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Avian Disease

Appraisal of Day-Old Vaccination with Recombinant HVT-IBD-ND vaccine versus challenge with Very Virulent Infectious Bursal Disease (vvIBDV) In Broiler Chickens

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ABSTRACT

Very virulent Infectious bursal disease (vvIBD) is a highly contagious disease of young chickens and still causing devastating economic losses in the Egyptian poultry industry consists of high mortality alongside with severe bursal damage and immunosuppression. This study aimed to assess the effectiveness of day-old vaccination with rHVT-IBD-ND vaccine against challenge with vvIBDV in broilers at 23 days of age. Four groups (N=30) were present in this study in which G1 (Vaccinated challenged), G2 (Vaccinated non-challenged), G3 (non-vaccinated challenged) and G4 (Negative control). Results revealed that absence of clinical signs; mortalities and gross lesions in G1, G2 and G4 compared to G3 which suffered from moderate clinical symptoms and the mortality were (6.6%). Moreover, the bursal body weight ratio (BBR) and bursal index (BI) were significantly reduced ($p \leq 0.01$) at 7 dpc in G3 (0.55 & 0.37) less than G1 (1.45 & 0.79) respectively. The serological immune response in the vaccinated groups (G1 and G2) was significantly higher ($p \leq 0.01$) than in non-vaccinated groups (G3 and G4) at 7 dpc and the mean ELISA titers were (13092, 12157, 6230 and 750) in G1, G2, G3 and G4, respectively. In addition, Histopathological examination revealed a significant reduction in the bursal lesions and mean severity index in G1 which recorded MLS of (1.5) in comparison with G3 significantly higher MSI (2.5). based on the results of the current study, it could be concluded that vaccination of broiler chickens with rHVT-IBD-ND vaccine at one-day-old provides complete clinical protection against vvIBDV beside partial protection from bursal tissue damage.

Keywords: Very virulent IBDV, Vaxxitek® rHVT-IBD-ND, Bursal body weight ratio, bursal index, clinical protection and Bursal damage.

1. INTRODUCTION

Infectious bursal disease (IBD) is one of the most significant illnesses encountering the worldwide poultry industry with severe implications on the birds' immunity. The disease is caused by infectious bursal disease virus (IBDV) which is a non-enveloped highly resistant virus that belongs to genus *Avibirnavirus* within the *Birnaviridae* family (WOAH, 2024). The virus contains a double-stranded RNA genome comprised of two segments (A and B segments) that encodes five viral proteins from which the VP1 is encoded in segment B which represents the viral

polymerase, while the structural proteins VP2, VP3, and VP4 as well as the regulatory protein VP5 are encoded in segment A. VP2 gene is the primary immunogenic domain and the most important pathogenicity determinant (Mahgoub, 2012; Eterradossi and Saif, 2013). Two serotypes among IBDV isolates are present from which Serotype-1 viruses are pathogenic to chickens but serotype 2 viruses are non-pathogenic (McFerran et al., 1980; Eterradossi and Saif, 2013). IBD virus has a high affinity to replicate in bursal B- cells making extensive Bursal damage represented by lymphocytic depletion and necrosis with subsequent immunosuppression due to bursal atrophy which high opportunity to secondary infections (Lukert and Saif, 1997; Van den Berg et al., 1991; Kumar et al., 2002; Jackwood et al., 2012). Very virulent IBD strains (genotype A3B2) were responsible for typical IBD signs, lesions and high mortality occurred in Egypt in the past years and still causing a threat to the poultry industry. However, novel variant IBDV (A2dB1b) was firstly isolated by (Legnardi et al., 2023) and found to be related to Chinese strains followed by another study by (Salaheldin et al., 2024) which described the same results. The disease is mainly controlled through effective vaccination. However, many commercially available vaccines are used in poultry industry which could provide complete or partial clinical protection against IBD infections; some of them have many drawbacks specially modified live vaccines. Modified live vaccines (MLV) vaccines unlikely can cause a varied degree of bursal damage with subsequent immunosuppression can occurs (Abd El-Razik., 2004; Sultan et al., 2006 & 2012; Muller et al., 2012). In addition, they need proper timing to be administered as they may be neutralized by the presence of MDA and may revert to virulent strain. Upon that, there is a great demand for IBDV vaccine which is safer and alleviates these drawbacks.

This study aimed to assess the protective efficacy of day - old vaccination of (Vaxxitek® rHVT- IBD-ND) vaccine contra challenge with recent vvIBDV isolate at 23 days of age in broiler chickens.

