A trial for preparation of tissue culture vaccine for Gumboro disease by using local isolate on Bursal gland

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Abstract

Thirty serial passages of local IBD virus on chicken bursal cell culture (CB) were prepared and titrated. After every 5 passages the virus was inoculated into ten 21 day-old chicken and observed for 21 days post infection then challenged with virulent IBD virus. The 20th passage induced complete loss of virus pathogenicity and gave 100% protection and used for preparation of live attenuated CB cell culture IBDV vaccine. The evaluation of the prepared vaccine was carried out for sterility, safety and potency. The potency test was performed by measuring humoral immune response as well as protection percentage against virulent IBDV. The neutralizing antibody titre in sera of chickens vaccinated with tissue culture vaccine was higher than those vaccinated with other commercial vaccine. The efficacy of the prepared vaccine was estimated for up to four months.

Introduction

IBD incidence is considered to be very high and occurred in all major poultry producing areas allover the world with high morbidity rate up to 100% and mortality rate of 25% or more (**3**). The use of vaccines to control the disease is a common practice by poultry industry.

The economic importance of IBDV will continue to be a very complex problem because of the recent field isolates of IBDV have been found to be antigenically different from previously isolated vaccinal strains of straned serotype I with 30-70% relatedness which provide an explanation for failure of maternal immunity and vaccination programmes against IBDV using conventional vaccines (**6**).

Therefore great attention was done for production of IBDV attenuated vaccines from field isolate of IBDV through serial passaging in embryonated eggs (**20**). Chicken embryo kidney cell (CEK) and chicken embryo fibroblast cells CEF (**16**).

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Chickens immunized with the cell culture IBDV showed less effect on the bursa than chicken vaccinated with the commercial vaccine (**16**). Cell culture adapted (IBDV) vaccine was effective as commercial vaccine in protecting against challenge.

The aim of the present study was preparation of bursal cell culture attenuated vaccine from field isolate of IBDV and evaluation of its efficacy and potency in young susceptible chickens.

Material and Methods

1-Virus strain:

IBDV bursal homogenate of local Egyptian isolate propagated on SPF (ECE) 3 passages then the 3rd passage propagated on (CEF) cells for 5 passages (**10**). The 5th passage was propagated serially on chicken bursal cell culture (CBC) for 30 passages.

2-Cells:

Chicken embryo bursal cells (CEB) 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 (WCS-Gibco) as growth medium or 2% NCS as in maintenance medium.

3-Media:

Nutrient agar, Sabouraud glucose, thioglycolate broth and Frey's media were used as described by (**1**), for testing sterility of the prepared vaccine.

4-Virus titration:

It was applied according to (**5**). The infectivity titers TCID50/ml were calculated according to (**12**).

5-Virus neutralization (VN) test:

It was applied according to (**14**).

6-Chicks:

Four hundred, twenty-one day old, of susceptible Hubbard chicks were used for vaccine evaluation.

7-Bursa/body weight ratio:

Random five chicks were chosen from vaccinated and unvaccinated groups five days post vaccination and five days post challenged. Slaughtered and collected bursas were weighed and mean organ (Bursa) body weight ratio was determined according to **(17).**

Results

Table (1): Propagation and titration of IBD virus on chicken bursal cells CB)

No. of passages	Time of harvesting post inoculation/days	Log_{10} $TCID_{50}$/ml
1	9	8
5	8	8
10	3	8
15	3	8
20	3	8
25	3	8
30	3	8

Table (2): The experimental infection of 21 days old chicks with propagated (IBDV) isolate on chicken embryo bursal cells (CEB)

No. of passage	Infectivity titer expressed in log_{10} $TCID_{50}$/ml	No. of chicken 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	6	60	3	2
15	8	10	2	20	3	1
20	8	10	0	0	3	0
25	8	10	0	0	3	0
30	8	10	0	0	3	0

Table (3): Challenge of chicks received propagated virus (IBDV) isolate on CEB cells

No. of passage	No. of inoculated chicks	Infectivity titer expressed in log_{10} $TCID_{50}$/ml	No.of dead chicks	Morbidity %	Mortality %	PM lesions	No. of challenge control	No. of dead chicks by virulent virus
1	-	8	-	-	-	-	3	3
5	-	8	-	-	-	-	3	3
10	4	8	4	40	40	Typical IBD lesions	3	3
15	8	8	2	20	20	Typical IBD lesion	3	3
20	10	8	-	0	0	-	3	3
25	10	8	-	0	0	-	3	3
30	10	8	-	0	0	-	3	3

Table (4): Experimental infection of 21 days old chicks by propagated IBDV isolate on CEB cells

No. of passage	Infectivity titer expressed in log_{10} $TCID_{50}$/ml	No. of chicken used	No. of dead chicks	Mortality %	No. of contact control	No. of dead contact control
18	8	10	1	10	3	-
19	8	10	-	0	3	-
20	8	10	-	0	3	-
21	8	10	-	0	3	-

Table (5): Challenge of 21 days old chicks received propagated IBDV isolate on CEB cells

