THE ROLE OF E.COLI INFECTION ON INFECTIOUS BURSAL DISEASE AFFECTED BROILER FLOCK AT NORTH SINAI GOVERNORATE.

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ABSTRACT

Several factors causing immune suppression in chickens would lead to immune deficiency, one of these significant factors are Escherichia coli (E. coli) infection. This study was performed to investigate the role of E. coli infection with virulent infectious bursal disease virus (IBDV) and IBD vaccination with intermediate plus strain vaccine in broiler chickens. The IBDv infected and/or IBD vaccinated birds with E. coli inoculation showed partially an increase in the humeral immuno -suppression. E.Coli was re-isolated from collected samples 48h post inoculation. Histopathological score of bursa Fabricius as well as E.coli lesion score and B: B wt ratio had significant variation when compared with control group. When the bursa of Fabricius (BF) of all groups were examined histopathlogically at 7 and 14 days post infection different degree of regeneration in their damaged lymphoid follicles were observed at 14 days when compared with control group. IBD infected chickens and inoculated with E. coli pre or concurrent to infection had lower antibody titers as compared to control groups. Mean whiles IBD vaccinated chickens inoculated with E. coli pre or concurrent to vaccination had significant difference in antibody titers. The chickens infected with E. coli pre or simultaneously IBD vaccination and challenged with NDV had significant mortalities as compared with vaccinated control group. Meanwhile IBD infected chickens inoculated with E. coli pre or simultaneously to infection and challenged with NDV had high mortalities as compared to control group. Mortality % ranged from 62, 48% and 60% respectevely, limited correlation between the level of humeral antibodies and the protection percentage against VVND challenge was observed.

INTRODUCTION

Protection of commercial chicken flocks against common viral diseases is dependent upon multiple vaccinations against these diseases.

Immunosuppressant caused by E. coli is the major interest because of the wide spread occurrence of infection in commercial chickens. Infection with

IBD was reported affect the course of variety of bacterial, protozoal and viral diseases of chickens. The effect of infection on the antibody response to NDV was one of the earliest observed immunosuppressive effects of IBD infection ***Allan et al., (1972).*** Infection with E. coli at an early age significantly compromises the immune responses of chickens, however; E.Coli infection in chickens is secondary localized or systemic disease occurring when host defenses have been impaired ***Nakamura et al., (1986)***, and immune response is based on the main organs as bursa of Fabricius and thymus gland. Vaccination failure is a serious problem facing poultry industry in Egypt. The immunosuppressive effect of some virus such as NewCastle disease (ND), Infectious bursal disease (IBD) and bacterial agents as E. coli causing the great economic loses in commercial flocks. Infectious bursal disease virus is an important pathogen which destroys the B lymphocytes of the bursa of Fabricius and causing immunosuppressive and death in aging 2 to 8 weeks. Escherichia coli (E. coli) infection is the most common secondary bacterial invaders followed the application of live vaccines as ND and IBD vaccine which are the most common vaccines used on large scale in commercial flocks ***Mazariegos et al., (1990)***. E. coli infection causes marked gross and microscopic bursal lesions leading to bursal atrophy which resulted transient humeral immunosuppression ***Hassan and Hassanein (1999).*** The objective of this study was to investigate the effect of experimental E. coli infection on the immune response to infection with virulent IBDVinfection (group 1) and IBD vaccination with inter mediate plus IBDV vaccine (group 2).

MATERIALS & METHODS

A- Materials

1- Chickens: A total of 600 day old broiler chicks were kindly supplied, from a local Hatcheries Company, reared in clean, disinfected separated rooms throughout the experimental period and were provided with feed and water ad libitum.

2- E. coli strain, E. coli serotype O55 : K59 was obtained from Bacteriological department Animal Health Research Institute, Doki, Cairo Identification, and classification was carried out according to ***Edwards and Ewing (1972)***. E. coli inoculums, an overnight broth culture was standardized to give bacterial suspension contained ($3x10^8$ CFU) viable organisms per ml. Each bird received 0.5 ml via intramuscular (I /M) inoculation ***Sojeka (1965)***.

