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## **STUDIES ON A RECENT IBD VIRUS FIELD VARIANT ISOLATE: (3) IMMUNOGENICITY OF THIS VARIANT STRAIN COMPARED WITH A CLASSICAL STRAIN IN BROILER CHICKENS**

(With 2 Tables and 3 Figures)

By

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دراسات عن عترة حقلية حديثة متحورة من فيروس الجبورو (٣)  
دراسة مناعية هذه العترة المتحورة مقارنة بعترة كلاسيكية  
من فيروس الجبورو في دجاج التسمين

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تم تحضير ثلاثة لقاحات زيتية مثبتة ضد مرض الجبورو وذلك باستخدام عترتين من فيروس الجبورو إحداهما كلاسيكية والثانية المحورة من الفيروس حيث تم تمرير كل عترة على حدة في أجنة بيض خال من المسببات المرضية لعدد ستة مرات متتالية. وقد تم معايرة هذه الفيروسات بعد تجميع كل عترة على حدة - في أجنة بيض خال من المسببات المرضية عند عمر عشرة أيام، ثم ثبتت هذه الفيروسات باستخدام فورمالين عند تركيز ٢%. وللقاحات الثلاث المحضرة، تمثل عترتي الجبورو المحورة والكلاسيكية حيث أن اللقاح الأول يحوى العترة الجبورو المحورة، واللقاح الثانى يحوى عترة الكلاسيكية شديدة الضراوة أما اللقاح الثالث فيحوى العترتين معاً. ولتقييم اللقاحات الثلاث فقد تم عمل دراسة مقارنة بينها وبين لقاح تجارى مثبت ضد مرض الجبورو وقد تبين أن اللقاحات الأربع نقية من الملوثات البكتيرية، كما أنها آمنة للاستعمال فى الدجاج. وقد تم دراسة فاعلية اللقاحات محل الدراسة فى دجاج التسمين عمر ٢ أسبوع حيث تم حقن مجموعات من دجاج التسمين عند عمر ٢ أسبوع بهذه اللقاحات، ثم عمل اختبار التحدى لهذه المجموعات باستخدام العترة المحورة والعترة الكلاسيكية من فيروس الجبورو، وقد تم قياس استجابة الدجاج المناعية لهذه التحصينات باستخدام اختبار الإليزا حيث وجد أنه يوجد استجابة مناعية معنوية لهذه اللقاحات محل الدراسة فى الدجاج المحصن مقارنة بالدجاج الغير محصن دون وجود فروق معنوية

فيما بين المجموعات المحصنة بأى من هذه اللقاحات. وبالنسبة لاختبار التحدى فقد أكدت الدراسة أن اللقاح المحضر من العترة المحورة لفيروس الجنبورو يحمى الدجاج المحصن به ضد فيروسات الجنبورو المحورة والكلاسيكية وقد تم الحصول على نفس النتيجة باستخدام اللقاح المحتوى على عترتى الجنبورو المحورة والكلاسيكية معاً. أما بالنسبة للقاح الجنبورو المحضر من العترة الكلاسيكية لفيروس الجنبورو فقد وجد أنه يحمى الدجاج المحصن به ضد فيروسات الجنبورو الكلاسيكية فقط وليس ضد العترات المحورة من الفيروس نفسه.

## SUMMARY

Three inactivated water-in-oil-in-water-emulsion infectious bursal disease virus (IBDV) vaccines were prepared from the Del/E related IBDV variant strain and a classical vvIBDV strain. Each IBDV strain were propagated for 6 serial passages in specific pathogen free (SPF) embryonated chicken eggs (ECE). The harvested IBD viruses were titrated in 10 day old ECE and inactivated using formalin at a final concentration of 0.2 % of the total volume. The first vaccine contained Del/E related IBDV variant strain, the second vaccine contained vvIBDV strain and the third vaccine contained both variant and vvIBDV strain. The 3 prepared IBDV vaccines are evaluated compared to a commercial inactivated IBDV vaccine, all the 4 vaccines proved to be sterile and safe. The efficacy of each vaccine was assisted by inoculation into a group of 14 day old broiler chicks via subcutaneous rout and challenged (2 weeks later) with Del/E related IBDV variant strain and the classical vvIBDV strain. The humeral immune response were determined using indirect ELISA, the geometric mean (GM) ELISA titer of the vaccinated chicken groups are significantly higher than the non vaccinated chickens, with no significant difference between the vaccinated chicken groups despite the variation in the IBDV strains. The protection was evaluated at 5 and 10 days post challenge, based on gross and microscopic lesions, bursal indices, proventricular/body weight ratio and bursl lesions scores. Vaccines made of variant IBDV alone or combined with vvIBDV protected chickens challenged with either the classical vvIBDV or the Del/E related IBDV variant strain while vaccines made of the classical IBDV strain alone protected chickens challenged with the classical vvIBDV strain but not against the Del/E related IBDV variant strain.

