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EFFICACY OF DAY-OLD OCULAR VACCINATION AGAINST VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS IN COMMERCIAL BROILERS

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SUMMARY

The effect of day old ocular vaccination with live intermediate infectious bursal disease virus (IBDV) vaccine was tested in commercial broiler chicks that have maternally derived antibodies (MDA) against infectious bursal disease virus (IBDV). Chicks were challenged with very virulent IBDV (vvIBDV) either at 24 days of age after being vaccinated at 1 and / or 14 days or at 31 days of age of those vaccinated at 1 or 14 and /or 21 days. The assessment of protection was determined by measuring, bursa / body weight (B: B) ratio, bursal index (BI), mean severity index (MSI) of bursal lymphoid tissue lesions and mortality rate at 7 days post-challenge (Pch), in addition, antibody response to IBDV at 14 days Pch.

Vaccination at 21, 14 & 21 and 1, 14 & 21 days of age protected 100% of vaccinated commercial broiler chickens only against mortality of vvIBDV. However, none of the different vaccination regimes protected commercial broiler chickens neither from bursal atrophy nor bursal lesions. Serum IBDV antibody levels, as monitored by Enzyme-Linked Immunosorbent Assay (ELISA), showed similar rates of decline among non-vaccinated and all the vaccinated groups and by day 35 PV, serum antibody level in non-vaccinated and vaccinated groups were below detectable levels. Results of these studies indicate that IBDV vaccination at one day of age via eye drop doesn't protected against mortality, bursal atrophy and lesions and doesn't cause accelerated IBDV specific MDA. Moreover, the serological examination of optimal vaccination time for each flock is required to control of vvIBDV in the field.

INTRODUCTION

Infectious bursal disease (IBD) is one of the most

important viral infections occurring in young chickens. The disease is caused by infectious bursal disease virus (IBDV), a member of Birnaviridae family (Lukert and Saif (2003). Two IBDV serotypes (1 and 2) that naturally infected chickens have been recognized. However, only strains of IBDV belonging to serotype 1 are considered pathogenic for chickens (McFerran et al., 1980; Jackwood and Saif, 1983; Jackwood et al 1985). IBDV is a lymphotropic pathogen with a special predilection for differentiating cells in the bursa of Fabricius. Infection can induce B-cells apoptosis, necrosis, and bursal atrophy with concomitant suppression of the humoral response (Sivanadan and Masheswaran, 1980; Muller, 1986; Jungmann et al., 2001). Damage to the bursa may occur with a severe inflammatory response such as the one described for standard IBDV strains (Lasher and Shane, 1994; Tanimura et al., 1995). However, atrophy of this organ may be induced with little or no inflammation (Tanimura et al., 1995).

In spite of intensive vaccination programs to prevent chickens from being infected with IBD, flocks infected with IBDV still occur throughout the world. The emergence of wide spectrum of IBDV strains (very virulent in Euorpe and recently in Egype and variants in USA) has resulted in the failure of protection by current IBDV vaccines in broiler even those having high titers of maternal antibodies (WU et al., 2001).

The IBD vaccination at one day old and its relation with the maternal antibodies have been previously reported. Lukert and Rifuliadi (1982) found that the IBD maternally immune chickens (one-day-old) given virulent and attenuated IBDV elicited active response to IBDV with high level by 10th weeks of age. This active response apparently was due to persistence of the virus until the maternal levels fall to a low point at 4-weeks.

Van den Berg and Meulemans (1991) concluded in their study that, even after intensive live vaccination and inactivated oil emulsion booster of parent hens, it is not possible to protect the progeny during the whole growing period and even when protecting against mortality, MDA may not prevent bursal damage. Moreover, Coletti et al. (2001), in Italy, evaluated the efficacy and safety of an IBDV intermediate vaccine used via in-ovo route. They found that the vaccine induced active immunity and protected SPF chickens from challenge but the protection was not complete in commercial chickens, as examined by bursal lesions, bursal index post challenge and vaccine immune response.

In Egypt, El-Sergany et al. (1974) reported for first time the occurrence of IBDV infection in commercial broiler chickens on the basis of pathological and serological examination, and Ayoub and Malek (1976) isolated and identified the causative virus. In 1990, El-Batrawi was the first to report the emergence of severe outbreaks of vvIBDV since summer of 1989 in vaccinated and

non-vaccinated chicken flocks of the foreign and native varieties associated with drastic mortalities. Subsequently several workers described similar outbreaks in various Governorates with severe pathological lesions and high mortalities up to 70% in replacement commercial layer pullets and up to 30% in meat-type chickens (Khafagy et al., 1990 and 1991; Ahmed, 1991; Sultan,1995; Hassan et al., 2002 and Fares, 2003).

