

Studies on a recent IBD virus field variant isolate: (2) Pathogenesis, pathogenicity and immunosuppressive potential of this virus in experimental infected chickens

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ABSTRACT

In the present study, the pathogenicity of the Del/E related infectious bursal disease virus (IBDV) is studied in broiler chicks at 4 and 14 day old chicks, the inoculated chicks showed depression, off-food and diarrhea with whole body pallor, higher mortality, poor feed conversion, low weight gain, stunted growth and passage of undigested food in their droppings. Five chickens from each group were sacrificed at the 3rd, 7th, 14th and 21st day post inoculation (PI) and the post mortum examinations revealed enlargement of the proventriculus as indicated by significant higher proventriculus/body weight ratio of the inoculated groups compared with the control chickens. The affected proventriculi appeared with grey-white mottling at their serosal surface with thickened wall. The bursa of Fabricius, spleen and thymus are significantly atrophied as early as the 3rd day PI and persisted along the experimentation period. The histopathological examination of the proventriculus illustrates granulocytes aggregation in submucosa with glandular epithelial hyperplasia. The histopathological examination of bursa of Fabricius, spleen and thymus illustrate sever lymphocyte depletions.

The Del/E related IBDV strain was reisolated from the inoculated chickens in SPF-ECE at the 3rd and 7th day PI from the lymphoid and non-lymphoid tissues while at the 14th day PI the reisolation from kidney and liver was failed, but proventricular, bursal, splenic and thymus sample still contained the virus. At the 21st day PI the virus was reisolated from the bursa of Fabricius only. The present study confirm that the Del/E related IBDV strain was found to have sever immunosuppressive effect in the inoculated chickens as indicated by the significant reduction in HI antibody titre against NDV vaccine as well as the significantly lower protection percentage against challenge with VVNDV in these chickens.

INTRODUCTION

Abnormality in proventriculus had been described for the first time by (1). From that time many infectious and non-infectious causes had been incriminated, while the identification of infectious bursal disease (IBD) virus as a causative agent was described by many authors (2-4). This syndrome was named transmissible viral proventriculitis (TVP) which was clinically and pathologically described by (2,5,6,7,8).

It is well known that variant IBDV causing sever atrophy in lymphoid organs, bursa of Fabricius (BF), spleen and thymus, that resulting in sever immunosuppression to the infected birds (9,10). The economical impact of the TVP was recorded by (11,12).

Our study was carried out to investigate the pathogenesis, pathogenicity and immunosuppressive potential of the Del/E related variant IBDV strain, that was isolated and identified previously, in 4 and 14 days old commercial broiler chickens.

MATERIAL AND METHODS

Experimental chicks

A total of 250, one-day-old broiler Ross breed chicks were obtained as hatched. The breeder flock source of used chicks was vaccinated against IBD using live attenuated vaccine at 8 and 14 days and inactivated vaccine at 7, 18 and 35 weeks of age.

Viruses

Variant IBD virus strain related to Del/E strain was used. This strain was isolated by (13) and further identified using RT-PCR-RFLP by (4).

Velogenic viscerotropic NDV : A lyophilized virus was kindly obtained from ND department of Serum and Vaccine Research Institute, Abbasia, Cairo.

Clone 30 live attenuated NDV vaccine : Intervet Company of titre $10^{6.5}$ EID₅₀ / dose as titrated in the Central Laboratory for Evaluation of Veterinary Biologics.

Embryonated chicken eggs

SPF fertile chicken eggs were obtained from Koum Osheim, Fayoum Province. Eggs were embryonated and used for passage and titration besides reisolation of the used virus strain from the affected tissues (14,15). Virus titre was estimated (16).

Experimental infection

Eye drop and crop inoculation were used for chicks infection at 4 or 14 days of age where each chick received 0.5 ml containing 10^3 EID₅₀ of IBDV strain under test (8, 12, 17).

Histopathological examination

Tissue samples (proventriculus, bursa, thymus, kidney, liver and spleen) were collected from sacrificed chicks and kept in buffer formol saline for histopathological examination (18).

