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Poultry diseases

A Comparative Study on Winterfield 2512-G61 and Winterfield-2512 IBDV Immune-complex Vaccines in Commercial Broiler Chickens

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ABSTRACT

Infectious bursal disease (IBD) is a very dangerous immunosuppressive disease affecting poultry production worldwide. The good vaccination program that can overcome the maternal derived antibodies (MDA) in young chicks is the main method to control this disease. In this study, the immunogenicity and protective efficacy of two IBDV immune-complex (ICX) vaccines were evaluated against challenge with very virulent IBDV. A total of 240-day-old commercial broiler chicks (Cobb-500) were divided into 3 groups (80 birds in each group). The first and second groups were vaccinated subcutaneously (s/c) on day-1 with Winterfield 2512-G61 and Winterfield-2512 ICX vaccines, respectively, the third group left as non-vaccinated control group and all groups were challenged on day-35 with vvIBDV local field isolate (GenBank accession no.KX646373), The protection assessment based on mortality rate, clinical signs, postmortem gross lesions, bursa to body weight ratio (BBR), seroconversion and mean severity index (MSI) of histopathological lesion scores was evaluated. The results revealed that both vaccines provide complete protection against mortality and clinical signs after challenge with vvIBDV. In addition, the partial protection against bursal atrophy, the (BBR) were (0.76, 0.95 and 1.27) versus (0.49, 0.56 and 0.25) in Winterfield 2512-G61, Winterfield-2512 vaccinated and non-vaccinated groups before challenge and 7-days post-challenge, respectively. The results of this study indicate that the vaccination with Immune-complex vaccines can provide complete protection against mortality and clinical signs; they also provide partial protection against bursal atrophy and histopathological lesions and give adequate immune response against challenge with the Egyptian vvIBDV.

INTRODUCTION:

The infectious bursal disease virus (IBDV) is the causative agent of Gumboro disease, a highly contagious chicken immunodeficiency disease that causes great economic losses for poultry production worldwide (Pikuła et al., 2020). The virus is a member of the family Birnaviridae, a non-enveloped with a bi-segmented double-stranded RNA family (Muller et al., 1979). The disease was firstly discovered in the USA in 1962 (Cosgrove, 1962), and then it was first recorded in Egypt in 1974 (El-Sergany et al. 1974).

Vaccination is the main method to control IBDV in chickens worldwide, however the maternally

derived antibodies is the main obstacle to the vaccination process. (Sedeik et al. 2019), so new generation of vaccines such as immune complex (ICX) vaccines have been developed. They are mixture of a certain amount of polyclonal IBDV-specific antibodies obtained from the sera of hyperimmunized chickens and live intermediate plus IBDV (Whit-fill et al., 1995). The ICX vaccines are suitable for injection in fertilized eggs at day 18 of incubation with the Inovoject® machine or subcutaneously injection at the first day of age in the hatchery (Haddad et al., 1997 and Ivan et al., 2005). Both methods of vaccination allowed a more systematic and automated administration process than the conventional live vaccines that are usually given via the drinking water or by eye-drop in some rare cases. At challenge, the experimental protective efficacy of the ICX vaccines was identical to or better than that induced by live IBDV vaccines (Jeurissen et al., 1998). With both vaccines, the virus was coupled with B-lymphocytes, macrophages and follicular dendritic cells in the BF and spleen, although IBD virus complexing with specific antibodies caused a 5-days delay in virus detection (Jeurissen et al., 1998). In another study (Ivan et al., 2005), the virus was firstly discovered in the bursa of vaccinated SPF chickens on day-14 post-vaccination and on days 17 to 21 in chickens with maternally derived antibodies. The most remarkable thing was the low level of bursal and splenic B-lymphocytes depletion in chickens vaccinated experimentally with IBDV immune-complex vaccine (Jeurissen et al., 1998).

The in-ovo applied antigen is taken up by the embryo orally and transferred into the gastrointestinal system and the lungs, unlike in the situation following subcutaneous application. Therefore, it can be assumed that different types of cells, lymphoid tissues, and/or mechanisms may participate in the immune response against the same antigen that result in different patterns of virus replication. It should also be taken into consideration that ICX vaccines of different companies may contain different quantities of specific antibodies (in view of the confidentiality of the composition) (Jochemsen and Jeurissen, 2002). Also the IBDV coated with different units of antibodies showed different degrees of replication. Also, the quantity of antibodies in the IBDV ICX vaccines could affect the onset and degree of virus replication (Kumar and Charan, 2001). This study was designed to evaluate the immunogenicity and efficacy of two commercial infectious bursal disease immune-complex vaccines: Winterfield 2512-G61 and Winterfield-2512 against challenge with recent field vvIBDV isolate (GenBank accession no.KX646373) in commercial broilers.