Different vaccination regimes in commercial broiler chickens using live intermediate IBDV vaccines have been applied in the field including vaccination with one and/or two vaccine doses at day-one and 9 to 20 days of age. In the present study, evaluation was carried out to assess the relative effect of day-old vaccination in commercial broiler chicks with live-IBDV ìintermediateî vaccine via eye drop in protection against vvIBDV challenge.

MATERIALS AND METHODS

Chickens:

Sufficient, one-day-old commercial broiler (Ross) chicks were produced from a commercial hatchery (EL-Wadi Company), which possessed maternal antibodies against IBD, acquired from their parents that were vaccinated with live and inactivated oil emulsion IBDV vaccines according to a specific vaccination program. The chicks were floor reared under natural daylight in strictly isolated experimental rooms, previously cleaned and disinfected and were provided with commercial broiler starter ration. Water and feed were provided ad libtum. Chicks were used for the following purposes:

a-Serological follow up of maternally derived antibodies by ELISA to determine maternal antibody waning and the age at which the chicks become susceptible to experimental infection or vaccination. .

b-Laboratory vaccination experiments.

Reference antigens and antisera:

Known positive and negative precipitating antigens in the form of bursal homogenates and known positive and negative precipitating reference antisera against IBDV obtained from Intervet, Inter. B.V.Boxmeer, Holland, were used for the AGPT.

IBD viruses:

a- Commercial live IBD intermediate vaccine (Lukert strain, Bioimmune, U.S.A.) obtained from the local agency (Tradimpex Egypt), was used in vaccination.

b- A local field isolate of vvIBDV isolated and identified by Sultan (1995), in the form of bursal extract was diluted 1: 10 in phosphate buffer saline, which killed 72 % of 7-week-old susceptible commercial male chickens, was

passed once in 7-week-old susceptible egg-type male chickens for propagation and was used for challenge in the form of bursal homogenate given intraocular in a dose of 100µl / bird. The virus was designated as (S-95).

Blood samples:

Chicken blood samples for serological tests were collected. The collected samples were prepared and the sera were kept frozen at ñ20 until used.

ELISA kits:

Commercial ELISA kits ProFlock supplied by Synbiotics Corporation, 11011 via Frontera, San Diego. CA 92127. They were used for measuring maternal antibody decline to estimate accurately the time of early age vaccinations and to evaluate the vaccine responses.

Agar gel precipitation test:

The test was used to detect of IBDV antigen (s) in the cloacal bursa of affected chickens as described by Wood et al. (1979).

Laboratory vaccination experiments:

For this purpose, commercial broiler chicks, from one hatch was used. The maternal antibody waning in those chicks was followed up at different intervals starting from 1 day up to 44 days of age. They were examined individually by ELISA. Twelve groups, each of which was 10 chicks were vaccinated and/or challenged at different ages according to the experimental design in the following table:

The experimental design of determination of the serological response and degree of protection following vaccination of IBD-susceptible commercial broiler chicks with live "intermediate" vaccines via eye drop and challenge with vvIBDV:

Groups Treatment	Vaccination regimes		IBDV Challenge (Age /days)	Assessment of protection			
	Freq.	Age/ days		Observation for 14 days Pch	Serology	Antigen detection	Histopathology (SI)
Challenged – Vaccinated	1X 1X 2X	1 14 1 , 14	24	1-clinical signs. 2-Mortality rate. 3-Gross lesions. 4-B: B* ratio for survivors at 7 days Pch	1-Follow up of maternal derived antibodies (MDA) 2-Seroconversion at 14 days Pch.	Pool of bursal homogenates of dead birds	Lesion scores for survivors at day 7 Pch.
	1X 1X 1X 2X 3X	1 14 21 14 , 21 1,14,21	31				
Challenged non-vaccinated	-- --	-- --	24 31				
Non-treated	--	--	--				

Freq.: Frequency.
P ch.:Post-challenge.
SI: Severity index of bursal lymphoid tissue lesions (Sharma et al., 1989).
B: B ratio= Bursal body weight ratio Sharma et al. (1989).
X= Number of vaccinations.
* vvIBDV isolated and identified in 1995 (Sultan, 1995).

