Efficacy of Intermediate and intermediate plus Infecti Bursal Disease Virus (IBDV) Vaccines against Very Viru IBDV (vvIBDV).

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Abstract

Laboratory experiment was designed to evaluate the control of virulent infectious bursal disease virus (vvIBDV) infection by using "intermediate", "2-intermediate plus", (2512) IBDV vaccines or e infection with vvIBDV at 15 or 25-day-old commercial white egg-type r chickens which have significant maternal antibody levels and challe with vvIBDV isolate after 7 days post vaccination or early infection. Clir signs, mortality rate, gross lesion, bursal body weight ratio, bursal ir and histopathological lesion of bursa ,thymus , spleen, Harderian gland cecal tonsils were recorded .

The results of the protection against challenge at 22-day-old with vvI field isolate either after vaccination with live IBDV vaccines "intermed or intermediate plus" or after previous infection with vvIBDV at 15-day revealed that mortality were 6.67%, 13.3%, 13.3%, 0.0% and 13 versus 6.67%, 6.67%, 26.7%, 6.67% and 13.3% when challenged at day-old. These results confirm that high level of MDA interfere vaccine efficiency. The results showed that none of the three vacc protected commercial egg type chickens neither from bursal atrophy bursal lesions. Also the results suggested that the serolog examination of optimum vaccination time for each flock is require effectively control IBDV in the field.

Introduction

Infectious bursal disease (IBD), is an acute highly contagious infection of young chickens described first by Cosgrove (1962) in Delmarva area. The disease leading to direct and indirect signifi economic losses to the world wide poultry industry (Chettle et al., 1 Van Den Berg et al., 1991). The direct economic losses of IBD is du morbidity and mortality rate while the indirect impact is due immunosuppression of infected birds (Allan et al., 1972; Ivanyi and Mo 1976; McNulty et al., 1979; McIlory et al., 1993; Kumar et al., 2002; Ka et al., 2004 and Wither et al., 2005) .

The etiological virus of the disease belongs to the recently descr family Birnaviridae (Brown, 1986; Van Den Berg, 2000 and Rautensch et al., 2003). Tow distinct serotypes I and II have been identified (Jackw and Saif 1983, and McFerran et al., 1980). Serotype I produces clir disease and distinct lesions in bursa of fabricus (BF) with musc hemorrhage and serotype-2, which infected both chickens and turkeys was recorded as non-pathogenic for both species.Several investiga especially in the USA have reported antigenic variation among the isol

of serotype-1 IBDV. These antigenic variants were also reported through the use of a selected panel of neutralizing monoclonal antibodies (Mabs). Furthermore, in1986 very virulent (vv) strains of IBD have emerged in Europe, which can cause up to70% flock mortality in laying pullets and 100% in specific pathogen-free (SPF) chicken (Chettle et al., 1989 and Van Den Berg et al., 1991).

IBD can be controlled both by live and inactivated vaccines. According to virulence, there were four kind of live serotype I vaccines: intermediate plus or hot, intermediate, mild intermediate, and attenuated mild strains. The protective efficacy of IBDV vaccines is traditionally evaluated in SPF chickens. But under field condition, residual maternal antibody (MA) levels may interfere with vaccines efficacy. Under experimental condition, it was demonstrated that intermediate IBDV vaccines may break through residual MA and induce protective immunity, but mild vaccines not cause the disease. Over all, successful IBDV vaccination depends on the time of vaccination, the vaccine strain, the MDA status of the flock, as well as the epidemiological field isolate. (Tuskamoto et al., 1995, and Rautenschlein et al., 2005). In addition control of IBDV via adequate management and sanitation (Van Den Berg and Meulemans, 1991 and Van Den Berg, 2000), so control policy based on vaccination is considered the principle method used for control of IBD in chickens and was initially based on immunization of broilers and replacement pullets with various commercial serotype-1 live vaccines of the mild and intermediate types, and in breeder pullets either the inactivated oil-emulsion vaccines were used to boost immunity at the point of lay. Ideally, an IBD vaccine should elicit a prompt long lasting protective antibody response against virulent field strains, with lake of injury to the immune system.

In Egypt (in the summer of 1989), severe outbreaks of very virulent IBD (vvIBDV), similar to those reported in European countries in both vaccinated and non-vaccinated flocks, and were associated with high mortalities up to 70% in replacement layer pullets and 30% in meat-type birds (El-Batrawi, 1990; Ahmed, 1991 and 1993; Khafagy et al., 1991). The incidence of IBD virus infection and its associated disease problems were still common in Egypt in spite of the routinely applied vaccination program Elham El-Ebiary et al. (2001) and Nadia et al. (2001).