2. MATERIALS AND METHODS

2.1. IBD vaccines

The recombinant (Vaxxitek® rHVT- IBD-ND) vaccine obtained from a local agent of Boehringer Ingelheim Animal Health USA Inc.'s was used in this study. Chicks were vaccinated at one- day- old through s/c injection.

2.2. IBD challenge virus

A local field isolate of vey virulent IBDV (vvIBDV) under GenBank accession no. of (KX646373) was supplied by Prof. Dr. Hesham Sultan was used by a dose of 100µl/ bird containing ($10^{3.5}$ EID_{50}) through oculonasal route at 23 days of age after being titrated according to (Reed and Muench, 1938).

2.3. Experimental design

One hundred and twenty-one-day-old cobb commercial broiler chicks were divided into 4 groups designated as: G1, G2, G3 and G4 with 30 birds each. G1 and G2 were vaccinated with rHVT-IBD-ND vaccine at one-day-old through s/c route, while, G3 and G4 were kept non-vaccinated. Moreover, G1 and G3 were challenged with vvIBDV at 23 days of age through oral route. All birds were observed for 7 days post challenge (dpc)for the presence of clinical signs and mortalities **(Fig. 1)**.

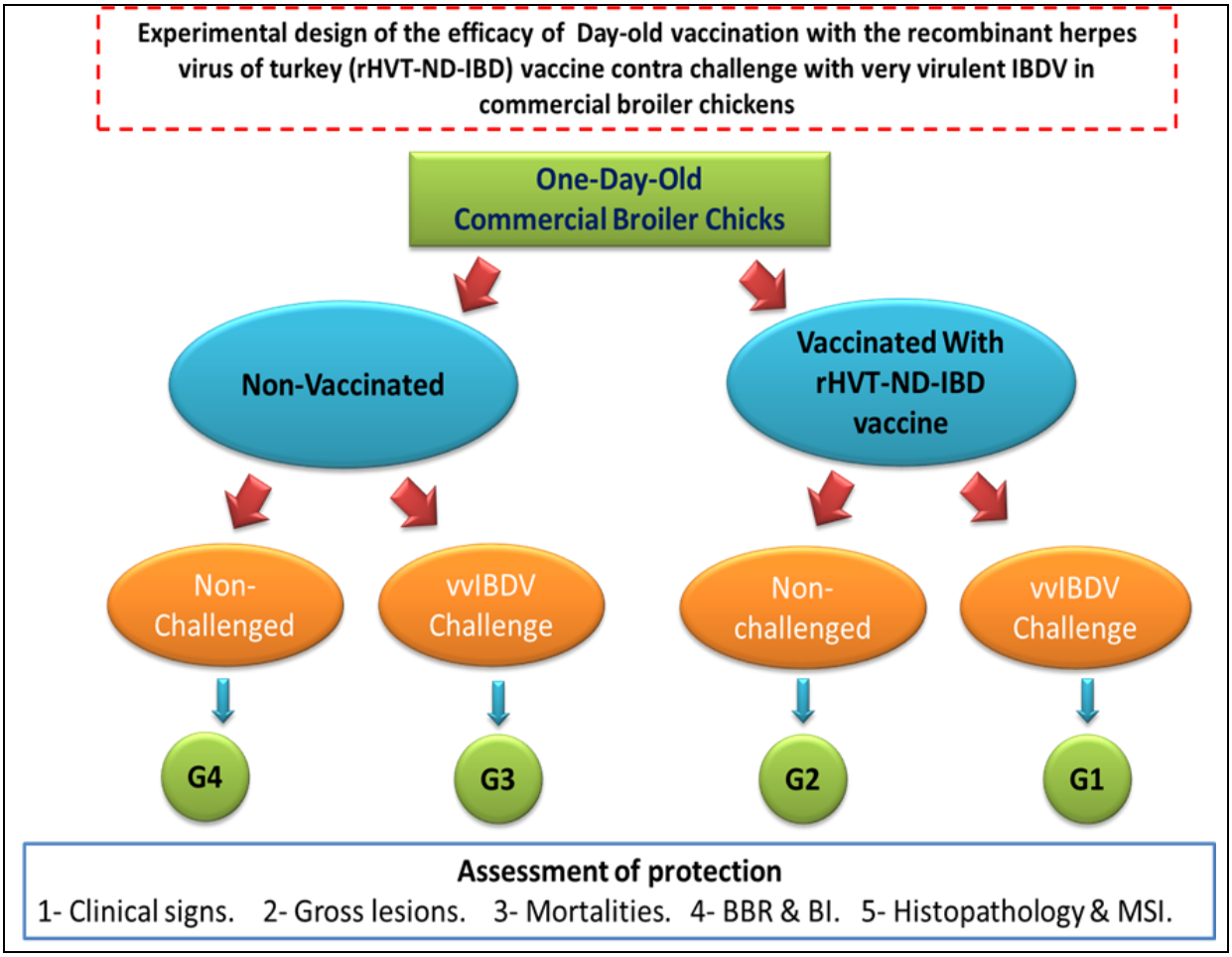

Fig. (1): Experimental design of the efficacy of Day-old vaccination with the recombinant herpes virus of turkey (rHVT-ND-IBD) vaccine contra challenge with very virulent IBDV in commercial broiler chickens**.**

2.4. Bursal body weight ratio (BBR) and bursal index (BI)

Bursal body weight ratio (BBR) and bursal index (BI) were calculated weekly after vaccination at 7, 14, 21 days of age and at 7 dpc. The BBR and BI were calculated according to (Lucio and Hitchner, 1979) as following: BBR was determined for each chicken by dividing the bursal weight on the body weight of the same bird. While the Bursal index (BI) = Mean B: B ratio of challenged chicks/ Mean B: B ratio of uninfected group.

2.5. Serology

Blood samples (N = 10) were collected from all groups from wing vein at 1, 7, 14, 23 and 30 days of age for estimation of the antibody response to IBD using BD + ELISA test. After collection of the whole blood, it left undisturbed to clot at room temperature for 15-30 minutes. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes and serum was separated. ELISA test was performed using (ProFlock® IBD plus ELISA kit, Symbiotic Corporation, 11011 via Frontex, San Diego, CA 92127) before and after challenge according to the manufacturers' instructions.

