M.T	Mean	G.M.T	Mean	G.M.T
A	A1	10	3.5±0.167c	11.3	4.5±0.268c	22.6	4.2±0.25c	18.4
	A2	10	4.5±0.307b	22.6	5.4±0.266b	42.2	5.2±0.2b	36.8
	**(c-ve)	10	1.4±0.29a	2.6	1±0.298a	2	0.5±0.167a	2>
B	B1	10	4.4±0.22a	21.1	5.4±0.267a	42.2	5.3±0.213a	39.4
	B2	10	4.7±0.26a	26	5.5±0.223a	45.3	5.4±0.221a	42.2
	**C-ve	10	1.1±0.273b	2.1	0.8±0.167b	2>	0.6±0.162b	2>
	B3	10	4.9±0.267c	29.9	6.1±0.268c	68.6	6.9±0.3c	119.4
	B4	10	5.8±0.25b	55.7	6.7±0.3b	104	7.5±0.268b	181
	**C-ve	10	1.1±224a	2.1	0.8±0.298a	2>	0.6±0.167a	2>
	B5	10	6.3±0.213a	78.8	7.8±0.25a	222.9	8.2±0.29a	294.1
	B6	10	6.6±0.221a	97	8±0.21a	256	8.5±0.307a	362
	**C-ve	10	1.2±0.273b	2.3	0.9±0.298b	2>	0.8±0.371b	2>
C	C1	10	5.5±0.167b	45.3	6.6±0.267b	97	7.4±221b	168.9

* Means with different superscripts are significant at P≤ 0.05
**C-ve: Non vaccinated non infected
G.M.T of maternal immunity = 12.1

E. coli infection after primary ND vaccination and before revaccination showed significant reduction in antibody PV, where the GMT in infected vaccinated group (B3) was 119.4 at 3 weeks PV, while in vaccinated non infected (B4) was 181. Also positive ELISA OD values in infected vaccinated group (B3) at 3 weeks PV were 4 positive dilutions, while in birds vaccinated only were 5 positive (Table 3). *E. coli* has harmful effect on the immune system and the immune response especially when the infection of chickens occurs before vaccination at all ages *(13, 32).* The ND vaccinated birds only showed higher Phagocytic indices than vaccinated infected birds at 1, 2, and 3 weeks PV (Table 5). Proliferation of lymphocytes in the vaccinated birds coupled with an absence of resistance towards a Th2 polarization of the immune response in birds free of *E. coli* infection *(33, 34).*

After challenge, the protection % was 75% in infected vaccinated group (B3) and 87.5% in vaccinated only (B4). Previous study showed that the protection % were 70-75%, 81- 100% and 70% in challenged chickens *(35-37).*

Infection with *E. coli* before revaccination with killed ND vaccine (B5), the GMT at 3 weeks PV was 294.1 lower than in vaccinated non infected birds (B6) 362. Also ELISA OD values showed non significant decrease in antibody titer, but positive at all dilutions (A5) at 3 eeks PV, (Table 3). Secondary NDV vaccination administered 14 days later failed to induce immunity against *E. coli* when chickens were infected 1 or 5 days after the vaccination ***(32,38)***.

The phagocytic indices recorded in infected vaccinated group and in vaccinated non infected were 2.92, 3, 2.2 and 3.4, 3.2, 3.1 at 1, 2, and 3 weeks PV respectively, similar finding were recorded using several vaccinations programs ***(39)***. The protection % was 87.5% and 100 % in infected vaccinated (B5) birds and vaccinated non infected (B6) respectively, similar results were recorded for the inactivated oil adjuvant vaccine, ***(40-42)***, (100%, 83,3%, and 84-100% respectively).

E. coli infection after ND vaccination (C1) has no effect on antibody titer. The GMT, ELISA values and phagocytic index showed no differences in ND antibody titer (Tables 2, 3, 5). The immune response not affected when chicken vaccinated before *E. coli* infection ***(32)***. On the other hand the protection % was 75% in infected group (C1) and 87.5 % in birds vaccinated non infected (B4) ***(43)***.