MATERIALS AND METHODS

Virus and vaccines:

Two immune-complex vaccines, i) Winterfield 2512-G61 (Transmune®, batch no. 2203H4D1KNHE) (Ceva, France) obtained from local agency (Ceva, Egypt) and ii) Winterfield 2512 (Bursa-Plex®, serial no. 278841) (Zoetis Inc, USA) obtained from local agency (International free trade corporation, Egypt) contain a specific volume of IBDV antisera with 24 antibody units mixed with a specific volume of virus suspension containing 100 mean embryo infectious dose (EID_{50}) of IBDV. They were all administered s/c to one-day-old chicks at the hatchery.

The previously identified and characterized local field isolate of vvIBDV **(GenBank accession no. KX646373)** was supplied by birds and rabbits medicine department (Faculty of Veterinary Medicine, University of Sadat city, El-Minoufia, Egypt). The virus was used for the challenge at a dose of 100µl containing $10^{3.5}$ egg infective dose (EID_{50}) per bird (50µL via the ocular route and 50 µL using nasal drops) at 35-days of age.

Experimental design:

One-day-old commercial broiler (Cobb-500) chicks (n=240) were purchased from commercial hatchery that had maternal derived antibodies against IBDV acquired from their parents which evaluated by ELISA. Chicks were divided into 3 groups (80 birds in each group). Two groups, (G1) and (G2) were subcutaneously vaccinated on day-1 with Winterfield 2512-G61 and Winterfield-2512 immune-complex vaccines, respectively at a volume of (0.2ml/chick). The chicks of the third group (G3) were injected subcutaneously with (0.2ml) of phosphate buffered saline (PBS) as a control. All groups were challenged on day 35 with 100µl of $10^{3.5}$ (EID_{50}) of vvIBDV **(GenBank accession no. KX646373)** per bird (50 µL via the ocular route and 50µL using nasal drops). Serum samples

were collected on days 7,14,21,28,34 post-vaccination and on day-7 post-challenge (10 chicks from each group) for antibodies monitoring. Six bursal samples were collected from each group on days 34 post-vaccination and on day-7 post-challenge for evaluation of bursa to body weight ratio (BBR) and histopathologic examination. The birds were observed for clinical signs, mortality rates and gross lesions for 7 Days post challenge as shown in **(Table 1).** All experimental procedures with laboratory animals were approved by the ethical committee of Sadat City University and Animal Health Research Institute.

Table 1. Experimental design of the efficacy of IBDV immune complex vaccines against recent vvIBDV (GenBank accession no.KX646373) in commercial broilers in Egypt.

Group NO.	NO. of birds	Vaccination regime			Assessment of protocol
		Age/ day	type	Route/ dose	
G1	75	1	Winterfield 2512-G61	S/C	1- Clinical signs. 2- Mortality %. 3- Gross lesions. 4- B/BR. 5- Seroconversion. 6- Histopathology.
G2	75	1	Winterfield -2512	S/C	
G3	75	---	---	---	

B/BR= Bursal body weight ratio **(Sharma et al., 1989)**
ELISA= Enzyme linked immunosorbent assay
S/C = Subcutaneous

Bursa to Body Weight ratio:

Six birds from each group were selected randomly on days 34 post-vaccination and on day-7 post-challenge, weighed and taken for a P.M. examination. Bursae were collected and weighed individually to calculate the bursa/body weight ratio. The ratio was calculated according to the equation of = bursa weight (gram)/bird weight (gram) X 1000. The bursa/body weight index was also calculated according to the equation of = (Bursa/body weight ratio of each bird)/(Mean Bursa/body weight ratio of uninfected control birds) according to **(Sharma, et al., 1989).**

Serology:

Enzyme-linked immunosorbent assay (ELISA) test was performed by using Commercial indirect ELISA kits (ID-VET, France) to determine the maternal derived antibodies (MDA) and antibody response of the vaccines in serum samples collected on days 7, 14, 21, 28, 34 post-vaccination and on day-7 post-challenge. According to the manufacturer's instructions, if ELISA titer is lower than 875, the IBD-immune status was considered negative.

