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Protective Efficacy of IBDV Winterfield H-2512 and SYZA-26 Immune-complex Vaccines against Recent Egyptian Very Virulent IBDV in Commercial Broiler Chickens

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ABSTRACT

Infectious bursal disease is a highly contagious disease that affects chickens and results in huge economic losses worldwide. This study aimed to compare protection obtained by vaccination with two different IBDV immune-complex (ICX) vaccines. 225 one- day-old broiler chicks were divided in to 3 groups (75 birds in each group). Group 1 and 2 were vaccinated with Winterfield H-2512 and SYZA-26 ICX vaccines, respectively, according to manufacturer's instructions, the third group left as a control group. 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. Our results revealed that chickens vaccinated with immune-complex vaccines (G1 and G2) were completely protected clinically with no mortality compared with control non vaccinated chickens (G3) that showed typical IBDV clinical signs with (30%) mortality. In addition the BBR was significantly lower in SYZA-26 vaccinated birds than Winterfield H-2512 vaccinated and non-vaccinated birds, at 1-day pre-challenge, but at 7-days post-challenge the BBR was significantly lower in the non-vaccinated challenged group than the vaccinated challenged birds. The results of this study indicate that both Immune-complex vaccines can provide complete protection against mortality and clinical signs, but the Winterfield H-2512 ICX vaccine had a lesser effect on the bursa and higher immune response than the SYZA-26 ICX vaccine against challenge with the Egyptian vvIBDV.

INTRODUCTION

Infectious bursal disease is one of the most economically important contagious diseases of young chickens caused by infectious bursal disease virus (IBDV) and characterized mainly by high mortalities, damage in bursa of Fabricius and immunosuppression (Eterradossi and Saif, 2013). This virus is a member of the family Birnaviridae: a non-enveloped with a bi-segmented double-stranded RNA (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). The Variant and vvIBD virus strains are the most dangerous antigenic mutants of IBDV that threaten chickens, causing high economic losses and vaccination failure because their

irreversible immunosuppressive effect on the young chicks (Withers et al., 2005).

IBDV is a very resistant virus and can survive in poultry houses after proper disinfection so isolation and sanitation is not sufficient for poultry production to control the virus (Benton et al., 1967). Therefore, the vaccination control policy is the main method of controlling IBD in chickens, serotype-I live vaccines of the mild and intermediate types used for vaccination of replacement pullets and broiler and the inactivated oil-emulsion vaccines were used to maintain immunity till the laying time in breeder pullets (Gao et al., 2011), but the interference of MDA with vaccine uptake remains a major problem in vaccination against IBD using live vaccines (Block et al., 2007). In order to help in effective prevention of IBDV, new vaccines technologies have been developed and introduced into the market (Meeusen et al., 2007), Vaccines consisting of a mixture of a certain amount of IBDV-specific antibodies and live intermediate plus IBDV called immune-complex (ICX) vaccines are a new generation of IBD virus vaccines (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). The differences between in-ovo vaccinations or the vaccination at hatch are that the tissue distribution was more extensive and virus levels in tissues were higher if the vaccine was given in embryonated eggs (Sharma, 1986). Also Komine et al., (1995) demonstrated that the vaccine efficiency and virus detection after vaccination depend on the mode of application.

It had been supposed that the IBDV-specific antibodies in the ICX shield the vaccine IBDV from maternal antibodies and other mechanisms that lead to reduction of the viral load available for induction of the immune response (White et al., 1975). Also it was suggested that the ICX vaccine virus can infect the chickens either after the maternal antibodies decayed or before by breaking through the maternal antibodies at a certain stage of antibody decay (Haddad et al., 1997). The IBDV coated with different units of antibodies showed different degrees of replication. Consequently, the amount of antibodies in the IBDV-ICX could exert an influence on the onset and degree of virus replication (Kumar and Charan, 2001). The present study aimed to evaluate the efficacy of two commercial infectious bursal disease immune-complex vaccines, Winterfield H-2512 and SYZA-26 against challenge with recent Egyptian field isolate of vvIBDV (GenBank accession no.KX646373) in commercial broilers.

