IMMUNOLOGICAL STUDIES ON THE LOCAL INFECTIOUS BURSAL DISEASE VIRUS (IBD) ADAPTED ON SPECIFIC PATHOGEN FREE EMBRYONATED CHICKEN EGGS SPF (ECE)

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Abstract

Local isolate of (IBD) virus [EID_{50} 10^6/ml] was propagated for three passages on (SPF) embryonated chicken eggs (EID_{50} $10^{6.5}$/ml) followed by three passages on SPF chickens (EID_{50} 10^8/ml), then, for hundred and ten serial passages on SPF embryonated chicken eggs prepared and titrated (EID_{50} 10^7/ml) after every 5 passages. The virus was inoculated into 21 days old chickens and observed for 21 days post-infection, then, challenged with virulent IBD virus. Passages (110, 77, 55) were used for preparation of live attenuated vaccines mild, intermediate, hot (IBDV) vaccines on SPF (ECE), respectively. The evaluation of the prepared vaccines was carried out for sterility, safety and potency. The potency was performed by measuring humoral and cellular immune response, as well as, protection percentage against virulent IBDV.The Efficacy of the prepared vaccine was estimated for up to six months.

INTRODUCTION

Infectious Bursal Disease Virus (IBDV) is of considerable economic interest for poultry industry worldwide.

The vaccine is used for control of the disease which is a common practice in the poultry industry.

However, the use of vaccines, the incidence of IBD virus infection and associated disease problems are still common.

Since 1987, there had been an increasing number of acute cases of IBDV with high mortality even in older chickens, being more evident with highly virulent IBD virus. The highly virulent IBDV has similar antigenicity to the classical strains. It can establish infections at levels of maternal antibodies that were protective against classical strains.

Therefore, there is a great attention to produce IBDV attenuated vaccines from field isolate of IBDV through serial passages in embryonated eggs (Yamaguch *et al .,* 1996).

The aim of the present study is propagation and adaptation of IBDV field isolate on SPF(ECE)for prepartion of live attenuated vaccine and evaluating the efficacy and potency of the vaccine in young susceptiable chickens.

MATERIALS AND METHODS

1-Virus Strain: IBDV bursal homogenate of local Egyptian isolate with titer of 10^6(Nadia *(*2001).

2-Chicks: Four hundreds 21 days old hubbard chickens susceptible to IBD virus were used for vaccine evaluation .

3-Embryos: Embryonated chickens specific pathogen-free (SPF) eggs were obtained from Ministry of Agriculture Koum Osheim, Fayoum, Egypt. At zero days, eggs were kept under observation in the incubator for 9-10 days, and were used for propagation and titration of IBD virus.

4-Media: Nutrient agar, Sabauraoud`s glucose, thioglycolate broth and Frey`s media were used for testing sterility of the prepared vaccines.

Methods

1-Propagation of IBD virus was carried out on SPF (ECE)for three passages, followed by three passages on SPF chiken, then, for hunderd and ten passages with titration of each five passages on SPF (ECE). It was applied according to Jackwood *et al*. (1984).The infectivity titers EID_{50} /ml were calculated according to Reed and Maench(1938).

2-Preparation of different types of vaccine. Live attenuated IBD virus on SPF (ECE) was used[the passage 55, p 77, p110] according to Isac *et al*. (2002).

3-Vaccination of chicks

a**.** Each bird was vaccinated with one dose of the live attenuated prepared vaccine [10^5 EID_{50} of each vaccine contained in 0.3 ml physiological saline].

b. Safety test

Each bird of 21 days old [10 chicks for each group] was installed intraocularly (1/0) with 10 field dose/bird with each prepared vaccine. The chicks were observed for 21 days post-inoculation, and three birds/group were weekly subjected to bursal/ body index and histopathological examination.

4-Challenge test: It was carried out according to Saif-Edin *et al* . (1996).

The previous vaccines were tested for sterility according to OIE , for immunological effect with serum neutrilization test (Rossiter *et al* ., 1985), lymphocyte blastogenesis assay(Garn *et al* ., 1994),bursal body weight ratio (Tasi and Saif, 1992), for histological examination (Nakamura *et al* ., 1990), for protection and

keeping quality (Saif-Edin *et al .,* 1996). These tests were carried out and compared with those vaccinated with (B.vac, D 78,228 E).

RESULTS

Table 1. Infectivity titers of IBDV isolation on SPF (ECE).

NO. of passages	Infectivity titer in Log 10 [EID_{50}/ML]
1st passage	6.1
2nd passage	6.2
3rd passage	6.5

Table 2. Infectivity titers of propagated [IBDV] isolation [SPF] young chicks.