Histopathologic examination:

Tissue samples (Bursa of Fabricius) were fixed in 10% buffered formalin (**Bancroft et al., 1996)**, processed for histology by routine procedures, embedded in paraffin, sectioned using a microtome into slices and stained with hematoxylin and eosin (H&E). The severity of bursal lymphoid tissue lesions (Mean severity index) (MSI) were scored from zero to 4 according to lymphoid necrosis and/or lymphocytic depletion of the lymphoid follicles according to **Sharma et al. (1989)** as follows, 0 = less than 5% of the lymphoid follicles (per field) affected, 1=5 - less than 25% of the lymphoid follicles (per field) affected, 2= 25 - less than 50% of the lymphoid follicles (per field) affected, 3= 50 - less than 75% of the lymphoid follicles (per field) affected. 4= more than 75% of the lymphoid follicles (per field) affected.

Statistical analysis:

The significance of differences between individual treatments and corresponding control were determined by data analysis using (ANOVA) where the significance level was set at $p \leq 0.05$.

Results:

Clinical signs and mortality:

No mortalities or clinical signs were recorded in all groups, either vaccinated against vvIBD or not, till the challenge age. The non-vaccinated challenged group showed depression, anorexia, ruffling feathers, whitish diarrhea and soiled vents from the third day post challenge and 10% mortality but the vaccinated challenged groups did not exhibit clinical symptoms or mortalities during this period **(Table 2).**

Gross lesions:

All bird taken for post-mortem examination before challenge did not show any gross lesions, but the dead birds of the non-vaccinated challenged group showed hemorrhage on the thigh and pectoral muscles, hemorrhage at the proventriculus-gizzard junction, bursa covered with gelatinous exudate and an enlarged spleen but the vaccinated challenged birds did not exhibit any macroscopic lesions.

The vaccinated birds showed significant (P≤0.05) decrease in BBR than the non-vaccinated birds before challenge, that was (0.76, 0.95 versus 1.27) in winterfield 2512-G61, winterfield-2512 vaccinated and non-vaccinated group, respectively. On day-7 post challenge there was significant (P≤0.05) decrease in BBR of non-vaccinated challenged group than the vaccinated challenged groups, that was (0.49, 0.56 versus 0.25) in winterfield 2512-G61, winterfield-2512 vaccinated and non-vaccinated group, respectively **(Table 2).**

Histopathology:

Before challenge, the non-vaccinated group did not show any significant histopathological lesions but significantly moderate (P≤0.05) lesions were observed in bursa of the vaccinated groups, the lesion ranged in all vaccinated group from interfollicular edema, hyperplasia of the linning epithelium and mild lymphocytic depletion and necrosis **(Fig. 2-4).** The MSI were significantly (P≤0.05) higher in vaccinated bird than the non-vaccinated birds, that were (0.6, 0.55 versus 0.2) in winterfield 2512-G61, winterfield-2512 vaccinated and non-vaccinated groups, respectively **(Table 2).**

On day-7 post challenge, the non-vaccinated challenged group showed severe lymphocytic depletion and necrosis, but the vaccinated challenged groups showed mild to moderate histopathological lesions (**Fig. 5-7)**. The MSI of winterfield 2512-G61 vaccinated birds was higher than the winterfield-2512 vaccinated birds but the MSI of the non-vaccinated challenged birds was significantly (P≤0.05) higher than the vaccinated challenged groups, that were (1.5, 1 versus 3) in winterfield 2512-G61, winterfield-2512 vaccinated and non-vaccinated groups, respectively **(Table 2).**

Table 2. Mortality rate, Bursal body weight ratio and mean severity index of commercial broiler chickens vaccinated with winterfield 2512-G61 or winterfield-2512 immune-complex vaccines and non-vaccinated group, challenged on day-35 with local field isolate of vvIBDV (GenBank accession no.KX646373).

