COMPARATIVE STUDIES ON DIFFERENT STRAINS OF INFECTIOUS BURSAL DISEASE VIRUS FOR PREPARATION OF HIGH QUALITY INACTIVATED OIL EMULSION VACCINE

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ABSTRACT

An immunological comparative study was done using 4 vaccinal strains of infectious bursal disease virus (IBDV) [BursaVac-M, D78, IBD-Blen and 228E] in order to prepare an inactivated oil emulsion IBDV vaccine. Experimental vaccines prepared using the fore mentioned strains pools. The antibody response was measured using serum neutralization test (SNT) and enzyme linked immunosorbent assay (ELISA) tests. Obtained results revealed that the most efficient vaccines were IBD-Blen and BursaVac-M. The results were confirmed after challenge test using the virulent IBDV field strain.

INTRODUCTION

IBD is an acute highly infectious viral disease of young chickens first described by **(Cosgrove, 1962)**. The disease cause high economic losses for poultry industry due to its immunosuppressive effect, high morbidity and mortality rates **(Fargher et al., 1974)**.

In Egypt many outbreaks were reported in either vaccinated and non vaccinated chicken flocks with high losses reached to 70 % in layer pullets and 30 % in meat broilers **(Khafagy et al., 1990 and Saif Edin and Mousa, 1996)**.

The failure to control the disease inspite of using commercially available vaccines has let us to study the serological response of some IBDV vaccinal strains in order to prepare an inactivated vaccine with efficient immunological response when it is possible.

MATERIAL AND METHODS

*** IBD viruses:**

*** Vaccinal strains:**

a- Mild strain (BursaVac-M).

b- Intermediate strains (D78 strain).

e- Hot strain (IBD-Blen and 228E strain).

All these strains were obtained from Newcastle disease Department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

• Virulent strain :

Local virulent strain (108 EID_{50}/ml) was kindly supplied by **Hala El-Makaky (1996)**, Department of Newcastle disease, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

• Tissue culture adapted IBDV :

It was kindly obtained from **Nadia (2001)**, Department of Newcastle disease, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo; and was used for SNT.

• Chickens :

One hundred and fifty day-old Hubbard chicks obtained from the United Company for Poultry Production.

• Preparation of inactivated oil emulsion IBDV vaccines :

Five types of vaccines using different types of IBDV strains (BursaVac-M, D78, IBD-Blen, 228E and pool of these strains) were prepared respectively. These four strains were propagated in SPF embryonated chicken eggs separately according to **Hitchner (1970)**, the collected viruses were inactivated using formalin 0.2 % in final concentration, the fluids were left on a magnetic stirrer at room temperature for 24 hours (**Li et al., 1986**). The four strains beside the pool of them (quarter from each strain) were used in preparation of five inactivated oil emulsion IBDV vaccines using paraffin oil (white light oil resella 17) having 106 EID_{50}/dose for each according to **Thayer et al. (1983)**.

Prepared vaccines were subjected to sterility, stability and safety tests according to Standard International Protocols as described by **British Veterinary Codex (1970) and Code of American Federal Regulation (1985)**.

Experimental design:

One hundred and fifty day-old Hubbard chicks were obtained and reared in isolated and disinfected wire floored cages. Fifteen random serum samples from these chicks were tested for maternal antibodies against IBDV by SNT and found susceptible to experimental infection after 21 days. The chicks were divided into six equal groups each of 25 chicks and were treated as follow:

Group 1 : Vaccinated with inactivated oil emulsion IBDV vaccine strain BursaVac-M.

Group 2 : Vaccinated with inactivated oil emulsion IBDV vaccine strain D78.

Group 3 : Vaccinated with inactivated oil emulsion IBDV vaccine strain IBD-Bien.

Group 4 : Vaccinated with inactivated oil emulsion IBDV vaccine strain 228E.

Group 5 : Vaccinated with inactivated oil emulsion IBDV vaccine of the pooled four strains.

Group 6 : Control non-vaccinated.

Each bird was vaccinated with 0.5 ml of vaccine S/C in the dorsal aspect of the neck. This dose contained at least 10^6 EID_{50}

Ten random blood samples were taken from each group weekly till 9 weeks post vaccination. Serum was separated from blood and tested for antibodies against IBD using the following serological tests.

1- Serum neutralization test :

This test was carried out according to the method described by **Ferreia (1976)**. The titer was expressed as the reciprocal of the highest serum dilution which neutralize 100-200 tissue culture ID_{50}.

2- ELISA test :

This test was carried out on a potent ELISA kits (IDEXX Inc., USA) according to the method described according to instructions of the producing company. The titer equation was calculated as follow.

$$\text{Log}_{10} \text{ titer} = (1.09 \times \log_{10} S/P) + 3.36$$

$$\text{Titer} = \text{anti} \log_{10}$$

3- Challenge test :

Twelve birds from each group three weeks post vaccination were subjected intraocularly to 10^3 EID_{50}/dose of virulent IBDV. The chicks were observed for 10 days post challenge. Dead birds during this period were collected for PM examination.

RESULTS AND DISCUSSION

Sterility test on the prepared vaccines proved that they were free from bacterial and fungal contamination. They were also safe for inoculated chickens after two weeks observation period.