Serum samples

Blood was collected from sacrificed birds for serum to determine IBD maternal antibody using ELISA kit (IDEXX Laboratories).

Haemagglutination Inhibition (HI) test

Beta procedure HI test was used to measure the serological response to NDV vaccine using 4 haemagglutinating units (19). HI titres were determined in all chickens, and the geometric mean titre (GMT) was calculated for each group.

Performance data

Both body weight, body weight gain, feed intake and feed conversion were weekly calculated in all groups.

Organ weight index

The weight of proventriculi, bursae, thymus and spleens of sacrificed birds were calculated (15, 20)

Statistical analysis

The obtained data were statistically compared using T-test (21)

Experimental design

Ten chicks out of the used (250 chicks) were sacrificed at 1 day of age to determine maternally derived immunity in their sera. The

rest of birds (240) were randomly divided into 4 equal groups (1-4); 60 chicks each. Chicken groups were kept on deep litter in isolated pens and feed commercial balanced ration adlibitum.

At the 4th and 14th day of age chicks of groups 1 and 3 were eye dropped and crop inoculated respectively each with 0.5 ml containing 10^3 EID₅₀ of IBD virus, while birds of groups 2 and 4 received sterile saline and kept as negative controls. Each group was then subdivided into 2 subgroups (a and b) each of 30 chicks. Five chicks from subgroup (a) of each group were randomly collected, weighed and sacrificed at 3, 7, 14 and 21 day post inoculation. The sacrificed birds were examined for macroscopic lesions and proventriculus, bursa, thymus, kidney, spleen and were weighed and taken for histopathological examination. Weekly body weight and feed intake were calculated.

At the 15th day of age, the chickens of subgroup (b) in all chicken groups were vaccinated ocularly against ND using clone 30 vaccine (Intervet Co.). Blood samples were collected just prevaccination and weekly for 2 weeks post vaccination. At the 2nd week post vaccination the vaccinated chickens in all groups were challenged intramuscularly using velogenic viscerotropic NDV at dose of 10^6 EID₅₀ per bird. Ten chickens from the subgroup (a) of the 4th group which were non-vaccinated and non-inoculated were challenged with velogenic viscerotropic NDV at dose of 10^6 EID₅₀ per bird. The challenged chickens were observed for 14 days post challenge and the mortalities due to ND were recorded.

RESULTS

A) Pathogenicity of the Del/E related IBDV variant strain in 4 day old broiler chickens

The signs of infection of chickens with the Del/E related IBDV showed general signs of depression, off-food and diarrhea, with whole body pallor, higher than average mortality, stunted growth, poor feed conversion and passage of undigested feed in their droppings, also poor flock production performance data, poor feathering, pale shanks and increased condemnations.

The lesions observed in proventriculi of sacrificed inoculated chickens on the 7th day post infection illustrate its enlargement (Fig. 1A) and Table 1. the outer surface of the affected proventriculus is discoloured with a grey-white mottled appearance Fig. 1A1, thickened wall to be 3 mm with white lobular pattern Fig. 1A2. The lumina of some proventricular lobules were distended with clear fluid that exuded in cut and the mucosa was covered with thick tenacious mucous exudates Fig. 1A2. On day 14 post inoculation, the proventriculi appeared enlarged, mottled with grey-white plaques and the proventricular isthmus was wide and flaccid, in cut the preventricular wall appeared thickened and the proventricular glands were flattened without distinct orifices Fig. 1A2.

On the 7th day PI, the BF, thymus and spleen of the inoculated chickens appeared atrophied as confirmed by the significant lower organ weight/body weight ratio, BF/BW ratio, spleen/BW ratio and thymus/BW ratio, compared with that of the non-inoculated chickens. The atrophy of these organs persisted till the end of the experimentation period Table 1.