Groups	Mortality %		BBR		MSI	
	Pre-challenge	7dpch	Pre-challenge	7dpch	Pre-challenge	7dpch
G1	0	0	0.76±0.16c	0.49±0.12b	0.60±0.05a	1.5±0.02b
G2	0	0	0.95±0.11b	0.56±0.13a	0.55±0.04b	1±0.01c
G3	0	10	1.27±0.13a	0.25±0.12c	0.2±0.04c	3±0.02a

G1= vaccinated with winterfield 2512-G61 ICX vaccine. **G2**= vaccinated with winterfield-2512 ICX vaccine

G3= non-vaccinated control group

Means within the same column of different litters are significantly differ at ($P < 0.05$)

IBD challenge virus=Oculnasal challenge at 35 day of age with 100µl/bird contain 103.5 EIDS-50 of IBDV local field isolate

B:BR= Bursal body weight ratio**(Sharma et al., 1989)**

MSI= mean severity index **SI**= severity index of bursal lymphoid tissue lesion **(Sharma et al., 1989)**

7Dpch= 7-days post-challenge

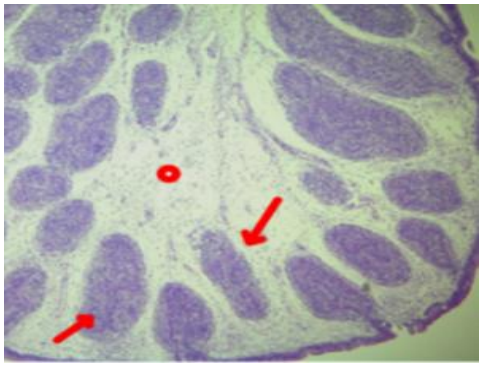

Fig. 2. Histopathological lesions of bursae of fabricius of G1 (Winterfield 2512-G61) on day 34 post vaccination showed depletion of lymphocytes, compressed follicles (arrow) and interstitial edema with inflammatory cells infiltration (circle) H&E X50.

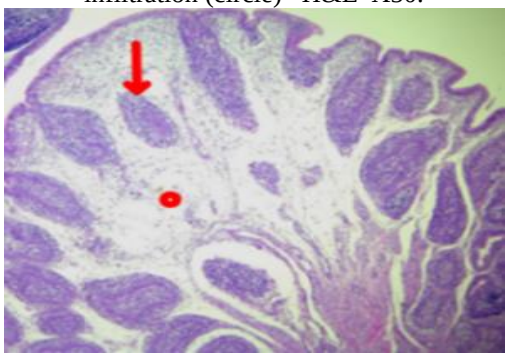

Fig. 3. Histopathological lesions of bursae of fabricius of G2 (Winterfield-2512) on day 34 post vaccination showed depletion of lymphocytes, compressed follicles (arrow) and interstitial edema with inflammatory cells infiltration (circle) H&E X50.

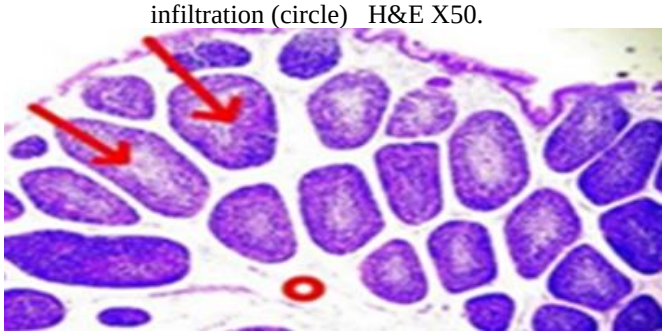

Fig. 4. Histopathological lesions of bursae of fabricius of G3 (Control) on day 34: Apparently normal follicles (arrow) with mild edema (circle) H&E X50.

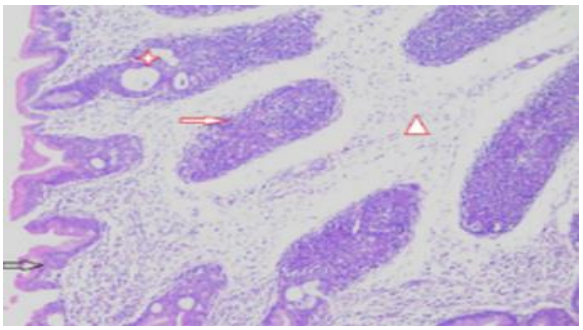

Fig. 5. Histopathological lesions of bursae of fabricius of G1 (Winterfield 2512-G61) 7 days post challenge showed hyperplasia of epithelium (black arrow) with cysts (star), depletion of lymphocytes (red arrow), compressed follicles and interstitial edema with inflammatory cells infiltration (triangle) H&E X100.