Material and Methods

Chickens:

Sufficient, one-day-old commercial egg-type (L.S.L) male chicks were produced from a commercial hatchery (El-Wadi hatcheries), which possessed MA 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 day light in strictly isolated experimental rooms, previously cleaned and disinfected and were provided with commercial layer starter ration. Water and feed were provided adlibtum. Chicks were monitord for IBDV-

specific MDA by agar gel precipitation test (AGPT) adn enzyme li immunosorbant assay (ELISA) to determine MDAwaning and the ag which the chicks become susbtable to expermental infection or vaccina

Reference antigens and antisera:

Aknown positive and negative precipitating antigen in the forr bursal homogenates and known positive and negative precipit reference antisera against IBDV obtained from Intervet, Inter. B Boxmeer, Holland, were used for the AGPT.

IBD viruses:

a- Three types of commercial live IBDV vaccines one "intermediate" (strain) and two "intermediate plus" "hot" vaccines represented "intermediate plus-1" and" intermediate plus-2" (2512 strain) obtained the local agencies, were used in vaccination studies.

b- A local field isolate of vvIBDV (El-Ataway 2006) in the form of b extract was diluted 1: 10 in phosphate buffer saline, which killed 45% week-old susceptible commercial male chickens, was passed once week-old susceptible egg-type male chickens for propagation and was in vaccination studies as challenge virus.

NewCastele disease vaccines:

B-1 Type, lasota strain live ND (NewCastle disease) vaccine obtained the local agencies, was used in vaccination studies.

ELISA kits:

Commercial ELISA kits ProFlock supplied by Synbiotics Corporation, 1101 Frontera, San Diego. CA 92127, were used for measuring IBDV antib Application and interpretation of the test were carreid out according to the instruct the kits manufacturers.

Samples for histopathological examination:

Bursa of Fabricius, spleen, thymus, cecal tonsils and Harderian glan experimentally infected and control birds were fixed in neutral buffered formalin solution.

Agar gel precipitation test:

The test was used to demonstrate the presence of antibodies to IB examined chicken sera and for detection of IBDV antigen (s) in the cl bursa of affected chickens as described by Wood et al. (1979).

laboratory vaccination experiment:

Determination of the serological response and degree of prote following subsequent ocular vaccination with live "intermediate", "interme plus-1", "intermediate plus-2 IBDV vaccines (2512 strain), or infection vvIBDV field isolate in 15 or 25-day-old commercial white-egg type chickens and challenged with vvIBDV 7-days later. For this purpose, suff one-day-old, commercial egg-type male chicks, from one hatch was used maternal antibody waning in those chicks was followed up at 7 days age t days-age. They were examined individually by AGPT and ELISA. Chicks vaccinated and/or challenged at different ages according to the experir design in the following table:

Experimental design of laboratory vaccination experiments:

Gr. No.	IBD Vaccination [1] regime			IBDV [2] challenge		Assessment of protection			
	Bird No.	Age / day	Type	Bird No.	Age/ day	Observation for 7 day post. Vacc. And post. Chall.[3]	Serolog[6]	Antigen detection	Histo - Pathology (SI)[7]
1	30	15	A	15	22	1- clinical signs 2- mortality % 3- gross lesions 4- B:B; ratio [4] 5- B:B; index [5] for survivors at 7 days Pch[8]. and 7 days post – vaccination	sero – conversion at 7 days Pch[8]. and 7 days post-vaccination	pool of bursal homogenate of dead birds	lesion score for survivors at 7 days post-vaccination and 7 days Pch.
2	30	15	B	15	22				
3	30	15	C	15	22				
4	30	15	v	15	22				
5	30	15	v	15	22				
6	30	15	I	15*	--				
			B						
			D						
			--						
			--						
7	30	25	A	15	32				
8	30	25	B	15	32				
9	30	25	C	15	32				
10	30	25	v	15	32				
11	30	25	v	15	32				
12	30	25	I	15*	--				
			B						
			D						
			--						
			--						

(1) = Field dose/bird via oculo-nasal route.
(2) = The chickens were subjected to oculo-nasal challenge with 100 ul / bird.
(3) = post-challenge.
(4) = Bursal body weight ratio. (Sharma et al., 1989).
(5) = Bursal body weight index. (Lucio and Hitchner, 1979).
(6) = Serological tests were used (AGPT- ELISA).
(7) = severity index of bursal lymphoid tissue lesion (Sharma et al., 1989).
(8) Pch = post challenge.
* = birds which were non vaccinated non challenged.