Table 3. The effect of *E. coli* infection on immune response post ND vaccination using ELISA test

Group	Sub group	Age of bird (days) /PVs (weeks)	Mean of OD value at different serum dilution					
			1:100	1:200	1:400	1:800	1:1600	1:3200
A	A1	14 (1 PV)	0.362*	0.316*	0.288	0.224	0.186	0.162
		21 (2 PV)	0.434*	0.348*	0.301*	0.228	0.202	0.186
		28 (3 PV)	0.408*	0.362*	0.279	0.209	0.181	0.164
	A2	14 (1 PV)	0.422*	0.364*	0.322*	0.261	0.208	0.188
		21 (2 PV)	0.578*	0.454*	0.368*	0.308*	0.240	0.203
		28 (3 PV)	0.524*	0.408*	0.337*	0.296	0.225	0.198
B	B1	25 (1 PV)	0.420*	0.392*	0.340*	0.286	0.235	0.186
		32 (2 PV)	0.575*	0.462*	0.382*	0.308*	0.280	0.245
		39 (3 PV)	0.569*	0.452*	0.366*	0.298	0.248	0.186
	B2	25 (1 PV)	0.438*	0.398*	0.348*	0.294	0.242	0.200
		32 (2 PV)	0.586*	0.465*	0.390*	0.322*	0.282	0.251
		39 (3 PV)	0.580*	0.455*	0.373*	0.306*	0.266	0.208
	B3	25 (1 PV)	0.488*	0.408*	0.306*	0.262	0.214	0.196
		32 (2 PV)	0.508*	0.468*	0.394*	0.316*	0.286	0.244
		39 (3 PV)	0.638*	0.512*	0.406*	0.337*	0.298	0.206
	B4	25 (1 PV)	0.508*	0.442*	0.364*	0.301*	0.247	0.206
		32 (2 PV)	0.585*	0.504*	0.412*	0.387*	0.302*	0.237
		39 (3 PV)	0.687*	0.535*	0.442*	0.396*	0.308*	0.289
	B5	21 (1 PV)	0.523*	0.413*	0.348*	0.301*	0.268	0.204
		28 (2 PV)	0.592*	0.506*	0.420*	0.375*	0.299	0.259
		35 (3 PV)	0.807*	0.620*	0.506*	0.444*	0.367*	0.308*
	B6	21 (1 PV)	0.575*	0.425*	0.378*	0.336*	0.289	0.224
		28 (2 PV)	0.642*	0.554*	0.486*	0.405*	0.342*	0.298
		35 (3 PV)	0.854*	0.686*	0.598*	0.485*	0.372*	0.323*
C	C1	25 (1 PV)	0.501*	0.410*	0.334*	0.300*	0.266	0.201
		32 (2 PV)	0.562*	0.492*	0.401*	0.356	0.301*	0.198
		39 (3 PV)	0.613*	0.500*	0.422*	0.372*	0.303*	0.288

O; optical ensity at wave length 405 an cut off value was 0.3 (+ve)
Non vaccinate negative control (C-ve) showe negative O value below 0.3
* mean positive results

Chickens vaccinated and revaccinated using killed *E. coli* vaccine at 14 and 28day old (E) showed high positive OD value at 3 weeks PV and the positive dilutions were at all dilutions, while single vaccination (D) showed 5 positive dilutions at 3 weeks PV (Table 4). Booster vaccination with *E. coli* vaccine (E) gave 100% protection against *E. coli* challenge, while single vaccination (D) and negative control (G) showed 90% and 60% respectively. Phagocytic indices showed no differences between single vaccination (D) and booster vaccination (E) (at 1, 2, and 3 Weeks PV. (Table 5). Vaccination per os once at 7 or twice at 7 and 21 days resulted in good protection, Chicks exhibiting high antibody titers by ELISA were well protected against challenge ***(44,45).*** Oil emulsified *E. coli* vaccine was protective against infection ***(10, 46).***

Significant increase in weight of bursa fabricius at 24, 48, and 72 hr PI with *E. coli* in comparison with non infected control, while at 5, 7, 10, and 14d PI there was no difference between infected and non infected, (Table 6). The relative weights of the bursa and thymus reduced rapidly to minimal relative weights at 8 days after inoculation. At 14 days after inoculation, both bursa and thymus had normal relative weights and histological structures ***(8).***