3- Newcastle disease virus (NDV) challenge strain, field velogenic viscerotropic NDV100% mortality in susceptible chicks was used propagated in

embryonated chicken eggs; the allantoic fluid titrated to inoculation.

Chickens were challenged by I/M route with 0.5ml dilution of allantioic fluid containing 10^7 EID_{50}/ml. ***Abou-Elkhair et al., (1998)***.

4- Infectious bursal disease virus (IBDV) strain, bursal homogenate containing virulent local strain of (IBDV) isolated from clinical causes.

The titer was expressed as the 50% embryo infective dose (EID_{50}) per ml. The inoculated dose was 10^3 EID_{50} / bird ***Okoye and Uzoukwu, (1990).***

5-ND vaccine, both live (Biotype B1 strain and B1 type, LaSota strain) ND vaccine (intervet international B.V. Buxomer- Holland) were used for vaccination of birds It was given as recommended by the manufacturer.

6-IBDV vaccine, A freeze dried live intermediate commercial vaccine (intervet international B.V. Buxomer- Holland) was used and given as recommended by the manufacturer company.

7-Serum samples for immunoassay and organs such as bursa Fabricius for histological examination.

B- Methods

1- Bioassay, chickens from group 1, 2 were challenged with NDV two weeks post lasota vaccination and kept under close observation at end of experiment. Post mortem (P.M.) examination, lesions of bursa of all sacrificed chickens were weighed and each bursa was compared with its whole carcass body weight and expressed as Bursa: Body weight (B: Bwt) ratio were calculated by the formula, organ weight in grams X 100/ total body weight in grams and then expressed as the arithmetic mean for each group of birds and evaluated stastically as described by ***Mazariegos et al., (1990).*** Lesions of pericarditis and perihepatitis (P+P) and air sacculitis were also recorded, clinical sign and mortality are investigated

2- Immunoassay, to investigate the possible effect of the E. coli infection on the humoral immunity before and simultaneously vaccination and infection of IBDV, individual serum samples were tested at 14, 21. 28.36 and 42 days of age weekly intervals and tested by Enzyme linked immunosorbent assay (ElISA) and Haemagglutination inhibition (HI) test ***Beard and Wilkes (1973).***

- ELISA test, Samples two hundred eighty was diluted 1:500 in buffer saline and assayed by IBD antibody ELISA kits (Lab. diagnostics, Inc; Maine, USA) ***Abdel – Alim et al., (2003).***

- HI test, ND antibody titer was determined by the HI test using the standard procedures in micro titer plates using 4 HA units ***Stephan et al., (1998).***

3- Bacteriological examinations: Blood samples collected from birds 48 hours post I/M inoculation with E. coli suspension as well as carcasses of sacrificed chicks at 14, 21, 28, 36 and 42 days of age were examined bacteriological to reisolate the inoculated E. coli strain ***Nakamura et al., (1992).***

4- Histopathology: Bursa Fabricius (BF) glands of sacrificed chickens at 14, 21, 28, 36 and 42 days of age were collected and fixed in 10% buffered formalin, processed and stained with H&E. Bursal lesions were subjectively scored according to the method described by ***Rosales et al., (1989)*** 1=. No lesions, 2= Focal, mild cell necrosis depletion in few follicles, 3= Multifocal 0.5 to 0.3 of the follicle show atrophy and 4= diffuse atrophy of all the follicles, in 75-100% of follicles. Statistical analysis, the average B: Bwt ratio of inoculated groups was compared with those of control groups for statistical significance using analysis of variance test. ***Sendecor and Cochran, (1980).***

5- Experimental design is shown in table (1)

Group 1- A total 300 day old chickens were used in this group, twenty randomly collected chicks were sacrificed and serum samples were collected for measuring the maternally acquired immunity (MAI) against ND and IBDV. At 23 days of age, 280 chicks were divided randomly into 7 equal subgroups (40 chicks / subgroup). E. coli was inoculated via I/M route with O.5 m1/bird $3x10^8$ CFU) at 23 days (subgroup 1), 27 days (subgroups 2 and 5) and 31 days of age (subgroup 3).chicks of subgroup4, 6 and 7 left uninoculated controls, at 27 days old chickens of subgroups I, 2, and 3 were infected with IBDV by nao – ocular rote each bird received 10^3 $ELD_{50,}$ chickens of subgroup 4, 5,6 and 7 kept without IBD infection, subgroup 4 control of vaccine, subgroup 5 control of E.coli infection ,sub group 6 control of NDV challenge and subgroup 7 as blank control.