2.6. Histopathology

Bursal samples were collected from all groups at 7 dpc and fixed in 10% formalin solution according to (Bancroft et al., 1996) for histopathological examination and the mean severity index (MSI) were calculated according to (Sharma et al., 1989).

2.7. Statistical data analysis

Data were analyzed using One- way ANOVA test followed by Duncan's new multiple range test to determine the significance of differences between individual treatments and corresponding controls. A probability (p) value ≤ 0.01 was considered statistically significant. The data were obtained by using SPSS 11.0 software (SPSS Inc., Chicago, IL).

3. RESULTS

3.1. Clinical signs, gross lesions and mortalities

No clinical symptoms, gross lesions or mortalities were recorded in all vaccinated and non-vaccinated groups till the age of challenge. On the other hand, at 7th dpc with vvIBDV G1 (vaccinated challenged) and non- challenged groups (G2 and G4) showed no clinical symptoms, and no mortality was recorded (0%, 0% and 0%, respectively), in comparison to G3 (non-vaccinated challenged) which revealed slight depression, anorexia and ruffling feather with 2/30 (6.6%) mortalities**.** All dead birds showed hemorrhages on the thigh and/or pectoral muscles, enlarged kidneys and the bursa was covered with *gelatinous exudates* **(Table 1).**

Table 1. Mortality percentage at 7th days post challenge of broiler chickens vaccinated or non-vaccinated with rHVT-IBD-ND and or challenged with vvIBDV at 23rd days of age.

Group No.	Vaccination regime		Challenge[2] at 23 days of age	Mortality[3]	
	Type	Age/day		No.	%
G1	rHVT-IBD-ND[1]	1	++	0/30	0^{A}
G2	rHVT-IBD-ND	1	--	0/30	0^{A}
G3	-----	--	++	2/30	6.6^{B}
G4	-----	--	--	0/30	0^{A}

1- HVT-ND-IBD= vector vaccine against Marek's disease (MD), infectious bursal disease (IBD) and Newcastle disease (ND), administrated by subcutaneous injection in the hatchery.
2- IBD challenge virus= oculonasal challenge at 23rd day of age with 100µl/bird contain 103.5 EIDS-50 of IBDV local field isolate.
3- Mortality at 7th days post challenge.
4- Means different litters within the same column are significantly different at (P < 0.01).