*Key words: Vaccination, IBD virus, broiler chickens.*

## INTRODUCTION

Infections bursal disease virus (IBDV), a member of the birnavirus group is the etiological agent of a highly contagious immunosuppressive disease of young chickens (Saif., 1991). The bursa of fabricious (BF) is the target organ of infection by IBDV, (Lukert and Saif., 1991). Variant IBDV strains have been implicated as a cause of the transmissible viral proventiculitis (TVP) in broiler chickens (Bayyari *et al.*, 1995; Huff *et al.*, 2001; Newberry, 1996 and Amer and Nassif, 2005). In Egypt Del/E IBDV variant strains; as detected by an antigen capture ELISA Kit (Hussien *et al.*, 2003) and RT/PCR- RFLP assay (Amer and Nassif 2005); was suspected to be the cause of TVP.

The economical impact of the TVP could be summarized in higher than average mortality, stunted growth, poor feed conversion rate and passage of the undigested feed in the faeces of the affected chickens (McNulty, 1991 and Goodwin, 1993).

Because vaccination is currently the most effective control measure of IBDV, there is continuous need to develop efficacious vaccines for commercial poultry production.

The objective of the present study is to prepare a combined and single inactivated vaccines from the variant Del/E strains and a classical vvIBDV strain previously isolated by Nassif 2001. And evaluate it in comparison with a commercial inactivated IBDV vaccine.

## MATERIALS and METHODS

### **Embryonated chicken eggs (ECE)**

Specific pathogen free (SPF) ECE were obtained from Nile SPF eggs, Kom, Oshiem, Fayoum, Egypt, and used for the propagation and titration of IBDVs.

### **Broiler chicks:**

Two hundred -one day old broiler chicks are obtained from El-wady company, reared in a good ventilation and clean pens, water and feed are supplied adlibidum.

### **IBDVs:**

Two strains of IBDV, a classical vvIBDV and a variant Del /E IBDV, were used. The vvIBDV was previously isolated and identified by Nassif 2001. The Del/E variant IBDV was detected and identified by Amer and Nassif 2005.

**Adaptation of IBDVs to ECE:** (Hassan and Saif., 1996)

Each IBDV strain (0.1 ml) was inoculated into a group of ECE (11 days old) via the chorioallantoic route. Eggs were incubated, observed daily and those with dead embryos during the first 24 hours post inoculation (PI) were discarded. On the third day, eggs containing dead embryos and those still surviving were chilled to 4°C.

Embryos, and chorioallantoic membranes (CAMs) were harvested and examined for the presence of IBDV characteristic lesions. The CAMs and the embryonic viscera were washed, homogenized and suspended in phosphate buffered saline (PBS, pH 7.2) the suspension was frozen and thawed in 3 cycles then centrifuged and the supernate was filtered and inoculated into another batch of SPF embryos and six serial passages were completed in a similar manner.

**Titration of IBDVs in SPF embryos:**

The egg adapted vvIBDV and Del/E variant IBDV were titrated in 10-day-old SPF embryonated eggs. The virus titer was determined as the EID<sub>50</sub> (Lukert and Saif., 1991).

