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EFFECT OF INFECTIOUS BURSAL DISEASE HATCHERY VACCINES ON H5 AVIAN INFLUENZA VACCINATION IMMUNITY IN COMMERCIAL BROILERS

By

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ABSTRACT

The following study was carried out to demonstrate the role of IBD vaccine as immunosuppressant and its effect on immune system of birds. Immunosuppression plays an important role in diminishing the ability of the immune system to respond to vaccines making birds less -immune to HPAI. IBD vaccines vary in their adverse effect on the immune system. In this study, four hundred commercial broiler chicks were divided randomly into four groups; - (ve) control G1, + (ve) control G2, HVT IBD vector vaccinated G3 and immune complex IBD (based on intermediate plus H2512 strain) vaccinated G4. Immune complex and HVT vector IBD vaccines were evaluated in terms of their immunosuppressive effect on the ability of commercial broilers to respond to a vaccination program against HPAI of H5 origin. The vaccination program was based on a H5 Fowl pox vector AI vaccine administered at day old followed by administration of an inactivated Re5 H5N1 avian influenza vaccine at day 10 of age. Vaccine take and humoral immune response was measured by Hemagglutination inhibition (HI) test. Mean titers of study groups showed the immune-complex vaccinated group was significantly lower ($P<0.05$) mean HI titers at 35 and 42 days of age than mean titres of the HVT vector IBD vaccine and the +ve control groups. Macroscopic and microscopic monitoring parameters used to evaluate any adverse effect on the bursa of Fabricius by the IBD vaccines in this study revealed a significant adverse effect of immune-complex vaccine in comparison to HVT vector IBD vaccine and the control group that could explain the serological variance. Results indicate that immune complex IBD vaccines may affect the efficacy of AI vaccination programs.

INTRODUCTION

Highly pathogenic avian influenza (HPAI) remains an economical threat to the poultry industry in Egypt. Vaccination using commercially available vaccines remains one of the most important tools to control mortality, clinical signs and shedding of the field viruses. Egypt is endemic with HPAI H5N1 **(Hagag *et al.,* 2014).** HPAI H5N8 has been recently reported in Egypt as well **(OIE, 2017).** Since 2006, clade 2.2.1 of highly pathogenic avian influenza (HPAI) H5N1 viruses has been widely circulating in Egypt, causing massive economic losses in the Egyptian poultry industry **(Aly *et al.,* 2008).** The HPAI H5N8 virus of clade 2.3.4.4 has been recently detected in wild birds and domestic poultry in Egypt **(Kandeil *et al.,* 2017)**. Potent AI vaccines, when properly used, can prevent disease and death, increase resistance to infection, reduce field virus replication and shedding, and reduce virus transmission, but do not provide"sterilizing immunity" in the field **(Swayne,2006)**.

Re5 H5N1 vaccine is proven to completely protect chickens and significantly reduce virus shedding of H5N1 **(Grund *et al.,* 2011)** and H5N8 **(Kandeil *et al.,* 2018).**

The immunogenicity of vaccines in young chicks with maternally derived antibodies (MDA) depends on the vaccination scheme and the type of vaccine used in their parent flocks. The heterologous prime-boost with live recombinant FP-vectored vaccine with H5 avian influenza gene insert (FP-AI) then inactivated AI vaccine in birds with MDA may at least partially overcome MDA interference on inactivated vaccine **(Richard-Mazet *et al.,* 2014).** Previous studies have shown that minimum specific HI serological titers were associated with protection in challenge studies when the vaccine and field viruses were genetically and antigenically similar **(Eggert *et al.,* 2010).** Very virulent infectious bursal diseases (vvIBDV) continue the presence of in intensively vaccinated flocks in Egypt **(Metwally *et al.,* 2009)**. Vaccination has remained essential because of the economic significance of the disease and the high prevalence of IBDV. Different modified live vaccines (MLVs) containing classical or variant viruses are commercially available, and are classified according to their degree of attenuation as "mild", intermediate", "intermediate plus" and "hot" IBD vaccines. Other types of vaccines have been developed which are less sensitive to the interference of passive immunity as immune complex (IC) vaccine that is used for in-ovo or for subcutaneous. Day old vaccination, in which the "intermediate plus" vaccine virus is complexed with antibodies. Vectored viral vaccines expressing proteins of IBDV have also been described as

