The Immunosuppressive Effect of *E. coli* in Chickens Vaccinated with Infectious Bronchitis (IB) or Infectious Bursal Disease (IBD) Vaccines

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Abstract: *Escherichia coli O78* infection 4 days before vaccination has adverse effect on immune response of chickens post vaccination with Mass type Infectious Bronchitis vaccine at 14 day old. The ELISA values of infected vaccinated group (B) were (0.366, 0.307) at serum dilution (1:100, 1:200), (0.412, 0.388, 0.307) at (1:100, 1:200, 1: 400) and (0.484, 0.406, 0.362, 0.308) at (1:100, 1:200, 1:400, 1:800) while only vaccinated group (D) the titer increased (0.408, 0.386, 0.322) at (1:100, 1:200, 1: 4 00), (0.522, 0.436, 0.362, 0.304) at (1:100, 1:200, 1:400, 1:800) and (0.625, 0.586, 0.508, 0.467, 0.351) at (1:100, 1:200, 1:400, 1:800, 1:1600) post vaccination with 1, 2 and 3 weeks respectively. Also phagocytic index in vaccinated non infected group was (2.08, 1.67, and 1.47) while in infected vaccinated group was (1.03, 0.98, and 0.86), respectively. Infection with *E. coli* post IB vaccination showed no differences in antibody titer and phagocytic index in both infected and non infected groups. Also *E. coli* infection 3 days before Infectious Bursal Disease vaccinations and before revaccination caused high decrease in ELISA antibody titer and also decrease in the protection percent 70% mean while it was 90% in vaccinated non infected. [Journal of American Science 2010; 6(9):762-767]. (ISSN: 1545-1003).

Key words: *E coli O78*, Immunosuppression, Vaccination, ELISA, Phagocytic index, Protection

Introduction:

Colibacillosis is a widespread diseases resulting in economic losses *(Barnes and Gross, 1997).* Avian colibacillosis starts as a respiratory infection (airsacculitis) frequently followed by generalized infections which manifested by perihepatitis, pericarditis, and septicemia (*Gross 1994; Pourbakhsh et al 1997.; and Ewers et al 2003).* Clinically apparent E. coli infection is generally indicative of immunosuppression in poultry *(Mc Gruder and Moore 1998).* E. coli infection damaged the immune systems of the chickens including lymphocyte depletion in both bursa and thymus *(Nakamura et al 1986 and 1990.* The aim of this work was to study the immunosuppressive effect of *E. coli* infection on vaccination against Infectious Bronchitis (IB) and Infectious Bursal disease (IBD) vaccination.

Materials and Methods:

1. Experimental birds: One hundred apparently healthy day old chicks, Cobb breed obtained from El Kahera Poultry Company (Egypt) were used in this study. All chicks were reared in floor pens under hygienic conditions.

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

3. Vaccines

a. IBV vaccine strain Mass type: Schering- Plough Animal Health .Mill sboro, Delawar, USA. Ser No/9094/07.

b. Gumboro vaccine (Nobilis strain D_{78}- IBD vaccines) Intervet Each dose contains at least 4.0 $\log_{10}$ $TCID_{50}$ of the Gumboro Intermediate strain D_{78} as primary vaccination. Batch/Lot: 6844DJ01.

c. IBD Blen- IBDV vaccine (Ceva SANT. Animal), as live attenuated hot strain as booster vaccine. Batch No/ 4511R3U2A

4. Challenge strains:
4.1. Bursal homogenate (virulent IBDV) with EID_{50} $10^{4.8}$/0.5ml.
4.2. *E. coli* serotype used was O_{78}: K80 (F103).
These strains were kindly supplied from the Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University.

5. Media: MacConky's agar, Nutrient agar, and Nutrient broth *(Oxoid).*
6. Antibiotic media: MacConky's Novobiocine agar, and Nutrient Novobiocine broth

I. 7. Fertile chicken eggs: One hundred and eighty fertile embrynated chicken eggs, used for titration of the viral

strains and vaccines, were obtained from hatchery located at Sharkia Governorate, Egypt

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

9. Materials used for assessment of phagocytic activity:

9.1. Reagents and Buffer: Hank`s solution and Phosphate buffered Saline (PBS) pH 7.4 (*Cruickshank et al, 1975).*

9. 2 Anticoagulant: Heparin ampoules: (5000 i.u.)

9. 3. *Candida albicans (C. albicans)*: kindly supplied by Department of Bacteriology, Mycology and Immunology, Faculty of Vet. Medicine, Zagazig University, Egypt.