Materials and methods:

Virus and vaccines:

Two immune-complex vaccines, i) Winterfield H-2512 (Gallivac-BDA®, serial no. LK042B) (Boehringer Ingelheim, INC., Germany) obtained from local agency (International free trade corporation, Egypt) and ii) SYZA-26 (Novamune®, batch no. 007HR1) (Ceva, France) obtained from local agency (Ceva, Egypt).

The previously identified and characterized local field isolate of vvIBDV (GenBank accession no.KX646373) was supplied by Dr. Hesham Sultan prof. of poultry diseases department of birds and rabbits medicine, Faculty of Veterinary Medicine, Sadat City University. The virus was used for the challenge at a dose of 100µl containing $10^{3.5}$ egg infective dose (EID_{50}) per bird.

Experimental design:

225 one- day-old ross broiler chicks were purchased from commercial hatchery that had maternal derived antibodies against IBDV. Chicks were divided into 3 groups (75 birds in each group). Two groups, (G1) and (G2) were subcutaneously vaccinated on the first day at hatch with Winterfield H-2512 and SYZA-26 immune-complex vaccines, respectively according to manufacturer instructions. Chicks of Group 3 were injected subcutaneously with (0.2ml) of phosphate buffered saline (PBS) as a control. All groups were challenged at 35-day of age with 100µl of $10^{3.5}$(EID_{50}) of vvIBDV **(GenBank accession no. KX646373)** per bird (50 µL via the ocular route and 50µL via nasal route). The birds were observed for clinical signs,

mortality rates and gross lesions for 7 Days post challenge as shown in **(Table 1)** The MDA waning in these chicks were followed from one-day-old until 42-day of age by ELISA.

Table (1): Experimental design for assessment of protection of commercial broiler chickens vaccinated on one-day-old with immune-complex vaccines against challenge with vvIBDV local field isolate **(GenBank accession no. KX646373)** at 35-day of age.

Group NO.	NO. of birds	Vaccination regime			Assessment of protocol
		Age/ day	type	Route/ dose	
G1	75	1	Winterfield H-2512	S/C	1- Clinical signs. 2- Mortality %. 3- Gross lesions. 4- B/BR. 5- Seroconversion. 6- Histopathology.
G2	75	1	SYZA-26	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 and 42 of age. 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).

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 $p \leq 0.05$ in BBR and MSI but was at $p \leq 0.01$ in ELISA results.

Results:

Clinical protection (clinical signs and mortality):

All groups either vaccinated or not did not show any clinical signs or mortalities before the age of challenge. The non-vaccinated challenged group showed the typical clinical picture of IBDV such as huddling together, depression, ruffling feathers, anorexia, whitish diarrhea, and soiled vents from the third day post challenge and 30% mortality but the vaccinated challenged groups did not exhibit clinical signs or mortality during this period **(Table 2).**

Pathology and histopathology:

Dead birds showed typical IBD gross lesions, like hemorrhage on the thigh and 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 bursa to body weight ratio (BBR) of commercial broiler chickens in G1 and G2 were 1.1 and 0.86 respectively versus 1.35 in G3, at 1-day pre-challenge, but on day-7 post-challenge the BBR in G1 and G2 were 0.67 and 0.62 respectively versus 0.29 in G3 **(Table 2 & Fig. 1).**

Before challenge, no histopathological lesions (either lymphocytic depletion and/or necrosis) were observed in the non-vaccinated birds, but significantly moderate (P≤0.05) lesions were observed in bursa of the vaccinated groups, the lesion ranged in all vaccinated group from mild lymphocytic depletion, interfollicular edema and hyperplasia of the linning epithelium **(Fig. 4-6).** The Mean severity index (MSI) in G1 and G2 were (0.6 and 0.8) respectively versus (0.3) in G3 **(Table 2 & Fig. 2)**

On day-7 post challenge, the non-vaccinated challenged group showed significant severe histopathological lesions than the vaccinated challenged groups (**Fig. 7-9**). The MSI in G1 and G2 were (1 and 0.5) respectively versus (2.5) in G3. **(Table. 2 & Fig. 2)**