NO. of passages	Infectivity titers in Log10 [EID_{50} /ml]
1ST passage	7
2nd passage	7.5
3rd passage	8

Table 3. Propagation and infectivity titers of further propagated IBDV isolate on SPF(ECE).

NO.of passages	Route of inoculatin	NO.of Inoculated egggs	NO.of Dead eggs	NO.of Postive eggs	Positive lesions of Inoculated ECE[IBDV]	Log10 EID_{50}/ml
1	C.A.M	5	5	5	5/5	8
5	C.A.M	5	5	5	5/5	8
10	C.A.M	5	5	5	5/5	8
15	C.A.M	5	5	5	5/5	8
16	C.A.S	5	5	5	5/5	8
20	C.A.S	5	5	5	5/5	8
25	C.A.S	5	5	5	5/5	8
30	C.A.S	5	5	5	5/5	8
34	C.A.S	5	5	5	5/5	8
35	C.A.S	5	4	5	5/5	8
40	C.A.S	5	4	5	5/5	8
45	C.A.S	5	4	5	5/5	8
50	C.A.S	5	4	5	5/5	8
55	C.A.S	5	4	5	5/5	8
60	C.A.S	5	4	5	5/5	7.75
64	C.A.S	5	4	5	5/5	7.75
65	C.A.S	5	4	5	5/5	7.50
68	C.A.S	5	4	5	5/5	7
69	C.A.S	5	1	5	5/5	7
70	C.A.S	5	0	5	5/5	7
75		5	0	5	5/5	7
77		5	0	5	5/5	7
78		5	4	5	5/5	7
79		5	4	5	5/5	7
80		5	5	5	5/5	7
85		5	5	5	5/5	7
90		5	5	5	5/5	7
93		5	5	5	5/5	7
94		5	4	5	5/5	7
95		5	4	5	5/5	7
96		5	4	5	5/5	7
97		5	4	5	5/5	7
98		5	3	5	5/5	7
99		5	3	5	5/5	7
100		5	2	5	5/5	7
105		5	2	5	5/5	7
110		5	2	5	5/5	7

C.A.M (Chiroallantoic Membrane).

C.A.S (Chiroallantoic Sac).

Positive lesion of inoculated embryonated eggs with IBDV embryos Subcutaneous oedema , Haemorrhages,Liver necrosis, and Death.Chiroallantouc membrane ,Haemorratgic,Oedematous.

Table 4. Experimental infection of 21 days old chicks by propagated IBDV isolate on SPF (ECE).

NO.of passages	Infectivity titer in Log 10 EID50/ml	NO. of chicks used	NO. of dead chicks	Mortality %	NO. of contact control	NO .of dead contact control
1	8	10	10	100%	3	3
5	8	10	10	100%	3	3
10	8	10	10	100%	3	3
15	8	10	8	80%	3	3
20	8	10	8	80%	3	3
25	8	10	5	50%	3	3
30	8	10	0	0%	3	0
35	8	10	4	40%	3	0
40	8	10	0	0%	3	0
45	8	10	0	0%	3	0
50	8	10	2	20%	3	0
55	8	10	0	0%	3	0
60	7.75	10	0	0%	3	0
65	7.50	10	0	0%	3	0
70	7	10	0	0%	3	0
75	7	10	0	0%	3	0
80	7	10	0	0%	3	0
85	7	10	0	0%	3	0
90	7	10	0	0%	3	0
95	7	10	0	0%	3	0
100	7	10	0	0%	3	0
110	7	10	0	0%	3	0

Table 5. The challenge of chicks inoculated with the propagated IBDV isolate on SPF (ECE).

NO. of passages	NO.of inoculated chicks with virulent virus	NO. dead chicks	Morbidity %	Mortality %	P/M lesions	NO.of Challenge control	NO. of dead challenge control
15	2	0	0	0	Not found	3	3
20	2	0	0	0	Not found	3	3
25	5	0	0	0	Not found	3	3
30	6	2	33	33	Typical IBDV lesions	3	3
35	10	2	33	33	Typical IBDV lesions	3	3
40	10	0	0	0	Not found	3	3
45	10	0	0	0	Not found	3	3
50	8	2	25	25	Typical IBDV lesions	3	3
55	10	0	0	0	Not found	3	3
60	10	0	0	0	Not found	3	3
65	10	0	0	0	Not found	3	3
70	10	0	0	0	Not found	3	3
75	10	0	0	0	Not found	3	3
80	10	0	0	0	Not found	3	3
85	10	0	0	0	Not found	3	3
90	10	0	0	0	Not found	3	3
95	10	0	0	0	Not found	3	3
100	10	0	0	0	Not found	3	3
110	10	0	0	0	Not found	3	3

Table 6. Experirnental infection of chicks inoculated with propagated IBDV isolate on SPF (ECE) and their challenge.

