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PREPARATION AND LABORATORY EVALUATION OF LIVE ATTENUATED INFECTIOUS BURSAL DISEASE (IBD) VIRUS VACCINE FROM RECENT EGYPTIAN ISOLATE PROPAGATED ON CHICKEN EMBRYO FIBROBLAST (CEF) CELL CULTURE.

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SUMMARY

Very virulent IBDV was propagated for 3 passages in SPF-ECE followed by 3 passages in young susceptible chicks then for 3 passages in SPF-ECE then for further 12 passages in SPF-ECE. The first passage of last propagation was further propagated for 3 passages on Vero cells and for 60 passages in CEF- cell culture.

The passage 40 of propagated IBDV proved to be safe and highly immunogenic by virus neutralization test. It still elicited high neutralizing antibody titers (18 log2) for a stationary phase about 7 months (the end of the experiment) and high values of lymphocyte blastogenesis (0.560 versus control 0.03). Vaccinated chicks well protected against challenge with highly virulent IBDV after 3 weeks post-vaccination (PV). The prepared vaccine has superior potential immunogenic effect than commercial live mild, and intermediate plus (hot) vaccines. The vaccine is effective even when preserved for 8 months (the end of experiment) at - 20°C.

INTRODUCTION

Different types of live IBDV vaccines are used for controlling IBDV infections. In term of virulence, live vaccines range from mild, intermediate to intermediate plus (lukert and saif 1997). Although these kinds of live vaccines are neutralized by maternal antibodies, the intermediate vaccines are superior to the mild vaccines in giving immunity to commercial chickens with maternal antibodies because intermediate vaccines are less affected by maternal antibodies. However, intermediate vaccines vary in virulence, some of them can induce severe bursal atrophy and immuno - suppression in birds with no maternal antibodies or low maternal antibodies (Mazariegos et al

1990) Till now it is difficult to control IBD with mild vaccines. This low efficiency of mild vaccines may have occurred for two reasons. First maternal antibodies might have interfered with vaccine (Wood et.al. 1981 and Tsukamoto et al. 1995). Second, the immunity induced by these mild live vaccines might be simply insufficient (Edison 1982, Giambrone & Yu 1982 and Kissling & Henk 1983). Intermediate and avirulent strains overcome maternal virus neutralizing antibodies titers of 250 and less than 100 respectively (lucio & Hitchner, 1979 and Skeeles et.al 1979 b). Vaccination with a more invasive vaccine does not provide better immunity when all antibodies are weaned (Vieltiz et al. 1991). The intermediate plus IBDV vaccines are antigenically related to highly virulent IBDV field strains which caused up to 100 % mortality in SPF-chicks and were able to protect chicks against challenge. Birds were protected as soon as 4 days after vaccination (Van Den Berg et.al 1991). Intermediate plus vaccine strains overcome maternal VN antibody titers of 500 (Skeels et al 1979 b). However, a cell-culture-adapted IBDV vaccine was as effective as a commercial vaccine in protecting against challenge. Chickens immunized with the cell-culture-adapted IBDV showed less effect on the bursa than chickens vaccinated with the commercial vaccine (Skeeles, et.al. 1979 a). The production of a cheap, safe, potent and effective vaccine from recent local Egyptian isolate of IBDV propagated on tissue culture cells and has the ability to protect chickens from the highly virulent IBDV is considered a desired objective in vaccine production. The aim of the present study is to prepare a protective and suitable CEF-propagated vaccine against IBDV.

MATERIAL AND METHODS

Chicks : seven hundred Hubbard 21days old chicks were used for vaccine evaluation.

Virus : IBDV bursal homogenate from natural outbreaks in broilr flocks located at El-Fayoum governorate, Egypt (1997) was used in these studies. The virus was obtained from the Department of Newcastle disease, Veterinary Serum and Vaccine Research Institute Abbassia, Egypt. This virus was propagated in 15 SPF-ECE (SPAFAS, USA) for three times (5 SPF - ECE per each passage) with end titers 6.3 $\log_{10}$ then propagated again three times in 3 weeks old chicks (10 chicks per each passage and 10 control non-infected. This virus was designated Fc-97 as the capital litter (F) denote to the locality at which the broilers were reared (Fayoum) and the small litter (c) denote to the host from which the virus was isolated (chicken) and the number 97 denote to the year in which the outbreaks were occurred. Furthermore, the bursal homogenate was propagated for successive passages in brain of mice 3 weeks old and subjected for further passages in SPF-ECE and young chicks as mentioned above this virus was named FM-97 as the capital litter (M) denote to the initial host for propagation (mice). Both Fc-

97 and FM-97 were further propagated in SPF-ECE for 12 passages, and the first passage of last propagation was propagated for further 3 passages in Vero cells then for 60 passages in CEF-cell culture.