Significant increase in spleen weight (at 1, 2, 3, 5 day PI respectively, in comparison with control, While at 7, 10, and 14d PI with *E. coli* there was no difference between infected and negative control, (Table 6). Macroscopic lesions of colibacillosis were observed in all inoculated birds, also moderate to severe lesions of airsacculitis, pericarditis, perihepatitis, and splenic hypertrophy were observed ***(5).***

Table 4. Evaluation of immune response post kille E. coli vaccine using ELISA test

Group	Sub group	Age of bird (days) / PVs(weeks)	Mean of O value at ifferent serum ilution					
			1:100	1:200	1:400	1:800	1:1600	1:3200
D		21 (1 PVs)	0.442*	0.354*	0.296	0.249	0.118	0.114
		28 (2 PVs)	0.574*	0.486*	0.408*	0.325*	0.269	0.180
		35 (3 PVs)	0.720*	0.606*	0.528*	0.415*	0.345*	0.233
E		35 (1 PVs)	0.653*	0.547*	0.434*	0.360*	0.331*	0.236
		42 (2 PVs)	0.748*	0.664*	0.541*	0.442*	0.353*	0.317*
		49 (3 PVs)	0.896*	0.737*	0.623*	0.514*	0.418*	0.348*

O: optical ensity at wave length 405 an cut off value was 0.3 (+ve)
Non vaccinate negative control (C-ve) showe negative O value below 0.3 * : mean positive results

Table 5. Result of Phagocytosis% , phagocytic index, and challenge test

Group NO	Subgroup	At 7th day post vaccination		At 14th day post vaccination		At 21st day post vaccination		Challenge test	
		Phagocytosis		Phagocytosis		Phagocytosis		Protection %	
		%	Index	%	Index	%	Index	ND	E. coli
A	A1*	13.5	1.76	11.36	0.983	9.7	0.889	12.5%	
	A2	23.4	2.03	20.1	1.43	15.8	1.09	25%	
	**C-ve	9.2	1.02	9	0.94	8.5	0.86		
B	B1*	24.2	2	22.8	1.36	18.9	1.03	50%	
	B2	25.78	2.25	23.51	1.45	20.44	1.23	50%	
	B3*	24.4	1.98	20.64	1.33	18.8	1.11	75%	
	B4	30.67	2.36	26.37	1.9	23.2	1.8	87.5%	
	**C-ve	9	1.03	8.8	0.98	8.2	0.86	0	
	B5*	31.2	2.92	33.54	٣	٢٩	2.2	87.5%	
	B6	36.38	3.4	38.41	3.2	35.67	3.1	100%	
	**C-ve	9	1.1	8.6	0.87	8.5	0.9	0	
C	1*	30.04	2.27	25.87	1.85	23.05	1.78	75%	
D		36.04	3.02	37.05	2.68	35.8	2.52		90%
E		36.62	3.04	37.8	2.8	36.06	2.65		100%
G	C-ve	9	0.98	8.8	0.85	8.8	0.87		60%

Gr(G): **C-ve Non vaccinated non infected *Infected with E. coli

Table 6. Effect of E. coli infection on immune organs weight

Organs	Group	The mean wieghts			
		24hr PI	48hr PI	72hr PI	Five day PI
Bursa of fabricius	1	0.150±0.041*	0.126±0.0073*	0.102±0.0079**	0.091±0.029
	2	0.1017±0.0061	0.080±0.028	0.075±0.0049	0.081±0.0041
Spleen	1	0.101±0.038**	0.223±0.094**	0.234±0.0117**	0.184±0.038*
	2	0.040±0.006	0.114±0.015	0.111±0.0083	0.141±0.018

* Means with superscripts are significant at P≤ 0.05
Gr (1): infected with E. coli O_{78} at 5 day old ($1x10^5$ cfu).
Gr (2): non infected group (C-ve) **PI:** post inoculation

REFERENCES

1.Alexander DJ (2000): Newcastle Disease and other avian paramyxoviridae infection In Disease of poultry, 10th ed. Calnek, BW. Iowa State University Press Ames, IA. 10th Ed. PP541-569.

2.Barnes H J and Gross WB(1997): Colibacillosis In Disease of poultry 10th edCalnek BW Iowa State University Press Ames, IA. 10th Ed. PP131.