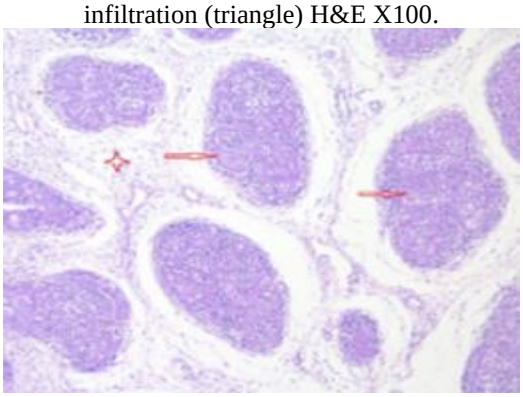

Fig. 6. Histopathological lesions of bursae of fabricius of G2 (Winterfield-2512) 7 days post challenge showed polyps of hyperplasia of lining epithelium (black arrow) with depletion of follicular lymphocytes (red arrow) and interstitial edema with inflammatory cells infiltration (star) H&E X100.

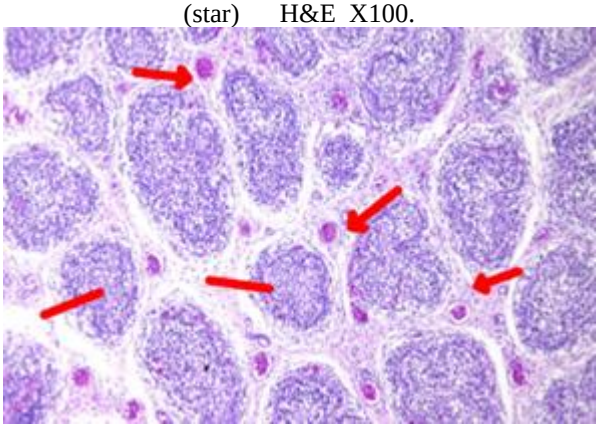

Fig. 7. Histopathological lesions of bursae of fabricius of G3 (Control) showed depletion of lymphocytes (line), interstitial edema with inflammatory cells infiltration and congested blood vessels (arrow) H&E X100.

Immune response:

The MDA of non-vaccinated birds waned and become negative from the third week of age till the time of challenge on day-35. The antibody titers of winterfield 2512-G61 vaccinated birds decreased gradually and start to increase from the 5th week of age, the titers were (4155±16.70, 2967±15.22, 2359±21.22, 2312±18.14 and 3198±17.14) on days 7, 14, 21, 28 and 34, respectively. The antibody titers of winterfield-2512 vaccinated birds decreased gradually and start to increase from the 4th week of age, the titers were (4369±19.50, 3056±18.19, 1996±11.12, 3415±12.19 and 4355±14.17) on days 7, 14, 21, 28 and 34, respectively **(Table 3 and Fig. 1).**

The serological response of the birds on day-7 post-challenge showed no significant differences ($P \leq 0.01$) between all vaccinated challenged and

non-vaccinated challenged groups, that were (5289±18.14, 5862±15.14 and 5046±14.17) in winterfield 2512-G61 and winterfield-2512 vaccinated challenged and non-vaccinated challenged groups, respectively **(Table 3 and Fig. 1).**

Table 3. Immune response of commercial broiler chickens vaccinated with winterfield 2512-G61 or winterfield-2512 immune-complex vaccines and non-vaccinated group, challenged on day-35 with local field isolate of vvIBDV (GenBank accession no.KX646373).

IBD vaccination regime			ELISA antibody titer					
Freq.	Age	Type	Age (days)					
			7	14	21	28	34	42
1x	1	Winterfield 2512-G61	4155±16.70C	2967±15.22B	2359±21.22A	2312±18.14B	3198±17.14B	5289±18.14B
1x	1	Winterfield-2512	4369±19.50B	3056±18.19A	1996±11.12B	3415±12.19A	4355±14.17A	5862±15.14A
--	--	Non-vaccinated Challenged	4459±15.17A	1456±12.22C	725±11.12C	663±7.18C	549±11.12C	5046±14.17C

Means within the same column of different litters are significantly differ at ($P < 0.01$)
Freq. = Frequency of vaccination.