Assessments of protection against IBDV challenge:

1-Clinical signs; mortality percentage and rate as well as postmortem gross lesions were recorded.

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

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

-Bursal index = Bursal weight / Body weight X 1000

-Bursa: body weight index = 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, spleen, thymus cecal tonsils and Harderian gla were fixed in 10% formalin solution , and then treated chemically with diffe concentration of alcohol and xylol. Paraffin sections were obtained by rota microtome. Tissue sections were stained with Harris hematoxyline and eo according to Bancroft et al. (1990).

a-The severity of bursal lymphoid tissue lesions were scored from 0 to 4 on basis of lymphoid necrosis and/or lymphocytic depletion according to Sharm al (1989).

b- The severity of spleen lymphoid tissue lesions were scored from 0 to the basis of lymphoid necrosis and/or lymphocytic depletion according to severity of histopathological changes (Henry et al., 1980).

c- The severity of thymus lymphoid tissue lesions were scored from 0 to the basis of lymphoid necrosis and/or lymphocytic depletion according to severity of histopathological changes (Henry et al., 1980).

d- The severity of HG lymphoid tissue lesions were scored from 0 to 4 or basis of lymphoid necrosis and/or lymphocytic depletion according to severity of histopathological changes (Dohms et al., 1988).

e- The severity of cecal tonsils lymphoid tissue lesions were scored fro to 4 on the basis of lymphoid necrosis and/or lymphocytic depletion accordir the severity of histopathological changes (Helmboldet and Garner, 1964).

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

6-Statistical analysis:

Whenever necessary, data were analyzed by the student's t-test or by ana of variance followed by application of duncan's new multiple range according to **SAS (1987)** to determine the significance of differences betv individual treatments and corresponding control.

Results

Decline of MDA of IBDV

Table (1) shows MDA waning of commercial white egg-type male chickens for studying serological response and degree of protection following vaccina of IBD vaccines. The maternal precipitins were not more detectable at 35 of age, whereas negative ELISA titers were detected at 49-day-old.

Age/days	Serological tests			
	AGPT [1] (Positives No./examined No.)		ELISA [2]	
	No.	%	Titer ± Sd [3]	%CV [4]
7	15/15	100	15580±10823	37.04
14	11/15	70	11395 ± 6447	30.87
21	6/15	40	8255 ± 6225	37.88
28	3/15	20	6700 ± 1105	38.60
35	0/15	0.0	2355± 1405	55.85
42	0/15	0.0	942 ± 814	45.30
49	0/15	0.0	0.0	--

1 = Agar gel precipitation test.
2 = Enzyme linked immunosorbant assay.
3 = Standard deviation.
4 = Coefficient of variance.

Protection against vvIBDV challenge:

Table (2): Results of the serological response following vaccination with A, B or C IBDV vaccines or infection with vvIBDV and challenge with vvIBDV 7-days later in 15 or 25-day-old–commercial white egg-type chickens.

Group treatment	IBDVacc.[1] regime		IBDV chall. Age/ day	Serological response		
	Age day	Type		AGPT[2] (Pos.no ./exam. No.)	ELISA[3] Range	Mean ± sd[4]
Vacc. non chall.	15	A	-	2/10	3698 - 9799	7294.33 ± 2141.61 ab
		B		4/10	4747 - 12634	
		B		4/10	5846 - 10971	8602.00 ±2893.13 a
		vvIBDV	-	0/10	1591 - 8525	8405.00 ± 2010.78 a
	--	--		5/10	5187 - 9200	4934.67 ± 2495.99 b
Non vac. Non cha						7219.17 ± 1911.15 ab
Chall. vac.	15	A		6/10	1856 - 3216	2425.67 ± 483.70 c
		B	22	9/10	2850 - 4481	3630.83 ± 6844.60
		C		0/10	2270 - 4488	bc
		vvIBDV		2/10	4377 – 7959	3485.17 ± 869.22 bc
Chall. non vac.	--	--	22	5/10	2612 – 5765	5787.67 ± 1278.02 a
	--	--	--	0/10	1396 - 8371	3809.00 ± 1294.00 b
Non vac. Non cha						5030.00 ± 2232.00 a
Vacc. non chall	25	A		2/10	0 - 4539	2559.30 ± 1823.87 c
		B	--	0/10	0 - 3734	2119.20 ± 1272.21 c
		C		0/10	1211 - 3705	2636.12 ± 1098.72 c
		vvIBDV		3/10	2927 - 10107	6394.24 ± 2645.83 a
	--	--	--	0/10	2671 - 3958	3366.80 ± 495.00 bc
Non vac. Non cha						
	[					7814.00 ± 2178.94 a
Chall. vac.		A	32	4/10	3951 - 10332	8884.33 ± 2719.08 a
		B		0/10	3879 - 11709	10523.33 ± 2582.90
	25	C		0/10	7215 - 13561	a
Chall. non vac.		vvIBDV		2/10	9633 - 13170	11425.17 ± 1244.52
		--	32	7/10	2712 - 15861	a
Non vac. Non cha	--	--	--	0/10	1874 - 4405	8802.00 ± 2575.00 a
	--					2647.00 ± 941.00 b