3.Dho- Moulin M and Fairbrother J M (1999): Avian pathogenic Escherichia coli (APEC). Vet Res 30(2-3): 299- 316.

4.Gross W B (1994): Disease due to Escherichia coli in poultry, p237-259. In C L Gyles (ed), Escherichia coli in domestic animal and man. CAB International Wallingford United Kingdom.

5.Pourbakhsh S A, Boulianne M, DoizeB M, Dozois CM Desautels C, and J M Fairbrother JM (1997): Dynamics of *Escherichia coli* infection in experimentally inoculated chickens. Avian Dis 41:221-233.

6.Ewers C, Janssen T, and Wieler L H (2003): Avian pathogenic Escherichia coli (APEC). Berl Munch Tierarztl Wochenschr, 116(9- 10): 381-95.

7.Mc Gruder E D and Moore G M(1998): Use of lipopolysaccharide (Lps) as a ppositive control for the evaluation of immunocompetentiating drugs candidates in experimental avian colibacillosis models. Research of Vet Sci 66:33-37.

8.Nakamura K, Imada Y, Maeda M(1986): Lymphocytic depletion of bursa of Fabricius and thymus in chickens inoculated with Escherichia coli. Vet Pathol. 23(6):712-7.

9. Nakamura K, Yuasa N, Abe H, and Narita M (1990): Effect of Infectious bursal disease virus on infections produced by Escherichia coli of high and low virulence in chickens. Avian patho. 19: 713- 721.

10.Kutkat M A and Mona A El- Shabrawy (2002): Laboratory study for immunization of broiler chickens against colibacillosis. J Egypt Vet Med Assoc. 60 2: 67 – 75.

11.Mona IH El Enbaawy and Sahar A Zou – ElFakar (2004): Response of broiler chickens to a whole cell microwaves irradiated E coli trivalent vaccine. J Egypt Vet Med Assoc. 64 1: 69 – 80.

12.Hassanein Z A, Mona M A, and Hassan M K (2001): Nature of humoral immune suppression induced by Escherichia coli infection in vaccinated chickens. J Egypt Vet Med Assoc. 61: 79-89.

13.Abdel-Fattah A M, Abdien H M F and Mohamed K M (2003): New Approach for Stimulation of Chickens Immune Response Against E Coli Infection and Newcastle Disease Vaccine. Zag vet J. 31(2) PP184-196.

14.Beard C W (1989): Serologic procedures in isolation and identification of avian pathogen (third edition). Pp 193. Edited by the American associatiation of Avian Pathologists.

15. ***Cruickshank R , Duguid J P , Marinion BP , and Swain R H (1975):*** Textbook of Medical Microbiology (12th ed) ES Living Stone Limited Edinburgh and London.

16. ***Hitchner B, Chairman S, Charks H, Purchase D O H, and Williams L E (1975):*** Isolation and identification of pathogens texas A and M. Univ. College Station Texas.

17. ***Lucy F L and LarryD P (1982)***: Ontogeny and line differences in mitogenic responses of chicken lymphocytes. Poultry science. 62: 579-584.

18. ***Leitner G, Melamed D, DrabkinN, and Heller E D (1990)*** An enzyme-linked immunosorbent assay for detection of antibodies against Escherichia coli: association between indirect hemagglutination test and survival. Avian Dis. 34(1): 58-62.

19. ***Barnhart E, Caldwell D, Crouch M, Byra J, Ccorrier D, and Hargis B (1999)***: Effect of lactose administration in drinking water prior to and during feed with drawl. Poultry Science. 78: 211-414.

20. ***Sambrook J, Fritsch E F, and Maniatis T(1989):*** Molecular coloning a laboratory manual. New York. Cold spring Harbor. Laboratory Press.

21. ***Reed I J, and Meunch H(1938)***: A simple method for estimating fifty percent end points. Am J Hyg. 27: 493- 496.

22. ***World Organization for Animal Health (OIE) (2004)*** In: Manual of diagnostic tests and vaccines for Terrestrial Animals 5th Edition. OIE, Paris, France (In Press).