Obtained results represented in Table (1) revealed that a highest titer for neutralizing antibodies (256) were recorded at 4^{th} week post vaccination (WPV) for group (1) and 4^{th} and 5^{th} WPV for

group (3), however a titer of 128 was recorded as early as 2 WPV for the former group, meanwhile the maximum titer (128) was recorded for the other groups persist for 8th, 7th and 6th WPV for groups (2), (4) and (5) in order. Regarding to the former neutralizing antibody results it could concluded that group (3) that vaccinated with IBD vaccine using IBD-Blen strain gave the best results followed by groups (1), (2), (4) and (5) that vaccinated with IBDV using BursaVac-M, D78, 228E and pooled strains respectively.

However results of ELISA antibody titers (Table 2) comes parallel to those of neutralizing antibody titers for groups (1), (3) and (5). The direct correlation obtained in this study between SNT and ELISA results agreed with that obtained by **Marquandt et al. (1980) and Sun et al. (1997)**, but noticeable differenee was recorded for the other two groups, (2) and (4), and this results comes in contact with that obtained by **Gerlach (1986)** where he stated that the ELISA results was not always reasonable with SNT results.

On judgment on the performed used strain for preparation of an excellent IBDV inactivated vaccine, it could be concluded that vaccine prepared by IBD-Blen and BursaVae-M strains were the most reliable strains for inactivated oil emulsion IBDV vaccine preparation. These results could be attributed to the presence of epitope 21 in the former two strains which present on very virulent IBDV as stated by **Mengel and Snyder (1994)**.

Serological response in this study were assured after the challenge with the virulent IBDV as presented in Table (3) where it recorded 91.6% for groups (1), (2) and (3), 83.2% for groups (4) and (5), and 16.8% for control none vaccinated group.

Table 1 : Serum neutralizing antibody titers in chickens vaccinated by different types of oil inactivated IBDV vaccines.

Chicken groups	Antibody titers / weeks post vaccination								
	1	2	3	4	5	6	7	8	9
1	32	64	128	256	128	128	128	128	64
2	32	64	128	128	128	128	128	128	64
3	32	128	128	256	256	128	128	128	64
4	16	64	64	128	128	128	128	64	64
5	32	32	128	128	128	128	64	64	64
6	0	0	0	0	0	0	0	0	0

Group (1) : chickens vaccinated by oil inactivated BursaVac-M IBDV vaccine.
Group (2) : chickens vaccinated by oil inactivated D78 IBDV vaccine.
Group (3) : chickens vaccinated by oil inactivated IBD-Blen IBDV vaccine.
Group (4) : chickens vaccinated by oil inactivated 228E IBDV vaccine.
Group (5) : chickens vaccinated by oil inactivated pool IBDV vaccine.
Group (6) : non-vaccinated control.
Antibody titer = the reciprocal of serum dilution which neutralize and inhibit the CPE of 100-200 TCID50 of IBDV.

Table 2 : Average mean ELISA antibody titer against IBDV in groups of vaccinated chickens.

Chicken groups	Antibody titers / weeks post vaccination								
	1	2	3	4	5	6	7	8	9
1	1381	1585	1862	2799	2543	2755	3184	3275	1907
2	1300	1668	1782	2142	2128	1966	2318	3122	1845
3	1488	1600	2341	2433	2936	2662	3950	3807	2398
4	1488	1823	2313	1912	2915	2363	3089	3214	1715
5	1300	1341	1394	1839	3275	2394	2442	3270	2368
6	220	275	170	215	273	273	290	139	157

N.B. Cut off value = 396.

Table 3 : Protection test against IBDV in chickens at 3rd week post vaccination.

Group	No. of birds	Mortality	Protection %
1	12	1/12	91.6
2	12	1/12	91.6
3	12	1/12	91.6
4	12	2/12	83.2
5	12	2/12	83.2
6	12	10/12	16.8

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الملخص العربى

محاولات مبدئية لتحضير لقاح ثلاثى مثبط زيتى ضد أمراض النيوكاسل والالتهاب الشعبى المعدى وتدنى البيض فى الدجاج

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فى دراسة مقارنة مناعية بين العترات المختلفة لڤيروس مرض الجمبورو (Bursa Vac-M, D78, IBD-Blen and 228E) وذلك لاختيار أفضلها مناعياً فى تحضير لقاح زيتى مثبط ضد مرض الجمبورو (ڤيروس غدة فابريشيس المعدى)، فقد تم تحضير أربعة لقاحات زيتية مثبطة من العترات السابقة بالإضافة إلى لقاح خامس يشمل خليط من هذه العترات. تم تحصين مجموعات مختلفة من الطيور بهذه اللقاحات وبعد دراسة رد الفعل المناعى باستخدام إختبارى التعادل المصلى والإليزا إتضح أن اللقاحات المحضران باستخدام العترتين IBD-Blen وBursa Vac-M أعلى كفاءة مناعياً من العترات الأخرى وقد عضد إختبار التحدى باستخدام العترة الحقلية شديدة الضراوة ضد مرض الجمبورو هذه النتائج.