**Vaccine preparation:**

**a-Virus inactivation:** (Wyeth *et al.*, 1981)

Each IBDV strain was inactivated using formalin in a final concentration of 0.2% of the total volume (virus – formalin mixture). The absence of residual infectivity of the inactivated virus was confirmed by inoculation into 10 day old ECE (0.2 ml/egg) via the allantoic sac. Three serial passages were completed in a similar manner, before the batch of the prepared vaccines were considered safe.

**b-Preparation of water in-oil-in-water emulsion (w-o-w-e) IBDV vaccines:** (Abdel-Wanees *et al.* 2004)

The double emulsion form of multiple emulsion (w-o-w-e) was prepared from a simple water in oil emulsion by re-dispersing it in an outer saline phase with aqueous phase emulsifier. Paraffin oil (white 300) white oil quality FDAIAL usp no 05200 Mobil, Aqueous phase emulsifier (tween 80) supplied by sigma of (HLB=15) and oil phase emulsifier arlacil (Span 80, supplied by Micbil, Aleyandrid (HLB =4.3) were used. The hydrophile – lipophile – Balance (HLB) of the surfactant mixture of the prepared batches were 9.36 determined as described by Schick (1966).

The prepared w-o-w-e IBDV vaccines have the following physical features:- The aqueous to oil ratio of 2:1, aqueous phase emulsifier concentration is 3% and the oil phase emulsifier concentration

is 10% of the oil phase and of viscosity of 1 second at 20°C for 0.4 ml flow times.

### **Evaluation of the prepared IBDV vaccines:-**

#### **1-Sterility test:**

The prepared vaccines were inoculated into nutrient agar, thioglycolate broth, PPLO media and sabaroud's dextrose agar media to assure their freedom from any bacterial, mycoplasma or fungal contaminations

#### **2- Safety test:**

A group of 3 week old chicks were used for each vaccine. Each chick was inoculated with double field dose via I/M route and the chicks, were observed for 2 weeks for any signs of local reactions, irritations or systemic reactions.

#### **3- Vaccines efficacy:**

Three IBDV vaccines were prepared:

Vaccine No.(1): contained the variant Del/E IBDV strain

Vaccine No.(2): contained the vvIBDV strain

Vaccine No.(3): contained both vvIBDV and variant Del/E strains

#### **Experimental designs:**

Efficacy of the 3 Prepared inactivated IBDV vaccines were examined compared with that of commercial inactivated IBDV vaccines (Newcevac, Intervet company) as follow:

One hundred and fifty -14 day - old chicks were used -, the chicks were divided into 5 groups, each group contained 30 chicks which represent 3 subgroups (a, b and c) each of 10 chicks. The experimental groups were vaccinated subcutaneously at the mid-dorsal region of the neck with 0.5 ml of the vaccine as follow:

Group 1: received vaccine No.(1) (at least  $10^{5.5}$  EID<sub>50</sub> per dose)

Group 2: received vaccine No.(2) (at least  $10^{5.5}$  EID<sub>50</sub> per dose)

Group 3: received vaccine No.(3) (at least  $10^5$  EID<sub>50</sub> of each strain per dose)

Group 4: received the commercial IBDV vaccine (at least  $10^6$  EID<sub>50</sub> per dose)

Group 5: none vaccinated control.

Blood samples were collected just prevaccination against IBD, at 7<sup>th</sup> day post vaccination (PV), 14<sup>th</sup> day PV, 21<sup>st</sup> day PV, 28<sup>th</sup> day PV and every 4 weeks for 2 months for detection of the active immune response against IBD using indirect ELISA.

At the 14<sup>th</sup> day PV the birds in subgroups "a" were challenged with  $10^4$  EID<sub>50</sub> of the vvIBDV per bird, while birds in subgroups "b"

were challenged with  $10^4$  EID<sub>50</sub> of the Del/E variant strain of IBDV via ocular and oral routes.

The challenged chickens were observed daily for any clinical signs and/or deaths.

