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EVALUATION OF TWO VACCINATION PROGRAMS TO CONTROL INFECTIOUS BURSAL DISEASE IN COMMERCIAL BROILERS

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SUMMARY

An experimental study was carried out to compare protection induced by two infectious bursal disease (IBD) vaccination programs in commercial broilers.

Chickens inoculated with live vaccine at 7 and 21 days old were protected against mortality but clinical signs of IBD were noted after challenge at 28 and 35 days old.

Chickens inoculated with both live vaccine (at 7 & 21 days old) and inactivated vaccine (at 8 days old) were better protected against challenge at 28 and 35 days of age. This vaccination program protected challenged chickens against both mortality and clinical IBD signs. However, the bursa: body weight ratios were lower than those of the control birds ($P<0.05$).

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious disease of chickens characterized by severe damage of the bursa of Fabricius and immunosuppression (Lukert & Saif 1991). In Egypt, IBD was first described by El-Sergany et al (1974) and Ayoub and Malek (1976).

During the last decade, outbreaks of an acute IBD with high mortality occurred in commercial broiler and pullet flocks throughout Egypt (Saif-Eldin et al.,1996). The disease causes heavy economic losses in poultry industry due to high mortality and consequences of immunosuppression. Although vaccination programs are used on intensive scale, IBD outbreaks are still reported from allover the country.

The evolution of acute IBD was attributed to antigenic variation in USA (Rosenberger et al., 1987) and very virulent classic IBD strains in Europe (Van den Berg et al., 1991), which led to significant changes in vaccination stratigies adopted to control the disease. Live intermediate vaccines are currently used more frequently than mild vaccines in chickens with high level of maternal immunity. These intermediate (less attenuated) vaccines are able to induce an active response in the presence

of higher levels of maternal immunity. The use of inactivated vaccines is a common practice in breeder and layer replacement. These inactivated vaccines are used to boost antibody levels in breeders primed with live vaccine. The objective of this study was to compare two vaccination programs for control of IBD in commercial broiler flocks.

MATERIALS AND METHODS

Chickens and housing: 350 broiler chicks were obtained from commercial source as one-day-old chicks. The chickens were housed in clean disinfected rooms and provided with feed and water ad libitum.

Vaccines: Two commercial IBD vaccines (Rhone-Merieax, France) were used in this study. Gumboral CT (Live vaccine, batch no. 53 F106) and Gumboriffa (inactivated vaccine, batch no. 50 P 831) were used according to the manufacturer's recommended procedures (Table 1). The live vaccine was titrated in chicken embryo fibroblast cells derived from 9 to 10 day-old SPF embryonating eggs (10^4 $CCID_{50}$/bird).

Challenge Virus: Virulent IBD field isolate that has been propagated in susceptible chicks was used for challenge (10^5 EID_{50}/ bird) and evaluation of protection.

ELISA Test: Blood samples (10 samples/group) were randomly collected from vaccinated and control groups at weekly interval to monitor the levels of IBD antibodies . Sera were diluted 1:500 and assayed by a commercial IBD antibody ELISA Kit (IDEXX laboratories, Inc., Maine, USA).

Experimental design: At seven-day-old, chicks were randomly divided into 7 groups consisting of 50 each, and reared in separate clean rooms. Chickens in groups IA, IB and 3 were vaccinated against IBD according to the vaccination program I (i.e. live vaccine at 7 & 21 days old and inactivated vaccine at 8 days (Table 1). Chickens in groups 2 & 4 were vaccinated with live vaccine only at 7 & 21 days old (program II). The last two groups were used as positive control (group 5 =nonvaccinated and challenged) and negative control (group 6 =non vaccinated and non challenged).

Challenge study: At 28 and35 days old, chickens (20 birds) from vaccinated groups (group IA, IB & 2) and unvaccinated group (group 5) were challenged through the eye drop route of 10^5 EID_{50} of the field IBD strain. Morbidity and mortality rates during the study were recorded. The remaining chickens were killed at 5 and 10 days post challenge and examined for gross IBD lesions.