9. 4. Media: Sabouraud`s dextrose broth *(Cruickshank et al, 1975), and* Roswell Park memorial institute medium (RPMI Medium 1640), (*Lucy and Larry, 1982)*

9. 5. Stain: Leishman stain (*Cruickshank et al, 1975).*

9. 6. Foetal calf serum

10. Materials used for ELISA (*Mockett and Darbyshire, 1981 and Snyder et al. 1986)*

11. Preparation of bacterial cultures for experimental infection: *E. coli* O_{78} was reconstituted in 5ml nutrient broth and incubated at 37C/ 24hr, then sub-cultured on MacConkey agar and incubated for 24hr at 37°C.

12. Preparation of Novobiocin E. coli marked strains *(Barnhart et al 1999).*

13. Titration: Bacterial titration (*Sambrook et al 1989) and* viral titration *(Reed and Meunch 1938).*

14. Experimental design(Table, 1): aimed to study the effect of *E. coli* O_{78} infection on IB and IBD immune response post IB and IBD vaccinations, at different ages (5, 10, 20 days). One hundred chicks one day old were divided into equal groups, each 10 birds and the nine kept as –ve control 20 birds (A, B, C, D, E, F, G, H, I).

15. Sera: blood samples were collected from wing vein (7, 14, 21 day post vaccination. Sera were separated and stored at -20 °C until used.

16. Heparinized blood: 2.5ml of blood was collected using heparin 50 I.U/ml blood, at 7, 14, 21 day post vaccination.

17. Evaluation of immune response:

17. 1. Enzyme-Linked Immunosorbent Assay (ELISA):
ELISA for IBV according to *Mockett and Darbyshire (1981)*
ELISA for IBDV according to Snyder et al. (1986)

17. 2. Detection of phagocytic activity: for evaluation of innate or non specific cell mediated immune response (*Wilkinson, 1977*).

$$\text{Phagocytic \%} = \frac{\text{Number of phagocytes containing } C.\ albicans.}{\text{Total number of counted phagocytes}} \times 100$$

$$\text{Phagocytic Index} = \frac{\text{Total No. of ingested yeast cells}}{\text{Total No of infected phagocytes}}$$

17. 3. Challenge test:
Groups E, F, G, H, and I were inoculated with bursal homogenate solution (vIBD virus) after 21 day post vaccination, $10^{4.8}$/0.1ml /bird, via eye instillation, birds were observed for 10 days after challenge, mortality and specific signs were recorded.

Results and Discussion:

E. coli infection 9 days before vaccination has no effect on the immune response. Positive ELISA values showed no characteristic differences in antibody titer when *E. coli* infection at 5 day old and IB vaccination at 14 day old (A). Also phagocytic index showed no differences, Tables (2, 3), these results agree with *Nakamura et al. (1986)* who recoded that *E. coli* infection may induce transient lymphocytic depletion of lymphoid tissues in the chicks for 5-7 days. While infection with *E. coli*, 4 days before vaccination with Mass type IB vaccine, has adverse effect on the immune response of chickens. The positive ELISA in infected vaccinated group (B) was (0.366, 0.307) at serum dilution (1:100, 1:200), (0.412, 0.388, 0.307) at (1:100, 1:200, 1: 400), and (0.484, 0.406, 0.362, 0.308) at (1:100, 1:200, 1:400, 1:800), while was higher in vaccinated non infected group (D) (0.408, 0.386, 0.322) at (1:100, 1:200, 1: 400), (0.522, 0.436, 0.362, 0.304) (1:100, 1:200, 1:400, 1:800), and (0.625, 0.586, 0.508, 0.467, 0.351) at (1:100, 1:200, 1:400, 1:800, 1:1600) PV with 1, 2, and 3 Ws respectively. Also phagocytic index was high in vaccinated non infected (2.08, 1.67, and 1.47) while in infected vaccinated group was lower (1.03, 0.98, and 0.86) at 1, 2, and 3 weeks post vaccination respectively, as shown in Tables (2 , 3). These results agree with *Van Dijk et al. (1980)* who mentioned that *E. coli* infection cause impairment of polymorphonuclear leukocytes (PMNLs) function, decreased phagocytic activity and ineffective opsonization, and *Krukowski and Smith,* (2005) who

recorded that the abnormalities in neutrophil functions as well as decrease in its number was due to recurrent or chronic cutaneous and bacterial or fungal infection that leads to neutrophil dysfunction.