Assessments of protection against IBDV challenge:

1- Clinical signs; mortality rate as well as post-mortem gross lesions were recorded.

2- Detection of IBDV antigen (s) in the cloacal bursa of dead birds.

3- Bursa: body weight ratio, and bursa: body weight index were calculated by the formulas given respectively by Sharma et al. (1989) and Lucio and Hitchner (1979) as follows:

-Bursa: body weight ratio (B: B) = Bursal weight / Body weight X 1000

-Bursa: body weight index (BI) = bursa/body weight ratio of infected chickens / mean bursal body weight ratio of uninfected chickens.

Chickens with bursa: body weight index lower than 0.7 were considered by Lucio and Hitchner (1979) to have bursal atrophy.

4-Histopathological examination: specimens of the bursae were fixed in 10% neutral formaline, and then treated chemically with different concentration of alcohol and xylol. Paraffin sections were obtained by rotatory microtome. Tissue sections were stained with Harris hematoxyline and eosine according to Bancroft et al. (1990).

The severity of bursal lymphoid tissue lesions were scored from 0 to 4 on the basis of lymphoid necrosis and/or lymphocytic depletion according to Sharma et al. (1989) as follows:

0= less than 5% of the lymphoid follicles (per field) affected,

1= 5-25% of the lymphoid follicles (per field) affected.

2= 25-50% of the lymphoid follicles (per field) affected.

3= 50-75% of the lymphoid follicles (per field) affected.

4= More than 75% of the lymphoid follicles (per field) affected.

5- Seroconversion to vaccination and/or infection was also followed up in those groups by ELISA.

Stastical analysis:

Wherever necessary data were analyzed by analysis of variance followed by application of Duncan's new multiple range tests after Steel and Torie (1960) to determine the significance of different between individual treatment and at corresponding controls.

RESULTS

Results of MDA waning and serological response:

Table (1) shows that MDA decline in commercial broiler chickens from IBD-vaccinated parents. The low means of ELISA titers were obtained by 35 (752±131.11). Moreover, instead, IBDV vaccination in all vaccination regimes, ELISA titers showed similar rates of antibody decline in all

vaccinated and non-vaccinated groups.

Results of mortality and degree of protection:

The mortalities in vaccinated groups of chickens, which were challenged at 24 or 31 days of age and vaccinated one time either at 1 or 14 or 21 days of age, were 2/10 , 3/10, 1/10, 1/10 and 0/10, respectively, versus 2/10, 2/10, 2/10, 2/10 and 2/10 in non-vaccinated challenged groups (Table 2 &3) while the mortalities in vaccinated group of chickens, which were vaccinated two times either at 1, 14 or 14, 21 days of age were 1/10 and 0/10, respectively, versus 2/10 and 2/10 in non-vaccinated challenged groups. However, the mortalities in vaccinated group of chickens, which was challenged at 31 days of age and vaccinated three times at 1, 14 and 21 days of age, were 0/10 versus 2/10 in non-vaccinated challenged groups, respectively, (Table 3).

Table (2& 3) show that bursa / body weight ratio, bursa index and bursal lymphoid tissue lesions. It is evident that a significant ($P < 0.05$) decrease in bursal body weight ratio was found between challenged vaccinated groups and non-challenged control groups, moreover, BI in all challenged groups was lower than reference normal value of 0.7 at all vaccination regimes.

Severity index mean score value for bursal lymphoid tissue lesions were almost similar slightly higher in challenged non vaccinated groups as compared with challenged-vaccinated at all intervals of vaccination regimes, except groups of chickens, which were challenged at 31 days of age and vaccinated either one time at 21 or two times at 14&21 or three times at 1,14 & 21 days of age (2.6, 2.0, &2.0 versus (4.0, 4.0 & 4.0) in challenged non-vaccinated groups, respectively.

IBDV preciptinogen could be detected in bursae of birds which died within four days post challenged but not in those which survived 7 days post challenge.

Positive antibody response to vvIBDV challenged, either at 24 or 31 days of age, was evident as judged by ELISA test in all vaccinated and non-vaccinated challenged groups at 14 days Pch (Table 3).