Histopathological findings of affected proventriculi of the Del/E related IBDV inoculated chickens revealed granulocyte aggregations in submucosa with glandular epithelial hyperplasia on the 3rd day PI, degeneration of the glandular epithelium on the 7th day PI, with sever proventricular necrosis at the 21st day PI Fig. 2a,b. The histopathology of BF revealed interfollicular proliferation of granulocytes and inflammatory cells aggregations at the 3rd day PI, intrafollicular lymphocyte depletion at the 7th day PI, sever fibrosis of follicles with cystic formation at the 14th day PI, sever lymphocyte depletion at the 21st day PI Fig. 3a,b. The histopathology of the thymus of the inoculated chickens revealed hemorrhage with mild degeneration at 3rd and 7th day PI, while sever lymphocytic depletion in the cortex was detected at 21st day PI Fig. 3d.

The histopathology of the spleen of inoculated chickens showed sever hemorrhage

at the 14th day PI with depletion in the white pulps and focal circumscribed round aggregation of lymphoid cells Fig 3c.

The histopathology of the kidney of the Del/E related IBDV chickens showed hemorrhage with hyperaemic intertubular blood capillaries and degenerated lining epithelial cells of the renal tubules with inflammatory cells aggregations Fig. 3E could be detected from the 7th day PI and persisted along the experimentation period.

The histopathology of liver of the inoculated chickens showed congested blood vessels with coagulative necrosis at the 7th and 21st day PI with bile duct proliferation Fig. 3E.

Pathogenesis of the Del/E related IBDV strain in broiler chickens:

Del/E related IBDV was reisolated in ECE from the lymphoid (BF, spleen, thymus and cecal tonsils) and non lymphoid (proventriculus, liver and kidney) of the inoculated chickens, either at the 4th or 14th day old, at the 3rd and the 7th day PI as shown in table 2. At the 14th day PI the IBDV was reisolated from lymphoid organs and proventriculus but not from liver nor kidney. At the 21st day PI the IBDV was reisolated from the BF only. These results were confirmed by the presence of histopathological changes in lymphoid and non-lymphoid organs of the inoculated chickens as shown in Figures 2 a , 2b 2c, 3 a, 3b, 3c, 3d, 3e and 3f.

Results of immunosuppressive potential of the Del/E related IBDV

The Del/E related IBDV strain induced sever immunosuppression in the inoculated chicken groups, as indicated by the significant reduction in the serologic response to NDV vaccines (HI antibody titre) in the inoculated chicken groups compared to that of the positive control chicken groups. The same results were obtained after the IBDV inoculated chicken groups were challenged by velogenic viscerotropic NDV, the protection percentage were 70% and 75% in group 1 and 3 respectively compared to 100% in positive control group as shown in Table 3.

Table 1. Pathogenicity of Del/E related IBDV strain in broiler chick groups that inoculated at the 4th or 14th day old as indicated by the organ/B.W. ratios and/or histological lesions scores of their proventriculus, BF, thymus and spleen

Groups	Age of inoculation	Inoculation	Days PI	Proventriculus		BF			Thymus/BW ratio	Spleen/BW ratio
				Prov/BW ratio	Histo. LS	BF/BW ratio	Histo. LS	Bursal index		
1	4 days	IBDV	3	1.25	2	0.2	3	0.57	0.465	0.120
			7	1.48	3	0.141	4	0.397	0.235	0.122
			14	1.14	4	0.110	4	0.29	0.130	0.130
			21	1.00	5	0.100	4	0.26	0.090	0.150
2	4 days	Saline control	3	1.26	1	0.350*	0	-	0.470	0.150
			7	1.00	1	0.355*	0	-	0.500*	0.175
			14	0.80	1.6	0.375*	0	-	0.570*	0.210*
			21	0.73	1	0.380*	0	-	0.650*	0.240*
3	14 days	IBDV	3	0.921	2.5	0.218	3	0.589	0.550	0.159
			7	0.925	3	0.155	4	0.401	0.300	0.155
			14	1.110	4	0.100	4	0.22	0.250	0.140
			21	1.130	3.5	0.08	4	0.177	0.190	0.105
4	14 days	Saline control	3	0.905	1	0.371*	0	-	0.600	0.200
			7	0.795	1	0.386*	0	-	0.605*	0.215*
			14	0.680	1	0.442*	0	-	0.612*	0.241*
			21	0.610	1	0.451*	0	-	0.595*	0.251*

PI: Post Inoculation.