At the 5<sup>th</sup> and 10<sup>th</sup> day post challenge (PC) five birds from subgroups "a" and "b" were euthanized then weighed, and necropsied. The BF and proventriculus of each bird also weighed and taken for histopathological examination

The protection percentage = 100- % of birds with bursal atrophy and bursal lesion score > 3 (Nassif, 2001)

#### **Histopathology:**

Methods of preparations, examination and interpretation of the results were carried out according to Tainmura *et al* (1995)

#### **Enzyme Linked Immuno Sorbent Assay:**

ELISA kits supplied from Idexx laboratories Inc. were used and the methodology was conducted according to manufactural instructions

#### **Organ/Body weight (BW) ratio:-** Tainmura *et al* (1995)

The BF and proventriculus of dead or euthanized birds are collected and weighed and the organ/BW ratio was determined as follow:

$$\frac{\text{Organ weight (gm)} \times 1000}{\text{Total body weight (gm)}}$$

#### **Bursal weight index:** (Lucio and Hitchner, 1979)

$$\text{Bursal weight index} = \frac{\text{BF/BW ratio of the challenged group}}{\text{BF/BW ratio of the non challenged group}}$$

Chickens with bursal index lower than 0.7 were considered to have bursal atrophy.

## **RESULTS**

#### **Adaptation of IBDVs to egg embryos:**

Embryos inoculated with vvIBDV and Del/E variant IBDV strains showed mortalities after 3 days in the first 3 passages only. Consequently the embryos were chilled after 72 hour PI in the subsequent passages. The CAMs of the dead embryos showed congestion and edema. The embryos showed subcutaneous

haemorrhage, dwarfing, heart palness, splenomegally and necrosis of liver and spleen.

**Titres of the IBDVs:**

The titre of vvIBDV and the Del/E related IBDV variant strain was  $10^6$  EID<sub>50</sub>/ml before inactivation.

**Evaluation of the prepared IBDV vaccines:**

**Sterility test:**

The results of sterility test revealed that the prepared vaccines are free from aerobic and anaerobic bacteria, mycoplasma and fungal contaminants.

**Safety test:**

No deaths, nor abscesses or any local reactions were observed in the inoculated chickens along the experimentation period that is means that the examined vaccines are safe.

**Potency test:**

The results of the experiment using the Del/E related IBDV inactivated vaccine vac. No. (1), vvIBDV inactivated vaccine vac. No. (2). And the combined in activated vaccines vac No. (3) are summerized in Tables 1, 2 and Figures 1, 2 and 3. The humoral immune response of the experimental chickens to vaccine No. 1 as measured by indirect ELISA (Table 1 and Fig.1) showed a significant ( $P < 0.05$ ) increases in GM ELISA titres in all vaccinated groups compared to the non vaccinated control group, from the 2<sup>nd</sup> week PV and continued to reach the peak at the 4<sup>th</sup> week PV then declined very slowly from the 5<sup>th</sup> week till the end of the experiment. Data in Table (1) showed that there is no significant difference in GM ELISA antibody titre between the vaccinated groups all over the experimentation period.

The results of challenge are summerized in Table (2), Figures 2 and 3 that showed that the chicken group that received vaccine No. 1 have 100% protection when challenged with either Del/E related IBDV strain or vvIBDV strain as indicated by the results of bursal indicis (no bursal atrophy) and proventricular /BW (Not higher than that of non challenged control) and histopathological score lesions of bursae and the histopathology of proventriculus. The same results are obtained for the chicken group received the combined IBDV vaccine vac. No. (3). On the other hand the chicken group, received vaccine No. (2) and that received vac. No. (4) showed 100% protection against challenge with the vvIBDV, while 70% and 60% protection were obtained respectively against the Del/E related IBDV starin, as indicated by bursal atrophy

bursal lesion score of 4 and significantly higher proventricular/BW ratio compared to the non challenged control.

In the control group the protection percentages were 30% for both vvIBDV strain and Del/E related IBDV variant strain, as indicated by bursal and proventricular indices and their histopathological findings at 5<sup>th</sup> & 10<sup>th</sup> days PC. Fig. 2 showed the histopathological finding of the bursa of non vaccinated and challenged chickens with Del/E related IBDV variant strain which represented as sever lymphocytic depletion and inter-follicular oedema, the histopathological score lesion is 4. Fig (3) showed the histopathological finding of the proventriculus in non vaccinated and challenged chickens with Del/E related IBDV variant strain which represented as sever epithelial hyperplasia of the mucosa with inflammatory cells infiltration with cellular damage in the glandular lumen and hyperplasia of the glandular alveoli.