Table (1): Results of waning of maternal derived antibody and serological response of commercial broiler chickens vaccinated one or two and / or three times with intermediate IBDV vaccine via eye drop:

Age / days	Vaccination regime		ELISA Titers		
	Frequency	Age / days	Range	means ± sd	% CV
1	-	-	9435 – 16229	13947 ± 634.25	16.62
7	- 1X	- 1	8346 – 15838 9385 - 11140	11741 ± 770.34 14282 ± 921.34	22.58 23.28
14	- 1X	- 1	3429 – 6115 1168 - 5607	4903 ± 811.21 3422 ± 645.37	18.19 46.10
21	- 1X 1X 2X	- 1 14 1 , 14	5753 – 7811 1384 – 7225 3970 – 8946 6863 - 9882	6479 ± 644.10 4329 ± 581.39 6267 ± 785.28 8292 ± 645.39	11.89 45.42 30.28 11.90
28	- 1X 1X 1X 2X 2X 3X	- 1 14 21 1 ,14 14, 21 1,14,21	18.88 – 5732 0 – 6191 3429 – 6115 1549 – 6819 1924 – 4860 0 – 8392 0 - 8381	3715 ± 814.72 2548 ± 702.08 4903 ± 825.42 5029 ± 864.71 3104 ± 644.35 3587 ± 778.62 5123 ± 609.33	34.28 81.61 18.19 38.45 33.73 89.30 61.56
35	- 1X 1X 1X 2X 2X 3X	- 1 14 21 1,14 14,21 1,14,21	0 – 2060 0 - 2051 0 – 1338 0 – 1636 0 – 3660 0 – 5116 0 - 1437	752 ± 131.11 773 ± 231.24 267 ± 212.73 1241 ± 351.77 1296 ± 192.83 1472 ± 251.75 715 ± 180.17	51.32 65.35 61.25 43.73 63.76 100.62 42.12
42	- 1X 1X 1X 2X 2X 3X	- 1 14 21 1,14 14,21 1,14,21	0 – 1517 0 – 1516 0 – 1125 0 – 1017 0 – 1046 0 – 1509 0 - 1621	303 ± 180.70 505 ± 187.18 225 ± 145.13 203 ± 153.14 209 ± 107.82 275 ± 133.45 324 ± 157.89	87.8 56.99 68.16 30.41 44.45 46.18 37.42

ELISA: Enzyme Linked Immunosorbent Assay.
X: Number of vaccination.
%CV: Coefficient of variation.
Sd.: Standard deviation.

Table (2): Results of mortality, bursal body weight ratio, bursal body weight index, severity index of lymphoid tissue lesions and serological response of commercial broiler chickens vaccinated one times with live intermediate IBD vaccine via eye drop and challenged at 24-days with vvIBDV .

Age of vaccination	Group treated	Mortality	Days post challenge			
			٧			14
			B.I Means ± Sd	B: BI	S I	ELISA titer Means ± Sd
1	Challenged- vaccinated	2 \ 10	0.729 ± 0.045[a]	0.509	4.0	1986 ± 619.5[a]
	Challenged- Non-vaccinated	2 \ 10	0.838 ± 0.122[a]	0.586	4.0	4370 ± 421.7[b]
	Non – Treated	0 \ 10	1.431 ± 0.301[b]	1.00	0.0	508 ± 45.72[c]
14	Challenged- vaccinated	1 \ 10	0.824 ± 0.150[a]	0.624	3.0	4942.0 ± 1064[a]
	Challenged- Non-vaccinated	2 \ 10	0.769 ± 0.108[a]	0.583	4.0	6347.2 ± 1415.1[a]
	Non – Treated	0 \ 10	1.32 ± 0.230[b]	1.00	0.0	218 ± 37.56[b]
1,14	Challenged- vaccinated	1 \ 10	٠,٨٦٠ ± ٠,١٣٦[a]	0.601	3.2	2022.6 ± 475.2[a]
	Challenged- Non-vaccinated	2 \10	0.838 ± 0.122[a]	0.586	4.0	2650.2 ± 541.2[a]
	Non - Treated	0 \ 10	1.431 ± 0.301[b]	1.00	0.0	508 ± 45.72[b]

*vvIBDV isolated and identified in 1995 (Sultan, 1995). B I: bursal index calculated after the formula of Sharma et al. (1989).
SI: severity index of bursal lymphoid tissue lesions after Sharma et al. (1989) Sd: standard deviation
B: BI: bursal body weight index calculated after the formula of Lucio and Hitchner (1979); values <0.7 indicated bursal atrophy
Any two means within the same age interval with the different superscripts are significantly different at $p \leq 0.05$.