Group -2 - A total 300 one days old chickens were used in this group twenty randomly collected chicks were sacrificed and serum samples were collected for measuring the maternally acquired immunity (MAI) against ND and IBDV. At .10 days of age, 280 chicks were divided randomly into 7 equal subgroups (40 chicks / subgroup). E. coli was inoculated via I/M route with O.5 m1/bird ($3x10^8$ CFU) at 10 days (subgroup 1), 14 days (subgroups 2 and 5) and 18 days of age (subgroup 3).chicks of subgroup 4, 6,and 7 left uninoculated controls, at 14 days chickens of subgroups I, 2, and 3 were vaccinated with IBD vaccine by nao – ocular rote ,chickens of subgroup 4, 5,6 and 7 kept without IBD infection, subgroup 4 control of vaccine, subgroup 5 control of E.coli infection ,sub group 6 control of NDV

challenge and subgroup 7 as blank control. Chickens of all groups 1and 2 (except those of subgroup 6) were vaccinated against ND at 10^{th} and 28^{th} day of age using hitcher B1 and lasota vaccine by eye drop route respectively. Chickens in group1 and 2 were challenged at 42 day of age with virulent ND virus by I/M route 0.5 ml/bird (10^7 ELD_{50}) subgroup 7 in each group left as unvaccinated uninfected and unchallenged control.

Table (1) - Experimental Design

Group 1							Group 2					
Sub G.	Treatment				Time of Treat.	NDV 42	Sub. G.	Treatment			Time of Treat.	ND V 42
	Sub G	E.coli	IBDV	ND Vac.				E.coli	IBD Vac.	ND Vac.		
1	40	At 23 day +	At 27 day +	10-28 days +	Pre	+	1	10 day +	14 day +	10-28 days +	Pre	+
2	40	27 day +	+	+	Conc.	+	2	14 day +	+	+	Conc.	+
3	40	31 day +	+	+	Post	+	3	18 day +	+	+	Post	+
4	40		-	+	Control Vac.	+	4	-	-	+	Control Vac.	+
5	40	27 day +	-	+	Control E.coli	+	5	14 day +	-	+	Control E.coli	+
6	40		-	-	Control NDV.	+	6	-	-	-	Control NDV.	+
7	40		-	+	Blank control	-	7	-	-	+	Blank control	-

Each bird inoculated with o.5ml/ $3x10^8$ CFUof E.coli broth culture
Each bird was inoculated via eye drop route LaSota ND vaccine
Each bird was inoculated via eye drop route with inter mediate IBD vaccine
Chickens were challenged via I/m route o.5ml/ 10^7 ELD50 of NDv challenges strains Each bird was challenged via eye drop route with 10^3 ELD50 of highly virulent filed IBDV strains

RESULTS

Serum samples were collected from 20 sacrificed chickens at 1 and 14 days old examined bacteriologically for free from E. coli and determination of ELISA titers against IBDV from group1 and 2. All samples gave positive antibody titers and free from E. coli. Result of immmunoassay, bioassay and bursal/ body weight ratios and lesion score were summarized in table (2, 3 and 4) fig.1. Chickens inoculated with E. coli at 23 days of age subgroup (1) followed by IBDV infection at 27 days old had marked decrease in NDV antibody response at 7 days post infection and antibody level increased significantly ($P< 0.05$) at14 days post infection. As shown in table

(4) significant decline of maternal antibody was noticed in subgroup 7 as compared with subgroup 4 similar trained was recorded in chickens of subgroup 2 which simultaneously inoculated with E. coli and IBDV infection at 27 days and chickens in subgroup3 inoculated with E. coli post IBDV infection at 31 days had significant decrease in antibody titer as compared with subgroup 4 vaccinated control.