potential IBD vaccines, using vectors such as fowl poxvirus, turkey herpes virus (HVT) fowl adenovirus, Marek's disease virus and Semliki Forest virus.

The immune-complex vaccine administered in ovo has been used successfully at farm hatcheries as well. It was also concluded that mild and intermediate vaccines are safer, in that they cause less bursal damage, than "hot" vaccines, but have a poor efficacy in the presence of MDA and against vvIBDVs. In contrast, less attenuated strains ("intermediate plus" and "hot" vaccines) can overcome higher levels of MDA, but they may cause more severe lesions in the bursa follicles, resulting in immunosuppression. These strains are not recommended for chickens younger than 10 days of age **(Van den Berg, 2000; Müller *et al.,* 2003)**. Live HVT recombinant vaccine (HVT+IBD) expressing the VP2 antigen of IBD virus produced protective immune responses in chickens better than the available attenuated viral strains and its use was recommended as a vaccine for IBDV **(Pradhan *et al.,* 2012).** The study aims to evaluate the immune suppressive effect of single shot hatchery IBD vaccines. It is namely HVT+IBD vector vaccine and immune-complex IBDV vaccine when either one of them is administrated to commercial broiler chicken on the humoral immune response to vaccination with FP-AI vector vaccine as priming at day old then with inactivated Re5 H5N1 vaccine at day 10 of age using Re-5 homologous antigen and expressed as HI units.

MATERIAL AND METHODS

1.Chicken.

Four hundred day old Commercial broiler chicks of Cobb 500 strain were obtained from the same breeder flock.The breeder flock was 52 weeks of age and was vaccinated at pre-lay with inactivated vaccines against ND (Ulster 2C strain), IB (Mass strain), IBD (VNJO strain), EDS (EDS76strain),REO (S1133strain)andHPAI H5N1(Re-5 H5N1 Strain) and at mid-lay with inactivated vaccine against HPAI H5N1(Re-5 H5N1 Strain) at the recommended dose and route of administration. The chicks were divided equally into 4 groups, 100 chicks per group and were reared on deep litter system in the same house separated with partitions. Chicken were fed on a balanced ration and received the same medication program and water was provided ad-libitum.

Group 1: Negative control (G1). This group was non-vaccinated to monitor weaning of MDAs and immune response in case of field exposure to the viruses under investigation.

Group 2: Positive control (G2). This group received the basic vaccination program and in addition, 0.2ml diluent / bird containing Pox AI and Mareks HVT and 0.2ml / bird diluent only both administered sub-cutaneously at day old in the hatchery. No IBDV vaccine was administered to this group.

Group 3: HVT+IBD vaccinated (G3). This Group received the basic vaccination program and in addition 0.2ml diluent / bird containing HVT+IBD and Pox AI according to the manufacturer recommendations and 0.2ml diluent only/bird both administered .

Sub-cutaneously at day old in the hatchery to monitor the immunosuppressive effect of HVT+IBD vaccine.

Group 4:Immune Complex IBD vaccinated (G4).This group received the basic vaccination program and in addition 0.2ml diluent / bird containing immune complex IBD vaccine and 0.2ml diluent / bird containing Pox AI and Mareks HVT according to the manufacturer recommendations both administered sub-cutaneously at day old in the hatchery to monitor the immunosuppressive effect of immune complex IBD vaccine.

Vaccination program:

The vaccination program applied to the different groups in the study is demonstrated in (Table 1).