Table (3): Results of mortality, bursal body weight ratio, bursal body weight index , severity index of lymphoid tissue lesions and serological response of commercial broiler chickens vaccinated two-times with live intermediate IBD vaccine via eye drop and challenged at 31-days of age with vvIBDV

Age of vaccination	Group treated	Mortality	Days post challenge			
			7			14
			B.1 Means ± Sd	B: BI	S I	ELISA titer Means ± Sd
1	Challenged- vaccinated	3 \ 10	0.657 ± 0.100[a]	0.498	4.0	ND
	Challenged- Non-vaccinated	2 \ 10	0.769 ± 0.108[a]	0.583	4.0	
	Non - Treated	0 \ 10	1.32 ± 0.230[b]	1.00	0.0	
14	Challenged- vaccinated	1 \ 10	0.618 ± 0.081[a]	0.515	3.0	
	Challenged- Non-vaccinated	2 \ 10	0.595 ± 0.139[a]	0.496	4.0	
	Non – Treated	0 \ 10	1.20 ± 0.294[b]	1.00	0.0	
21	Challenged- vaccinated	0 \ 10	0.741 ± 0.045[a]	0.561	2.6	10321 ± 2707[a]
	Challenged- Non-vaccinated	2 \ 10	0.769 ± 0.108[a]	0.583	4.0	6347 ± 1415.1[a]
	Non – Treated	0 \ 10	1.32 ± 0.230[b]	1.00	0.0	218 ± 37.56[b]
14, 21	Challenged- vaccinated	0 \ 10	٠.٧٢٢ ± 0.141[a]	0.547	2.0	9226.2 ± 607.2[a]
	Challenged- Non-vaccinated	2 \ 10	0.769 ± 0.108[a]	0.583	4.0	6347.2 ± 1415.1[a]
	Non - Treated	0 \ 10	1.32 ± 0.230[b]	1.00	0.0	218 ± 37.56[b]
1,14, 21	Challenge-vaccinated	0 \ 10	0.860 ± 0.049[a]	0.652	2.0	6228.8 ± 2013.9[a]
	Challenged- Non-vaccinated	2 \ 10	0.769 ± 0.108[a]	0.583	4.0	6347.2 ± 1415[a]
	Non – Treated	0 \ 10	1.32 ± 0.230[b]	1.00	0.0	218 ± 37.56[b]

*vvIBDV isolated and identified in 1995 (Sultan, 1995). B I: bursal index calculated after the formula of Sharma et al. (1989).
SE: standard deviation. SI: severity index of bursal lymphoid tissue lesions after Sharma et al. (1989).
B: BI: bursal body weight index calculated after the formula of Lucio and Hitchner (1979); values <0.7 indicated bursal atrophy.
ND: not done.
Any two means within the same age interval with the different superscripts are significantly different at $p \leq 0.05$