Chickens inoculated simultaneously at 14 days old with E. coli and IBD vaccine subgroup 2 had lower IBD antibody response at7days old post vaccination as compared with vaccinated chicks subgroup 4, moderate depression anorexia , ruffled feather , off food and diarrhea were noticed 48 h post E. coli inoculation in group 1 and 2 (subgroup 1,2,3and 5 from each) typical ND lesions were noticed in challenged group 1 and 2 (subgroup 1,2,3,4,5 and 6) and lesions of IBDV infection noticed in group 1 (subgroup 1,2 and 3) the severity of postmortem lesions was recorded as shown in table (3).The pathological alteration in form of inflammatory signs represented by edema and inflammatory cell infiltration in the stroma accompanied with congestion of bursal blood vessels and area of hemorrhages were detected in bursa of fabricius, the lymphoid follicles appeared atrophy with increase vaculation and cyst formation inside the follicles of infected group 1 (subgroup 1,2 and 3) Fig.1(C and D) .However, no pathological alteration were detected in BF of non infected, non treated birds subgroup 7, in group 2 vaccinated moderate pathological alteration in the form of hemorrhage Fig. 1(A and B). E. coli was reisolated from blood samples 48h post inoculation (PI) and from organs of sacrificed birds from group 1 and 2 (subgroup 1, 2, 3 and 5 each).

Post mortem (P.M.) and histopathological examination: The B: B.wt ratios, histopathological scores of bursa and as well as E. coli lesions pericarditis, perihepatites and air saculitis (P+P) were detected in scarified chickens at different age intervals are presented in table (3) Fig 1(E and F). Results of challenge studies, as shown in table (2), chickens inoculated with E. coli pre and concurrent IBD infection group 1 and vaccination groups 2 respectively had significant high mortality rates compared with those chickens inoculated with E. coli 4-days post IBD infection or vaccination.

Table (2): Results of bioassay of birds inoculated with E.coli Pre, concurrent and post IBDV infection and IBD vaccination.

Sub. G.	Treatment		Time Of Treat.	VVND Challenge									
				IBDV infection group 1					IBDV vaccination group 2				
	E. coli	ND Vac.		Dead No./ Total No.	Sur. With ND signs	No.of Total Pos.	Pos. %	Pro. %	Dead No./ Total No.	Sur. With ND signs	No.of Total Pos.	Pos. %	Pro. %
1	+	+	Pre	3/25	4	7	28%	62%	1/20	3	4	20%	80%
2	+	+	Conc.	8/25	5	13	52%	48%	5/20	2	7	35%	65%
3	+	+	Post	4/25	6	10	40%	60%	3/20	3	6	30%	70%
4	-	+	Control	4/25	2	6	24%	76%	2/20	1	3	15%	85%
5	+	+	Control	12/25	4	18	72%	28%	5/20	5	10	50%	50%
6	-	-	Control	24/25	0	25	100%	-	20/20	-	20	100%	0%

Details about groups and their treatment are shown in table (1)

Pos. = Positive Pro. = Protection Sur. = Survived Vac. = vaccine Conc. = Concurrent

Table (3): Results of Bursal-Body Weight ratio (B:B.wt), Bursa Fabricius Lesion Score (BF.L.Sc) and E.coli lesion score of chickens.

Sub. G.	Treatment		Time of Treat. IBDV	IBD Infection Group 1						IBD Vaccination Group 2					
				B/B.wt Ratio		E.coli Lesion score		BF.L.Sc.		B/B.wt Ratio		E.coli Lesion score		BF.L.Sc.	
	E.coli	ND Vac.		7 D PI	14 D PI	7 D PI	14 D PI	7 D PI	14 D PI	7 D PV	14 D PV	7 D PV	14 D PV	7 D PV	14 D PV
1	+	+	Pre	2.7 ± 0.36	2.4 ± 0.19	++	+++	2.4	2	4 ± 0.18	4.5 ± 0.39	+	+	3	1.8
2	+	+	Conc.	3.3 ± 0.52	1.7 ± 0.18	+	++	2.5	2.1	3 ± 0.27	3.1 ± 0.23	+	-	3.5	2.6
3	+	+	Post	3.6 ± 0.31	3.5 ± 0.35	+	+	2.3	1.8	2.4 ± 0.36	2.6 ± 0.25	+	-	1.54	0.5
4	-	+	control	3 ± 0.15	3.8 ± 0.24	-	-	1.6	1.2	3.5 ± 0.28	3.2 ± 0.34	-	-	1	-
5	+	+	control	4 ± 0.6	2.4 ± 0.34	+	++	2.5	2	1.8 ± 0.16	1.9 ± 0.14	+	+++	3.3	2
6	-	-	control	1.9 ± 0.4	4.6 ± 0.15	-	-	1	-	4.2 ± 0.23	4.1 ± 0.35	-	-	-	-
7	-	+	Blank control	5.2 ± 0.6	5.8 ± 0.35*	-	-	0	0	4.5 ± 0.92	5.5 ± 0.62*	-	-	-	-