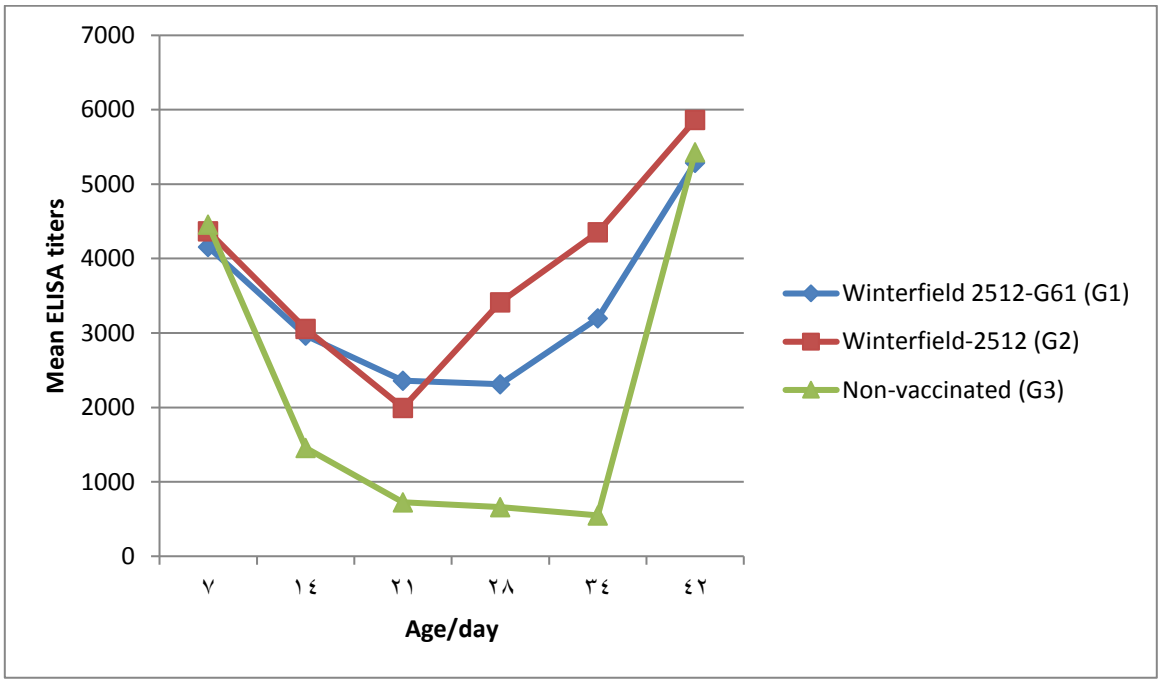

Fig. 1. Mean ELISA titers (±SD) of broiler chickens vaccinated with winterfield 2512-G61 or winterfield-2512 immune-complex vaccines and non-vaccinated group, challenged on day-35 with local field isolate of vvIBDV (GenBank accession no.KX646373)

Discussion:

Infectious bursal disease is a highly contagious immunosuppressive disease that affects chickens and result in huge economic losses every year. The half-life of the MDA and their homogeneity or heterogeneity are essential to determine the optimal time of vaccination (Block et al., 2007). So new generation of vaccines developed called immune-complex vaccines that contain mixture of the live attenuated strain of IBDV with specific antibodies.

In the present study we evaluated the efficacy of two types of ICX vaccines (winterfield 2512-G61 and winterfield-2512) against challenge with recent Egyptian local field isolate vvIBDV (GenBank accession no.KX646373) by assessing several parameters such as clinical signs, mortality rate, postmortem gross lesions, bursal

atrophy (BBR), MSI of histopathological lesions and IBD antibody titer.