1 = Infectious bursal disease virus.
2= Agar gel precipitation test.
3= Enzyme linked immunosorbant assay.
4 = Standard deviation.
Any two means within the same time interval with different superscript are significantly different at p ≤0.05.

Table(3): Result of determination the degree of protection following vaccina with A, B or C IBDV vaccines or infection with vvIBDV and challenge vvIBDV 7-days later in 15 or 25-day-old commercial white egg-type chickens

Group treatment	Vaccination regime[1]		IBDV Chall. age/day[2]	Assessment of protection						M SI[7]
	Age/day	Type		Mort[3] rate.	Mort. %	B:BR[4] Means ± sd	B:Bl[5] Mean	Bursal lymphocytic tissue lesion (Sl)[6]		
								Lymphocytic necrosis	Lymphocytic depl esion	
vac. non chall.	15	A		0/30	0.0	5.33±0.7	0.97	1.0	1.0	1.0
		B		0/30	0.0	1a	1.04	1.0	1.0	1.0
		C	--	0/30	0.0	5.70±1.1	0.98	1.0	1.0	1.0
		vvIBDV		0/30	0.0	5a	0.81	4.0	4.0	4.0
Non vac. Non cha	--	--	--	0/30	0.0	5.39±0.8 5a 4.74±0.9 3b 5.47±1.5 1a	1	0.0	0.0	0.0
Chall. vac.	15	A		1/15	6.67	2.47±0.7	0.48	3.0	3,0	3.
		B		2/15	13.3	8b	0.41	3.8	3.6	3.
		C	22	2/15	13.3	2.17±0.7	0.48	3.3	3.1	3.
		vvIBDV		0/15	0.0	5b	0.35	4.0	4.0	4.
Chall. Non vac.	--	-	22	2/15	13.3	2.51±0.8	0.51	4.0	4.0	4.
Non vac. Non cha	--	--	--	0/15	0.0	0b 1.83±0.4 3b 2.68±0.8 6b 5.20±0.8 7a	1	0.0	0.0	0.
vac. non chall.	25	A		0/30	0.0	4.80±0.9	0.89	2.1	1.9	2.
		B		3/30	10.0	8ab	0.66	3.6	3.8	3.
		C	--	0/30	0.0	3.55±0.7	0.81	3.0	3.0	3.
		vvIBDV		4/30	13.3	8b	0.42	4.0	4.0	4
Non vac. Non cha	--	--	--	0/30	0.0	4.37±1.2 1ab 2.27±0.4 0c 5.37±1.7 1a	1	0.0	0.0	0
Chall. vac.	25	A		1/15	6.67	1.58±0.2	0.42	3.7	3.9	3
		B	32	1/15	6.67	9c	0.45	3.6	3.8	3
		C		4/15	26.7	1.68±0.3	0.40	3.9	3.7	3
		vvIBDV		1/15	6.67	5c	0.40	3.0	3.0	3
Chall. Non vac.	--	--	32	2/15	13.3	1.51±0.2	0.63	4.0	4.0	4
Non vac. Non cha	--	--	--	0/15	0.0	2c 1.51±0.1 6c 2.37±0.3 9b	1	0.0	0.0	0

						3.37±0.40a				

(1) Field dose/bird via oculonasal route

(2) The chickens were subjected to oculonasal challenge with 100ul /bird of identified local field isolate in the form of bursal extract and observed for 7 days.