Table (1): Vaccination program of different study groups.

Age	Groups			
	G1 -ve control (no vaccination)	**G2 +ve control (No IBD vaccination)**	**G3 HVT+IBD**	**G4 Immune Complex IBD**
1 Day old	**No Vaccination**	**Diluent A Only 0.2 ml/bird**	**HVT+IBD & (FP-AI) in diluent A 0.2 ml/bird**	**Immune Complex in diluent B 0.2 ml/bird**
	No Vaccination	**(FP-AI)&HVT in diluent A 0.2 ml/bird**	**Diluent A Only 0.2 ml/bird**	**(FP-AI)+HVT in diluent A 0.2 ml/bird**
10 Days old	**No Vaccination**	**Re-5 H5N1 0.5ml/bird sub-cut**		

2. Vaccines.

IBD vaccines:

HVT+IBD: live cell associated vector vaccine against Mareks and IBD, 0.2 ml / bird at day old.

Immune complex: live vaccine against IBD, 0.2 ml / bird at day old.

Other vaccines:

Pox AI: vector live vaccine against AI H5, 0.2 ml / bird at day old alone or mixed with HVT + IBD.

MAREKS HVT: live cell associated vaccine against Mareks serotype 3, 0.2 ml / bird at day old alone and mixed with Pox AI.

RE-5 H5N1: inactivated- oil- emulsion reassortant vaccine against AI H5N1 was injected sub-cutaneous at 0.5 ml / bird on the 10^{th} day of age.

3. Vaccines Diluent.

Sterile diluent A, 0.2 ml / bird at day old:

- Used alone.
- Used to administer Pox AI.
- Used to administer mixed Pox AI and HVT + IBD.

Sterile diluent B, 0.2 ml / bird at day old used to administer Immune complex live vaccine against IBD.

Sampling:

1.Blood samples.

For serum collection, blood samples 1-2 ml, were collected individually from wing vein of 20 birds / group at 4, 14, 21, 28, 36 and 42 days of age using disposable 3ml syringe. All serum samples were labeled and stored at-20 ºC until serological examination within the following 3 minutes.

2.Bursa samples.

Individual bursa samples were collected by euthanizing five birds / group at 28 and 35 days of age. Samples were weighed to determine the bursa body-weight ratio and index, and then fixed in 10% formalin for histopathology examination.

Hemagglutination (HA) and Hemagglutination inhibition (HI) test:

The Hemagglutination (HA) and hemagglutination inhibition (HI) test were carried out following the recommendation of **(OIE-Manual, 2009).** The reagents required for the test are isotonic PBS (0.1 M); pH 7.0-7.2, citrated chicken red blood cells (RBCs) was taken from SPF chicken. Cells were washed three times in PBS before use as a 1% (packed cell v/v) suspension. Positive and negative control antigens and antisera were run with each test, as appropriate.

Bursa body weight ratio and index:

Bursa body weight ratio was carried out according to **Sharma *et al.*, (1989)**. Collected bursae were weighed and the organ/body weight ratio was determined as follows:

$$\text{Organ/body weight ratio} = \frac{\text{Organ weight (gm)}}{\text{Body weight (gm)}} \times 1000$$

$$\text{B:B index} = \frac{\text{Mean B:B ratio of challenged bird}}{\text{Mean B:B ratio of un-infected control}}$$

B: B indices lower than 0.7 was considered atrophied.

Histopathology:

Tissues were fixed in 10% neutral buffered formalin, dehydrated in graded alcohols, cleared with xylene, and infiltrated and embedded in paraffin. Embedded tissues were sectioned at 4 to 6 um and stained with hematoxylin and eosin (H&E). The severity of the microscopic lesions was graded based on the extent of the lymphoid depletion/ necrosis, epithelial hyperplasia and cystic degeneration. Scores of 0 to 4 were used to indicate relative degree of severity, a score of 0 indicated absence of lesions, and scores 1 to 4 were for 25%,25 to 50%, 50 to 75 % and 75% of follicles affected,respectively **(Jackwood *et al.*, 2011).**

2.3. [Statistical analysis.