Cells : chicken embryo fibroblast (CEF) cells were prepared from SPF-ECE and used for IBDV vaccine preparation and virus neutralization. The cells were supplemented with MEM (Gibco) with 10 % newborn calf serum (NCS-Gibco) as growth medium or 2 % NCS as in maintenance medium.

Media : RPMI-1640 (Gibco) was used for lymphocyte blastogenesis assay. Nutrient agar, Sabouraud glucose agar, thioglycolate broth and Frey's media were used as described by Cruickshank (1975). These media were used for testing sterility of the prepared vaccine.

Virus neutralization (VN) test : was applied according to Rossiter et. al ., (1985).

Lymphocyte blastogenesis assay : was applied according to Garn et. al., (1994).

Virus titeration : was applied according to Jackwood et. al., (1984) .

The infectivity titers $TCID_{50}$ /ml were determined according to Reed & Munch (1938).

RESULTS

When Fc-97 and FM-97 IBDV isolate were propagated on CEF cell culture for 60 passages and the fifth passage subsequently from first passage to the 60 passage was intraocular instillated in susceptible 21 day old chicks, the morbidity and mortality rates varied (Table 1). P1 and P5 of both Fc-97 and FM-97 IBDV isolate induced 100 % mortalities, P10 did not induce any mortalities, P15 and P20 induced 20 % mortalities in FM-97 but not in Fc-97, P25 induced 20 % moralities in both, P30 did not induce any mortalities in both Fc-97 & FM-97, P35 induced 40 % mortalities in Fc-97 and 20 % in FM-97, while from P40 to P60 no mortalities were observed. The passage of choice that will not induce post inoculation reactions or mortalities is not strictly defined therefore P39 to P 41 were used for determining the most safe passage that did not induce any morbidity or mortalities when intraocular instillated in susceptible chicks. The passage 39 induced 20 % mortalities by both Fc-97 and FM-97 but P40 and P41 did not induce any mortalities. Therefore the best safe passage of choice is P40 (Table 1). When survived chicks were challenged with the very virulent IBDV 21 days post intraocular instillation with the prepared passages the P40 of Fc-97 or FM-97 propagated on CEF cell culture offered good protection (Table 2). The prepared vaccine (P40 Fc-97 on CEF cell culture) when tested for sterility it proved to be free from any contaminants. When it was used for evaluating the im-

mune response (humeral and cellular) it was compared with other commercial vaccines (mild, intermediate and intermediate plus). The mean neutralizing antibody titers log_2 in sera of vaccinated chicks denote to the potency of the local prepared vaccine as it induced good humeral immune response from the first week post vaccination (PV) till the 28th weeks PV (8 & 18 log_2 respectively) but the commercial live mild intermediate and hot vaccines induced weak humeral immune response (2 - 11 log_2) when compared with the local prepared vaccine. The neutralizing antibody titers offered by the local prepared vaccines were still high in a stationary phase for about 7 months (the end of the experiment) with 18 log_2 mean neutralizing antibody titers while the live commercial mild vaccine induced humeral immune response graduated from 4 log_2 in 1 st week PV to 11 log_2 in the 6th week PV, then decreased gradually to 5 log_2 in 11th week PV to 2 log_2 in 22 week PV. The intermediate commercial vaccine is less to some extent in its induction of humeral immune response than the mild commercial but in great variation than the locally prepared vaccine. Furthermore the intermediate plus (Hot vaccine) induced very weak humeral immune response, that decreased gradually from 7 log_2 VN antibody titers in 3rd week PV to 4 log_2 VN antibody titers in 12th week PV then 2 log_2 in 22th PV (Table 3).