NO. of passages	Infectivity titer in Log10, EID50/ml	NO.of Chicks used	NO. of Dead chicks	Mortality	NO.of Contact control	NO.of dead Contact control	NO. of Inoculated Chicks with Virulent virus	NO.of Dead chicks	Morbidity	Mortality	P/M lesions	NO.of Challenge control	NO. of dead Challenge control
P50	8	10	2	20%	3	0	8	2	25%	25%	IBDV lesion	3	3
P51	8	10	2	20%	3	0	8	2	25%	25%	IBDV lesion	3	3
P52	8	10	2	20%	3	0	8	2	25%	25%	IBDV lesion	3	3
P53	8	10	0	0	3	0	10	0	0	0	Not found	3	3
P54	8	10	0	0	3	0	10	0	0	0	Not found	3	3
P55	8	10	0	0	3	0	10	0	0	0	Not found	3	3

Table 7. Safety test.

Group of vaccination	NO.of chicks used	Vaccination| chick 10fild close W.P.V			W.p.challanged		
		1ST Morb. morta	2nd Morb. morta	3rd Morb. morta	1ST Morb. morta	2nd Morb. morta	3rd Morb. morta
P55	10	0 0	0 0	0 0	0 0	0 0	0 0
P77	10	0 0	0 0	0 0	0 0	0 0	0 0
P110	10	0 0	0 0	0 0	0 0	0 0	0 0
control	10	0 0	0 0	0 0	6 6	4 4	- - - -

W.P.V =WEAK POST VACCINATION
W.P.CHALLANGED =WEAK POST CHALLENGED
MORB.= MORBIDITY
MORTA. =MORTALITY

Table 8. Sterility of prepared live attenuated IBDV.

media	Live propagated IBDV		
	P55	P77	P110
Nutrient ager	NC	NC	NC
Thioglycollate broth	NT	NT	NT
Saboraud glucose agar	NC	NC	NC
Frey's media	NC	NC	NC

NC = NO COLONIES ON THE USED MEDIUM
NT= NO TURBIDITY IN USED BROTH

Table 9. Mean neutralizing anti- body titers log_2 in sera of chicks vaccinated with locally prepared live attenuated vaccine SPF (ECE) compared with those vaccinated with B.vac, D78 and 228E.

Chicken group	Type of Vaccine used	Week post-vaccination																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	20	22	24
1	Live SPF (ECE) (p55)	5	6	7	7	7	8	8	8	7	7	7	6	6	6	5	5	5	4	4	4
2	Live SPF (ECE) (p77)	5	5	7	7	8	8	9	9	10	10	9	8	8	7	5	5	4	4	4	3
3	Live SPF (ECE) (p110)	6	7	8	8	12	11	11	12	12	12	12	12	12	11	11	10	10	10	9	9
4	Mild (B.vac)	4	5	6	7	8	8	11	10	10	9	9	8	8	7	5	5	4	4	4	3
5	Intermediate (D78)	5	5	7	8	7	8	10	9	9	9	8	7	6	6	5	4	4	3	3	3
6	Hot (228E)	5	6	7	7	7	7	8	8	8	8	7	7	6	5	4	4	4	3	3	3
7	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 10. Mean of lymphocyte blastogenesis of vaccinated chicks with locally prepared live attenuated vaccines on SPF(ECE)compared with those vaccinated with B.vac,D78 and 228E.

Chicken group	Type of vaccine used	Weeks after vaccination		
		1st	2nd	3rd
1	Live SPF (ECE) P55	0.489	0.366	0.290
	Live SPF (ECE) P77	0.429	0.338	0.320
	Live SPF (ECE) P110	0.442	0.266	0.590
2	Mild B.vac	0.402	0.302	0.400
3	Intermediate D78	0.443	0.330	0.275
4	Hot 228E	0.515	0.345	0.235
5	control	0.04	0.01	0.03

Table 11. Bursal body weight ratio in vaccinated chickens before and after challenge.

Chicken group	Type of vaccine used	Weight in bursal		Body weight		Bursal body weight ratio	
		5 days post vaccination	5 days post challenge	5 days post vaccination	5 days post challenge	5 days post vaccination	5 days post challenge
1	Live SPF (ECE) P55	0.2000	0.4021	180	220	1.111	1.8277
2	Live SPF (ECE) P77	0.1800	0.3390	190	240	0.9478	1.4125
3	Live SPF (ECE) P110	0.1130	0.3260	190	250	0.5947	1.3040
4	Mild B.vac	0.09120	0.2650	170	220	0.5362	1.2045
5	D78	0.1900	0.3420	170	230	1.109	1.4089
6	228E	0.2100	0.3521	180	220	1.1744	1.6000
7	CONTROL	0.2030	0.3600	190	250	1.0711	1.4400

Table 12.The rate of protection of locally prepared live attenuated IBDV vaccine after preservation at-20°C.