On the other hand *E. coli* infection post IB vaccination showed no differences in phagocytic index in both infected and non infected (1.85, 1.58, and 1.42) and (2.08, 1.67, and 1.47) at 1, 2, and 3 weeks post vaccination respectively, (Table, 3). These results agree with *Ariaans et al. (2008)* who recorded that broilers inoculated with IBV H120 vaccine or virulent M41 and challenged 5 days later with *E. coli 506* showed no impairment in phagocytic capacity and recruitment, so enhanced colibacillosis after IBV infection or vaccination is caused at least by altered innate immunity and less by impairment of phagocytic cell function. In case of Infectious bursal disease (IBD), *E. coli* infection 3 days before vaccination at 8 and 16 days old (E) caused high decrease in ELISA antibody titer (0.407, 0.346, 0300), (0.468, 0.402, 0.357, 0.303) and (0.582, 0.468, 0.392, 0.318), while the titer was higher in vaccinated non infected birds (H) (0.538, 0.421, 0387, 0.302), (0.681, 0.567, 0.488, 0.421, 0.362), and (0.774, 0.682, 0.568, 0.461, 0.402, 0.364) at serum dilution (1:100, 1:200, 1:400, 1:800, 1:1600) post vaccination with 1, 2, and 3 weeks respectively. On the other hand the effect of *E. coli* infection was not significant after primary vaccination and before revaccination by 5 days (F), the titer was (0.480, 0.402, 0.343), (0.608, 0.516, 0.412, 0.333), and (0.708, 0.633, 0.500, 0.406, 0.326) at serum dilution (1:100, 1:200, 1:400, 1:800, 1:1600) post vaccination with 1, 2, and 3 weeks respectively. Phagocytic index decreased in infected vaccinated group (E), (1.03, 0.94, and 0.86) and in group infected before revaccination (F), (1.3, 1.12, and 0.98) when compared with non infected vaccinated group (H) (2.08, 1.67, and 1.47). The protection percent was 70% in infected vaccinated group (E), 80% in infected revaccinated group (F) and 90% in non infected vaccinated group (H), these results agree with *Tsukamoto et al. (1995)*; *Aly and Hasanean (1998)* who recorded that inoculation of *E. coli* by I/M route, but not by the oral route, caused temporary bursal lymphoid depletion for 7 to 14 days.

The IBD immune response post vaccination was not affected when chicken infected with *E. coli* post vaccination (G), (table, 2). Also phagocytic index showed no significant differences, (1.85, 1.58, and 1.42) in infected and increased in non infected vaccinated group (2.08, 1.67, and 1.47) at 1, 2, and 3 weeks post vaccination respectively. The protection percentage in both was 90%, Table (3). These results similar to that mentioned by *Pitcovski et al. (2001)* who stated that *Escherichia coli* post immunization with commercial vaccine against infectious bursal disease virus had no effect on antibody titer which determined by ELISA and their resistance to challenge with virulent IBDV.

Table (1): Shows experimental design of the infected and vaccinated groups

Group	No of birds	Infection			Vaccine			Serum sample time	Challenge test
		Type	Age	Route	Type	Age	Route		
A	10	*E. coli* O_{78} 1×10^5 cfu/ ml	5d	I/M	Mass type IB vaccine	14d	I/ N	21, 28, 35d	-
B	10		10d	I/M		14d	I/ N	21, 28, 35d	-
C	10		20d	I/M		14d	I/ N	28, 35, 42d	-
D	10	—	—	—		14d	I/ N	21, 28, 35d	-
E	10	*E. coli* O_{78} 1×10^5 cfu/ ml	5d	I/M	Nobilis D78- IBD vaccines * (Intermediate)	8d	Eye drops	23, 30, 37d	38d
					IBD Blen- IBDV vaccine@	16d	DW		
F	10		10d	I/M	Nobilis D78- IBD vaccines	8d	Eye drops	23, 30, 37d	38d
					IBD Blen- IBDV vaccine	16d	DW		
G	10		20d	I/M	Nobilis strain D78- IBD vaccines	8d	Eye drops	23, 30, 37d	38d
					IBD Blen- IBDV vaccine	16d	DW		
H	10	-	-		Nobilis strain D78- IBD vaccines	8d	Eye drops	23, 30, 37d	
					IBD Blen- IBDV vaccine	16d	DW		
I	20	-	C-ve					21, 28,35d 23, 30, 37d	

cfu colony forming unit
I/M intra muscular
I/N intra nasal
DW drinking water
*Intermediate strain of IBD as primary vaccine
@Hot strain of IBD as booster vaccine
C-ve control group unvaccinated not infected with E coli

