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EVALUATION OF DIFFERENT INFECTIOUS BURSAL DISEASE VACCINES

(With 6 Tables)

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تقييم لقاحات مرض الجمبورو المختلفة

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لقد اتضح من خلال دراسة تقييم أنواع مختلفة من اللقاحات المستخدمة لمقاومة مرض التهاب حويصلة فابريسي في كتاكيت في عمر أسبوع وأسبوعين أن الأجسام المناعية المكتسبة من الأمهات قد أثرت على حماية الكتاكيت عند العدوى بعترة شديدة الضرواة على عمر ٤ أو٥ أسابيع. تم قياس الاستجابة المناعية بعد التحصين بمدى الوقاية من العدوى الصناعية والفحص السيرولوجي للأجسام المضادة باختباري الترسيب والاليزا وقياس دليل حويصلة فابريسي بعد أسبوع من العدوى. وقد أعطي اللقاح الحي (288E strain) أعلى حماية (٦٠%) بالمقارنة باللقاح الحي (Bur 706) أو الميت أو التحصين المزدوج باستخدام اللقاح الحي (Bur 706) مع اللقاح الميت أو لقاح جمبورال (Gumboral CT) الحي والتي أعطت جميعها حماية أقل (٤٠- ٥٠%) عند تحصين الكتاكيت في عمر أسبوع. وجميع هذه النسب تعتبر غير مرضية . وكانت الاستجابة المناعية أفضل عند تحصين الكتاكيت في عمر أسبوعين. وقد أعطى اللقاح الحي (228E strain) أعلى حماية (٩٠%) متبوعا بالتحصين المزدوج باستخدام اللقاح الحي. (Bur 706) متبوعا باللقاح الميت (٨٠%). وقد أعطى كل من اللقاح الحي (Bur 706) , اللقاح الميت (٧٠%) حماية بينما لقاح جمبورال أعطى أقل نسبة حماية (٦٠%). وتعتبر جميع نسب الحماية غير مرضية باستثناء الحماية المكتسبة من التحصين باللقاح الحي (Bur 706) .

SUMMARY

The evaluation of different commercial infectious bursal disease virus (IBDV) vaccines in one and two weeks old commercial egg-type male

chicks indicated that maternal antibodies interfered with the development of satisfactory protection when challenged with very virulent IBDV field strain at 4 or 5 weeks of age. Immune response against IBD was estimated by protection rate against challenge and seroconversion using the agar gel precipitation (AGP) and enzyme-linked immunosorbant assay (ELISA) tests. The less attenuated 228 E strain was higher in protection (60%) than Bur 706, inactivated vaccine, and the combined vaccination with BUR 706 and inactivated vaccine which gave (50%) protection, while Gumboral CT was the lowest in protection rate (40%) when vaccination was done at one week of age. Vaccination of 2 weeks old chicks gave better immune response in all vaccinated groups. The less attenuated 228E strain was higher in protection (90%), followed by combined vaccination with BUR 706 and inactivated vaccines (80%), the inactivated vaccine (70%), Bur 706 (70%) and Gumboral CT (60%).

Key words: *Infectious bursal disease vaccines*

INTRODUCTION

Since the first report of infectious bursal disease (IBD) in Egypt by El-Sergany et al. (1974) and Ayoub and Malek (1976), it continues to be a major problem in commercial poultry flocks, particularly after the emergence of the very virulent form of the disease in 1989 in vaccinated flocks (El-Batrawi, 1990). Protection of chicks in early life from IBD virus (IBDV) infection is tried through breeder booster vaccination (El-Batrawi, 1990; Mousa and Saif Edin, 1990). Maternal antibodies have proved to be a problem in the timing of vaccination programs (Hitchner, 1971; Winterfield et al., 1980). Winterfield and Thacker (1978) reported that an intermediate vaccinal strain induced moderate bursal lesions and have the ability to overcome the maternal immunity. Mousa et al. (1988a) reported that an apathogenic IBDV isolate (T-73) recovered from turkeys was highly protective after vaccination at 4, 8 and 12 days of age in commercial chicks possessing maternal antibodies against IBDV. Mousa et al. (1988 b) evaluated the efficiency of different IBDV vaccines and found that strain 1/65/PV (Biogumboro, Bio. Pharmaceutical Research Production Lab., Italy), strain Winterfield 2512

(CEVA lab. inc. overland park, KS France), Vineland and Univax were efficiently immunogenic in birds possessing no detectable maternal immunity, but their immune response was not sufficient in chicks with high levels of maternal immunity. On the other hand, strain D78 (Intervet) vaccine produced moderate bursal lesions, with no immunosupressive effect and was immunogenic in both immune and susceptible chicks. Goddard et al. (1994) reported that there was no benefit in administering a live vaccine either alone or in addition to an inactivated oil-emulsion vaccine to commercial layer chicks with maternally derived antibodies. Abou Zeid et al. (1995) concluded that a locally prepared Gumboro disease vaccine was safe and efficient in protecting the vaccinated birds against Gumboro disease. CAO-Yong Chang et al. (1998) reported that inactivated vaccines should not be used in parent birds and that young broilers should be vaccinated with live IBDV vaccines at 1 day of age and revaccinated at 8 and 15 days. Savic et al. (1998) observed that the highest titer of antibodies in broilers was achieved when they were revaccinated at 12 days with live vaccine after the first vaccine. Zorman-Rojs and Cajavec (1998) reported that live vaccines can not protect broilers against very verulent IBDV strains.