(3) Mort. =mortality.

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

(5) B: B= Bursal body weight index. (Lucio and Hitchner, 1979).

(6) SI=Severity index of bursal lymphoid tissue lesions (Sharma et al., 1989).

(7) MSI=Mean severity index.

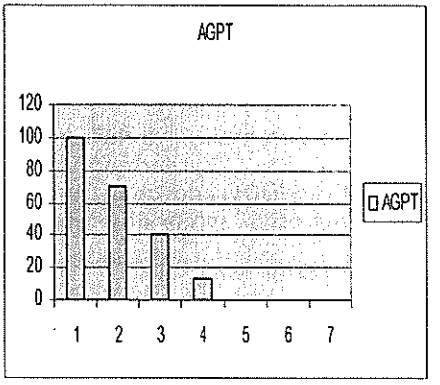
Any two means within the same time interval with different superscript are significantly different at p ≤0.05.

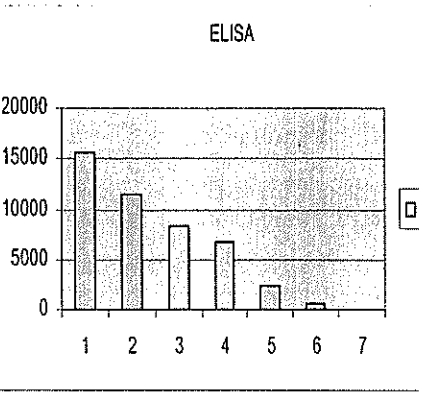
Table (4): Results of the histopathological examination following subsequent ocular vaccination with live "intermediate", "2-intermediate plus" (2512 strain) IBDV vaccines or infection with vvIBDV and challenge with vvIBDV 7-days later in 15 or 25–day-old commercial white egg-type chickens.

Group treatment	IBD Vacc. Regime [1]		IBDV chall.	Histopathological examination Lesion scores					
	Age/ day	Type	Age/ day	BF [2]	Sp. [3]	Th. [4]	HG [5]	CT. [6]	TM [7]
Vacc. non chall.	15	A	--	1.0	2.0	0.0	1.0	1.0	1.0
		B		1.0	1.0	0.0	3.0	2.0	1.4
		C		1.0	2.0	0.0	1.0	1.0	1.0
	--	vvIBDV	--	4.0	3.0	1.0	3.0	2.0	2.6
Non treated		--		0.0	0.0	0.0	0.0	0.0	0.0
Chall. vac.		A		3.0	2.0	3.0	1.0	1.0	2.0
	15	B	22	3.7	2.0	1.0	2.0	1.0	1.94
		C		3.2	1.0	1.0	1.0	1.0	1.44
		vvIBDV		4.0	2.0	2.0	1.0	2.0	2.2
Chall. non vac.	--	--	22	4.0	3.0	2.0	3.0	0.0	2.4
	--	--	--	0.0	0.0	0.0	0.0	0.0	0.0
Non treated									
Vacc. non chall	25	A	--	2.0	0.5	1.0	2.0	0.0	1.1
		B		3.7	2.5	2.0	3.0	3.0	2.8
		C		3.0	1.0	2.0	2.0	1.0	1.8
		vvIBDV		4.0	3.0	3.0	4.0	4.0	3.6
	--	--	--	0.0	0.0	0.0	0.0	0.0	0.0
Non treated									
Chall. vac.		A		3.8	1.0	1.0	1.0	0.0	1.36
	25	B	32	3.7	2.0	0.0	1.0	0.0	1.34
		C		3.8	2.0	1.0	0.5	0.0	1.46
		vvIBDV		3.0	1.0	2.0	1.0	0.5	1.5
Chall. non vac.	--	--	32	4.0	3.0	3.0	2.0	0.0	2.4
	--	--	--	0.0	0.0	0.0	0.0	0.0	0.0
Non treated									

1 =IBDV = Infectious bursal disease virus.
2 = lesion score of the examined BF according to (Sharma et al., 1989).
3 = lesion score of the examined spleen according to (Henry et al., 1980).
4 = lesion score of the examined thymus according to (Henry et al., 1980).
5 = lesion score of the examined HG according to (Dohms et al., 1988).
6= lesion score of the examined cecal tonsils according to Helmboldet and Garner (1964).
7 = TM: total means lesion scores of the examined lymphoid organs.