**Table 1:** Humoral immune response of the experimental broiler chickens to the 3 prepared inactivated IBDV vaccines in comparison with a commercial inactivated IBDV vaccine as measured by indirect ELISA.

Vaccine	GM ELISA titre						
	Pre vaccination	Post vaccination					
		2 <sup>nd</sup> W	3 <sup>rd</sup> W	4 <sup>th</sup> W	8 <sup>th</sup>	12 <sup>th</sup>	16 <sup>th</sup>
Vac No. 1	1800	1530	2250	4100	3100	2500	2000
Vac No. 2	1800	1580	2800	4450	3350	2700	2100
Vac No. 3	1800	1600	2750	4150	3200	2500	2050
Vac No. 4	1800	1650	2810	4210	3300	2800	2000
Non vac. Control	1800	912	840*	650*	515*	540*	460*

GM: Geometric mean

\* Significant at P < 0.05

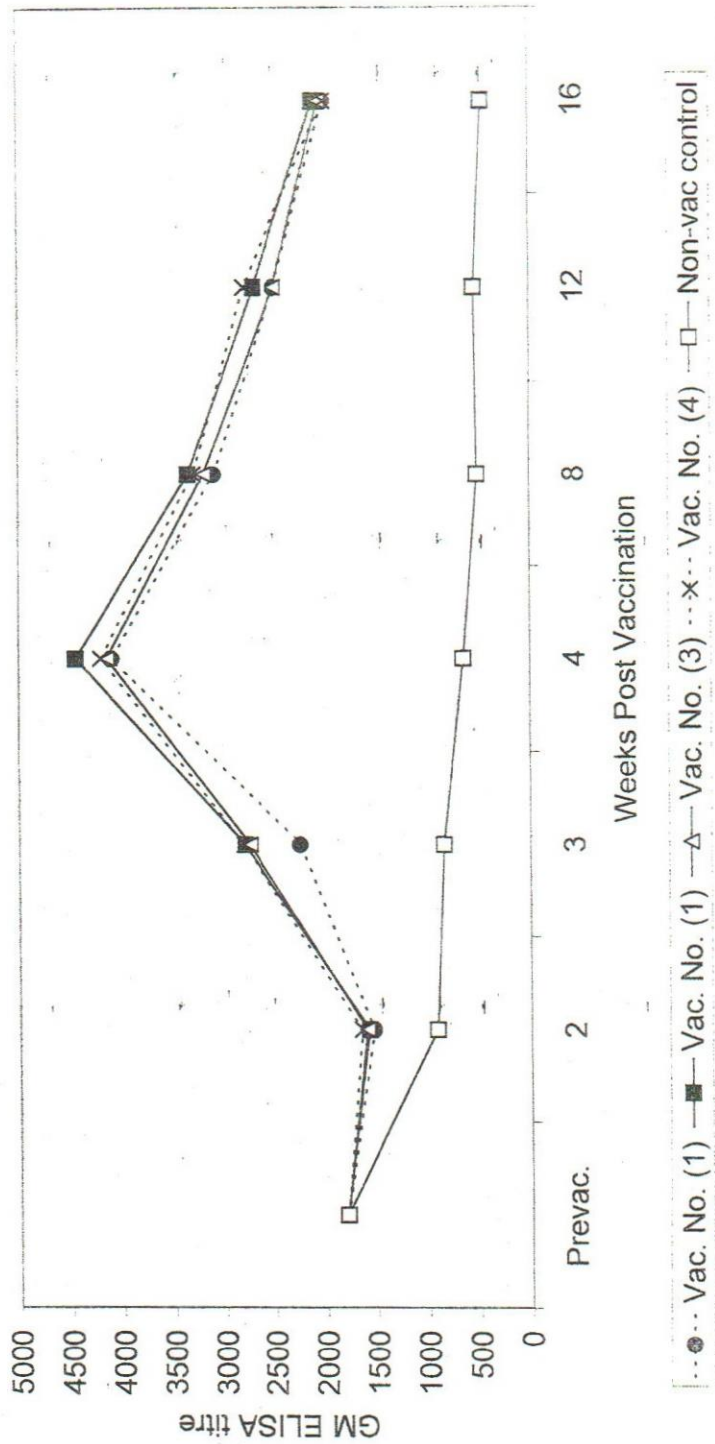


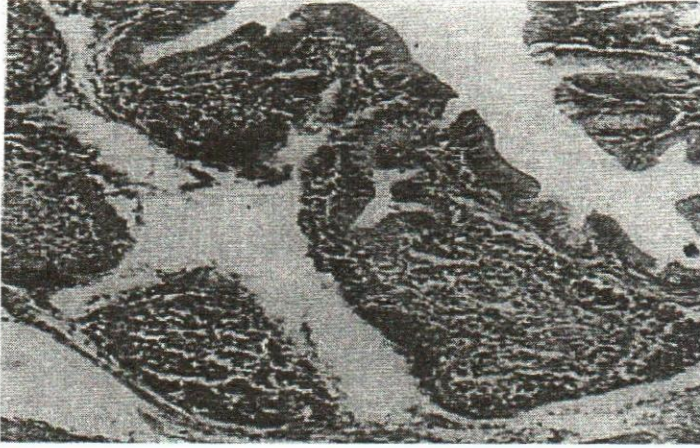
**Table 2:** Evaluation of the protection percentage of the 3 prepared IBDV vaccines in comparison with a commercial IBDV vaccine.

Vaccine	Challenge virus	Bursal index at days post challenge		Bursal lesion scores at days post challenge		Proventricular / BW ratio at days post challenge		No. of birds with bursal lesion score $\geq 3$	Protection %
		5	10	5	10	5	10		