Table 2. Mortality rate, Bursal body weight ratio and mean severity index of commercial broiler chickens vaccinated with winterfield H-2512 or SYZA-26 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	1.1 ± 0.12^{b}	0.67 ± 0.13^{a}	0.6 ± 0.04^{b}	1 ± 0.03^{b}
G2	0	0	0.86 ± 0.16^{c}	0.62 ± 0.12^{a}	0.8 ± 0.03^{a}	0.5 ± 0.02^{c}
G3	0	30	1.35 ± 0.11^{a}	0.29 ± 0.14^{b}	0.3 ± 0.04^{c}	2.5 ± 0.03^{a}

G1= vaccinated with winterfield H-2512 ICX vaccine.
G2= vaccinated with SYZA-26 ICX vaccine
G3= non-vaccinated control group
Means within the same column of different litters are significantly differ at (P < 0.05)
Mort. = mortality
B: BR= Bursal body weight ratio **(Sharma et al., 1989)**
MSI= mean severity index **(Sharma et al., 1989)**
7Dpch= 7-days post-challenge

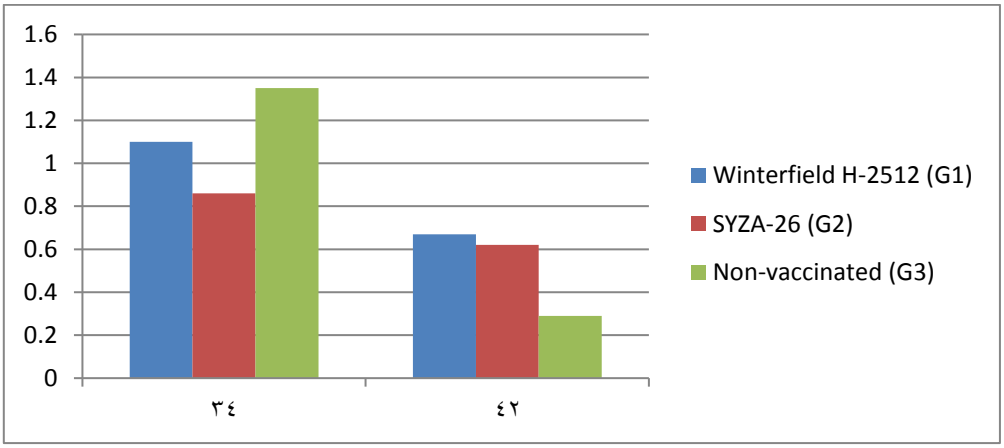

Fig. 1. Bursa to body weight ratio of broiler chickens vaccinated with winterfield H-2512 or SYZA-26 immune-complex vaccines and non-vaccinated group, at 1-day pre-challenge and 7-days post-challenge with local field isolate of vvIBDV (GenBank accession no.KX646373)