This work was planned to evaluate four different commercial IBDV live and inactivated vaccines in 7 and 14 days old commercial chicks possessing variable antibody levels.

MATERIAL and METHODS

Commercial chicks:

One day-old commercial male Hyline chicks were obtained from Hyline parents immunized four times with live IBD vaccine during the growing period and boostered at 18 weeks of age with inactivated IBD oil adjuvant vaccine. The chickens were kept on a wirenet floor in complete isolation for vaccination trials and challenge.

IBD vaccines:

Four commercial IBD vaccines were used for vaccination of the experimental chicks:

A- Live IBDV vaccines:

1- Gumboral CT:

It is a live freeze-dried vaccine against Gumboro disease (RHONE MERIEUX). Each vaccine dose contained at least 10^3 EID_{50} of attenuated IBDV.

2- BUR 706:

It is a freeze dried modified live vaccine against IBD, (RHONE MERIEUX). Each vaccine dose contained at least $10^{4.0}$ EID_{50} of attenuated IBDV.

3- Less attenuated IBDV vaccines (intermediate plus):

It is a live vaccine (strain 228E, Intervet). Vaccine was a field virus isolated from a non-vaccinated flock of broiler chickens. Each vaccine dose contained at least 10^4 EID_{50}.

B-Inactivated IBDV vaccine:

Inactivated IBDV vaccine (RHONE MERIEUX) was used for vaccination by intramuscular route.

Newcastle disease virus (NDV) vaccine:

Hitchner B1 vaccine (CEVA) was used for vaccination of all birds used in this study against ND via drinking water according to the manfacture's recommendations.

Challenge IBDV:

A very virulent IBDV (VVIBDV) field isolate was provided by Dr. S. Mousa, Dept. of Poultry Dis., Fac. of Vet. Med., Assiut Univ. It was used at a dose of 100 chicken infective dose 50 (CID_{50}) intraocularly to challenge the vaccinated experimental chicks.

Chicken embryos:

10 days old embryonated chicken eggs were provided by the farm of Fac. of Agriculture, Assiut Univ. for propagation of the challenge virus by the chorio-allantoic membrane (CAM) route.

Agar gel precipitation test (AGPT):

The test was carried out according to the method of Hirai and Shimakura (1972) using bursal homogenate from birds infected with IBDV strain (D.78) as antigen.

ELISA test:

Serum samples were assayed at a final dilution of 1:500 for antibodies to IBDV, using a commercial ELISA system (Flock-chek Agritech system, Portland, Maine). The test procedure followed the

directions supplied with the kits and ELISA titetrs were logarithmically transformed.

Histopathological examination:

Paraffin sections were prepared from the bursae, stained with hematoxylin and eosin and examined microscopically. All sections were then scored from zero to 5 for lesions according to the criteria of Rosales et al. (1989): 0 = no detectable lesions, 1 = less than 25% of lymphoid follicles affected, 2 = 25-50% of lymphoid follicles affected, 3 = 50-75% of lymphoid follicles affected and, 4 = greater than 75% of lymphoid follicles were involved.

Experimental design:

Evaluation of different live and inactivated IBDV vaccines as well as combined vaccination with live and inactivated vaccines.

I- In one week old chicks:

A number of 300, one- week-old commercial chicks were divided into 6 equal groups. Birds of group 1, 2, 3 were vaccinated by eye drop with BUR 706, Gumboral CT and less attenuated (strain 228E) IBD vaccines, respectively. Group 4 was vaccinated intramuscularly (I/M) with inactivated vaccine. Group 5 was vaccinated with live intermediate (BUR 706) vaccine by eye drop, then revaccinated with inactivated vaccine I/M at 2 weeks of age. Group 6 served as non vaccinated control.

II- In two weeks old chicks:

A number of 300, two weeks old commercial chicks were divided into 6 equal groups. Birds of group 1, 2, 3 were vaccinated via eye drop with BUR 706, Gumboral CT and less attenuated (strain 228E)IBD vaccines, respectively. Group 4 was vaccinated I/M with inactivated vaccine. Group 5 was vaccinated with live intermediate (BUR 706) vaccine by eye drop, then revaccinated with inactivated vaccine I/M at 3 weeks of age. Group 6 served as non vaccinated control.