3.2. Bursal body weight ratio (BBR) and Bursal index (BI)

Bursal body weight ratio (BBR) and bursal index (BI) were calculated weekly after vaccination at 7th, 14th, 21st days of age and at 7th days post challenge. Results revealed that the BBR was (1.7, 1.9 and 1.7) in IBD vaccinated groups (G1 & G2) versus to (1.6, 2.07 and 2.09) in IBD non-vaccinated groups (G3 & G4) on day 7, 14 and 21, respectively **(Table 2).** Moreover, the BBI was (1.08, 0.92 and 0.89) in vaccinated groups (G1 and G2) in comparison with (1,1 and 1) in non – vaccinated groups (G3 & G4) on day 7, 14 and 21, respectively **(Table 2).**

While at 7th dpc with vvIBDV, G1 (vaccinated challenged) showed (1.45 & 0.79) for BBR and BI, respectively. In addition, G2 (vaccinated non-challenged) showed (1.93 & 0.98) for BBR and BI, respectively, Moreover, G3 (non-vaccinated challenged) showed (0.55 & 0.37) for BBR and BI, respectively, and G4 (non-vaccinated non-challenged)

showed (2.5 & 1.08) for BBR and BI, respectively **(Table 2) and (Fig. 3).**

Table 2. Bursal body weight ratio (BBR) and Bursal index (BI) of broiler chickens vaccinated or non-vaccinated with rHVT-IBD-ND and or challenged with vvIBDV at 23rd days of age.

Group No.	Vaccination regime	Challenge[2] at 23 days of age	BBR[3]				BI[4]			
	Type		7	14	21	7dpc	7	14	21	7dpc
G1	rHVT-IBD-ND[1]	++	1.7^{A}	1.9^{A}	1.7^{A}	1.45^{A}	1.08^{A}	0.92^{A}	0.82^{A}	0.79^{A}
G2	rHVT-IBD-ND	--	1.7^{A}	1.9^{A}	1.7^{A}	1.93^{B}	1.08^{A}	0.92^{A}	0.82^{A}	0.98^{B}
G3	-----	++	1.6^{A}	2.07^{A}	2.06^{B}	0.55^{C}	1^{A}	1^{A}	1^{B}	0.37^{C}
G4	-----	--	1.6^{A}	2.07^{A}	2.06^{B}	2.5^{D}	1^{A}	1^{A}	1^{B}	1.08^{D}

1- HVT-ND-IBD= vector vaccine against Marek's disease (MD), infectious bursal disease (IBD) and Newcastle disease (ND), administrated by subcutaneous injection in the hatchery.
2- IBD challenge virus= Oculo-nasal challenge at 23rd day of age with 100µl/bird contain 103.5 EIDS-50 of IBDV local field isolate.
3- BBR= Bursal body weight ratio (Sharma et al., 1989).
4- BI=Bursal index (Lucio and Hitchner, 1979), considered bursae to be atrophied if the index was less than 0.7.
5- Means different litters within the same column are significantly different at ($P < 0.01$).

3.3. Serological immune response to IBD

The antibody response to IBD was estimated using BD+ELISA beginning from one day old till the 7th dpc with vvIBDV. Results demonstrated that all vaccinated and non-vaccinated groups showed high level of maternal derived antibodies with mean ELISA titer was (10980) for all groups. Moreover, the serum antibody levels started to decrease by age beginning from the 7th day of age and continued to decrease till 14 days of age due to the waning of MDA which recorded a mean titer of (7230 & 5545) at 7 and 14 days, respectively for G1, (7230 & 5537) at 7 and 14 days, respectively for G2, (7230 & 5075) at 7 and 14 days, respectively for G3, while G4 recorded a mean ELISA titer of (7230 & 5068) at 7 and 14 days, respectively. Furthermore, a significant increase in the antibody level was observed in the vaccinated groups (G1 and G2) at 23 days of age which recorded (9883 & 8460) for G1 and G2, respectively due to the effect of rHVT-IBD-ND vaccine, while the non- vaccinated groups (G3 and G4) showed continuous declining of the antibody levels which showed (2853 & 2537) for G3 and G4, respectively. On the other hand, there was a significant increase in the serological immune response in the vaccinated groups (G1 and G2) in comparison with non-vaccinated groups (G3 and G4) at 7 days post challenge and the mean ELISA titers were (13092, 12157, 6230 and 750) in G1 (vaccinated challenged), G2 (vaccinated non-challenged), G3 (non-vaccinated challenged) and G4 (non-vaccinated non-challenged), respectively **(Table 3 and Fig. 2).**

Table 3. Serological immune response of broiler chickens vaccinated or non-vaccinated with rHVT-IBD-ND and or challenged with vvIBDV at 23 days of age.