PI = Post infection PV = Post vaccination LSc = lesion score BF = Bursa fabricius

*** Average bursal lesion score of examined (5 bursa)**

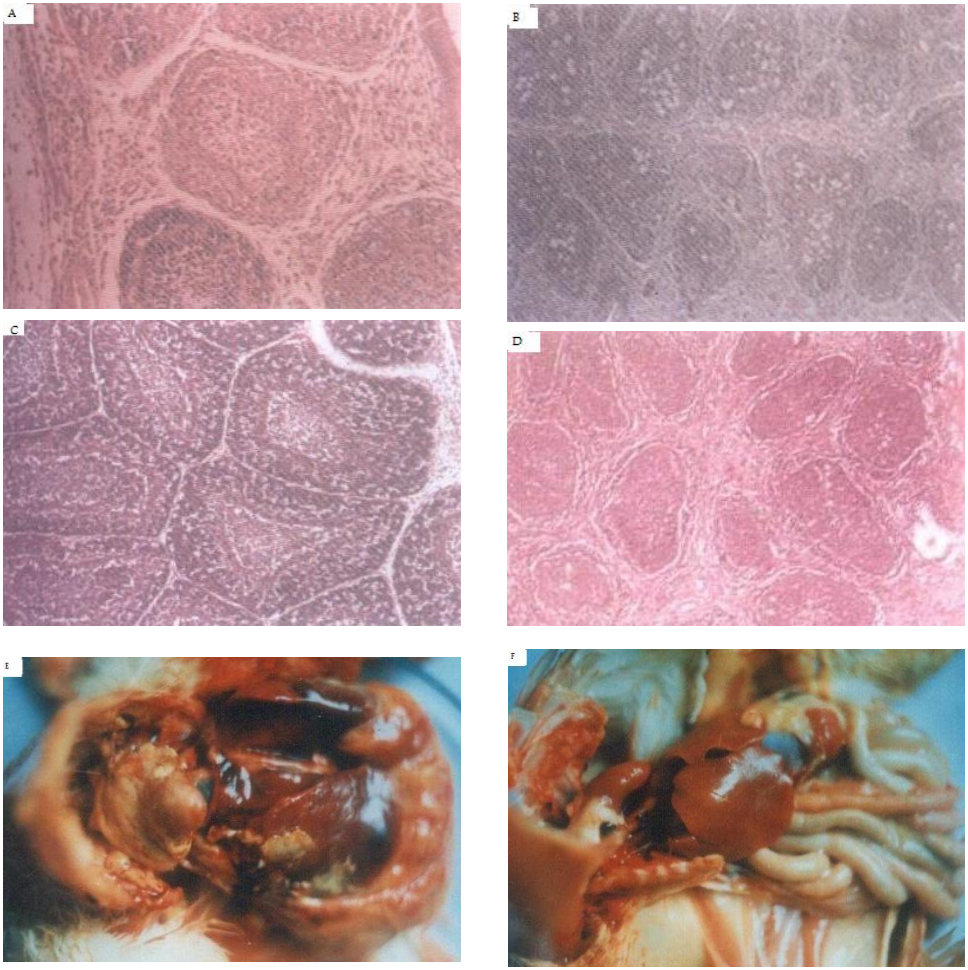

Fig.1

A- Bursa of Fabricius (BF) of chickens 7 days post IBD vaccination showing edema and inflammatory cells infiltration in stroma notice the absence of lymphoblast cell and presence of reticular fibers in germinal center (H&E X 150).