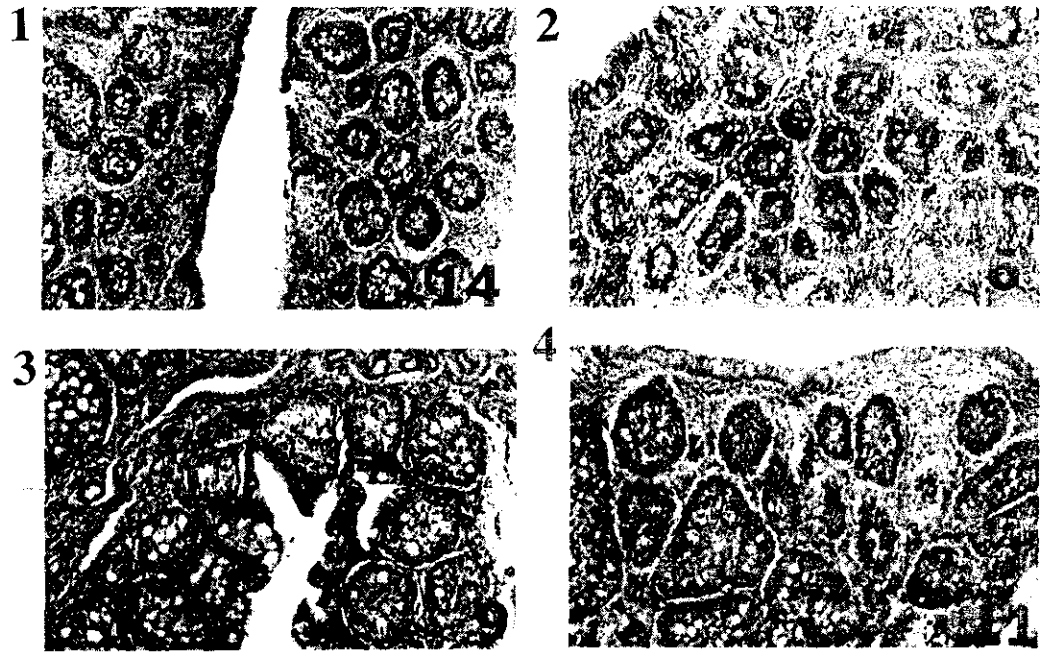

Fig. (1): Bursa of 31-day-old commercial broiler chicken experimentally infected with vvIBDV and vaccinated at one day old, showing severe edema and lymphocytes deple tion (H & E X100).

Fig.(2): Bursa of 24-day-old commercial broiler chicken experimentally infected with vvIBDV after vaccination at one day old, showing severe edema and lymphocytes depletion (H & E X).

Fig.(3): Bursa of 24-day-old commercial broiler chicken experimentally infected with vvIBDV and vaccination at 1, 14 day of age, showing severe hemorrhages, edema and lymphocytes depletion (H & E X100).

Fig.(4): Bursa of 31-day-old commercial broiler chicken experimentally infected with vvIBDV and vaccination at 1, 14 day of age, showing severe edema and lymphocytes depletion (H & E X100).

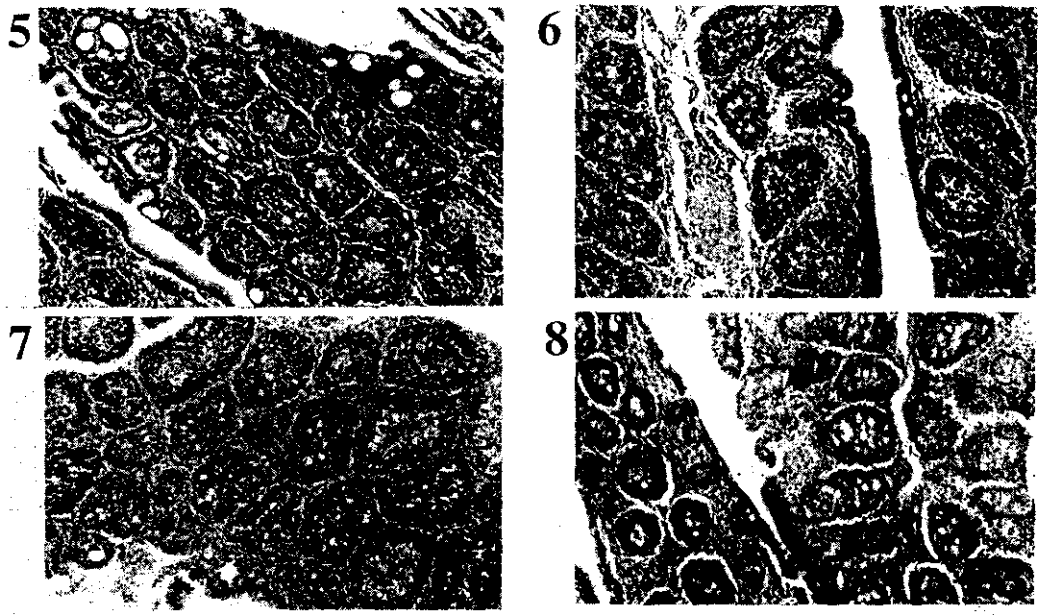

Fig.(5): Bursa of 31-day-old commercial broiler chicken experimentally infected with vvIBDV and vaccination at 1, 14 & 21 day of age, showing moderate edema and lymphocytes depletion (H & E X100).

Fig.(6): Bursa of 31-day-old commercial broiler chicken experimentally infected with vvIBDV and vaccinated at 21 day old, showing moderate edema and lymphocytes depletion (H & E X100).

Fig.(7): Bursa of 31-day-old commercial broiler chicken experimentally infected with vvIBDV vaccinated at 14 &21 day old, showing moderate edema and lymphocytes depletion (H & E X100).