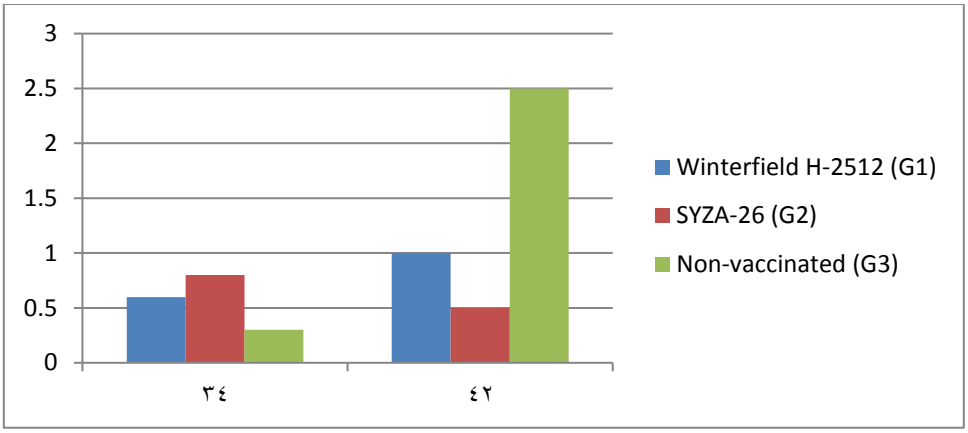

Fig. 2. Mean severity index of broiler chickens vaccinated with broiler chickens vaccinated with winterfield H-2512 or SYZA-26 immune-complex vaccines and non-vaccinated group, at 1-day prechallenge and 7-days post-challenge with local field isolate of vvIBDV (GenBank accession no.KX646373).

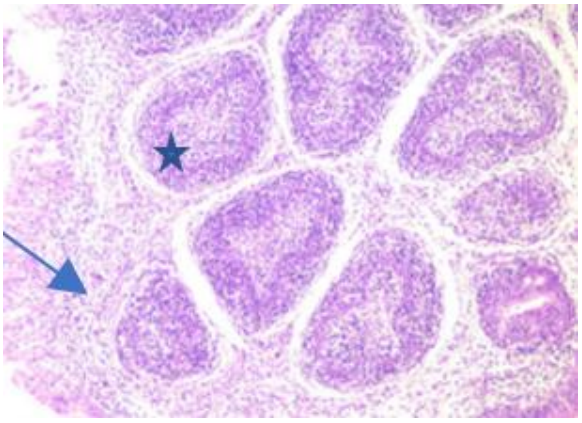

Fig. 4. Histopathological lesions of bursa of fabricius of G1 (Winterfield H-2512) on day-34 post vaccination showing hyperplasia of lining epithelium, interfollicular edema with inflammatory cells infiltration (arrow) , depletion of lymphocytes (star) H&E X100.

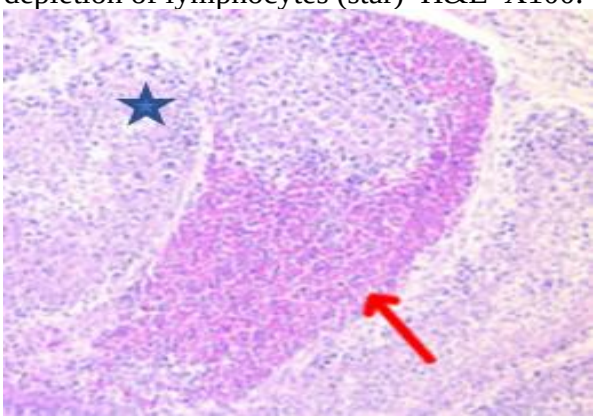

Fig. 5. Histopathological lesions of bursa of fabricius of G2 (SYZA-26) on day-34 post vaccination showing depletion of lymphocytes in cortex and medulla with granulocytes infiltration (arrow) and proliferation of corticomedullary epithelium (star) H&E X200.

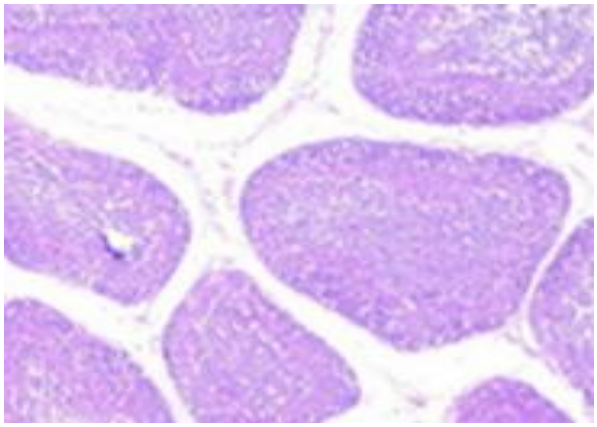

Fig. 6. Histopathological lesions of bursa of fabricius of G3 (Control) on day-34 post vaccination (Control non-vaccinated): apparently normal architecture H&E X100.