Group No.	Vaccination regime	Challenge[2] at 23 days of age	IBD immune response BD+ Mean ELISA titer				
	Type		1	7	14	23	7dpc*
G1	rHVT-IBD-ND[1]	++	10980^{A}	7230^{A}	5545^{A}	9883^{A}	13092^{A}
G2	rHVT-IBD-ND	--	10980^{A}	7230^{A}	5537^{A}	8460^{B}	12157^{B}
G3	-----	++	10980^{A}	7230^{A}	5075^{B}	2853^{C}	6230^{C}
G4	-----	--	10980^{A}	7230^{A}	5068^{B}	2537^{D}	750^{D}
G1	rHVT-IBD-ND[1]	++	10980^{A}	7230^{A}	5545^{A}	9883^{A}	13092^{A}

1- HVT-ND-IBD= vector vaccine against Marek's disease (MD), infectious bursal disease (IBD) and Newcastle disease (ND), administrated by subcutaneous injection in the hatchery.
2- IBD challenge virus= Oculo-nasal challenge at 23rd day of age with 100µl/bird contain 103.5 EIDS-50 of IBDV local field isolate.

3- Means different litters within the same column are significantly different at ($P < 0.01$).

***- dpc=** days post challenge with vvIBDV at 23 days of age.

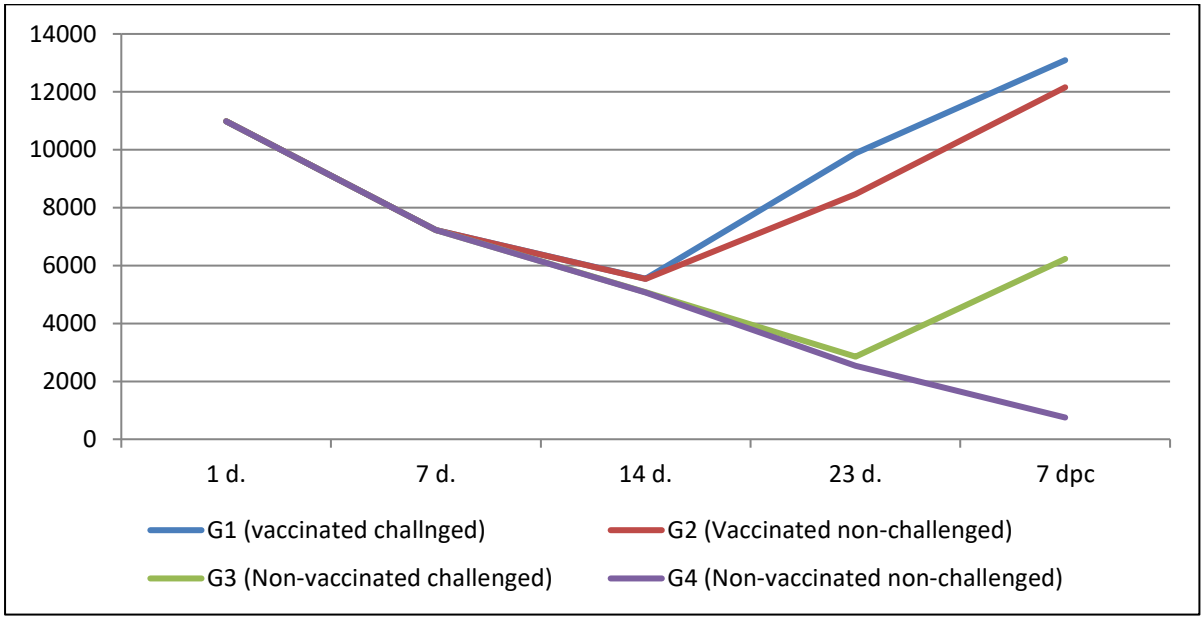

Fig. 2. Serological immune response using ELISA for IBD of broiler chickens vaccinated or non-vaccinated with rHVT-IBD-ND and or challenged with vvIBDV at 23rd days of age.

3.4. Histopathology and Mean severity index (MSI)

Bursae of Fabricius were collected from all vaccinated and non- vaccinated groups at 7th dpc and mean severity index (MSI) of bursal tissues were calculated. Results revealed a significant reduction in the bursal lesions and mean severity index (1.5) was recorded in G1 (vaccinated challenged) which showed mild lymphocytic depletion with micro cysts formation beside with inter-follicular edema, slight inflammatory cells infiltration along with connective tissue formation, in comparison with G3 (non-

vaccinated challenged) which showed a severe lymphocytic depletion and necrosis and compressed bursal follicles with cysts formation along with inter-follicular edema and high inflammatory cells infiltration with significantly higher MSI (2.5). On the other hand, G2 (vaccinated non-challenged) and G4 (non-vaccinated non-challenged) showed apparently normal follicles with mean severity index recorded (0) as shown in **(Table 4 and Fig. 3 & 4).**

Table 4. Bursal lesions and Mean severity index of bursal tissue (MSI) at 7th dpc for broiler chickens vaccinated or non-vaccinated with rHVT-IBD-ND and or challenged with vvIBDV at 23rd days of age.