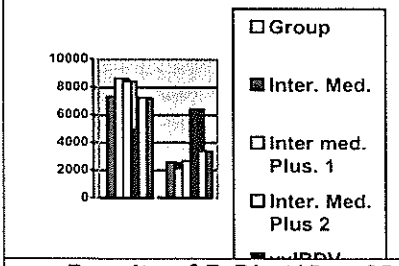
Waning of MDA in commercial white egg-type male chickens used f pathogenicity studies of IBDV local field isolate from 1 to 7-weeks age.

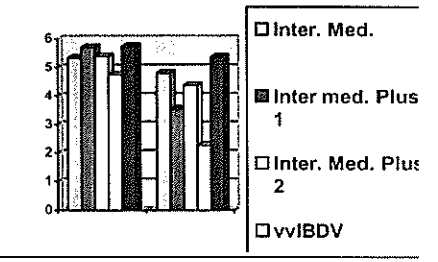




Results of the Serological Response at15 or 25-days

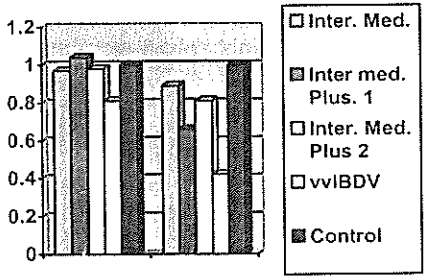
Results of B:BR at 15 days



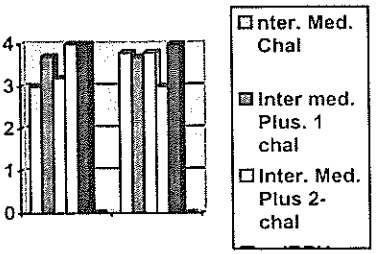


Results of B:BI at15 or 25-days

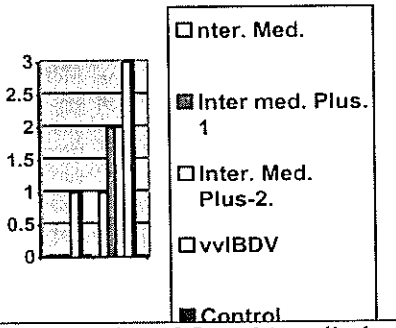
Results of Bursal Lesion S Pch.at 22 or 32 days

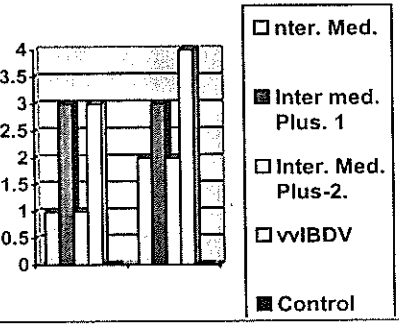


Results of Thymic lesion scores at 15 or 25-days

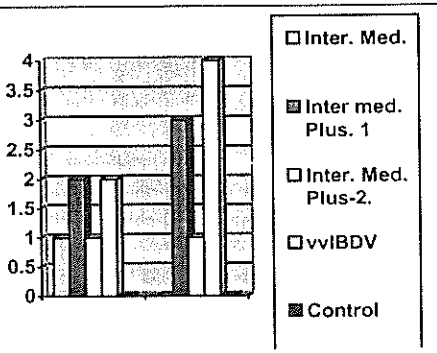


Results of HG lesion scores at 15 o 25-days





Results of Cecal tonsils lesion scores at 15 or 25-days



Dscussion

The aim of this studies was to investigation the control of circu vvIBDV infection by vaccination, For this purpose, laboratory vacci experiment was designed to determine the degree of protection to inf with vvIBDV following vaccination with A, B or C or following early inf with vvIBDV field isolate at 15 or 25-day-old age in susceptible comm white egg-type male chickens having residual MDA.

The results of the protection against challenge at 22-day-old with v field isolate either after vaccination with A, B or C or after previous inf with vvIBDV at 15-day-old revealed that mortality were 6.67%, 1 13.3%, 0.0% and 13.3% versus 6.67%, 6.67%, 26.7%, 6.67% and when challenged at 32-day-old. These results confirm that MDA int with vaccination (Table 7) as previously emphasized by others (Mus al., 1979; Lucio and Hitchner, 1980; Witerfield et al., 1980, Wyeth, and Solano et al., 1985).