Chicken group	Type of vaccine used	Temp. of Storage	Protection for every month after preservation											
			1st month		2nd month		3rd month		4th month		5th month		6th month	
			Prot	Prot. %	Prot.	Prot. %	Prot.	Prot.%	Prot.	Prot.%	Prot.	Prot. %	Prot.	Prot.%
1	Live Prepared P55	-20°C	5/5	100	5/5	100	5/5	100	5/5	100	5/5	100	5/5	100
2	Live Prepared P77	-20°C	5/5	100	5/5	100	5/5	100	5/5	100	5/5	100	5/5	100
3	Live Prepared P110	-20°C	5/5	100	5/5	100	5/5	100	5/5	100	5/5	100	5/5	100
4	control	------	0/2	0	0/2	0	0/2	0	0/2	0	0/2	0	0/2	0

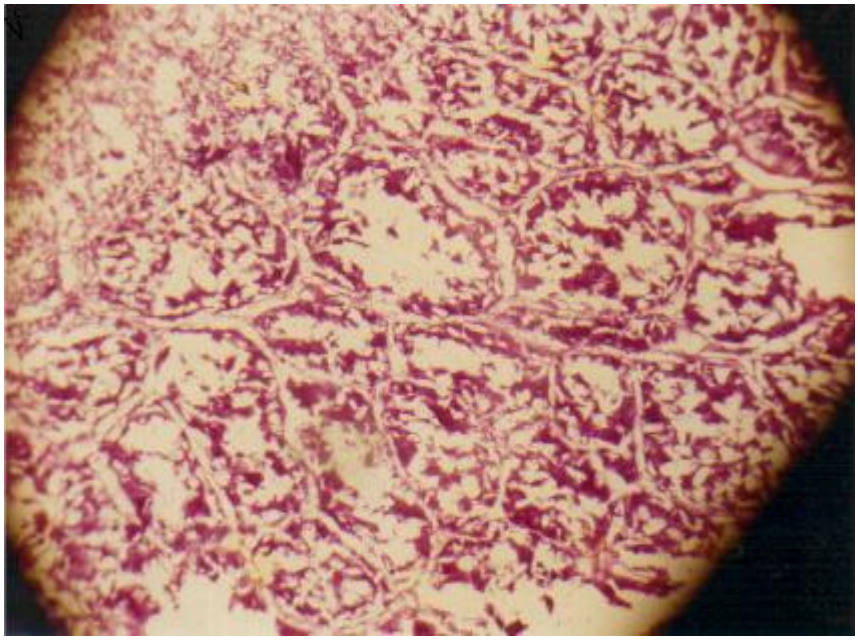

Fig 1. Histopathological section of the bursa of chicken 5 days post - vaccination with locally prepared live attenuated IBDV vaccine on SPF ECE P55(X l00).

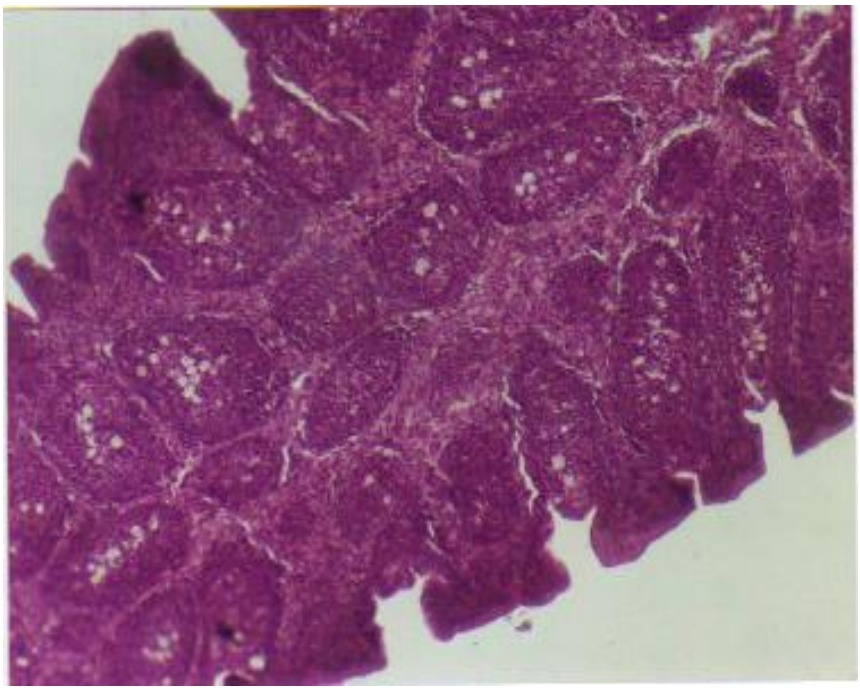

Fig 2. Histopathological section of the bursa of chicken 5 days post-vaccination with locally prepared live attenuated IBDV vaccine on SPF ECE P77 (X l00).