Group No.	Vaccination regime	Challenge at 23rd days of age	Assessment at 7th dpc		
	Type		Bursal lymphocytic tissue lesion		Mean Severity Index (MSI)[1]
			Lymphocytic depletion	Lymphocytic necrosis	
G1	rHVT-IBD-ND	++	1.4	1.6	1.5^{A}
G2	rHVT-IBD-ND	--	0	0	0^{B}
G3	-----	++	2.5	2.5	2.5^{C}
G4	-----	--	0	0	0^{B}

1- MSI= mean severity index of bursal lesions according to (Sharma et al., 1989).

2- Means different litters within the same column are significantly different at ($P < 0.01$).

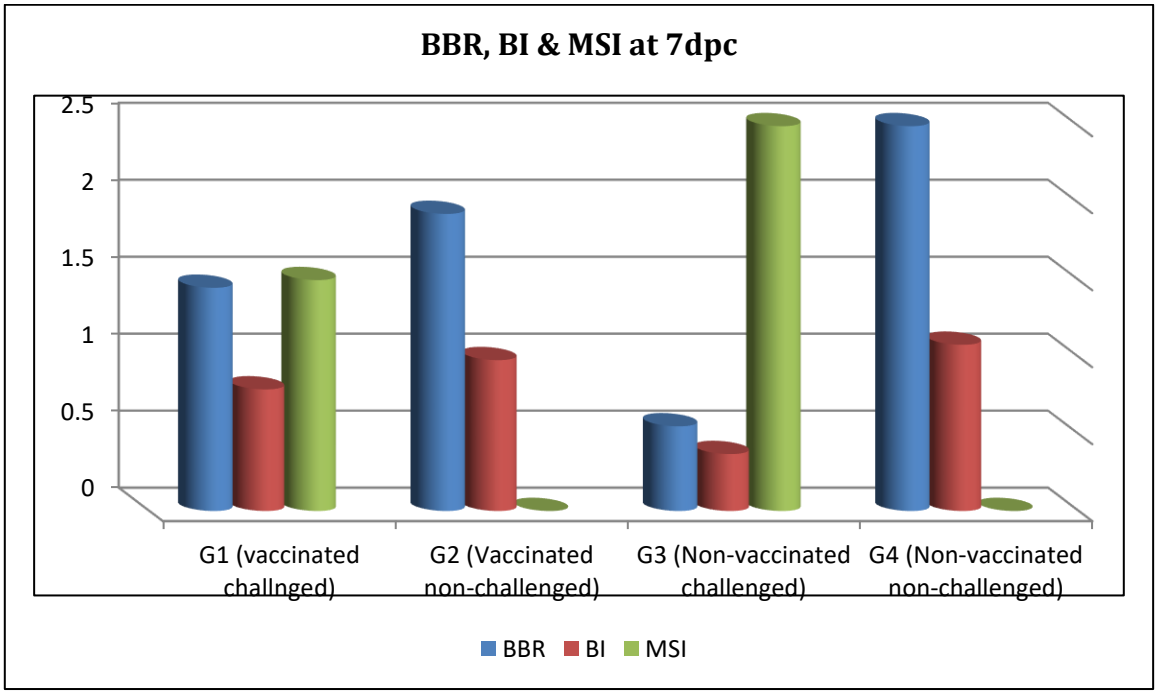

Fig. 3. Bursal body weight ratio, bursal index and mean severity index of bursal tissue at 7th dpc of broiler chickens vaccinated or non-vaccinated with rHVT-IBD-ND and or challenged with vvIBDV at 23rd days of age.