The results of serological response (ELISA) following vaccination w B or C or early infection with vvIBDV at 15-days-age 7294.33±2141.61, 8602.00±2893.13, 8405.00±2010.78 4934.67±2495.99 respectively, but it were 2425.67±4 3630.83±6844.60, 3485.17 ±869.22 and 5787.67±1278.02 respec when challenged 7-days later. Moreover, it was 2559.34±18 2119.20±1272.21, 2636.12±1098.72 and 6394.24±2645.83 respec when vaccinated at 25-days-age. In addition, it were 7814.00±21 8884.33±2719.08, 10523.33±2582.90 and 11425.17±1244.52 respec when challenged 7 days later. So we concluded that high titer of following vaccination with B or C vaccines than A revealed that the B vaccines were more immunogenic according to Van Den Berg et al. (Furthermore, the ELISA titer of non treated birds were 7219.17±19 5030.00±2232.00, 3366.80±495.00 and 2647.00±941.00 at 15, 22, 2 32-days-age of vaccinated or challenged groups respectively, sim table (1) revealed that decrease level of MDA which have role in prot of birds from early infection (mortality % of birds infected with vvIB 15-days-age were 0%) according to Wyeth and Chettle (1982).

In the present study, the Bursal body weight ratio (B:BR) and Bursal in of vaccinated birds at 15-days-age with A, B or C or early infectio vvIBDV were "5.33±0.71, 0.97", "5.70±1.15, 1.04", "5.39±0.85, 0.9 "4.74±093 and 0.81", respectively. Versus it were "4.80±0.98, 3.55±0.78, 0.66", "4.37±1.21, 0.81" or "2.27±0.40 and 0.42" respec following vaccination at 25-days-age due to residual MDA accord (Tuskamoto et al., 1995, and Rautenschlein et al., 2005). Differen effectiveness between vaccines A, B or C must be related to the ba existing between their efficiency and their safety. More re pathogenicity allows the use of vaccines B or C, as shown in table (BBR, BI and MSI of vaccinated bird with B were 3.55±0.7, 0.66 an respectively. Versus 4.80±0.98, 0.89 and 2.0 in vaccinated birds days-age with vaccine A respectively, but were 5.70±1.15, 1.04 a

versus 5.33±0.71, 0.97 and 1.0 in vaccinated birds at 15-days-age (residual MDA) similar to Coletti et al. (2001).
So decrease level of MDA that indicated the protection increase 7 days PV. And high level of MDA interfere with vaccine efficiency these results may support that vaccination can be helpful when in one flock of multiple-house farms with the same level of MDA. These results indicated the agreement with (Tuskamoto et al., 1995, and Rautenschlein et al., 2005). And the best vaccination time of IBDV (De Wit, 2003).

Since protection against mortality might not be considered as absolute criterion of efficiency of the tested vaccine other parameter reflecting protection against bursal atrophy were included in the experiment, BBR and BI revealed that there were no significant difference between vaccinated and non vaccinated birds in vaccinated bird at 15-days-age. But there were significance difference between vaccinated and non vaccinated birds in vaccinated bird at 25-days-age. Table (3) agreement with Rautenschlein et al. (2005).

None of the three vaccines protected commercial egg type chickens neither from bursal atrophy nor bursal lesions (Table3). These results suggested that the serological examination of optimum vaccination time for each flock is required to effectively control IBDV in the field (Tuskamoto et al., 1995). Moreover, in comparison with A and B,C vaccines induced bursal atrophy revealed that B and C induced bursal atrophy with high possible lesion score and A induced moderate bursal atrophy at 7-days PI (Table3) especially at 25-days-age. The best protection against mortality was induced by B vaccines. We speculated that better protection with more virulent strains due to more systemic stimulation on the basis of severe bursal atrophy and lesions that have been previously reported by Rautenschlein et al. (2003).

Riks et al. (2001) concluded that two main factors influence the correlation between the potency assay of IBDV vaccines in young chickens and the protection against IBDV challenge. These are the strain used in the vaccine and the virulence of IBDV challenge strain. Moreover, the age of vaccinated birds and the time of antibody assay are of minor importance.

In this study, the histopathological examination of lymphoid organs. So the MSI of the BF and total means (TM) of examined lymphoid organs Table(4) in vaccinated birds with live "intermediate", "intermediate plus-1", "intermediate plus-2" or early infection with vvIBDV field isolate at 15-day-old were "1.0, 1.0", "1.0, 1.4", "1.0, 1.0" and "4.0 and 2.4" respectively, versus "2.0, 1.1", "3.7, 2.8", 3.0,1.8" and "4.0, 3.6" respectively, in vaccinated bird at 25-day-old due to residual MDA (Rautenschlein et al., 2005) .