Mean differences were analyzed by SPSS ver. 20 (SPSS, Inc., Chicago.Il.USA). Two way analysis of variance (ANOVA) and LSD test for post hoc comparison were used. The level of significance was set at $p < 0.05$

RESULTS AND DISCUSSION

Table (2): Effect of 2 different IBD vaccines on mean HI titers (log2) against AI H5 vaccines in broiler chicken (4 - 42 days of age) using homologous antigen.

Group/Age in days	4	14	21	28	35	42
G2	5.60a	5.05a	5.00a	5.45a	6.25a	7.25a
G3	5.60a	5.05a	5.10a	5.45a	6.55a	7.15a
G4	5.60a	4.95a	4.75a	4.75a	5.35b*	5.6b*

***Means with a different letter within the same column are significantly different at p value ≤0.05.**

G2: Not vaccinated against IBD (Diluent only)

G3: HVT+IBD vaccine at day old

G4: Immune complex vaccine at day old.

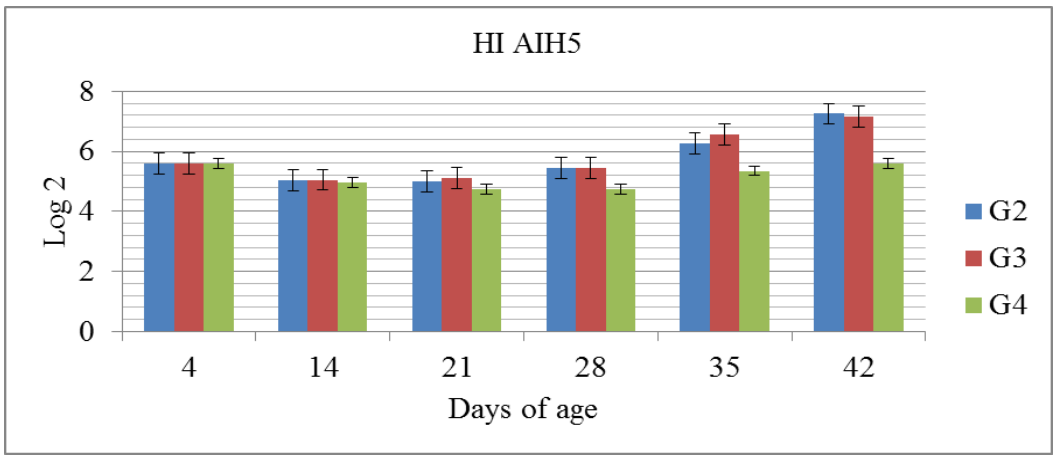

Fig. (1): Effect of two different IBD vaccines on mean HI titers (log2) against AI H5 vaccines in broiler chicken (4 - 42 days of age) using homologous antigen.

Table (3): Effect of two different IBD vaccines on mean bursa weight in grams, mean bursa-body weight ratio and mean bursa body weight index of broiler chicken at 28 and 35 days of age.

Group\Age	Mean BBW at 28 days of age index			Mean BBW at 35 days of age index		
Parameter	Mean BW/g	Mean BBW ratio	Mean BBW index	Mean BW/g	Mean BBW ratio	Mean BBW index
G2	2.02	1.15	1.00	2.70	1.23	1.00
G3	2.32	1.35	1.17	2.74	1.17	0.95
G4	1.98	1.21	1.06	1.52b*	0.84b*	0.68b*

***Means with a different letter within the same column are significantly different at $p \leq 0.05$.**

G2: Not vaccinated against IBD (Diluent only)

G3: HVT+IBD vaccine at day old

G4: Immune complex vaccine at day old

BBW: bursa body weight

BW: bursa weight

Table (4): Effect of two different IBD vaccines on mean bursa lesion score of broiler chicken at 28 of age.