B- Bursa of Fabricius of chickens 14 days post IBD vaccination showing connective tissue proliferation stroma notice the vacuolation of lymphoid follicles (H&E X 150).

C- Bursa of Fabricius of chickens 7 days post IBD infection showing proliferation of fibrous connective tissue in stroma and atrophy and vacuolation of lymphoid follicle (H&EX60).

D- bursa of Fabricius of chickens 14 days post IBD infection showing increase in size of lymphoid follicle and appearance of considerable number of lymphocyte at peripheral (H&E X60).

E- Experimental infected chicks with E.coli subgroup 5 shown fibrineous pericardities perihepatits and air saculites.

F- Control of broiler chickens subgroup 7 show normal organs.

DISCUSSION

Bursa of fabricius and thymus are considered as central lymphoid organ that play essential role in immune response, immunization of commercial chicken flocks is the principle method adopted to control common viral diseases e.g. ND, and IBD, numerous factors can cause suboptimal vaccine response ***Allan et al., (1972).*** Damage of BF due to viral (IBD) or bacterial (E. coli**)** agents has direct adverse effect on humeral immune response ***Nakamura et al., (1992)*** reported that marked lymphocytic depletion in bursa and was noticed due to experimental inoculation with E. coli. This observation was confirmed by the work of ***Hassan and Hassanein (1999).*** In the present study experimental inoculation of E. coli induced marked bursal lymphocytic depletion for about two weeks PI. Results of the present study revealed that E. coil infection caused humeral depression furthermore inoculation of E. coli pre and concurrently with IBD infection resulted in significant high mortalities when infected chickens were challenged with VVNDV similar results have been noticed in previous study ***Nakamura et al., (1986)***. Inoculation of E.coli 4 days pre IBDV infection had adverse effect on immune response, most research on E. coli infection was focused on its effect as secondary invader that complicate other primary viral or bacterial infection similar results have been noticed in previous study ***Wyeth (1975).***

In this study the role of E. coli as pre, concurrent and post infective agent was evaluated in vaccinated chickens. The results pointed out the possible immunosuppressive role of E. coli and its adverse effect on the immune response to NDV and IBDV vaccinations, and in this investigation the immunosuppressive effect of IBDV infection, IBD vaccination and E. coli was evident as indicated by significant lower B: Bwt ratio table (2) and histopathological lesion score table (3) of infected and vaccinated groups when compared with that of blank control group, these results coincid with those of ***Hassan and Hassanein, (1999).***

The effect of pre, simultaneously and post inoculation of E. coli on the level of ND antibodies was studied in group 1 with IBDV infection revealed that the HI titer of all subgroup had arranged of geometric means varied from (0.14and 4.1), in group 2 with IBD vaccination the HI titer of all subgroup geometric means ranged between (0.15 and 4.85). Chickens inoculated with E. coli concurrent and post IBD infection group 1 and IBD vaccination

groups 2 respectively had significant high mortality rates compared with those chickens inoculated with E. coli 4-days pre IBD infection or IBD vaccine. Lesion of IBD virus are detected in infected subgroup mainly at subgroup inoculated with E.coli pre ,concurrent or post as subgroup 1,2 and 3 when compared with subgroup 4. Significant low of IBD antibody was noticed at 36 days age old chickens inoculated simultaneously at 28 days old with E. coli and IBD infection decreased antibody titer more than subgroup vaccinated and inoculated with E.coli which had lower IBD antibody response at 36 days old as compared with subgroup 4.

Regarding results of VVNDV challenge and HI geometric means it could be observed that there was a limited correlation between the level of humeral antibodies and the protection percentage. The later might be attributed to presence of other means of protective tools such as cell mediated immune response. In addition, the bioassay studies using VVNDV challenge proved the potency of NDV strain where infection resulted in 100% mortality in ND unvaccinated immune compromised subgroup (6).

Moreover, the efficacy of used NDV vaccines well documented as the protection percentage ranged (50% and 85%) in vaccinated control group's table (2 and 3), these result agreement with ***Nakamura et al., (1992).***

Generally speaking from results of group 1 and 2 it was observed that the E. coli infection partially increase the humeral immune suppression when inoculated pre, concurrent or post IBDV infection or IBD vaccination.