23. ***Snyder D B, Marquardt W W, Mallinson E T, Russek- Cohen E, Savage P K, and Allen DC (1986)***: Rapid serological profiling by enzyme- linked immunosorbent assay. IV. Association of infectious bursal disease serology with broiler flock performance. Avian Dis. 30: 139- 148.

24. ***Wilkinson PC (1977):*** Techniques in Clinical Immunology'' ed By Thompson RA, Publications. PP201, USA.

25. ***Tamhane A C and Dunlop D D (2000):*** Statistics and data analysis from elementary to intermediate. Upper saddle River, USA.

26. ***Rakhmanina I A, Shubin V A, and Groshevy G A(1979):*** Influence of 8 mixed infection(with infectious bronchitis virus and E. coli) on the immune response of chicks to ND vaccine. Vliyomie Smeshannei Infek Siiu molodnyakapitis. Trudy vessoyuznogo Instituta Eksperimental No. 1 Veterinarii 133-138.

27. ***Marquardt WW, Snyder DB, Savage PK, Kadavil SK, and Yaneey FS (1984):*** Antibody response to Newcastle disease virus given two different routes as measured by ELISA and hamoagglutination-inhibtion test and Associated Tracheal Immunity. Avian disease 29(1): 71-77.

28. ***Hassan M, and Hassanein Z A (1998)***: Effect of Escherichia coli infection on the immune response of chickens to Newcastle diseases virus vaccine. J Egypt vet Med Ass. 59(1): 75-91.

29. ***Adel O (2004):*** Effect of some immune stimulants on the immune response postvaccination against Newcastle disease in chickens. M. V Sc. Thesis, Faculty of Veterinary Medicine, Zagazig University.

30. ***Mohamed H R (2008):*** Immunological Studies on Escherichia coli Infections in Broilers. M. V. Sc. Thesis, Faculty of Veterinary Medicine, Department of Bacteriology and immunology, Zagazig University,

31. ***Kapczynski D and King D J(2005):*** Protection of chickens against overt clinical disease and determination of virus shedding following vaccination with commercially available Newcastle disease virus vaccines up on challenge with high virulent virus from California 2002 Exotic Newcastle disease outbreak. Vaccine, 23: 3424-3433.

32. ***Ramadan H A A(2003):*** Effect of Ecoli infection and its treatment on immune response for Newcastle disease vaccines in

chickens. M. V. Sc. Thesis, Faculty of Veterinary Medicine, Zagazig University.

33. ***Kohase M, Moriya H, Sato TA, Kohno S, and Yamazaki S (1986)***: Purification and characterization of chick interferon induced by viruses. J Gen. Virol. 67: 215-218.

34. ***Wertz K, Buttner M, Mayr A, and Kaaden OR (1994):*** More than one component of the Newcastle disease virus particle is capable of interferon induction. Vet Microbiol. 39(3-4): 299-311.

35. ***Elamin M A G, Khalafalla A I, and Ahmed S M (1993):*** Observations on the use of Komarov strain Newcastle disease vaccine in Sudan. Tropical Animal Health and Production. 25(3): 151-154.

36. ***Ismail A, El- Sabbagh A, El TaherA M, Hassan N M, Ibrahim K, Ibrahim S N, and Barhoum N (1981):*** Immune response to KND vaccination in chickens. Zag Vet J, 3:67-76.

37. ***Orajaka L J E and Ezema W S (2004):*** The effect of Newcastle Disease virus vaccines on local hens. Nigerian. J, 25(2): 60-65.

38. ***Huang H J, and Mastsumoto M (2000)***: Nonspecific innate immunity against Escherichia coli infection in chickens induced by vaccine strains of Newcastle disease virus. Avian Dis. 44(4): 790- 796.

39. ***Afifi M A, Zou-El Fakar S A, and El Kady M F (1998)*** Immune response of chickens vaccinated with Newcastle disease vaccines using different vaccination programs. Vet Med. J, Giza. 46(2): 209-222.