Moreover, it were "3.0, 2.0', "3.7, 1.94", 3.2, 1.44" and 4.0 and 2.2' respectively, when challenged at 22-day-old which were vaccinated at 15-days-age. And it were "3.8, 1.36", 3.7, 1.34", 3.8, 1.46", and "3.0 and 1.5" respectively, in challenged birds at 32-days-age which were vaccinated at

25-day-old, which revealed that non of intermediate nor intermediate IBDV vaccines prevent lymphoid changes according to (Tuskamoto 1995, and Sultan et al., 2006-b). Also vaccinated birds at 15-days-age live "intermediate", "intermediate plus-1", "intermediate plus-2"or infected with vvIBDV field isolate and non treated group revealed th significant difference between vaccinated and none vaccinated at 15-age except group was early infected with vvIBDV (1^{ST} challenge). Tab similar finding have been reported by (Rautenschlein et al., 2005), showed that there were significant difference between vaccinated an vaccinated birds at 25-days-age (Table 8) similar finding have reported by (Rautenschlein et al., 2005).

Histopathological examination of the spleen of the infected groups vvIBDV at 15, 22, 25 and 32-day-old had high lesion score which we but it were 0.0 in non treated groups. While at vaccinated groups at 1 25-days-age with "intermediate", "intermediate plus-1" and "interme plus-2" ranged between 1.0 and 2.0 due to low vaccine effect than vv on the spleen according to (Helmbolt and Garner, 1964, and Henry 1980).

In thymus examination, in vaccinated groups with " intermec "intermediate plus-1", "intermediate plus-2" or infection with vvIBDV days-age it were 0.0, 0.0, 0.0 and 1.0, respectively. While it were 1.0 2.0 and 3.0 when vaccinated at 25-day-old due to low level of according to (Helmbolt and Garner, 1964; Henry et al., 1980, and Sh et al., 1989). On the other hand, there is only challenged groups ha lesion scores when challenged at 22and 32-days-age due to its viru (Henry et al., 1980). In HG examination, the groups vaccinatec "intermediate", "intermediate plus-1", "intermediate plus-2" or infectio vvIBDV at 15-day-old it were 1.0, 3.0, 1.0 and 3.0, respectively. In adc when vaccinated at 25-day-old it were 2.0, 3.0, 2.0 and 4.0, respec (MDA level). While these were 3.0, 3.0, 4.0 and 2.0 in challenge vaccinated groups at 15, 22, 25 and 32-day-old, respectively. according to (Survashe et al., 1979, and Dohms et al., 1988).

In the cecal tonsils examination of lesion scores, the groups vaccinated challenged with vvIBDV it were 2.0, 0.0, 4.0 and respectively. But in non treated groups it were 0.0 according to (Hel and Garner, 1964). Moreover, in vaccinated groups with "intermec "intermediate plus-1", "intermediate plus-2" or infection with vvIBDV days-age it were 1.0, 2.0, 1,0 and 2.0, respectively. But it were 0.0 1.0 and 4.0, respectively when vaccinated at 25-days-age due to MDA Since protection against mortality might not be considered as ab criterion of efficiency of the tested vaccine other parameter refl protection against bursal atrophy were included in the experimen bursal indices revealed that there is no complete protection against I atrophy provided by either intermediate plus or intermediate vaccine s finding have been reported by (Mousa et al., 1988-b; Van Den Ber Meulemans, 1991; Sultan, 1995, and Sultan et al., 2006-b).

Nevertheless, in the present situation, some restrictive problems still remain first of all, due to its high resistance of disinfection and environmental factors; pathogenic IBDV generally survives in contaminated premises. Then, the birna virus are subjected to mutation; the intensive use of live IBDV vaccines strains with increased virulence. Moreover, the use of vaccine with increasing pathogenicity (intermediate plus" for prophylaxis may be dangerous as they are more invasive and immunosuppressive.We think as already emphasized by Kibenge et al. (1988-b and 1990) and Van Den Berg and Meulmans (1991), that recombinant vaccines mad in fowl pox, pigeon pox or turkey herpes virus vectors could be an alternative for the future as their advantages are: lack of residual pathogencity, lack of interference with MDA, no risk of selecting variants, differentiation between infected and vaccinated birds and polyvalent vaccination.

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