Table (2): The effect of *E. coli* infection on immune response post IB and IBD vaccination using ELISA test:

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	21 (1 PV)	0.405*	0.385*	0.316*	0.255	0.176	0.124
	28 (2 PV)	0.480*	0.418*	0.355*	0.301*	0.266	0.193
	35 (3 PV)	0.620*	0.581*	0.500*	0.460*	0.348*	0.288
B	21 (1 PV)	0.366*	0.307*	0.288	0.246	0.182	0.162
	28 (2 PV)	0.412*	0.388*	0.307*	0.201	0.165	0.128
	35 (3 PV)	0.484*	0.406*	0.362*	0.308*	0.266	0.205
C	21 (1 PV)	0.403*	0.369*	0.302*	0.222	0.193	0.151
	28 (2 PV)	0.478*	0.421*	0.358*	0.302*	0.258	0.187
	35 (3PV)	0.618*	0.575*	0.502*	0.448*	0.312*	0.249
D	21 (1 PV)	0.408*	0.386*	0.322*	0.288	0.216	0.192
	28 (2 PV)	0.522*	0.436*	0.362*	0.304*	0.271	0.209
	35 (3 PV)	0.625*	0.586*	0.508*	0.467*	0.351*	0.295
E	23 (1 PVs)	0.407*	0.346*	0.300*	0. 245	0.206	0.155
	30 (2PVs)	0.468*	0.402*	0.357*	0.303*	0.262	0.197
	37 (3 PVs)	0.582*	0.468*	0.392*	0.318*	0.272	0.209
F	23 (1 PVs)	0.480*	0.402*	0.343*	0.268	0.206	0.189
	30 (2 PVs)	0.608*	0.516*	0.412*	0.333*	0.293	0.256
	37 (3 PVs)	0.708*	0.633*	0.500*	0.406*	0.326*	0.289
G	23 (1 PVs)	0.520*	0.408*	0.361*	0.301*	0.241	0.189
	30 (2 PVs)	0.667*	0.550*	0.464*	0.398*	0.342*	0.288
	37 (3 PVs)	0.768*	0.660*	0.551*	0.434*	0.392*	0.335
H	23 (1 PVs)	0.538*	0.421*	0.387*	0.302*	0.257	0.202
	30 (2PVs)	0.681*	0.567*	0.488*	0.421*	0.362*	0.289
	37 (3 PVs)	0.774*	0.682*	0.568*	0.461*	0.402*	0.364*

OD: optical density at wave length 405 and cut off value was ≥ 0.3 positive
Group (D): control +ve Vaccinated with IB v not infected with *E coli*
Group (H): control +ve Vaccinated with IBD v not infected with *E coli*
Unvaccinate negative control (I) showed negative OD value below 0.3
*positive OD value

Table (3): Result of Phagocytosis post IB and IBD vaccination, and Challenge test with VVIBDVs, 3 weeks post IBD vaccination:

Group NO	One week post vaccination		Two weeks post vaccination		Three weeks post vaccination		Rate of	
	Phagocytosis		Phagocytosis		Phagocytosis		Mortality	Protection
	%	Index	%	Index	%	Index		
A	25	2.02	21.8	1.65	20.2	1.4	-	-
B	16.7	1.03	13.4	0.98	10.45	0.86	-	-
C	24.48	1.85	22.02	1.58	20.01	1.42	-	-
D	26	2.08	22.6	1.67	20.8	1.47	-	-
E	18.7	1.03	16.4	0.94	14.45	0.86	**30%**	**70%**
F	26.8	1.3	21.54	1.12	19.46	0.98	**20%**	**80%**
G	30.48	1.85	28.02	1.58	23.82	1.42	**10%**	**90%**
H	32	2.08	28.6	1.67	24.8	1.47	**10%**	**90%**
I	9.2	1.05	8.8	0.9	8.8	0.86	**40%***	

Group (D): control +ve Vaccinated with IB v only and not infected with *E. coli*
Group (H): control +ve Vaccinated with IBD v only and not infected with *E. coli*
Group (I): control –ve unvaccinated not infected with *E. coli*
*mortality was not more than 40% may be due to the presence of maternal antibodies to IBD (up to 5 weeks).

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