Vac. No.1	Variant IBDV	1.00	0.964	1.0	1.5	0.820 $\pm$ 0.003	0.781 $\pm$ 0.02	0/10	100%
	VvIBDV	0.992	0.947	1.0	1.2	0.801 $\pm$ 0.009	0.790 $\pm$ 0.005	0/10	100%
Vac. No.2	Variant IBDV	0.615	0.437	2.8	3	1.5 $\pm$ 0.11	1.71 $\pm$ 0.20	3/10	70%
	VvIBDV	0.997	0.988	1.0	1.2	0.800 $\pm$ 0.05	0.740 $\pm$ 0.08	0/10	100%
Vac. No.3	Variant IBDV	0.999	0.979	1.0	1.2	0.850 $\pm$ 0.003	0.785 $\pm$ 0.009	0/10	100%
	vvIBDV	1.00	0.965	1.0	1.5	0.845 $\pm$ 0.008	0.800 $\pm$ 0.008	0/10	100%
Vac. No.4	Variant IBDV	0.611	0.420	3	3	1.9 $\pm$ 0.3	1.95 $\pm$ 0.4	4/10	60%
	vvIBDV	0.915	0.899	1	1.2	0.811 $\pm$ 0.003	0.800 $\pm$ 0.009	0/10	100%
Non vac. A,b	Variant IBDV	0.451	0.308	4	4	2.0 $\pm$ 0.325	2.11 $\pm$ 0.420	7/10	30%
	vvIBDV	0.514	0.361	4	4	0.800 $\pm$ 0.005	0.750 $\pm$ 0.009	7/10	30%
Non vac. c		ND	ND	0	0	0.850 $\pm$ 0.09	0.860 $\pm$ 0.07	-	-

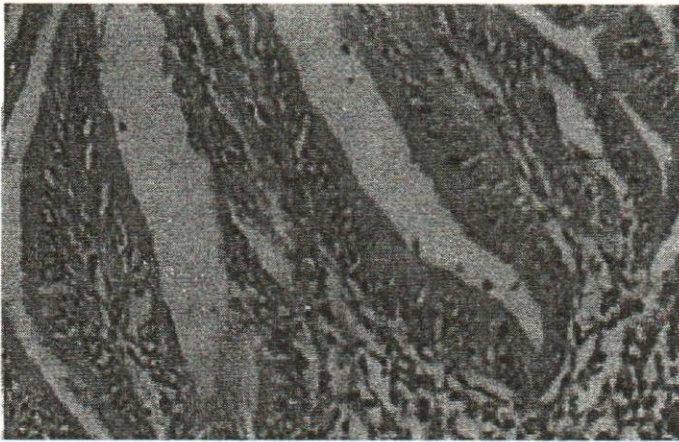
ND: Not Done

Fig. (1): Humoral immune response of the experimental broiler chickens to the 3 prepared inactivated IBDV vaccines in comparison with a commercial inactivated IBDV vaccine as measured by indirect ELISA