.Histopathology: The bursae were collected from all vaccinated and control groups at 5 & 10 days post challenge. The specimens were fixed in 10% neutral formalin, sectioned and stained with hematoxylin and eosin . Scoring of the bursal microscopic lesions, ranged from 1 to 4, were subjectively conducted according to the method described by Rosales et al. (1989a).

Table (1): IBD vaccination programs used in this study.

Vaccination program	vaccine used	Dose/bird	Route	Age of chickens at vaccination (days)
I	Gumboral CT	10^4 CCID^{50}	Oral	7
	Gumboriffa	0.3 ml	I/M[2]	8
	Gumboral CT	10^4 CCID50	Oral	21
II	Gumboral CT	10^4 CCID$_{50}$	Oral	7
	Gumboral CT	10^4 CCID50	Oral	21

[1]CCID$_{50}$ = Cell culture infective dose 50.
[2]I/M = Intramuscular injection.

Statistical analysis: The average bursa/body weight (B/BW) ratios of the vaccinated birds were compared with those of control groups for statistical significance using analysis of variance followed by Fisher's least significant difference.

RESULTS

1- Monitor of IBD antibody levels in vaccinated and control chickens by ELISA test:

The mean ELISA titers (10 birds/ group) are illustrated in figures 1 & 2. The results indicate that day-old chickens had high maternal antibody levels (6870 IDEXX units). This maternally derived IBD antibody persisted in the chickens until 35 days of age.

Chickens vaccinated with the live vaccine (program 2) did not show significant increase in ELISA titer than those of the control (Fig.1). The vaccinated and challenged chickens had slight increase in antibody titer which did not last more than 7 days. On the other hand, chickens inoculated with both live and inactivated vaccines (program I) had higher levels of antibody than the control nonvaccinated birds (Fig.2). The increase of antibody levels started about 14 days post inoculation of the inactivated vaccine. Marked immune response was noticed in vaccinated challenged chickens as shown by the increase of antibody titer (Fig.2).

2- Clinical and pathological observations after challenge of vaccinated and control groups:

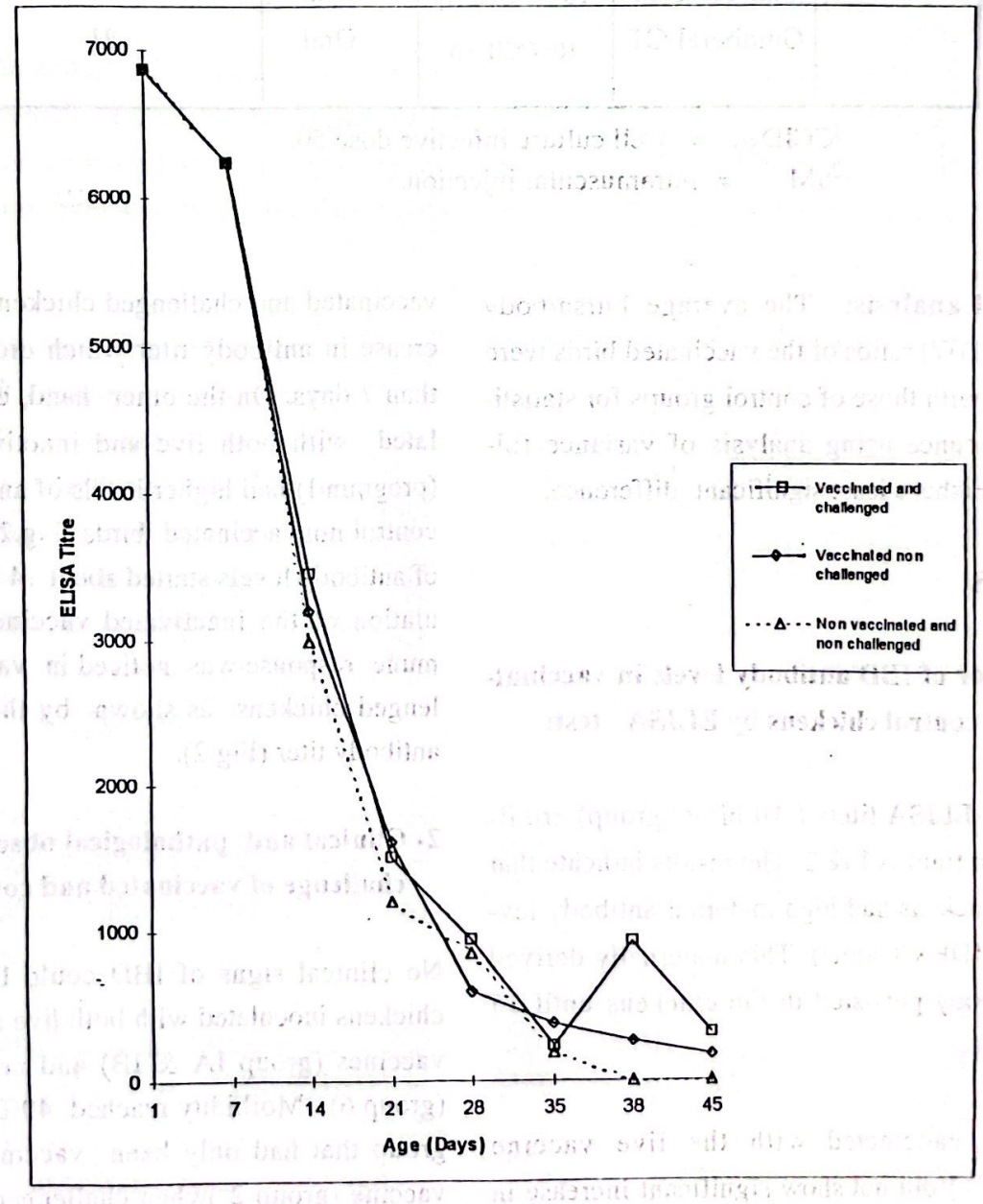
No clinical signs of IBD could be observed in chickens inoculated with both live and inactivated vaccines (group IA & IB) and negative control (group 6). Morbidity reached 40% & 50% in the group that had only been vaccinated with live vaccine (group 2)when challenged at 28 and 35 days old , respectively. However, unvaccinated

challenged chickens (group 5) had 50% and 90% morbidity rates at 28 and 35 days old, respectively (Table 2).

Mortality rates in the unvaccinated challenged chickens (group 5) ranged from 4% to 40% at 28 and 35 days old (Table 2). Groups vaccinated with either live and / or inactivated vaccines (groups IA, IB and 2) did not show mortalities after challenge at 28 and 35 days old. As could be expected, no mortality was observed in the negative controls (group 6).

Clinical signs (prostration, ruffled feathers and diarrhea) and P.M. lesions (muscular and / or proventricular haemorrhages, renal injury, bursal

Fig. (1): IBD antibody of chickens vaccinated according to vaccination program II.



oedema and haemorrhages) observed in affected chickens within 3-4 days post challenge were consistently typical of an IBDV infection. Also, at no time did the negative controls (group 6) showed signs of IBD.

Comparison between the bursa: body weight ratios (B/BW) of the challenged groups (Table 3) revealed that both group 5 (non vaccinated & challenged) and group 2 (live vaccine only) had the lowest B/BW ratios when compared with other groups ($P<.05$). Chickens vaccinated with both live & inactivated vaccines had B/BW ratios higher than those inoculated with live vaccine only but lower than the negative control (group 6). The data presented in this experiment showed that

Fig. (2): IBD antibody of chickens vaccinated according to vaccination program I.