On regarding lymphocyte blastogenesis of locally prepared vaccine it well activated lymphocyte blastogenesis as it produced high values (0.31) in the first week PV and still that at 3rd week PV (0.568) then decreased in the 4th week PV to (0.240). The mild commercial vaccine also activate the lymphocyte blastogenesis but in less extent than the local prepared vaccine. The intermediate and intermediate plus (Hot) vaccines activate the lymphocyte blastogenesis in the 1st week PV then decreased gradually from the 2nd week PV till the 4th week PV (Table 5). The histopathological examination of bursae of vaccinated chicken with the locally prepared vaccine (5 days PV) indicated that this vaccine is of mild type one as it did not deplete the lymphocyte from the bursae. and not induce bursal atrophy (Fig. 1 & 2). The live CEF-cell culture propagated vaccine is effective after 8 months of preservation at -20°C. It offered 100 % protection when vaccinated chickens were challenged with very virulent IBDV (Table 4)

Table (1) Experimental infection of 21 day old chicks with the propagated Fc-97 and FM-97 IBDV isolates on CEF cells.

No. of passage	Fc-97						FM-97					
	Infectivity titer expressed in log10 $TCID_{50}$/ml	No. of Chicks used	No. of dead chicks	Mortality %	No. of contact control not challenged	No. of dead contact control	Infectivity titer expressed in log10 $TCID_{50}$/ml	No. of Chicks used	No. of dead chicks	Mortality %	No. of contact control not challenged	No. of dead contact control
1	8	10	10	100	3	3	7.5	10	10	100	3	3
5	8	10	10	100	3	3	7.5	10	10	100	3	3
10	8	10	0	0	3	3	7.5	10	0	0	3	3
15	8	10	0	0	3	3	7.5	10	2	20	3	3
20	8	10	0	0	3	0	7.5	10	2	20	3	0
25	8	10	2	20	3	0	7.5	10	2	20	3	0
30	8	10	0	0	3	0	7.5	10	0	0	3	0
35	8	10	4	40	3	0	7	10	2	0	3	0
39	8	10	2	20	3	0	7	10	2	20	3	0
40	7.8	10	0	0	3	0	7	10	2	20	3	0
41	7.8	10	0	0	3	0	7	10	0	0	3	0
45	7.8	10	0	0	3	0	6.8	10	0	0	3	0
50	7.2	10	0	0	3	0	6.8	10	0	0	3	0
55	7.2	10	0	0	3	0	6.8	10	0	0	3	0
60	7.2	10	0	0	3	0	6.8	10	0	0	3	0

Table (2): Challenge of 21 days old chicks received propagated Fc-97 and FM-97 IBDV isolates on CEF cells.

No of passage	Fc-97								FM-97							
	No of challenged chicks	Infectivity titer expressed in log10 TCID50/ml	No. of dead chicks	Morbidity %	Mortality %	PM lesions	No. of Challenged control	No. of dead control by virulent virus	No. of inoculated chicks with virulent virus	Infectivity titer expressed in log10 TCID50/ml	No. of dead chicks	Mortality %	Mortality %	PM lesions	No. of Challenged control	No. of dead control
1	-	8	-	-	-	-	-	-	-	7.5	-	-	-	-	-	-
5	-	8	-	-	-	-	-	-	-	7.5	-	-	-	-	-	-
10	10	8	6	60	60	Typical IBD lesion	3	3	10	7.5	2	20	20	Typical IBD lesion	3	3
15	10	8	4	40	40	Typical IBD lesion	3	3	8	7.5	0	0	0	Typical IBD lesion	3	3
20	10	8	0	0	0	Typical IBD lesion	3	3	8	7.5	2	-	25	Typical IBD lesion	3	3
25	8	8	2	25	25	Typical IBD lesion	3	3	8	7.5	2	25	25	Typical IBD lesion	3	3
30	10	8	2	20	20	Typical IBD lesion	3	3	10	7.5	0	0	0	Typical IBD lesion	3	3
35	6	8	0	0	0	Typical IBD lesion	3	3	8	7.5	0	0	0	Typical IBD lesion	3	3
39	8	8	2	25	25	Typical IBD lesion	3	3	8	7	0	0	0	Typical IBD lesion	3	3
40	10	7.8	0	0	0	Not found	3	3	10	7	0	0	0	Not found	3	3
41	10	7.8	0	0	0	Not found	3	3	10	7	0	0	0	Not found	3	3
45	10	7.8	0	0	0	Not found	3	3	10	7	0	0	0	Not found	3	3
50	10	7.2	0	0	0	Not found	3	3	10	6.8	0	0	0	Not found	3	3
55	10	7.2	0	0	0	Not found	3	3	10	6.8	0	0	0	Not found	3	3
60	10	7.2	0	0	0	Not found	3	3	10	6.8	0	0	0	Not found	3	3

Typical IBD lesions = enlargement and hemorrhagic bursa.