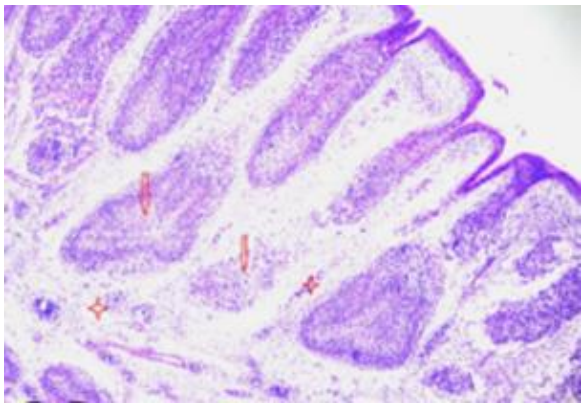

Fig. 7. Histopathological lesions of bursa of fabricius of G1 (Winterfield H-2512) 7 days post challenge showing depletion of lymphocytes in cortex and medulla, compressed follicles (arrow) and interstitial edema with inflammatory cells infiltration (star) H&E X50.

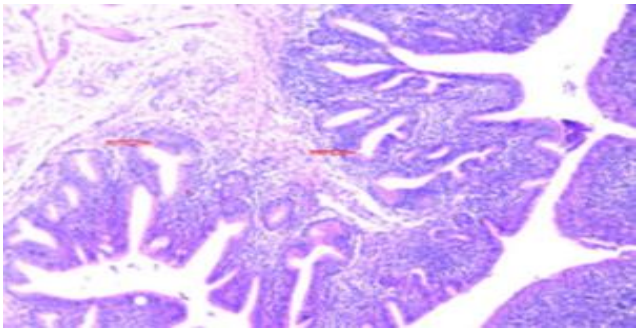

Fig. 8. Histopathological lesions of bursa of fabricius of G2 (SYZA-26) 7 days post challenge showing hyperplasia of lining epithelium and epithelization (arrow) H&E X100.

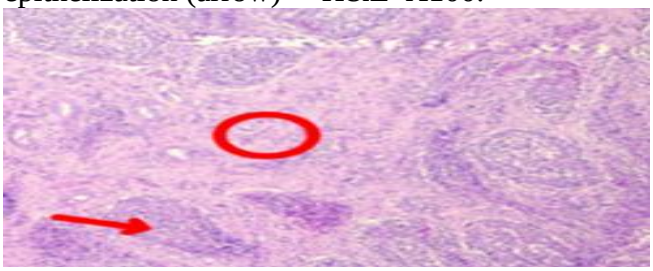

Fig. 9. Histopathological lesions of bursa of fabricius of G3 (Control) 7 days post challenge showing showing depleted follicles (arrow) interstitial connective tissue proliferation (circle) H&E X100.

Immune response:

The waning of maternal antibody in commercial broiler chickens used for vaccination experiment judged by ELISA titers, which decreased gradually and became negative the third week of age till the time of challenge. The winterfield H-2512 ICX vaccine induced a significant ($p \leq 0.01$) increase in IBDV antibodies compared to the SYZA-26 ICX vaccine that were (2278 vs 2126), (2481 vs 1360) and (3589 vs 1666) on days 21, 28 and 34 respectively **(Table 3 and Fig. 3).**

On day-7 post-challenge there were no significant differences ($P \leq 0.01$) in seroconversion between all vaccinated challenged and non-vaccinated challenged groups, that were (5896, 5657 and 5237) in G1, G2 and G3, respectively **(Table 3 and Fig. 3).**

Table 3. Immune response of commercial broiler chickens vaccinated with winterfield H-2512 or SYZA-26 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 H-2512	4105±19.25^{c}	2998±15.25^{b}	2278±15.22^{a}	2481±16.19^{a}	3589±15.16^{a}	5896±12.13^{a}
1x	1	SYZA-26	4437±17.34^{b}	3012±12.41^{a}	2126±16.17^{b}	1360±10.14^{b}	1666±16.17^{b}	5657±17.14^{b}
--	--	Non-vaccinated Challenged	4589±14.18^{a}	1198±13.32^{c}	614±10.15^{c}	576±8.12^{c}	512±12.14^{c}	5237±15.13^{c}

Means within the same column of different litters are significantly differ at ($P < 0.01$)

Freq. = Frequency of vaccination.