**Fig. 2:** BF of the non-vaccinated broiler chickens that challenged with the Del/E related IBDV variant strain at 5<sup>th</sup> day post challenge showing interfollicular oedema, lymphocytic depletion and esinophils aggregation in the follicular cortical portion (H & E, 40x).



**Fig. 3:** Proventriculus of non-vaccinated broiler chickens that challenged with the Del/E related IBDV variant strain at 5<sup>th</sup> day post challenge showing severe epithelial hyperplasia of the mucosa with inflammatory cells infiltration with cellular damage in the glandular lumen and hyperplasia of the glandular alveoli (H & E, 40x).

## DISCUSSION

Our study was conducted in 3 steps, the first step was detection, isolation and identification of a new variant IBDV strain using RT/PCR-RFLP assay, that contributed to be the cause of TVP in broiler chickens. The results confirm that the isolated IBDV is a variant strain related to the Del/E variant IBDV. In the second stage we study the pathogenicity and immune suppressive potential of this variant strain and in the present study we try to complete the previous work by preparation of inactivated w-o-w-e IBDV vaccine from our Del/E related IBDV strain in a trial to overcome, face and even prevent the spread of TVP in broiler chickens in Egypt.

In the present study 3 inactivated IBDV vaccines were prepared; vaccine No. (1) contained the Del/E related strain; vaccine No.(2) contained the vvIBDV and vaccine No. (3) contained both IBDV stains. To evaluate the efficacy of the prepared vaccines a commercial inactivated IBDV vaccine was used. The humoral immune response of the experimental chickens, as detected by indirect ELISA, showed significant increase in GM ELISA antibody titre in all vaccinated Groups, compared with non vaccinated Control group, along the experimentation period from the second week PV (Table 1). Our results agreed with that obtained by Amal *et al.*, 2002.

No significant differences were detected between the levels of GM antibody ELISA titre of the vaccinated chicken groups.

We could explain this result by the similarity of IBDV titers, before inactivation, in the 4 vaccines despite the variation in the IBDV strains contained in these vaccines. The 2 IBDVs either the classical or the variant strains are related to serotype 1 of IBDV, so they shared a group of common epitopes which contribute common antigen. So the obtained result are logic.

The results of challenge test (Table 2) revealed that the chicken groups received vaccine made of variant Del/E IBDV strain either vaccine No. 1 or vaccine No. 3, have 100% protection against challenge with either Del/E related variant IBDV strain or vv IBDV strain as detected by bursal indices proventricular/B.W ratio and histopathological lesion score of BF and proventriculus. While the chicken groups received vaccine made of classical IBDV strain have 70% and 60% protection against challenge with Del/E related variant IBDV strains and 100% protection against challenge with vvIBDV strain.

Our results, agreed with that obtained by Saif, 1994; Nassif 2001 and Ismail and Saif 1994 who found that inactivated IBDV vaccine made of variant IBDV protected chickens against challenge with either standard or variant viruses even at high doses, while inactivated IBDV vaccine made of the standard strains protected chicken against challenge with the standard strains at high or low doses and only against the low challenge dose of variant strain but not against the high dose.

It was concluded that the addition of the Del/E related IBDV vaccine to all the vaccine preparation containing inactivated IBDV (either univalent Gumboro, or ND+G, ND+G+IB or ND+G+IB+Reo that applied to breeders will be helpful in protection of progeny against both IBD and TVP. Also continuous and regular surveys on the IBD outbreaks in Egypt is necessary to isolate and identify the new strains of the IBDV, that could be circulate in the field, to be always ready and standby for continuous prevention and control of the IBD in Egypt.

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