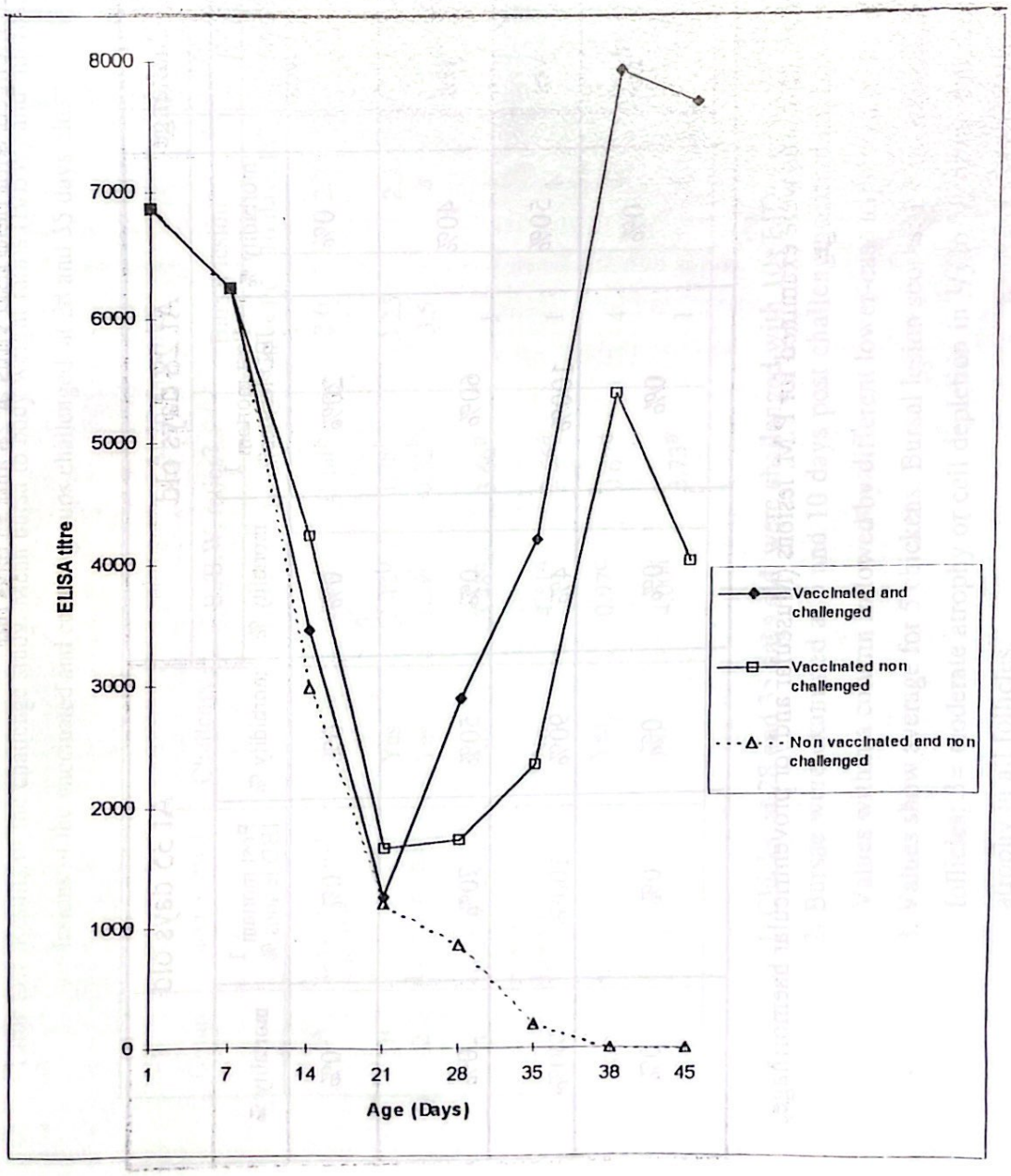


Table (2): Results of the challenge study. PM lesions, morbidity and mortality rates (%) of the vaccinated and control groups challenged with field IBD virus at 28 and 35 days old.