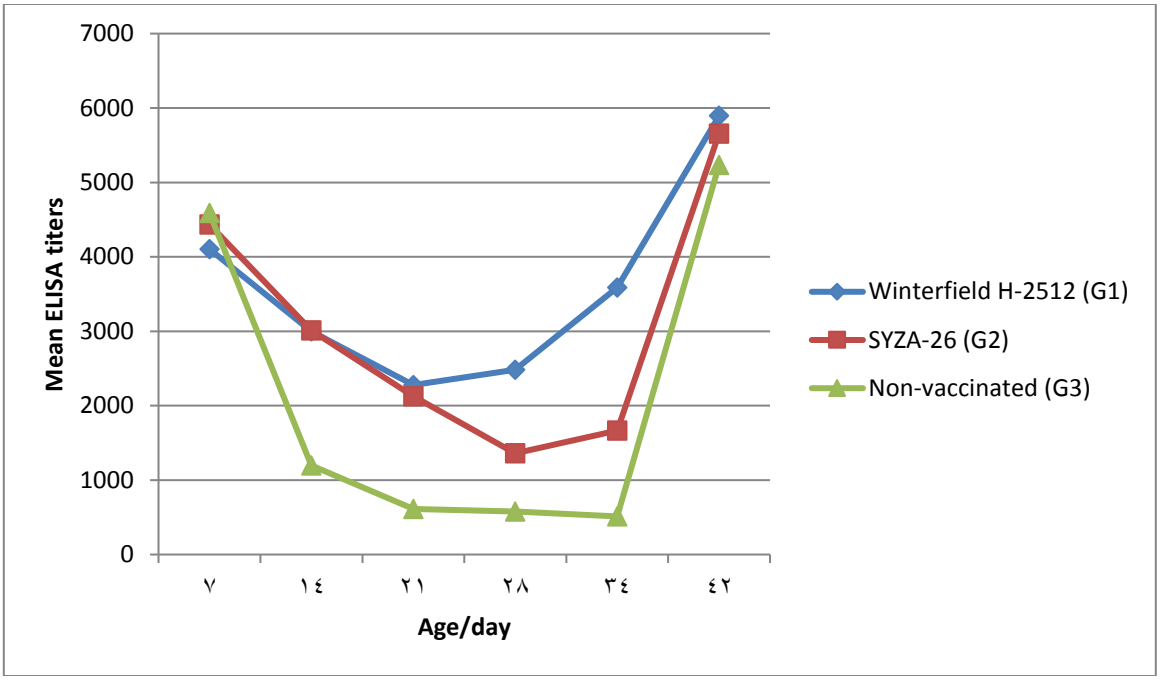

Fig. 3. Mean ELISA titers of broiler chickens vaccinated with winterfield H-2512 or SYZA-26 immune-complex vaccines and non-vaccinated group, challenged on day-35 with local field isolate of vvIBDV (GenBank accession no.KX646373)

Discussion:

There are a lot of vaccination programs for the control of IBD that differ in the vaccine strain, timing and route of administration, frequency, and vaccine interference by MDA. The half-life of the MDA and their homogeneity or heterogeneity are essential to determine the optimal time of vaccination (Block et al., 2007). However, many farmers apply different IBD vaccination strategies, especially live vaccines, without determining the MDA titers; therefore in this study the efficacy of some commercial ICX vaccines was evaluated in commercial broiler chickens against challenge with recent local field vvIBDV isolate, the assessment of protection based on clinical signs, mortality percentage, postmortem gross lesions, Bursa body weight ratio, seroconversion and mean severity index (MSI) of histopathological examination.

The vaccinated groups had zero mortalities and did not show any clinical symptoms or postmortem gross lesions before challenge, but on day-7 post challenge the non-vaccinated challenged group showed acute typical clinical signs, and gross lesions with 30% mortality in comparison with the vaccinated challenged groups which had no clinical signs, gross lesions or mortalities. Several studies showed the same results 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 (Haddad et al., 1997; Palya et al., 2004; Sameeh, 2017 and Gharam, 2019).