In all experiments ten serum samples were collected from each group at the time of vaccination and every week post-vaccination and subjected to AGP and ELISA tests. All birds were challenged at the 4th week of age (experiment I) or the 5th week (experiment II) with VVIýBDV by eye drop method. They were observed for clinical signs and mortalities were recorded. One week after challenge, survivor birds were killed, weighed immediately and the bursae were removed and

weighed. Bursa/body weight ratios and bursa/body weight indexes were calculated after the fromula of Lucio and Hitchner (1979) as follows:

$$\text{B/B weight index} = \frac{\text{Bursa/body weight ratio of infectd birds}}{\text{Bursa/body weight ratio of control group}} \times 100$$

The bursae were fixed in buffered formalin for histopathological examination.

RESULTS

The results of evaluation of different commercial live and inactivated IBDV vaccines in one week old commercial chicks which have mean ELISA titers of maternal antibodies (1494-1770) and challenged with VVIBDV field strain at the 4th week of age are shown in Tables (1, 2 and 3). They indicated that the less attenuated (228 E) strain vaccine was higher in protection rate (60%) and mean bursa body weight index (3.8) as well as lower bursal lesion score (2) in comparison to the other vaccines and non vaccinated challenged control. Mean ELISA titers at 4 weeks of age (just before challenge) were negative in all experimental groups.

The results of evaluation of different commercial live and inactivated IBD vaccines in two weeks old commercial chicks which have moderate mean ELISA titers of maternal antibodies (920-1060) and challenged at the 5 th week of age are shown in Tables (4, 5 and 6). They indicated that there was better immune response in all vaccinated groups as compared to vaccination at one week of age, and the less attenuated (228 E) vaccine was again higher in both protection rate (90%) and mean bursa body weight index (3) and lower in bursal lesion scores (1) than the other vaccines. Mean ELISA titers at 5 weeks of age (just before challenge) ranged between 1018 and 7890 in the vaccinated groups.

DISCUSSION

In spite of various vaccination programs adopted against IBD, infection continued to be a major problem in commercial flocks in

Egypt. Usually the breeders are vaccinated 2-4 times with attenuated live IBD vaccines during the growing period followed by inactivated vaccine at 18-20 weeks of age. It was obvious that the passively transfered antibodies interfere with the development of vaccinal immunity.

In this study the main concern was to evaluate the protectiveness of some commercially available IBDV vaccines in one and two weeks old commercial egg-type chicks against a VVIBDV field strain.

The evaluation criteria were based on the degree of protection, bursa body weight index, bursal lesion scores and seroconversion as judjed by AGP and ELISA tests.

The results of vaccination with different live and inactivated vaccines at one week of age in chicks possessing high mean ELISA titers (1494-1770) of maternal antibodies and challenged 3 weeks later indicated that the less attenuated (228 E) strain was comparatively higher in protection (60%) than BUR 706 (50%), inactivated (50%), combined live and inactivated vaccine (50%) and Gumboral CT (40%). Moreover, declining antibody levels were noticed at all intervals following vaccination with any of the vaccines used. These results are unsatisfactory and document the interfering effect of high maternal antibody levels on the development of active vaccinal immunity regardless of the type of the vaccine used.

Administration of the vaccines to two weeks old chicks of the same hatch which possessed moderate mean ELISA titers (920-1060) of maternal antibodies and challenged 3 weeks later resulted in comparatively better immune response in all vaccinated groups, but the less attenuated vaccine strain (228E) gave satisfactory and superior protection (90%) to the other vaccines used (protection less than 90%). Lucio and Hitchner (1979) found that porgeny from breeders vaccinated with oil emulsion IBDV vaccines had maternal immunity sufficient to protect them for 4-5 weeks. Such maternal immunity could prevent effective immunization with live IBDV vaccines (Lucio and Hitchner, 1980; Wood et al., 1981; Sharma, 1985). The immune response may also be prevented because of the negative feed-back effect on the immune system exerted by the existing antibody (Subba Rao et al., 1978). Also Savic et al. (1998) and Zorman-Rojs and Cajavec (1998) mentioned that live vaccines can not fully protect broilers against IBDV.

In the present study the less attenuated IBD vaccine (228 E) caused some damage to the bursae of chicks with low titers of maternal

antibodies. This result confirms the so-called (intermediate virulence) of this type of vaccines (Rosales et al., 1989; Tsukamoto et al., 1995).

Relatively high but unsatisfactory levels of protection (<90%) were obtained by intermediate vaccines (Bur 706 and Gumboral CT) given at 2 weeks of age which may be attributed to the ability of these strains to overcome residual maternal antibody to some extent. Similar results were reported by Hitchner (1971); Winterfield and Thacker (1978); Mousa et al. (1990); Mazariegos et al. (1990) and Tsukamoto et al. (1995).