Histo. LS: Histopathological Lesion Score.

* Significant at $P < 0.05$

Table 2. Pathogenesis of the Del/E related IBDV strain in commercial broiler chicken via re-isolation attempts from different organs of the inoculated chickens at various ages post inoculation (PI)

Chicken group	Age of Inoculation days	Age PI	Organs						
			BF	Provent.	spleen	Thymus	C.T.	Liver	Kidney
I	4	3	+ve	+ve	+ve	+ve	+ve	+ve	+ve
		7	+ve	+ve	+ve	+ve	+ve	+ve	+ve
		14	+ve	+ve	+ve	+ve	+ve	-ve	-ve
		21	+ve	-ve	-ve	-ve	-ve	-ve	-ve
II	4	3	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		7	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		14	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		21	-ve	-ve	-ve	-ve	-ve	-ve	-ve
III	14	3	+ve	+ve	+ve	+ve	+ve	+ve	+ve
		7	+ve	+ve	+ve	+ve	+ve	+ve	+ve
		14	+ve	+ve	+ve	+ve	+ve	-ve	-ve
		21	+ve	-ve	-ve	-ve	-ve	-ve	-ve
IV	14	3	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		7	v	-ve	-ve	-ve	-ve	-ve	-ve
		14	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		21	-ve	-ve	-ve	-ve	-ve	-ve	-ve

C.T. : ceecal tonsils

Table 3. The immunosuppressive potential of the Del/E related IBDV strain in broiler chicken via estimation of HI titer and the protection percentage against NDV vaccine

Chicken group	Age of inoculation	NDV vaccine	Mean log 2 HI titer			Protection percent
			Prevac.	7 dpv	14dpv	
1	4 th	Clone 30	3.2±0.2	5.1±0.11	6.5±0.15	70%
2	Saline control	Clone 30	3.4±0.11	6.5±0.15	8.2±0.16	100 %
3	14 th day	Clone 30	3.3±0.2	5.5±0.67	*6.9±1.0	75 %
4 (A)	Saline control	Clone 30	3.3±0.2	*6.6±0.3	8.3±0.2	100 %
4 (C)	Saline control	Non vaccinated control	3.3±0.1	1±0.5	0	0 %