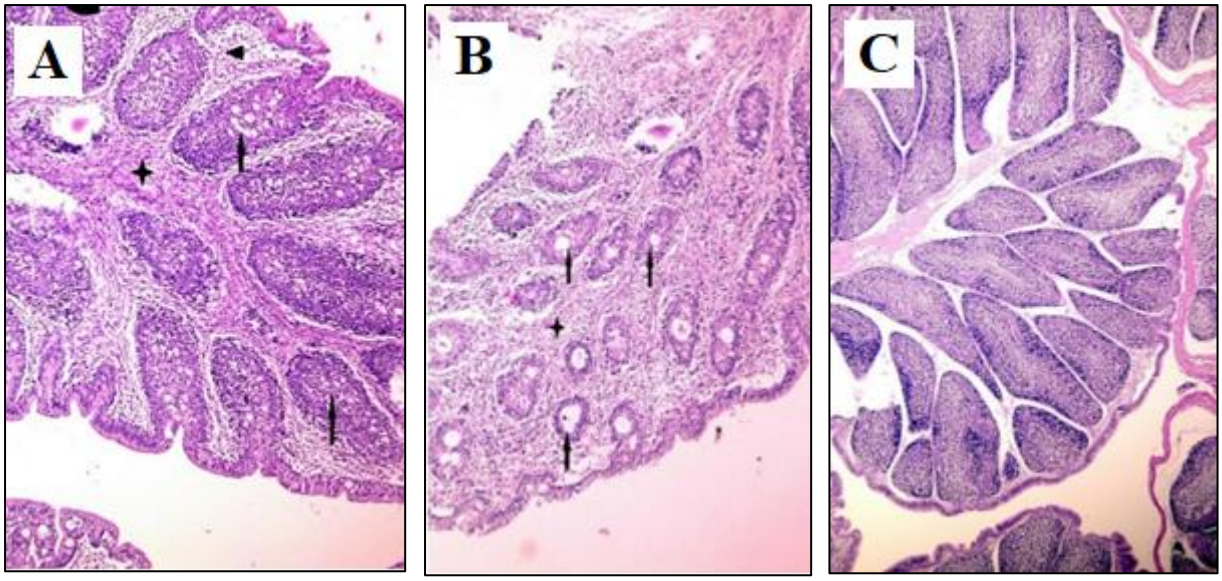

Fig. 4. Histopathological lesions of bursae of Fabricius 7 days post challenge, **(A):** G1 (vaccinated challenged) showed slight depletion of lymphocytes with micro cysts formation (arrow), and inter-follicular edema and inflammatory cells infiltration (triangle), with connective tissue formation (star) (H&E x 100). (**B**): G3 (non-vaccinated challenged) showed severe lymphocytic depletion and necrosis with compressed follicles, cysts formation (arrow), and inter-follicular edema with inflammatory cells infiltration (H&E x 100). (**C**): G4 (control non-vaccinated non-challenged) showed apparently normal architectures (H&E x 100).

4. DISCUSSION

Very virulent Infectious bursal disease (vvIBD) is a highly contagious disease of young chickens that still causing a devastating economic loss in worldwide poultry industry consists of high mortality alongside with severe bursal damage which in turn lead to severe immunosuppression and increase susceptibility to secondary infections (Van den Berg et al., 1991; Kumar et al., 2002; Jackwood et al., 2012). The disease is mainly controlled through effective vaccination. However, many commercially available vaccines are used in poultry industry which could provide complete or partial clinical protection; some of them have many drawbacks specially modified live vaccines. MLV vaccines unlikely can cause a varied degree of bursal atrophy with subsequent immunosuppression can occurs (Abd El-Razik., 2004; Sultan et al., 2006; Muller et al., 2012; Sultan et al., 2012). In addition, they need proper timing to be administered as they may be neutralized by presence of MDA and may revert to virulent strain. Upon that, this study aimed to evaluate the protective efficacy of day-old vaccination of (Vaxxitek® rHVT-IBD-ND) vaccine contra challenge with recent vvIBDV isolate at 23 days of age in broiler chickens.

No clinical symptoms, gross lesions or mortalities were recorded in all vaccinated and non-vaccinated groups till the age of challenge suggesting that all groups were not subjected to a field infection with IBDV during the experiment till the time of challenge with vvIBDV at 23 days of age. On the other hand, at 7th dpc with vvIBDV at 23 days of age, the vaccinated groups either challenged (G1) or non-challenged (G2) in addition to the negative control group (G4) revealed complete clinical protection from clinical symptoms and no recorded mortality in comparison to the non-vaccinated challenged group (G3) which showed slight depression, anorexia and ruffling feather with 6.6% mortalities**.** All dead birds showed hemorrhages on the thigh and/or pectoral muscles and the bursa was covered with gelatinous exudates**.** These results indicated that day-old vaccination with rHVT-IBD-ND vaccine conferred complete clinical protection against clinical signs, mortalities and postmortem gross lesions produced by vvIBD challenge virus which come in agreement with the previous studies by (Bublot et al., 2007; Sultan et al., 2012; Rashid et al., 2013; Roh et al., 2016; Gelb et al., 2016; Yakout, 2024; Wang et al., 2024).