The results of protection rate obtained by combined live intermediate and inactivated vaccines given at 2 and 3 weeks of age, respectively, were better than when given at 1 and 2 weeks (experiment I) or when each vaccine was given alone. Similar results were reported by Goddard et al. (1994).

Rosenbusch et al. (1990) found that the protection rates against challenge was higher after vaccination with inactivated vaccines than after live vaccines.

The results of microscopic examination of bursae of survivor birds 1 week post-challenge to evaluate the extent of bursal damage by bursa/body weight index and bursal score lesions were similar to those of Rosales et al. (1989). Certain degree of bursal damage was evident in all vaccinated groups one week following challenge with VVIBDV regardless of the vaccine used.

It could be concluded that IBD outbreaks still occur in spite of using different IBDV vaccines and vaccination programs resulting in variable losses in chicks. The determination of IBDV maternal antibody levels is important for prediction of the suitable time of vaccination. Sound management and biosecurity doubtless play a decisive role in preventing or at least minimizing early and heavy exposure to the field virus before optimal vaccinal immunity have time to develop.

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Table 1: Protection rate, bursa body weight index and bursal lesion score in chicks vaccinated with different IBD vaccines at one week of age and challenged at 4 weeks of age

Group	Type of vaccine	Morbidity %	Mortality %	Protection %	Mean B/BW* index 1 wk P chl	Bursal lesion score 1 wk P chl.
1	Bur 706	60	50	50	2.6	3
2	Gumboral CT	60	60	40	2.9	3
3	Strain 228E	50	40	60	3.8	2
4	Inactivated	70	50	50	2.8	3
5	Bur 706+ inactivated**	60	50	50	2.7	3
6	Non vaccinated control	70	70	30	2.1	4

** B/BW = bursal body weight ; wk P ch = week post-challenge*
*** Inactivated vaccine was given at 2 weeks of age*

Table 2: Results of AGP test in chicks vaccinated with different IBD vaccines at one week of age and challenged at 4 weeks of age.

Group	Type of vaccine	%Positive in AGP test Age in weeks			
		1	2	3	4
1	Bur 706	90	90	60	0.0
2	Gumboral CT	80	70	50	0.0
3	Strain 228E	70	70	50	0.0
4	inactivated	90	90	40	0.0
5	Bur 706+ inactivated	80	70	30	0.0
6	Non vaccinated control	80	70	30	0.0

Table 3: Mean ELISA titersa in commercial chicks vaccinated with different IBD vaccines at one week of age and challenged at 4 weeks of age

Group		Mean ELISA titers			
		1	2	3	4
1	Bur 706	1494	520	334	398
2	Gumboral CT	1684	499	290	260
3	Strain 228E	1770	511	610	596
4	Inactivated	1695	1540	930	547
5	Bur 706+ inactivated*	1560	1493	670	543
6	Non vaccinated control	1695	1534	643	337

Table 4: Protection rate, bursa body weight index and bursal lesion score in chicks vaccinated with different IBD vaccines at 2 weeks of age and challenged at 5 weeks

Group	Type of vaccine	Morbidity %	Mortality %	Protection %	Mean B/BW index 1 wk P chl	Mean bursal lesion score 1 wk P chl.
1	Bur 706	60	30	70	2.0	2
2	Gumboral CT	50	40	60	2.6	3
3	Strain 228E	20	10	90	3	1
4	Inactivated	70	30	70	1.8	2
5	Bur 706+ inactivated*	40	20	80	2.6	2
6	Non vaccinated control	80	85	15	1.7	4

** Inactivated vaccine was given at 3 weeks of age*

Table 5: Results of AGP test in chicks vaccinated with different IBD vaccines at 2 weeks of age and challenged at 5 weeks of age

Group	Type of vaccine	Positive in AGP test			
		Age in weeks			
		2	3	4	5
1	Bur 706	70	30	20	0.0
2	Gumboral CT	50	40	60	50
3	Strain 228E	60	40	80	70
4	Inactivated	70	50	30	20
5	Bur 706+ inactivated	70	40	30	30
6	Non vaccinated control	60	30	10	0.0

Table 6: Mean ELISA titers in commercial chicks vaccinated with different IBD vaccines at 2 weeks of age and challenged at 5 week of age

Group	Type of vaccine	Mean ELISA titers			
		Age in weeks			
		2	3	4	5
1	Bur 706	941	560	780	1018
2	Gumboral CT	1060	1531	2430	2898
3	Strain 228E	989	1700	2933	3605
4	inactivated	980	660	3980	6400
5	Bur 706+ inactivated	920	890	4750	7890
6	Non vaccinated control	1003	520	425	330