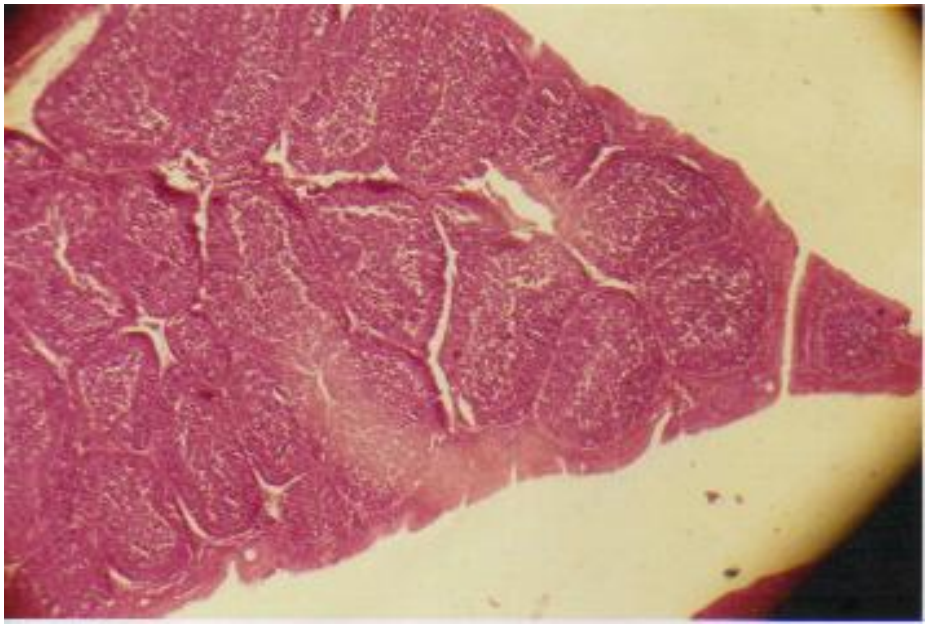

Fig 3. Histopathological section of the bursa of chicken 5days post-vaccination with 1ocal1y prepared live attenuated IBDV vaccine on SPF ECE P110 (X l00).

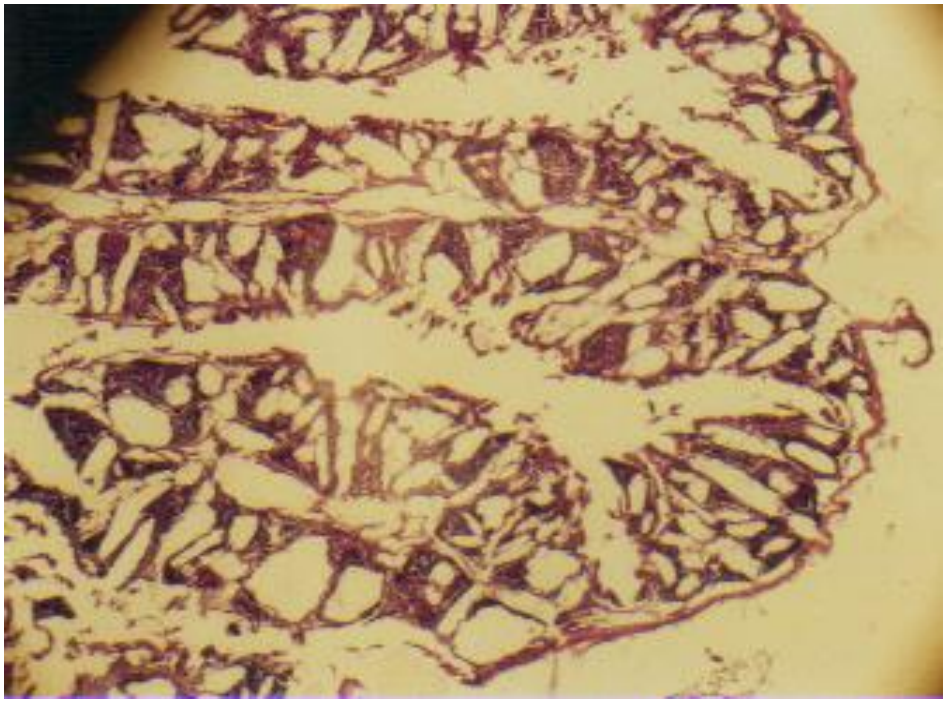

Fig 4. Histopathological section of the bursa of chicken challenged with IBDV 5 days post-experimental infection (X l00).