• Significant at < 0.05

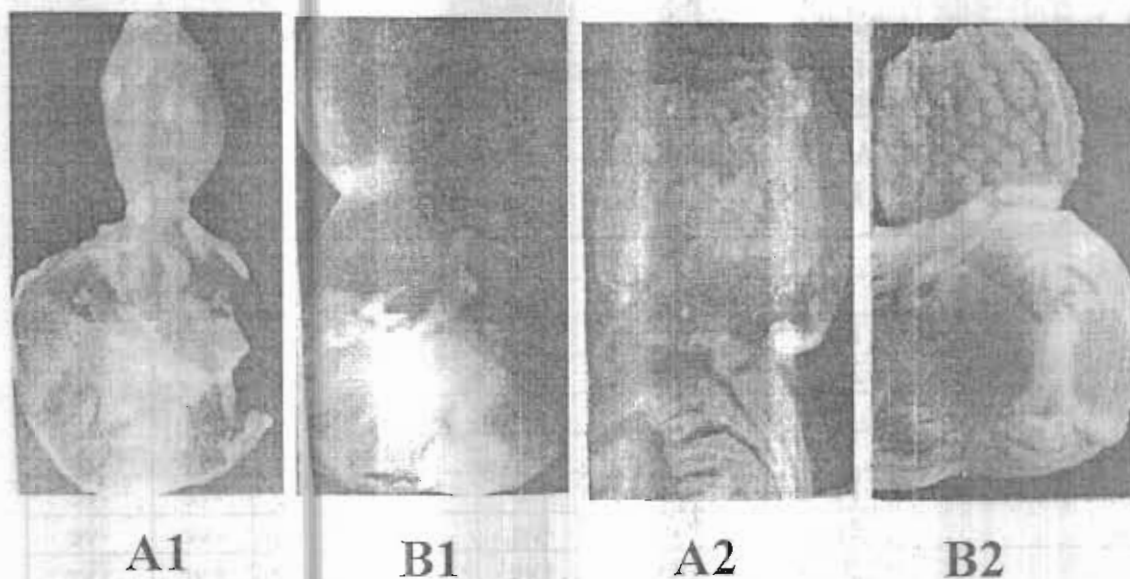


Fig. 1.

A1: Proventriculus of Del/E related IBDV inoculated chicken at the 14th day PI showing marked enlargement with grey-white mottling at the serosal surface.

A2: Cut-section of A1 proventriculus showing thickened wall covered with thick tenacious mucous exudates, the proventricular glands were flattened with non-distinct papillary orifices.

B1, B2: Proventriculi of saline controls showing normal thickness and size.

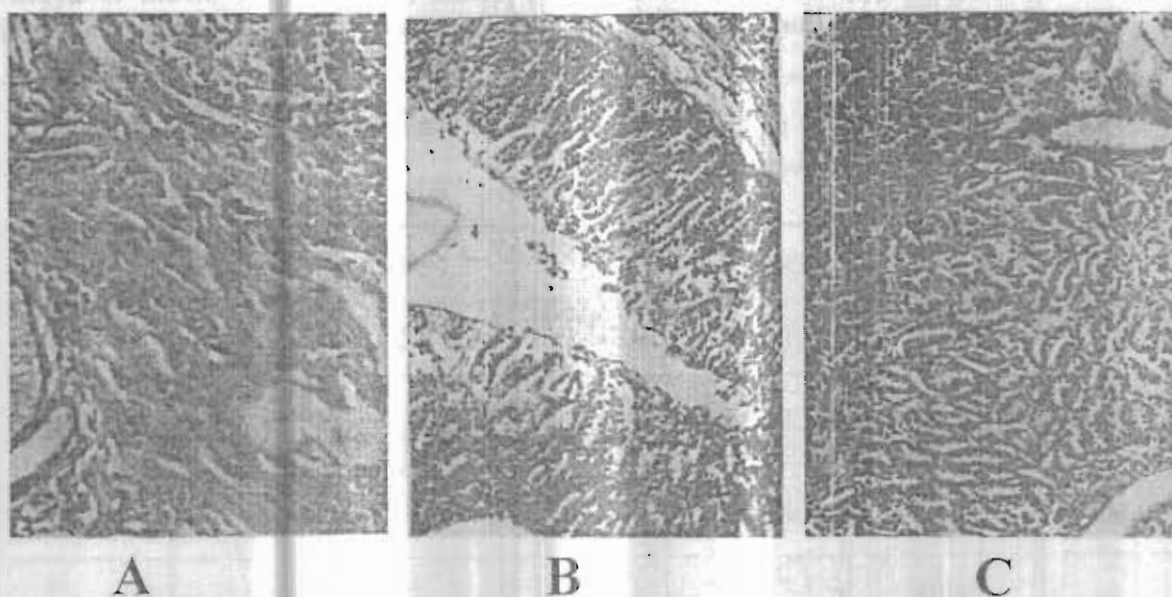


Fig. 2. Proventriculi of chickens inoculated with Del/E related IBDV strain showing:

A. Mild granulocytes aggregation in submucosa at 3rd DPI (H and E, x 160)

B. Moderate degeneration of the glandular epithelium at 7th DPI (H and E, x100)

C. Severe degeneration of the glandular layer at 14th DPI (H and E, x100)

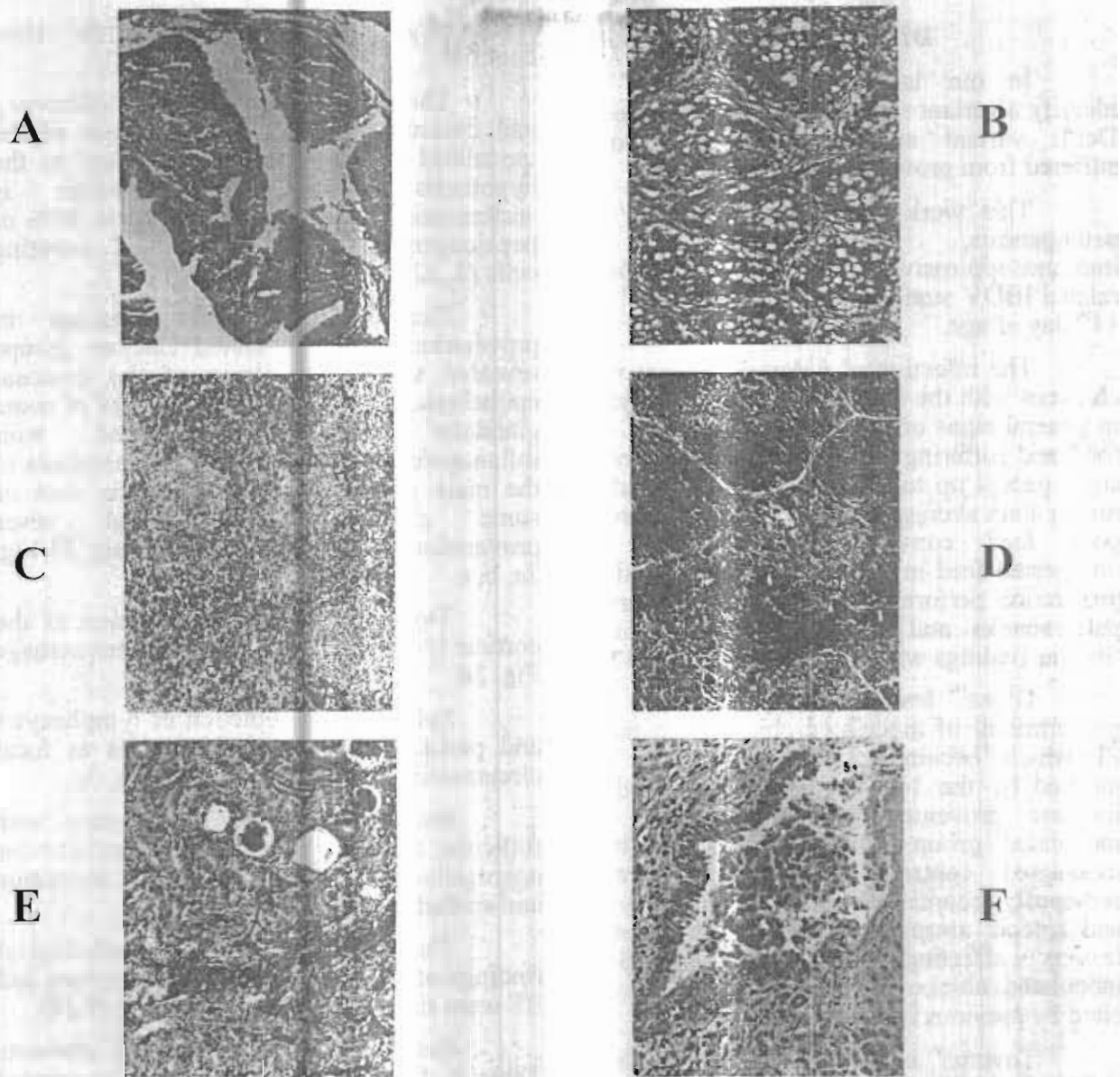


Fig. 3.