Group No	Vaccine used	Challenge	At 28 days old			At 35 days old		
			morbidity %	Post mortam [1] IBD lesions%	mortality %	morbidity %	Post mortam [1] IBD lesions %	mortality %
1 (A & B)	(Program I) Live & inacti-vated vaccines	yes	0%	20%	0%	0%	0%	0%
2	(Program II) live vaccine	yes	40%	60%	0%	50%	70%	0%
5	none	yes	50%	100%	4%	90%	100%	40%
6	none	no	0%	0%	0%	0%	0%	0%

1- At 5 and 10 days post challenge, chickens were examined for P.M. lesions (Muscular and /or proventricular haemorrhage, renal injury and bursal oedema and haemorrhage.

Table (3): Results of the challenge study. Mean bursa to body weight ratios (B/BW) and microscopic bursal lesions of the vaccinated and control groups challenged at 28 and 35 days old.

Group	Vaccine used	Challenge	At 28 days old[1]				At 35 days old[1]			
			B./B.W. ratios[2]		Bursal lesion[3]		B./B.W. ratios[2]		Bursal lesion score	
			5 days PC	10 day PC	5 days PC	10 days PC	5 days PC	10 days PC	5 days PC	10 days Pc
IA	Live & inactiv vaccine	Yes	2.24^{b}	1.64^{b}	3.0	2.7	1.37^{b}	1.52^{b}	2.5	3.0
IB	" "	Yes	2.42^{b}	1.78^{b}	3.25	2.5	1.83^{b}	1.38^{b}	3.0	3.0
2	Live vaccine	Yes	1.59^{b}	0.82^{bc}	3.5	4	1.35^{b}	0.85^{bc}	3.25	4
3	live & inacti-vated vaccine	No	3.48^{a}	3.66^{a}	1	1	3.35^{a}	3.88^{a}	1	1
4	Live vaccine	No	4.51^{a}	2.56^{a}	1	1	3.79^{a}	3.44^{a}	1	1
5	none	Yes	0.97^{c}	0.67^{c}	4	4	3.92^{b}	0.67^{c}	4	4
6	none	No	4.00^{a}	3.73^{a}	1	1	3.62^{a}	3.89^{a}	1	1

1. Chicks at 28 and 35 days old were challenged with 10_5 EID_{50} virulent IBD virus via t he ocular.
2. Bursae were examined at 5 and 10 days post challenge (PC). Values show average of 10 chicks. Values within a column followed by different lower-case superscripts are significant ($P<0.05$).
3. Values show average for 5 chickens. Bursal lesion scores: 1 = no lesions; 2 = mild cell depletion in few follicles; 3= moderate atrophy or cell depletion in $^1/_3$ to $^1/_2$ of the follicles and 4= severe necrosis and atrophy in all follicles.

vaccination alone (group 3 & 4)) did not induce bursal atrophy since there was no significant difference with those left as negative control ($P<0.05$).

3- Histopathological examinations:

Bursal lesion scores (Table 3) of the vaccinated chickens with both live and inactivated vaccines (group IA & IB) and challenged at 28 and 35 days old ranged from 2 (mild, scattered cell depletion in few follicles) to 3 (moderate atrophy in $^1/_3$ to $^1/_2$ of the follicles). By comparison, lesion scores of vaccinated chickens with only live vaccine (group 2) ranged from 3 to 4 (severe necrosis and atrophy in all follicles). Severe bursal necrosis and atrophy were observed in all non vaccinated and challenged birds (group 5). No histologic lesions were noticed in the bursas of the vaccinated and non challenged birds (group 3 & 4) and the negative control (group 6).

DISCUSSION

Conventional control of IBD has relied on vaccination of parent stock followed by immunization of progeny after maternal antibody levels have waned to avoid interference with the live vaccine. However, the emergence of very virulent IBD (VVIBD) during the last decade caused heavy economic losses. The VVIBD virus is able to infect chickens at early age in the presence of maternal antibody titers too high to allow mild vaccine strains to replicate and elicit active immune reponse . Therefore, vaccines of intermediate virulence have been used more frequently in an effort to control VVIBD virus (Rosales et al., 1989b). These intermediate vaccines are able to be used earlier than the mild vaccines thus inducing active immunity earlier and so reduce the window of infection with VVIBD (Solano et al., 1986).