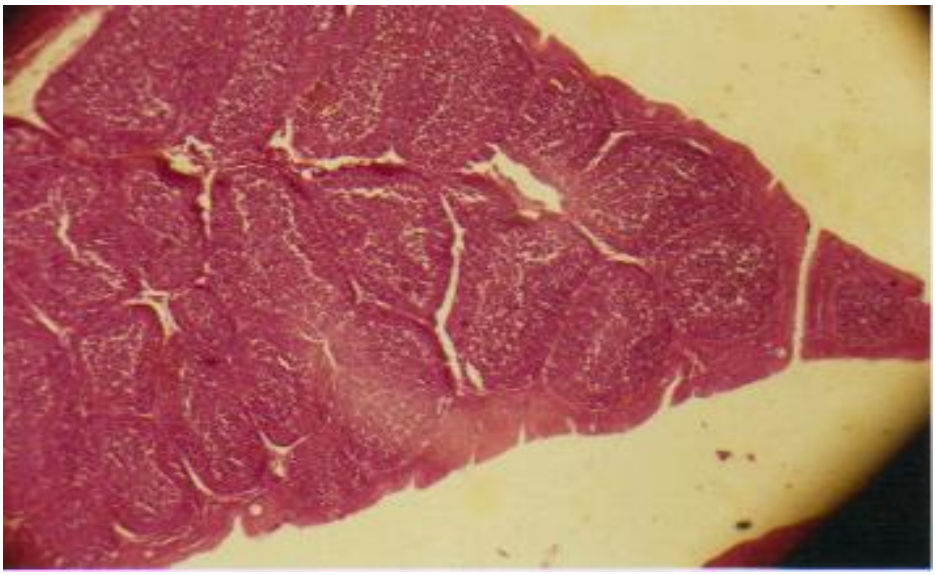

Fig 5. Histopathological section of the bursa of chicken non-vaccinated as control negative (X100).

DISCUSSION

The aim of present study was to prepare a safe and potent live attenuated IBD vaccine propagated on SPF (ECE) from local field isolate.

The scheme used for preparing the vaccine started with the propagation of a bursal hemogenate of the isolated IBD virus for three passages on SPF embryonated chicken eggs SPF (ECE), followed by further 3 passages on SPF chickens and followed by 110 serial passages on SPF (ECE).

It was noticed in Table 1,that there was an increase in infectivity titer EID_{50} from the first to the third passage with 0.4log 10 , while, in Table 2,there was an increase in infectivity titer (EID_{50}) from first passage in chicken to third passage with one log, and reached to 10^8 EID_{50}/ml.This result agreed with that obtained by Dutko *et al*. (1988).

In Table 3, it was noticed that infectivity titer EID_{50}/ml from p_1 till p_{59} was $10^{8.}$, while, p60 till p64 EID_{50} decreased and reached to $10^{7.75}$ and p65 till p_{69} EID_{50}, then, reached $10^{7.50}$ and from p70 till p110 EID_{50} it was stable 10^7.

In inoculated eggs from p1 till p15, inoculation of SPF (ECE) through chorio allantioc membrane and from p16 till p110 inoculation of SPF (ECE) through chorio allantioc sac and from p1 till p34, the number of dead inoculated embryonated eggs was 5 (5/5) to each passage .Also, we noticed that the mortality rate of inoculated embryos was changed with passages p35 till p68, the number of dead embrys in inoculated eggs with IBDV was 4 (4/5) to each passage and p69 the number of dead

inoculated eggs 1 (1/5) and from p70till p77 no deaths in inoculated eggs were noticed .

In p78, the number of dead inoculated eggs was 4 (4/5), and in p80 till p93 the number of dead inoculated eggs was 5 (5/5), and from p94 till p97, dead inoculated eggs were 4 (4/5), and in p 98 -p99 the number of dead inoculated eggs was 3 (3/5), and from p100 till p110 the number of dead inoculated eggs was 2 (2/5) to each passage . Positive lesion of IBDV in inoculated embryonated eggs was from p1 to p110 .The positive lesion of inoculated eggs with IBDV was as follows:

Embryos ➔edematous, haemorrhagic , liver necrosis and death.

Chorioallantoic membrane ➔ edematous, haemorrhagic. The results agreed with those obtained with RAO *et al*. (1978).