- 3A: BF of Del/E related IBDV inoculated chicks at 3rd day PI showing depletion and eosinophilic aggregation in the follicular cortical portion (H and E, x160)
- 3B: BF at 14th day PI showing severe depletion with cystic formation with interfollicular fibrosis (H and E, x160)
- 3C: Spleen at 7th day PI showing hyperaemic red pulps with depletion of lymphoid cells in white pulps besides appearance of circumscribed round focal aggregated lymphoid cells (H and E, x160)
- 3D: Thymus at 7th day PI showing severe hyperaemia in the medullary portion with lymphoid depletion in the cortex (H and E, x100)
- 3E: Kidney at 14th day PI showing hyperaemic intertubular blood capillaries with focal haemorrhages and inflammatory cell aggregation (H and E, x160)
- 3F: Liver at 21st day PI showing pronounced bile duct proliferation with coagulative necrosis (H and E, x 250)

DISCUSSION

In our last study, we isolate and identify a variant strain of IBDV that related to Del/E variant strain from broiler flocks suffered from proventriculitis (4).

This work was planned to study the pathogenesis, pathogenicity and immunosuppressive potential of the Del/E related IBDV strain in broiler chicks at 4th and 14th day of age.

The infection of 4-day-old commercial chickens with the Del/E related IBDV resulted in general signs of illness and depression, off-food and suffering from diarrhea. This general signs persist up to 4 days, whole body pallor, higher than average mortality, stunted growth, poor feed conversion and passage of undigested feed in their feces, also poor flock production performance data, poor feathering, pale shanks and increased condemnations. Similar findings were previously recorded (8).

Gross lesions were observed in proventriculi of inoculated chickens on day 7 PI which become markedly enlarged and mottled by the 14th day PI, as indicated by increase proventriculus/B.W. ratio of the inoculated group compared with the non-inoculated control. Similar results were previously recorded (2,7,8, 22). Bursal, thymic and splenic atrophy appears to be important lesions affecting Del/E related IBDV inoculated chickens. Similar findings were cited by previous investigations (5, 23).

Thymus atrophy in the inoculated chickens begin from the 7th day PI and persisted through the duration of the experiment as indicated by the significant lower thymus/BW ratio of the inoculated chickens compared with the non-inoculated control. Spleen atrophy of the inoculated chickens begin from the 3rd day PI as indicated by the significant lower spleen/BW ratio of the inoculated chickens compared with the non-inoculated control. Also, bursal atrophy begin earlier and persisted along the duration of the experiment as indicated by bursa/BW ratio and bursal index.

The cumulative feed conversion ratio of the Del/E related IBDV inoculated at 4th or 14th old chickens were higher, during the 3

weeks of observation, than that of the saline controls.

The poorer feed conversion efficiency and decreased the body weight gain of the inoculated chicken may be attributed to the hypothesis that proventriculitis is accompanied by destruction of up to 80% of pepsinogen and hydrochloric acid secreting cells (2, 22).

The histopathological findings in proventriculus of inoculated chicken groups revealed sever hyperplasia of the mucosal epithelium, with hyperplastic changes of some glandular alveoli accompanied with inflammatory cells aggregations hyperplasia of the main ductules and the common duct of some glandular alveoli and sever proventricular necrosis at the 21st day PI Fig. 2a, b, c.

Thymus; pronounced depletion of the cortical lymphoid tissue, and mild hemorrhage Fig. 3d.

Spleen; great depletion of lymphocytes and proliferation of lymphoid cells as focal circumscribed round aggregation Fig. 3c.

Bursa; Lymphocytic depletion with follicular atrophy and necrosis, interfollicular aggregation of granulocytes, cystic formation and epithelization Fig. 3a, b.

The results of histopathological findings of proventriculus, thymus, spleen and BF were similar to those obtained by (9 10).

Results of virus reisolation attempts Table 2. from organs of chickens inoculated with Del/E related IBDV at either 4 or 14 days of age indicate that the virus persisted in these tissues for at least 7 days PI. IBDV was consistently isolated from BF, proventriculus, spleen, thymus, liver, kidney and cecal tonsils at 3rd, 7th and 14th day PI, and persisted in the BF of all groups at the 21st day PI.