Group\Parameter	G2	G3	G4
Lymphocytic necrosis and depletion	**0**	**0**	**2**
Atrophy of Lymphoid follicles	**0**	**0**	**1**
Intra-follicular cyst	**0**	**0**	**0**
Inter-follicular fibrous connective tissue proliferation	**0**	**0**	**1**
Inflammatory cells infiltration	**0**	**0**	**1**
Overpopulation with lymphocyte	**0**	**2**	**0**

Means with a different letter within the same column are significantly different at $p \leq 0.05$.

G2: Not vaccinated against IBD (Diluent only)

G3: HVT+IBD vaccine at day old

G4: Immune complex vaccine at day old

0 indicated absence of lesions

1 = 25% of follicles affected

2 = 25% to 50% of follicles affected

3 = 50% to 75% of follicles affected

4 = 75% of follicles affected.

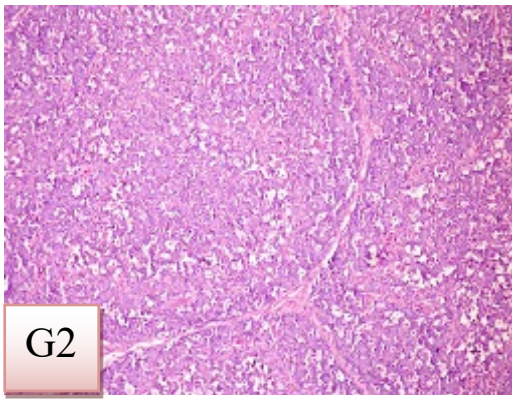

Fig (2): G2 Bursa 28-day of age of broiler chicken not vaccinated with any IBD vaccine, showing normal bursa histology.

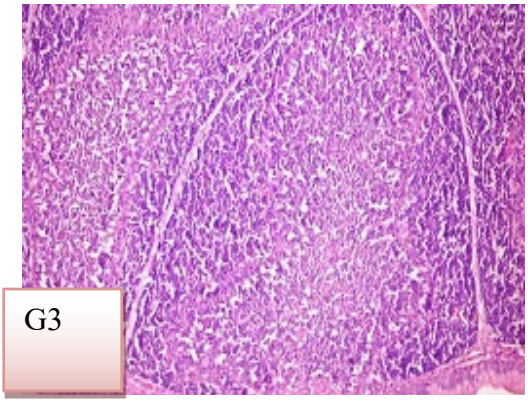

Fig (3): G3 Bursa 28-day of age of broiler chicken vaccinated with HVT + IBD at 1 day of age,showinghyperplasiaand hypertrophy of lymphoid follicles.

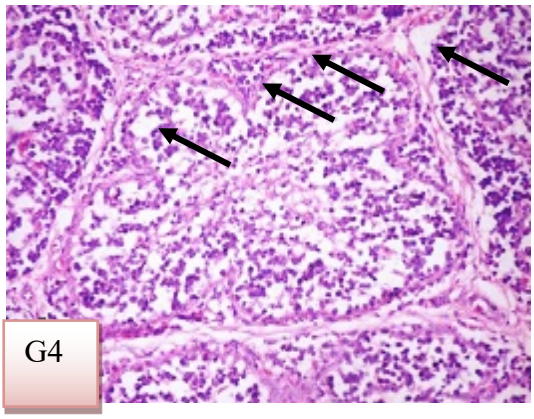

Fig (4): G4 Bursa 28-day of age of broiler chicken vaccinated with Immune complex IBD at 1 day old of age, showing lymphocytic necrosis, lymphocytic depletion associated with atrophy of lymphoid follicles, interfollicular fibrous connective.