In Table 4, when IBDV was propagated on SPF (ECE) for 110 passages and every five passage, it was inoculated in 10 susceptible 21 days old chicks. The morbidity and mortality rates varied: p1, p5 and p10 of (IBDV) isolate induced 100% mortalities, p15 and p20 induced 80%mortalities, P25 of IBDV isolate induced 50%mortalities, but p30 did not induce any mortality. In p35 it induced 40% mortalities, in p40 and p45 it did not induce any mortalities, and p50 induced 20% mortalities.

From p 55 to p 110, no mortalities were observed, and also the contact control chicken from p1 to p25 induced 100% mortality, but, from p30 to p110 contact control chicken did not induce any mortality, then variation in mortality rates by passages from p1 to p50 but not by passages after that (p55-p110) .This may be due to the variation in the epitopes of virulence being altered by further passages .These results are in agreement with those obtained by Michael *et al.* 1997, Rinaldi (1972) who reported that the IBDV lost its virulence by progressive propagation on SPF (ECE).

In Table 5, when inoculated chicks were challenged with the very virulent IBDV 21days post- inoculation with the prepared passages, the p15-p20-p25did not induce any mortality, but, in p30&p35 it induced 33% mortalities, while, in p40&p45 it did not induce any mortality, but, in p50 it induced 25% mortalities, in p50 to p110 it did not induce any mortality in chickens.

The prepared passage 55 on SPF (ECE) gave good protection to inoculated chickens. These results agreed with those obtained by Saif (1994).

In Table 6, the passage of choice that did not induce any mortality was which we used p50 to p55 for determining the most safe passage that did not induce any morbidity or no mortalities when inoculated in susceptible chicks. The p50-p51-p52 induced 20% mortalities and after challenge, it induced 25% mortalities, but, from

p53-p54-p55 it did not induce any mortality from inoculated passages or after inoculation with virulent virus.

Passage of choice which did not induce any mortality was p53 which gave 100% protection.This result agreed with that obtained by Chowd Hury *et al.,* (1996).

In Table 7, it was indicated that the prepared vaccines were safe when inoculated in susceptible chicks with ten times the field dose .It was noticed that there was no morbidity or mortalities in vaccinated chicks, while, all non -vaccinated control died after challenge .This result agreed with that obtained by Saif-Edine *et al.* (1996).

Table 8 confirms the sterility of prepared vaccines from any contaminant.

From results of Tables 3,4,5,6, we noticed that the passage of choice was p53 EID_{50}/ml 8log10, and the number of positive eggs [5] was 5/5 and number of dead inoculated eggs was 4[4/5]. In passages 70 to p77, the presence of other variation in infectivity titer EID_{50} 7log10 and number of positive lesion of inoculated eggs [5] 5/5 and number of dead inoculated (ECE) Zero [0/5] meant an increase in amount of collected eggs from inoculated embryonated eggs.

So, it is profitable to use p77 to inoculated chicks and to determine immune response in inoculated susceptible chicks. We made comparison between three passages 55-77-110 after inoculation in susceptible chicks, and determined immune response (humoral and cellular) compared with other commercial vaccines.

In Table 9, we mentioned the mean neutralizing antibody titers log 2 in sera of vaccinated chicks with the locally prepared vaccine [p110] which induced good humoral immune response from the first week post-vaccination till the 24^{th} week pv[6&9 log, respectively]and reached to maximum value in week 5 till week 13 (12log2). From week 14-15 it reached to 11log2 and in week 16 to 20 it reached to 10log2 and ended in week 24 (9log2).

In p77, the mean neutralizing antibody titers log2 reached from week (3) to 7log 2 and increased gradually till it reached to the maximum in week 9-10, then, reached 10log2, then, decreased to gradually till reached to 3log2 in week 24 ,while, in p55 mean neutralizing antibody titers log2 in sera of vaccinated chicks. We noticed that, from the first week ,it reached 5log2 gradually and increased till reached to maximum value in week 6-7-8 (8log2) and gradually decreased till it reached in week24 (4log2) .When compared with other commercial vaccines, we found that mild vaccine (B.vac) vaccine began with 4log2 in first week and gradually increased till reached to maximum in week 7 11log2 and ended with 3log2 in week 24 . Also, in intermediate vaccine (D78), it began with 5log2 in first week and gradually increased till reached to maximum in week 7 (10log2), then, gradually decreased till reached to 3log2 in week 24. The hot strain (228E) being the neutralizing antibody titers, began

from first week with 5log2 and reached to maximum in week 7 till 11 (8log2) then, decreased till reached 3log2 in week 24.

From the above results, we noticed that the mild vaccine was highly immunogenic as it induced high neutralizing antibody titers for about 6 months with 9log2 VN antibodies, and the lowest VN antibody titers were induced by intermediate and hot vaccines due to the highly destructive effect of B-CELLS by both vaccines (hot and intermediate).