Table (3): Mean neutalizing antibody titers log 2 of sera collected from vaccinated chickens with local and commercial vaccines

No. of passage	No. of challenged chicks	Weeks post vaccination																	
		1st	2nd	3rd	4th	5th	6th	7th	8th	10th	11th	12th	14th	16th	18th	1st	20th	22th	24th	28th
50	Live CEF	8	8	19	17	17	19	19	20	20	20	20	19	20	20	20	20	20	20	18
50	Live mild	4	5	5	7	8	11	8	7	6	6	5	5	5	4	4	3	2	2	2
50	Intermediate	5	5	6	8	8	11	6	6	6	5	5	5	4	4	3	3	2	2	2
50	Hot	5	6	7	7	7	7	6	6	6	5	5	4	4	4	3	3	2	2	2
50	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Locally prepared vaccines
* Imported vaccines.

Table (4): Rate of protection of locally prepared CEF cell propagated live attenuated Fc-97 vaccine after 8 months of preservation at -20°C

No. of chicken used	Vaccine used	3rd month		4th month		5th month		6th month		7th month		8th month	
		Protection	Prot. %	Protection	Prot. %	Protection	Prot. %	Protection	Prot. %	Protection	Prot. %	Protection	Prot. %
30	Live CEF attenuated	5/5	100	5/5	100	5/5	100	5/5	100	5/5	100	5/5	100
12	unvaccinated	0/2	0	0/2	0	0/2	0	0/2	0	0/2	0	0/2	0

Prot. = protection

Table (5): lymphocyte bloastogenesis of chickens vaccinated with live attenuated (locally prepared and imported) vaccines

Number of vaccinated chicken	Type of vaccine used	Lymphocyte blastogenesis values per Weeks post vaccination			
		1	2	3	4
50	Locally CEF-propagated	0.431	0.236	0.560	0.290
50	Mild	0.391	0.267	0.369	0.314
50	Intermediate	0.432	0.299	0.245	0.182
50	Intermediate plus	0.503	0.312	0.204	0.282
50	Control	0.04	0.02	0.03	0.06

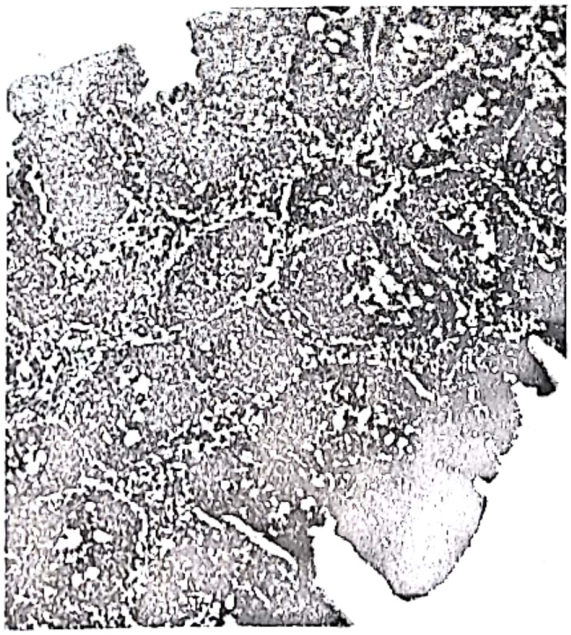

Fig (1) Bursa of of unvaccinated chick shows no lesions (stained with H & E).