40. ***Box P G (1975):*** Newcastle disease antibody level in chickens vaccinated with oil emulsion adjuvant killed vaccines. Vet Rec. 96: 108-111

41. ***Bastami M A, Amer M M, and Hamouda A S (1986):*** Comparative study on the immune response of chickens vaccinated intramuscularly with different Newcastle disease vaccines. Assuit Vet Med. J. 17(34): 223-229

42. ***Foltise R, Halvorson D V, and Sivanan dan V (1998):*** Efficacy of combined killed- in oil emulsion vaccine and live Newcastle disease vaccine in chicken. Avian Dis. 42:173- 178.

43. ***Yunis R, Ben-David A, Heller E D, and Cahaner A (2002):***Genetic and phenotypic correlations between antibody responses to *Escherichia coli* Infectious Bursal Disease Virus (IBDV) and Newcastle Disease Virus (NDV) in Broiler Lines Selected on antibody response to *Escherichia coli*. Poultry Science, 81: 302–308.

44. ***Melamed D, Leitner G, and Heller ED (1991):*** A vaccine against avian collibacillosis based on ultrasonic inactivation of E coli. Avian Dis. 35: 17 – 22.

45. ***Frommer A, Freidlin p J, Bock RR, Leitner G, Chaffer M, and Heller ED (1994):*** Experimental vaccination of young chickens with a live non pathogenic strain of E coli. Avian pathology, 23: 425 – 433.

46. ***Kariyawasam S, Wilkie B N, Hunter D B, and Gyles C L (2002):*** Systemic and mucosal antibody responses to selected cell surface antigens of avian pathogenic *Escherichia coli* in experimentally infected chickens. Avian Dis. 46:668–678.

الملخص العربى

تأثير عدوي الميكروب القولونى على الاستجابة المناعية للدجاج

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** الهيئة العامة للخدمات البيطرية بمحافظة الشرقية

أجريت هذه الدراسة لدراسة التأثير الضار للعدوى بالميكروب القولونى على الأستجابة المناعية للدجاج المحصن بلقاح مرض النيوكاسل باستخدام أختبارمنع التلازن، تحليل الاليزا، المناعة الخلوية، و التحدى. و كذلك تقييم التحصين بلقاح الميكروب القولونى الميت. كانت نسبة الحمايه فى إختبار التحدى للمجموعه المحصنة بجرعتين من لقاح الميكروب القولونى الميت ١٠٠٪ عند عمر ١٤ و٢٨ يوم بينما فى المحصنه بجرعه واحده عند عمر ١٤ يوم ٩٠٪ . أوضحت النتائج ان الأصابة بعدوى الميكروب القولونى قبل التحصين بيومين الى خمسة ايام تؤدى الى نقص فى رد الفعل المناعى حيث سجل الدجاج المعدى بالميكروب القولونى عند عمر ٥ أيام والمحصن بلقاح هيتشنرب١ عمر ٧أيام (A1) نقص معنوى فى المتوسط الهندسى للأجسام المناعية من تلك التى سجلت فى الدجاج المحصن فقط (A2) على مدار ثلاثة اسابيع بعض التحصين كان متوسط الأجسام المناعية فى الدجاج المصاب و المحصن(١١,٣، ٢٢,٦ ، و ١٨,٤) بينما كانت (٢٢,٦، ٤٢,٢، و ٣٦,٨) فى مجموعة الدجاج المحصن فقط بعد ١ ٢ و٣ أسبوع من التحصين على التوالى. كانت نسبة الحماية بعد إختبار التحدى بفيروس مرض النيوكاسل الضار فى المجموعة المعدية و المحصنة ١٢,٥٪ وفى المحصنة بالهتشنر فقط كانت ٢٥٪ كذلك أثبت تحليل الآليزا بعد (١، ٢، و٣ أسبوع من التحصين) ان مجموعة الدجاج التى حصنت فقط كان لديها نسب أجسام مناعية أعلى من تلك فى مجموعة الدجاج المعدية و المحصنة.

وجد إنخفاض معنوى فى المناعة الخلوية فى المجموعة المعدية والمحصنة من تللك المسجلة فى المجموغة المحصنة فقط وكان دليل الخلايا اللهمية(١,٦٧ ، ٠,٩٨٣ ، و ٠,٨٨٩) فى المجموعة المعدية والمحصنة بينما فى المحصنة فقط (٢,٠٣ ،١,٤٣ ،و١,٠٩) بعد ١ ،٢، و٣ أسبوع من التحصين على التوالى.