Our results are agreed with that obtained previously (24, 25)

Results of immunosuppressive potential Table 3. of Del/E related IBDV in inoculated chicken groups at 4th or 14th day PI relieved sever immunosuppression as indicated by significant reduction of HI antibody titres against NDV vaccine as well as significant reduction in protection percentage against

velogenic viscerotropic NDV in the IBDV inoculated chicken group compared with positive control group. These results could be explained due to the sever bursal atrophy as well as splenic and thymic atrophy that induced by the Del/E related IBDV in the inoculated chicken groups. Similar results were cited (9, 10).

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الملخص العربي

دراسات عن عترة حقلية حديثة متحورة من فيروس الجمبورو
(٢) دراسة القدرة الإمرضية ومدى التثبيط المناعي
الذي تحدثه هذه العترة المتحورة في الدجاج المعدي تجريبيا

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*المعمل المركزي للرقابة على المستحضرات الحيوية البيطرية ** كلية الطب البيطري - جامعة القاهرة

تم دراسة القدرة الإمرضية للعترة المتحورة من فيروس الجمبورو في مجموعتين من الكتاكيت الأولى عمر أربعة أيام والثانية أربعة عشر يوماً، وأظهرت الدراسة أن الكتاكيت في كلا المجموعتين أصيبت بخمول وفقدان الشهية، إسهال، شحوب في الجسم، زيادة في نسبة النفوق، ضعف معدل التحويل الغذائي، انخفاض الأوزان، تقزّجات ووجود عليقة غير مهضومة في زرق الطيور المصابة. ولقد بينت الصفة التشريحية التي أجريت على خمسة طيور عند اليوم الثالث والسابع والرابع عشر والواحد وعشرون بعد العدوى وجود تضخم معنوي في المعدة الغدية نسبة إلى وزن جسم الطائر الكلي في المجموعة المصابة مقارنة بمجموعة الضبط السالبة مع وجود بقع بيضاء رمادية اللون على السطح الخارجي للمعدة الغدية وأيضاً وجود زيادة في سمك جدار المعدة. ولقد أظهرت الصفة التشريحية أيضاً وجود ضمور معنوي في غدة كيس فابريشيس والطحال وغدة الثيماس. وبالنسبة للفحوصات الهستوباثولوجية فلقد بينت وجود تجمعات خلوية من خلايا الجراثيم في الطبقة تحت الطلائع للمعدة الغدية مع وجود تضخم وزيادة في الخلايا الغدية، وبالنسبة لكيس فابريشيس والطحال وغدة الثايمس فلقد أكدت هذه الفحوصات الهستوباثولوجية وجود ضمور واضمحلال شديد بها. من ناحية أخرى تم إعادة عزل فيروس الجمبورو المتحور من دجاج التجربة المعدي بالفيروس في اليومين الثالث والسابع بعد الإصابة من كل أعضاء جسم الطيور سواء الليمفاوية والغير ليمفاوية مثل كيس فابريشيس، الطحال، الغدة الثيموثية المعدة الغدية والكبد والكلى وتكرر إعادة العزل في اليوم الرابع عشر بعد العدوى من كل الأعضاء السابقة عدا الكبد والكلى وفي اليوم الواحد والعشرون لم تتجح إعادة العزل إلا من كيس فابريشيس وبالنسبة لأثر هذه العترة المحورة من فيروس الجمبورو مناعياً فلقد أثبتت الدراسة أن لها تأثير مثبط بشكل كبير على الجهاز المناعي للطيور وذلك باستخدام اختبار مانع التجلط حيث تبين نقص الأجسام المناعية المضادة للنيوكاسل وإيضاً نقص الحماية ضد اختبار التحدي باستخدام العترة الضارية من النيوكاسل في مجموعتي الدجاج المعدي بعترة الجمبورو مقارنة بمجموعة الضبط السالبة.