Fig.(8): Bursa of 24-day-old commercial broiler chicken experimentally infected with vvIBDV and vaccinated at 14 day old, showing severe edema and lymphocytes depletion (H & E X100).

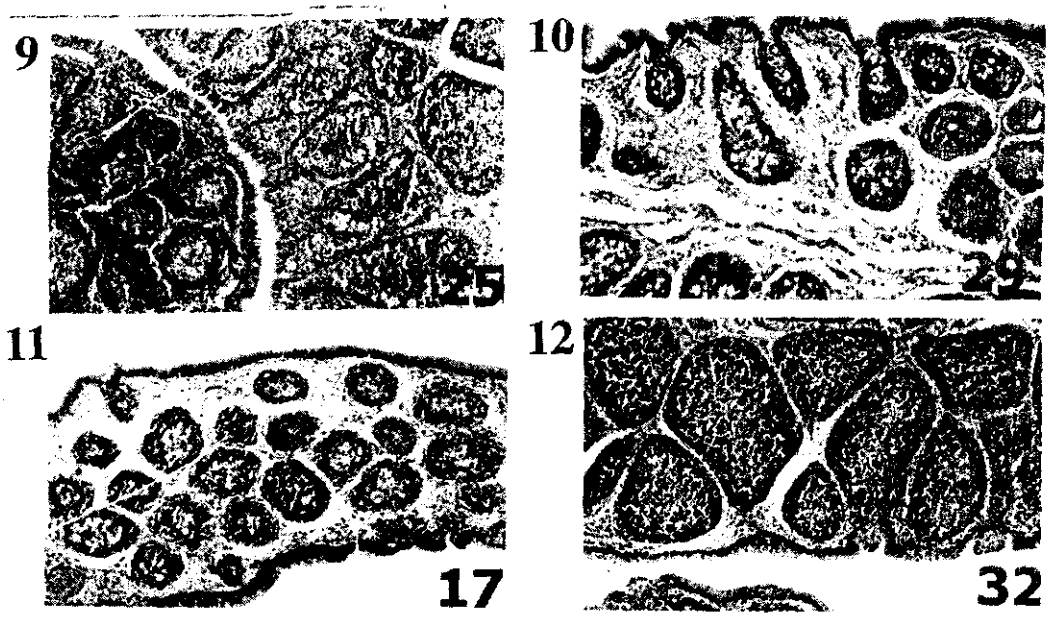

Fig.(9): Bursa of 31-day-old commercial broiler chicken experimentally infected with vvIBDV and vaccinated at 14 day old, showing severe edema and lymphocytes depletion (H & E X100).

Fig.(10): Bursa of 24-day-old commercial broiler chicken experimentally infected with vvIBDV (non-vaccinated) showing severe edema and lymphocytes depletion (H & E X100).

Fig.(11): Bursa of 31-day-old commercial broiler chicken experimentally infected with vvIBDV (non-vaccinated) showing severe edema and lymphocytes depletion (H & E X100).

Fig.(12): Normal bursa of 24-day-old commercial broiler chicken (non-infected non- vaccinated) (H & E X100).

DISCUSSION

Since 1987, acute IBD cause up to 30-60 % mortality in broiler and pullet flocks, respectively. These have been related to the emergence of a pathotype of IBDV known as very virulent virus (Box, 1989; Chettle et al., 1989; Van den Berg et al., 1991). IBD outbreaks with these characters appeared in Egypt and occurred since 1989 and have caused serious economic losses despite vaccination (El-Batrawi, 1990; Khafagy et al., 1991 and Sultan, 1995;El-Khiate, 2003).

In the present study, we analyzed the waning and the interference of MDA with different regimes of live intermediate IBDV vaccine in order to evaluate the optimal vaccination regime that could be given to the offspring, in addition, to investigate the development of immune response and build-up of protection in commercial broiler chickens following ocular vaccination with commonly used live IBDV intermediate vaccines. The evaluation of protection against vvIBDV challenge was assessment by the mortality rate, bursal/body weight ratio (B: B), bursal body weight index (BI) and the mean severity index (MSI) as relative criteria of effectiveness of tested vaccinal regime after vvIBDV challenge.

ELISA antibody mean titer reached a minimum level either in vaccinated or non-vaccinated experimental chicks by day 35 of age (Table-1).The results achieved in present study confirmed that MDA interfere with the development of active vaccination. Lukert and Rifuliadi (1982) in their study on the use of day- old vaccination in maternally immune chicks found that the vaccine had no effect on the level of MDA, moreover, the active immune response was observed at high level by the 10th week PV.