DISCUSSION

Table (2), Fig. (1) Demonstrate the effect of two different IBD vaccines on mean HI titers (log2) against AI H5 vaccines in broiler chicken (4-42 days of age) using homologous antigen. It can be noticed that, the mean titer for G2, G3 and G4 at 4 days of age was 5.6 log2 HI units then declined till it reached the lowest titer at 14 and 21 days of age for G3 and G2 respectively recording mean titer of 5.05 and 5 Log2 HI unit respectively.

Mean titers for both groups started to increase until reaching the highest value at 42 days of age recording 7.15 and 7.25 log2 HI unit for G3 and G2 respectively. G4 titers continued to decline until 28 days of age reaching a mean titer of 4.75 log2 HI units before it started to increase again at 35 days of age to reach the highest titer of 5.6 Log2 HI units at 42 days of age. These results indicate that although G2 and G3 showed slightly higher mean titers than G4 there was no statistical significant difference *(P<0.05)* among the groups until 28 days of age. However at 35 and 42 days of age mean titers of G4 (5.35 and 5.6 respectively) were significantly lower than G2 (6.25 and 7.25 respectively) and G3 (6.55 and 7.15 respectively) (P<0.05). Similar results were reported by **(Ismail *et al.,* 2014)** where sera measured by the variant A/chicken/Egypt/VRLCU67/2011 (H5N1) isolate showed significant difference (P<0.05) between mean HI titers of bird vaccinated by traditional IBDV vaccines and titers of those vaccinated with the HVT+IBD vaccine. The results could be explained by **(Aly *et al.,* 2012**) **and (Bublot *et al.,*2007)** who agreed thatHVT+IBD vaccine is non-immunosuppressive. **(Rautenschlein *et al.,*2011)** compared the effects on the humoral and cell-mediated immunity between HVT+IBD vector vaccine and an IBDV immune complex vaccine after in ovo vaccination of commercial broilers and concluded that immune complex vaccine reduced the number of circulating B cells in comparison to the HVT+IBD vaccinated and the non-inoculated control group. (Table 3) demonstrates the effect of two different IBD vaccines on mean bursa weight in grams, mean bursa body weight ratio, mean bursa body weight index of broiler chicken at 28 and 35 days of age. It is demonstrated in (Table 3) that no statistical significant difference (P<0.05) was found between G2 and G3 at 28 and 35 days of age. However, G4 was statistically significantly lower in mean bursa weight, ration and index (P<0.05) at 35 days of age when compared to G2 and G3. Similar results were reported by **Rautenschlein *et al.,*(2011)** when they compared the bursa to body weight ratio of

commercial broilers vaccinated either by HVT+IBD or an immune complex vaccine. They concluded that, the bursa to body weight ratio was not affected in the non-vaccinated and the HVT+IBD vaccinated group while immune complex inoculated birds showed a reduction of the bursa weight beginning at 21 days post hatch ($P<0.05$). (Table 4), Fig. (2- 4) demonstrate the effect of 2 different IBD vaccines on mean bursa lesion score of broiler chicken at 28 of age. It can be noticed that G2 and G3 are with similar lesion score while G4 records higher scores. This result agrees with **(Rautenschlein *et al.*, 2011)** who detected histopathological lesions only in the immune complex vaccinated group and not in the HVT+IBD or non-vaccinated groups. The macroscopic and microscopic parameters used to monitor the bursa Fabricius proved that in G4 the immune-complex vaccine had an adverse effect on the bursa of Fabricius. A result that may explain the significant low ($P<0.05$) serological findings as a response to vaccination at 35 and 42 days of age. A finding that was clear when compared to G2 (non -IBD vaccinated) and G3 (HVT+IBD vaccinated). However, previous studies showed that minimum specific HI serological titers were associated with protection in challenge studies when the vaccine and field viruses were genetically and antigenically similar **(Eggert *et al.*, 2010).** Further challenge studies are required to evaluate the impact of the statistically significant difference in HI titers between the different groups on the protection against mortality, clinical signs and change virus shedding.

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