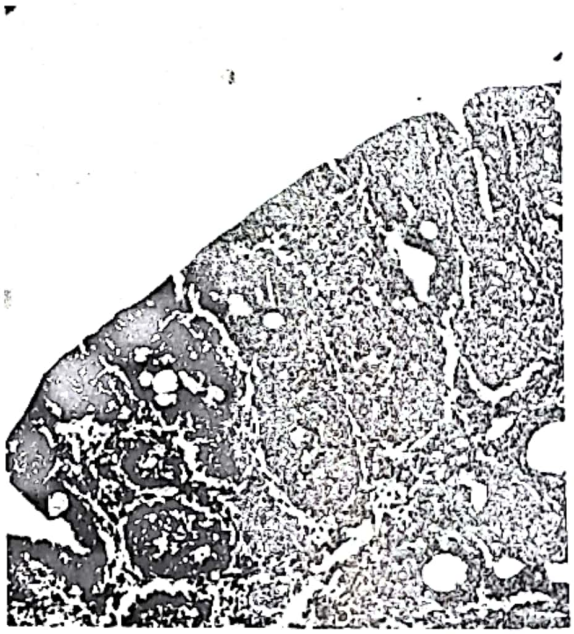

Fig (2) Bursa of vaccinated chick with CEF cell culture propagated vaccine shows mild depletion of lymphocyte (stained with H&E)

DISCUSSION

From the above mentioned results it is very clear that the P40 of both Fc-97 and FM-97 on CEF cell culture are safe and effective when used to vaccinate 21 day old susceptible chicks where there is no maternal antibodies. The survived chicks, post vaccination with CEF-cell culture adapted vaccine that challenged 21 days PV revealed that chicks received P10 - P30 showed from 20 - 60 % mortalities with typical clinical disease & bursal lesions of IBDV infection but chicks received P35 - 60 did not show clinical disease, bursal lesions or mortalities (Table 2). These results assure that the P40 of IBDV on CEF cell culture is safe and protective against highly virulent IBDV field isolate.

The variation in mortality rates offered by passages from 1 to 39 but not by passages after that (40 - 60) may be due to the variation in the epitopes of virulence that altered by further passages. These results are in agreement with those obtained by (Mandelli, et.al.; 1972, Skeels, et.al. 1979a, Saijo, et.al. 1990 and Tsukamoto, et.al. 1995) whom reported that the IBDV lost its virulence by progressive propagation on CEF cells. On other words, the adapted CEF-cell culture prepared vaccine showed reduction in the pathogenicity of vaccinated young chickens and did not kill any of them and this may be attributed to the mutagenesis of viral nucleic acid and antigenic diversity between the prepared cell culture adapted vaccine and the original field isolate that produced 100 % mortalities in challenged not vaccinated chickens. Therefore to assure this point protein electrophoresis analysis of propagated virus was needed. Tsai and Saif (1992) found that 30 - 40 passages in BGM-70 cell culture resulted in loss of pathogenicity and replication of IBDV variants, and when used as live vaccine it did not protect SPF chicken. On the other hand when it was used as inactivated preparation it maintained their antigenicity and immunogenicity as demonstrated by the immunofluorescence and VN tests and by the satisfactory protection induced by vaccinating SPF-chicken. The loss of replication in live vaccine and maintenance of antigenicity in inactivated vaccine denote to the mutation of replicative genes. Also Yamaguchi, et.al. (1996) observed antigenic diversity between the cell-culture-adapted HV-IBDV strains and classical strains by cross-VN test. The CEF cell culture adapted P40 of Fc-97 IBDV proved to be highly immunogenic as it induced high neutralizing antibody titers for about 7 months (the end of the experiment) with 18 $\log_2$ VN antibodies. On the other hand the commercial live mild vaccine induced satisfactory VN antibody titers 11 $\log_2$ at the 6th week PV then decreased gradually to 2 $\log_2$ at 22 week PV. The lowest VN antibody titers induced by hot vaccine followed by intermediate one are due to highly destructive effect of B-cells by both vaccines and this result was confirmed by lymphocyte blasto-

genesis (Table 5). The histopathological examination of bursae of vaccinated chickens with the CEF-cell culture-adaped vaccine revealed that the prepared vaccine is of miled type one because it did not induced any destructive effect in bursal B-lymphocytes follicles or bursal atrophy. The undestructive B-lymphocyte by miled type vaccines in chicken with low or without maternal antibodies were achieved by (Skeeles et.al. 1979 a , Wood et.al. 1981 and Tsukamoto et.al. 1995). Otherwise, the destructive effect, bursal atrophy and immunosuppression in one day old, 3 weeks old SPF-chickens and in chickens of low maternal antibodies were observed by (Mazariegos et.al. 1990, and Lukert & Saif 1997). From these results it could be concluded that the CEF-cell culture adapted locally prepared vaccine can be used safely in chickens haven't maternal antibodies or of low maternal antibodies for its potential immune response and satisfactory protection.

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