The effect of one day-of-age vaccination with IBDV alone or in combination with Marek's disease virus (MDV) in broiler chicks has been previously investigated (Knoblich et al., 2000). The results indicated that IBDV vaccination at 1 day of age does not cause accelerate IBDV-specific MDA decline as detected by ELISA but does appear to cause an accelerated decline in neutralizing IBDV-specific MDA.These serological findings strongly agree with our findings as shown in tables (1 and 2).Moreover, Wood et al. (1981) found that both high and low level of MDA prevented effective vaccination at 1 and 14 days of age, but by 28 days of age the vaccine was effective in birds of both initial antibody levels.

Indeed, 100% of birds vaccinated either one time at 21or two times at 14 & 21 and/or three times at1, 14 & 21 days of age versus 70-90% of bird vaccinated either one time at 1or 14 and/or two times at 1& 14 days only were protected. These mean that the "intermediate" IBDV vaccine strain is capable for breaking through moderate level of

MDA as previously reported (Van den Berg and Meulemans, 1991 and Kouwenhoven and Van den Bos, 1992). However, the highest mortality rate (3/10) was observed PV with ìintermediateî vaccines at 1 day of age and challenged at 31 day of age (Table-3) suggesting that a too early vaccination with strain might reduce significantly the protective effect of MDA (van den Berg and Meulemans, 1991).Whatever, the differences in effectiveness between the different vaccination regimes must be related to the MAD levels. These results confirmed that MDA interfere with vaccination (Table-2) as previously emphasized by others (Muskett et al., 1979; Lucio and Hitchner, 1980 Winterfield et al., 1980; Wyeth, 1980 and Solano et al., 1985). Also, Kouwenhoven and Van den Bos (1992) stated that the intermediate type vaccine could prevent IBD outbreak caused by a vvIBDV only to some extent and they failed in situations of highly infection pressure. Vaccination failures were due to the inability of the intermediate vaccine to break through MDA, as compared with the virulent virus, and deficient timing of vaccination. In addition, Aly et al. (1996), in their study, for evaluation vaccination of one-day-old SPF and commercial chicks showed that maternal antibodies interfered with vaccination with mild, intermediate, or inactivated type of IBD vaccines. Maternally immune nonvaccinated chicks challenged at 4-weeks of age showed better protection than those vaccinated at one day of age.

None of the different vaccinal regimes protected commercial broiler chickens neither from bursal atrophy nor bursal lesions (Table 2&3). These results suggested that the serological examination of optimum vaccination time for each flock is required to effectively control IBDV in the field (Tsukamoto et al., 1995; Rautenschlein, et al., 2003; Zouelfakar, et al., 1997; Riks et al., 2001).

The severity of microscopic lesions was correlated with bursal atrophy as measured with bursal body weight ratio (B: B) ratio and bursal body weight index (BI) (Tables 2&3).However, differences in the protection of the three regimes of IBDV vaccines were compared. The most significant differences were found in the protection against mortality.

Since protection against mortality is not enough criteria for judging the protection confirmed by the tested IBDV vaccine, protection against bursal lesions due to vvIBDV challenge was also considered in the experiment. Thus, bursal body weight mean ratio (B:B) determined for birds that survived vvIBDV challenge revealed no significant difference between vaccinated and nonvaccinated challenge groups. The results of determination of bursal body weight index (BI) as well as severity ndex (SI) of bursal lesions on the birds that survival challenge at 24 and 31 days of age may confirm this conclusion. All challenged groups, BI were less than normal reference (0.7) of Lucio

and Hitchner (1979) indicating bursal atrophy, and moderate to severe bursal lymphoid lesion score (2-4) were determined histologically.

We think, as already emphasized by Kibenge et al. (1988) and Van den Berg and Meulmans (1991), that recombinant vaccines made in fowl pox, pigeon pox or turkey herpes virus vectors could be an alternative strategy for the future as their advantages are: lack of residual pathogenicity, lack of interference with MDA, no risk of selecting variants, differentiation between infected and vaccinated birds and polyvalent vaccination.

In conclusion, administration of live intermediate IBDV-vaccine at day old of age via eye drop doesn't protect from mortality, bursal atrophy and bursal lesions in vvIBDV-challanged birds.

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