In Egypt, severe outbreaks of IBD are still reported despite extensive and repeated use of live intermediate vaccines. In an effort to control VVIBD, researchers investigated other stratigies as the use of hot vaccines (Aly et al., 1996) or inoculation of immune yolk (Ahmed & Abd Ellatif, 1996). Hot vaccine (228E) induced better protection but resulted in noticeable bursal lesions especially in chickens with low titers of maternal antibody. Uneven levels of maternal immunity are not uncommon which means that chickens have low level of maternal immunity would suffer from immunosuppression due to vaccination with hot vaccine strain. It is known that there is risk associated with the use of live vaccines attributed to residual virulence. Mazariegos et al. (1990) demonstrated that some intermediate vaccines were almost as pathogenic as a known virulent (Edgar strain) virus with respect to bursal atrophy and histological lesions. It is worth mentioning that hot vaccines have been discontinued in the market place in USA (Lukert & Saif 1991) . Trails of control of IBD outbreaks using egg yolk inoculation as emergency tool proved to be effective in delaying the appearance of signs and lowered mortalities (Aly et al., 1996). Inoculation of immune yolk should be conducted as early as possible i.e. within the first 24 hours post IBD infection. However, A major disadvantage of yolk inoculation is the possibility of contamination with vertically transmitted avian viruses which may lead to serious outcome. Therefore, it should be applied under strict hygienic precautions.

In this study, we investigated the use of inactivated and live vaccines in commercial broilers. As known, inactivated vaccines have been used extensively in breeder flocks as they produce a high level of immunity that is long lived and allows progeny protection through maternal antibody. As shown in tables 2 & 3, chickens vaccinated with live vaccine only at 7 & 21 days old were protected against mortalities when challenged at 28 & 35 old. However, clinical and P.M. examinations revealed that chickens had moderate to severe IBD lesions. Also, results of the extent of bursal damage evaluated by B/BW ratios and histopathology scoring revealed that live vaccine alone was not able to provoke satisfactory protection. In addition, monitor of IBD antibody levels (Fig.1) illustrated that vaccinated and challenged chickens had slight higher level of antibody which lasted for few days. These observations indicate that chickens used in this study which had high levels of maternal antibody did not show active immune response due to interference with live vaccine. The interference between maternal antibody and live vaccine is well documented (Lucio & Hitchner, 1979; Skeeles et al. 1979). Therefore, the results of this experiment emphasize that decision on which a flock may need vaccination must be based on monitoring of IBD antibody at day-old chicks and hence vaccination should be based on "a per flock basis" rather than a general vaccination program.

Chickens in groups IA & IB which received the combination live and inactivated IBD vaccines had the highest antibody titer (Fig.2) and were more resistant to challenge than those vaccinated with only live vaccine. Although chickens were protected against morbidity and mortality after challenge, the B/BW ratio was lower than that of vaccinated nonchallenged and blank control groups. Also, there was mild to moderate microscopic lesions. It is note worthy that intramuscular inoculation of the inactivated vaccine caused moderate to severe post vaccinal reaction at site of inoculation. Hence , it is recommended to inoculate inactivated vaccine subcutaneously in the neck region. Results of this research also demonstrate that there was marked increase in antibody titers recorded about 2 weeks post inoculation of the inactivated vaccine (Fig.2) which resulted in an antibody gap. To birdge this gap, it might be helpful to inoculate the inactivated vaccine as early as the first day of age.

In conculsion, the use of inactivated IBD vaccines in broiler flocks is considered as a positive step toward more effective vaccination programs against this serious disease. Vaccination of day-old chicks against IBD using inactivated vaccine and/or live vaccine will be the subject of future work.

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