The IBDV vaccinal effect on bursa of fabricius is one of the most important parameters used in evaluation the efficacy of these vaccines. The ICX IBDV vaccine contains hot strain of IBDV, which leads to high pathogenic effect on the Bursa of fabricius. The BBR in both vaccinated groups were significantly ($p < 0.05$) lower than the non-vaccinated control group before the day of challenge, the same results obtained by (Haddad et al., 1997) who studied the pathogenicity of IBDV ICX vaccine in presence of MDA against challenge with vvIBDV in comparison with classic live attenuated vaccine, Furthermore, Camilotti et al. (2016) compared the pathogenicity of IBDV ICX vaccine, vector vaccine and live attenuated vaccine, they noticed that the bursae of the chickens vaccinated with the ICX vaccine exhibited severe bursal atrophy.

On day-7 post challenge the BBR decreased in all vaccinated and non-vaccinated challenged groups, which indicated that the local field isolate cause severe bursal atrophy (Sultan, 1995), these results confirm the findings of (Conway et al., 2007) who noticed that the bursae were atrophied in all vaccinated groups 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 ICX vaccines. Moreover, the BBR were significantly ($p \leq 0.05$) lower in non-vaccinated challenged group than the ICX vaccinated challenged groups, these results agreed with (Corley et al., 2002) who noticed the significant decrease in BBR in non-vaccinated challenged birds than vaccinated challenged birds.

Both ICX vaccinated groups showed mild histopathological lesions in the bursa with significant ($p \leq 0.05$) increase of the MSI than the non-vaccinated control group at 1-day prechallenge, the same findings obtained by (Haddad et al., 1997) who noticed that the bursae of ICX vaccinated group showed histologic changes that consisted of diffuse follicular atrophy, cortical and medullary lymphocyte depletion, macrophage infiltration and epithelial infolding. The MSI on day-7 post challenge revealed highly significant differences ($p \leq 0.05$) between vaccinated and non-vaccinated challenged groups that was (1, 0.5 versus 2.5), 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).

Serologically, the MDA decreased gradually in the non-vaccinated control group and become negative at 21-days of age. The same results reported by (Sadrzadeh et al., 2005) who found that the MDA declined to negative level in the non-vaccinated groups at 21 days of age as MDA level on chicks usually wane after 7–14 days or within 15–20 days post hatching (Palya et al., 2004; Chansiripornchai et al., 2009 and Zorman et al., 2011). The ELISA antibody titers follow up in vaccinated non challenged groups indicated that the winterfield H-2512 vaccinated group antibody titers increased from the 4^{th} week post-vaccination and were significantly ($p \leq 0.01$) higher than the SYZA-26 vaccinated group (which slightly increased from the 5^{th} week post-vaccination) at days 21, 28 and 34 post-vaccination. These results are similar to the results obtained by (Whitfill et al., 1995; Johnston et al., 1997; Sameeh, 2017 and Gharam, 2019) who made studies on BDA-IBDV immune-complex vaccine and found that After maternal antibody levels waned between Day 21 to 28 of age, the BDA-IBDV vaccinates went on to develop high geometric mean antibody titers against IBDV especially after challenge with vvIBDV. Furthermore, (Felfoldi et al., 2019) reported that Serological results showed that the SYZA-26 ICX IBD vaccine-take was reached between 28 and 35 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 protective efficacy of the ICX IBDV vaccine against the challenge with a recent vvIBDV local field isolate was evaluated in this study. The ICX vaccines has provided protection for commercial broiler chicks against clinical signs, mortality but not against bursal atrophy after challenge with the vvIBDV strain. Also the winterfield H-2512 ICX vaccine give faster and higher immune response than the SYZA-26 ICX vaccine, so the vaccination with winterfield H-2512 ICX vaccine more effective than the vaccination with the SYZA-26 ICX vaccine in commercial broiler chickens.

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