Regarding to the effect of rHVT-IBD-ND vaccine on bursa, there were no significant difference in bursal body weight ratio (BBR) or bursa index (BI) between vaccinated and non-vaccinated groups before challenge suggesting that these birds were not subjected to any field infection before challenge. Moreover, these results ensuring that the rHVT-IBD-ND vaccine replicated without affecting bursal tissue or producing bursal lesions. These results agreed with (Bublot et al., 2007) who mentioned that HVT-IBD vaccine had negligible impact on the bursa of Fabricius when compared with IBD MLV and is able to protect chickens against various IBDV challenge strains including very virulent, classical, and USA variant IBDV even in presence of high MDA at the time of vaccination. The same results were also obtained by (Rashid et al., 2013) who revealed that the vaccination of VAXXITEK®HVT+IBD vaccine did not damage the bursa of broilers with higher protection levels specially at 21 and 28 days in comparison with IBD-

BLEN® vaccine. Moreover, the BBR and BI were significantly reduced in vvIBDV challenged groups (G1) and (G3) after challenge with vvIBDV at 23 days of age in comparison with non- challenged groups (G2) and (G4) which indicated that the local field isolate cause severe bursal atrophy as described by (Sultan, 1995). Furthermore, a significant reduction in the BBR and BI was observed after vvIBDV challenge in the non-vaccinated challenged group (G3) which showed (0.55 & 0.37) for BBR and BI, respectively in comparison with the vaccinated challenged group (G1) which recorded (1.45 & 0.79) for BBR and BI, respectively. In addition to the vaccinated non-challenged group (G2) which showed (1.93 & 0.98) for BBR and BI, respectively, clarifying that day- old vaccination with Vaxxitek® rHVT- IBD-ND vaccine can protect the birds from bursal atrophy after challenge with vvIBDV. These results came in agreement with (Bublot et al., 2007; Sultan et al., 2012; Roh et al., 2016; Yakout, 2024).

Serological response to IBD was screened using BD+ ELISA test at 1st, 7th, 14th, 23rd days of age and at 7th dpc with vvIBDV. ELISA is the most reliable test for estimation of IBD immune response due to its' high specificity and sensitivity which is commonly used for confirmation of the infection of the birds with IBDV (WOAH, 2024). The immune response to IBD expressed as mean ELISA titer was decreased by the age due to waning of MDA till the age of 23 days which started to increase in the vaccinated groups (G1 and G2) due to the effect of vaccination versus to continuous decline in the non-vaccinated groups (G3 and G4). On the other hand, there was a significant increase in the serological immune response in the vaccinated groups (G1 and G2) in comparison with non-vaccinated groups (G3 and G4) at 7 days post challenge and the mean ELISA titers were (13092, 12157, 6230 and 750) in G1, G2, G3 and G4, respectively. These results agreed with (Lee et al., 2015). Furthermore, the same results were obtained by (Rashid et al., 2013) who revealed that the vaccination of VAXXITEK® HVT+IBD could provide a higher protection levels specially at 21 and 28 days in comparison with IBD- BLEN® vaccine. Furthermore, (Gelb et al., 2016) mentioned that protection was not induced until 18th days post vaccination with Vaxxitek HVT IBD of SPF leghorns and continued to protect 22 DPV and 26 DPV.

Results revealed a significant reduction in the bursal lesions and mean severity index (1.5) was recorded in G1 (vaccinated challenged) which showed mild lymphocytic depletion in comparison with G3 (non-vaccinated challenged) which showed a severe lymphocytic depletion and necrosis and compressed bursal follicles with cysts formation with significantly higher MSI (2.5) These results indicated that Partial protection against bursal tissue damage were obtained after day-old vaccination with Vaxxitek® rHVT-IBD-ND vaccine in broiler chickens challenged with vvIBDV at 23rd days of age. The same results were obtained by (Rashid et al., 2013; Gelb et al., 2016; Roh et al., 2016).

5. CONCLUSIONS

This study concluded that vaccination of broiler chickens with rHVT-IBD-ND vaccine at one-day-old provides complete clinical protection against vvIBDV clinical signs, mortalities and gross lesions beside partial protection from bursal tissue damage.

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