These results agreed with those obtained by Lukert (1992). So, we recommend to use live mild vaccine in young age susceptible chicks.

In Table10,we noticed that lymphocyte blastogenesis of locally prepared vaccine activated lymphocyte blastogenesis, as it produced high values in first week post- vaccination in p110 (0.442)and still till 3rd (0.590),but, in p77 in first week (0.425) and to 3rd (0.320) and in p55 in first week (0.489)and to 3rd (290) and in mild B.V ac vaccine in first week (0.402) and in third week (0.400) and in D78 first week (0.443) and 3rd (0-275).

In 228E first week (0.515), 3rd (0.295) these results agreed with those obtained by Mayahi (1997).

The histopathological examination of bursae of vaccinated chickens with the SPF (ECE) revealed that the prepared vaccine from (p110) is of mild type because it did not induce any destructive effect in bursal B-lymphocyte follicles of bursal atrophy.

The prepared vaccine from (p77) is of intermediate type because it induced slight destructive effect on Bursal B-lymphocytes of follicles in chickens with low or without maternal antibodies, but, the prepared vaccine from p55 is of hot type because it induced destructive effect in bursal B-lymphocyte follicles in vaccinated chickens. These results agreed with those obtained by Tsukamoto *et al. (*1995).

From Table11, it is clear that the locally prepared live attenuated vaccines have no clear difference between the bursal body weight ratios of vaccinated chicks and control chicks.

In vaccinated chicks with locally prepared IBDV p110-p77 and p55, we found that the result of bursal body weight ratio after 5 days of vaccination challenged the first one nearly to mild vaccine (B.vac), but the second one challenged nearly to intermediate vaccine (D78) and the third challenged nearly to hot vaccine (228E).

This result agreed with that obtained by Nieper and Muller (1994). From these results and from histopatological examination of bursae of vaccinated chickens and also, results of lymphocytes blasogensis, we could classify IBDV propagated on SPF (ECE) as p110 (Mild vaccine), p77 (intermediate vaccine) and p55 (hot vaccine).

In Table 12, discussing the keeping quality of the prepared live IBD virus vaccines when stored at -20° C, it was clear that the vaccines were stable and potent for a period of 6 months where the protection reached 100%.This result agreed with Saif –Edin *et al.* (1996).

From these results, it could be concluded that adapted locally prepared vaccine on SPF (ECE) P110can be used safely in chickens which have not or of low maternal antibodies [one day old chicks, 3 week old chicks] .So, the preparation of live attenuated vaccine is safe, potent and immunogenic in young chicks.

P77, P55 can be used safely in older chickens at 45 days, but p55 can be used as inactivated vaccine.

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دراسة القدره المناعية للعترة المعزولة محليا" لفيروس التهاب غدة فابريشيس بعد تمريره١ علي آجنة البيض الخالي من المسببات المرضية

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تم تمرير العترة المعزولة محليا " من فيروس مرض التهاب غده فابريشبس ٣ تمريرات علي آجنه البيض الخالي من المسببات المرضية ثم ٣ تمريرات آخري علي الكتاكيت الخالية من المسببات المرضية ثم مائة و عشرة (١١٠) تمريرة علي آجنة البيض الخالي من المسببات المرضية و قد تم حقن كل خمس [٥] تمريرات في عدد (١٠) كتاكيت عمر الحادى و العشرين يوما"و تمت الملاحظة لمدة إحدى وعشرين يوما" بعد حقنها بالفيروس الضاري .

و قد وجد أن التمريرة رقم **٥٥** و٧٧ و١١٠ قد فقدت قدرتها المرضية و قد أعطت حماية للكتاكيت المحقونة بنسبة ١٠٠% و استخدمت لتحضير لقاح حي مستضعف لفيروس مرض التهاب غده فابريشبس .

و قد تم تقييم اللقاح المحضر من حيث النقاوة و الآمان و القدرة المناعية و قد تم قياس المناعة الخلوية و اجراء اختبار التحدي بأستخدام الفيروس الضاري.

و قد تم قياس المناعة للكتاكيت المحصنه بأستخدام اختبار المصل المتعادل و قد تم حفظ اللقاح عند –٢٠° م لمدة ستة شهور متتالية و قد تم اختبار قوة الصد في الكتاكيت المحصنة.

و توصي هذه الدراسة باستخدام اللقاح المحضر علي آجنه البيض الخالي من المسببات المرضية في السن الصغير من سن يوم الي خمس عشر يوما" بأستخدام اللقاح المستضعف p110 و اللقاح intermediate p77 في السن الكبير 45 يوما" و اللقاح hot p55 كلقاح مثبط.