The vaccinated groups had no mortalities and did not show any clinical signs or gross lesions before challenge, but the destructive effect of the ICX vaccines appeared in the bursal atrophy and the significant ($p \leq 0.05$) increase of the MSI of the histopathological lesions in comparison with the non-vaccinated group, these observations attributed to the effect of the intermediate plus IBDV strain in the ICX vaccines that make bursal atrophy, Several studies agreed with these results like (Kumar et al. 2000) who reported that the live IBD vaccines, either intermediate or intermediate plus, have a destructive effect with various degrees on the bursa, inducing transient immune suppression. Furthermore, Haddad et al., (1997) obsereved a decrease in the BBR of the ICX vaccinated bird, also the bursae of ICX vaccinated group showed histologic changes that consisted of diffuse follicular atrophy, cortical and medullary lymphocyte depletion, macrophage infiltration and epithelial enfolding. Another study reported that the ICX vaccinated commercial birds exhibited bursal depletion (starting on day-33), less severe than the vaccinated SPF chicks, in which depletion started 7 days post-vaccination (Iván et al., 2001).

On day-7 post challenge the non-vaccinated challenged group showed acute typical clinical signs, and gross lesions with 10% mortality in comparison with the vaccinated challenged groups which had no clinical signs, gross lesions or mortalities. The same results obtained by (Haddad et al., 1997) who demonstrated that the vaccination of broiler chickens with IBDV-ICX vaccine can fully protect them against the challenge virus because no acute signs or mortalities were observed after challenge and this also observed in other studies (Palya et al., 2004; Sameeh, 2017 and Gharam, 2019).

The BBR decreased significantly ($p < 0.05$) in ICX vaccinated and non-vaccinated challenged groups indicated that the local field isolate cause bursal atrophy (Sultan, 1995), these results confirm the findings of (Conway et al., 2007) who reported that the bursae of all vaccinated groups were atrophied post challenge with indices below 0.7, which indicated that none of these vaccines could prevent damage of the bursa of Fabricius, similar results were reported by (Chansiripornchai et al., 2009 and Gharam, 2019) for the immune-complex vaccines. Moreover, the BBR were significantly ($p < 0.05$) lower in non-vaccinated challenged group than the other vaccinated challenged groups, these results agreed with (Corley et al., 2001) who studied the protection of the in-ovo vaccinated SPF embryos by the IBDV-ICX vaccine, they noticed when compared control unchallenged birds, the IBDV-ICX vaccine caused significant bursal atrophy ($p<0.05$) and protected only 12.5% of the bird that were challenged.

The MSI on day-7 post challenge revealed highly significant differences ($p< 0.05$) between vaccinated and non-vaccinated challenged groups that was (1.5,1 versus 3), respectively, which indicated that the immune-complex vaccines give partial protection against bursal damage, similar results obtained by (Herczeg et al., 2011; Sameeh, 2017 and Gharam, 2019).

The waning of MDA was monitored in all vaccinated and non-vaccinated groups to actually detect the time at which the active immune response stimulated, the MDA of the non-vaccinated birds declined and became negative (according to the ELISA Kit manufacturer's protocol) on day-21, the same results reported by (Sadrzadeh et al., 2005) who found that the MDA declined in the non-vaccinated groups at 21 days of age as MDA level on chicks usually wane within 15–20 days post hatching (Palya et al., 2004; Chansiripornchai et al., 2009 and Zorman et al., 2011). The antibody titer of Winterfied 2512-G61 vaccinated group started to grow up in the fifth week post vaccination and was significantly ($P\leq 0.01$) lower than the titer of Winterfied-2512 vaccinated group which started to increase in the fourth week post vaccination, the same results obtained by (Palya et al., 2004) who studied the immunogenicity of (Winterfield 2512-G61) ICX vaccine and noticed that the antibody titer start to grow through the third week of age, but the significant increase was started from the beginning of the fifth week of age, also (Sedeik et al., 2019) noticed that the immunocomplex vaccine resulted in poor immune response at three weeks of age, which may be explained by the virus in the vaccine being still bound by some virus neutralizing factor resulting in poor active immunity (Bose et al., 2003) ; but they increased significantly at 28 days of age. On day-7 post challenge, significant seroconvertion ($p< 0.01$) occurred in all

vaccinated and non-vaccinated challenged groups, but there were no significant differences ($p< 0.01$) between each other that were the same observations of the studies carried out by (Sedeik et al., 2019). In conclusion, the use of ICX vaccines at one day old was safe and gave complete protection against mortality and clinical signs, also provide partial protection against bursal atrophy and histopathological lesion against the challenge with Egyptian vvIBDV isolate, but the winterfield-2512 ICX vaccine gave higher and earlier immune response than the winterfield 2512-G61 ICX vaccine.

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