This indicate that the immune suppression resulted from either inoculation of virulent IBDV, intermediate plus IBD vaccine and E. coli inoculation under the condition of the present study, as it only decrease the protection percentage in birds by IBDV infection more than birds by IBD vaccine, these result agreement with ***Okoye and Uzoukwu (1990)***. From aforementioned results it could be concluded that the depression in humeral immune response was lower in all inoculated subgroup with E. coli as compared with positive and blank control subgroup 4 and 7., Therefore E. coli infection had adverse effect on the immune response; such effect should be taken in consideration under field conditions when vaccinating E. coli infected chickens. Also, it was shown that E. coil infection caused temporary humoral immune depression that may last for 2 weeks under experimental conditions eventually control of E. coli in commercial flock could be help in

facing IBDV antigenic exposure and its immunosuppreive effect which is a hazard. Anticipating causative agent of serious economic impacts in poultry industry allover the world.

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الملخص العربى

دور الميكروب القولوني االعصوى فى عدوى مرض الجمبورو وأثرها فى قطعان التسمين بمحافظة شمال سيناء

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مركز البحوث الزراعية - معهد بحوث صحة الحيوان - معمل العريش

العوامل المصاحبة للتثبيط المناعى قد تؤدى الى نقص المناعة ومن هذه العوامل عدوى الميكروب القولوني العصوى (E.coli) وهى تسبب ضمور فى غدة الفابريسيس يؤدى الى تثبيط المناعة فى الطيور المصابة. ولذلك تم إجراء هذة الدر اسه لتوضيح دور الميكروب القولونى العصوى مع عدوى مرض الجمبورو (مجموعه أولى مقسمه إلى ٧مجموعات صغيره) ا و عدوى E.coli مع تحصين الجمبورو العتره المضاعفة (مجموعه ثانية مقسمه الى ٧ مجموعات صغيرة) . الدراسة أوضحت ان الطيور التى تم إجراء العدوى الصناعية لها بميكروب E.coli ومرض الجمبورو فى المجموعة الاولى او التحصين ضد مرض الجمبورو والعدوى المجموع الثانية حدث زيادة بالتثبيط المناعى بعد ٤٨ ساعة

تم إجراء إعادة عزل ميكروب E.coli من الطيور التى أجريت لها العدوى من العدوى من الصفة التشريحية و النتائج وجد ان معدل ظهور الأعراض لمرض E.coli ومرض الجمبورو واضحه اذا ما قورنت بالمجموعة الضابطة. تم اجراء الفحص الهستوباثولوجى لغدة الفابريسيس مع دراسة معدل

الاصابه بها ومعدل الاصابه لميكروب E.coli وكذلك معدل وزن غدة الفابريسيس مع الوزن الكلى لجسم الطائر وذلك بعد ٧ ايام من العدوى ، وجد بعد ١٤ يوم من العدوى تحسن فى نسبة الاصابة والاعراض المصاحبة لها وبخاصة غدة الفابريسيس هذا بالمقارنة مع المجموعة الضابطة.

تم اجراء الفحص السيرولوجى لقياس الاجسام المناعيه فى المجموعات المختلفة وجد ان العدوى الصناعية بميكروب E.coli قبل ومع الاصابه بالجمبورو تؤدى الى تقليل الاجسام المناعيه اذا ماقورنت بالمجموعه الضابطه بينما مجموعات الطيور فى العدوى بميكروب E.coli مع التحصين ضد مرض الجمبورو قبل ومع التحصين لوحظ تغير طفيف فى الاجسام المناعيه اذا ماقورنت بالمجموعه الضابطه. تم اجرء عدوى صناعيه بفيروس النيوكاسل بالعترة شديدة الضراوة وجد ان الطيور التى تم عمل عدوى صناعيه لها مع الجمبورو نسبة الوفيات بها عاليه اذا ماقورنت بالمجموعه الضابطة بينما المجموعة الثانيه من الطيور كانت الوفيات بها اقل من المجموعه الاولى وكانت نسبة الوفيات ٦٢,٤٨% والمجوعه الثانيه ٦٠% بالترتيب ومن الدراسه وجد ارتباط بين نسبة الوفيات والاجسام المناعية فى الطيور المصابة.