No. of passage	No. of inoculated chicks	No. of dead chicks	Morbidity %	Mortality %	PM lesions	No. of challenged control	No. of dead control by virulent virus
18	9	-	-	-	-	3	3
19	10	-	-	-	-	3	3
20	10	-	-	-	-	3	3
21	10	-	-	-	-	3	3

Table (6): Sterility of the prepared IBD vaccine

Media	Living attenuated CB cells propagated vaccine
Nutrient agar	No colony
Thioglycollate broth	No turbidity
Sabauraud's glucose agar	No colony
Grey's media	No colony

Table (7) log_2 mean neutralizing antibody titers in sera of chicks vaccinated with IBDV propagated on CB cells in comparison with those vaccinated with other common vaccines

Gr. No.	Type of vaccine used	Week post vaccination													
		1	2	3	4	5	6	7	8	9	10	11	12	14	16
1	Live bursal passage	5	5	7	8	10	10	12	12	12	12	12	12	12	11
2	Mild	3	4	5	5	7	10	8	7	7	6	6	5	5	5
3	Intermediate	4	4	5	6	8	9	7	6	6	6	5	5	5	4
4	Hot	4	5	6	6	7	7	6	6	6	6	5	5	4	4
5	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table (8) Rate of protection of locally produced CEB cell propagated live attenuated IBDV vaccine

Group	Type of vaccine used	1st month			2nd month			3rd month			4th month		
		No. of challenged chicks	Survived	Protection %	No. of challenged chicks	Survived	Protection %	No. of challenged chicks	Survived	Protection %	No. of challenged chicks	Survived	Protection %
1	Live attenuated IBD on CEB cells	5	5	100	5	5	100	5	5	100	5	5	100
2	Control	2	0	0	2	0	0	2	0	0	2	0	0

Table (9) Keeping quality of prepared IBD virus vaccine (months)

Group	Type of used vaccine	Temp. of storage	Protection %							
			1 month		2 months		3 months		4 months	
			S	%	S	%	S	%	S	%
1	Live attenuated IBD on CB	- 20 °C	5/5	100	5/5	100	5/5	100	5/5	100
2	Unvaccinated	-	0/2	0	0/2	0	0/2	0	0/2	0

S = survived % = protection percent

Table (10): Bursa/body weight ratio in vaccinated chickens before and after challenge

Chicken group	Type of vac. used	Weight of bursa		Body weight		Bursa/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 bursal	0.1	0.295	140	290	0.7142	1.0172
2	control	0.140	0.3190	145	210	0.9655	1.5190

Figure (1) Histopathological section of bursa of chicken vaccinated with locally prepared live attenuated IBD vaccine (CB) propagated 5 days post vaccination (magnification power 100x)

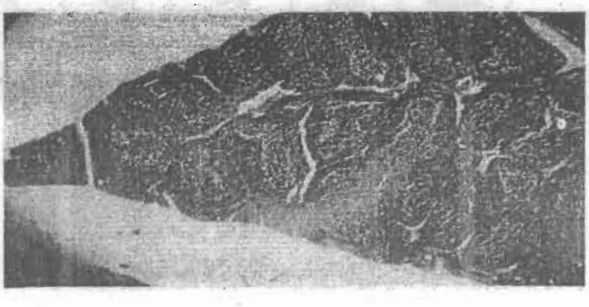

Figure (2): Histopathological section of bursa of chicken non-vaccinated control negative (magnification power 100x)

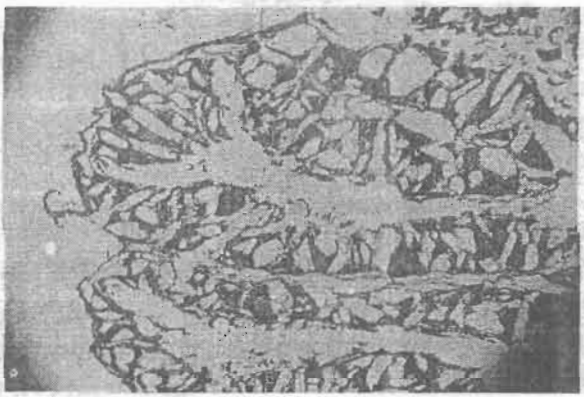

Figure (3) Histopathological section of bursa of chicken challenged with vvIBDV 5 days post experimental infection (magnification power 100x)

Discussion

The aim of the present study was to prepare a safe and potent live chicken bursal (CB) cell propagated vaccine from local field isolate. The scheme used for preparing the vaccine starting with the propagation of the isolated IBD virus on SPF embryonated chicken eggs (ECE) followed by further 5 passages on chicken embryo fibroblast cell culture (CEF) and followed by 30 serial passages on on chicken bursal cells (CB).

It was noticed in Table (1) that no changes in the titre of the propagated virus from the 1st till the 30th passages, while the harvesting time was declined by serial passages from 9 and 8 days and fixed at 3 days from 10th to 30th passage.

Tables (2 & 3) showed that the IBDV lost its virulence gradually from the 1st to 15th passage and completely from 20-30 passage but still immunogenic to the susceptible chicks. These results are in agreement with those obtained by **(8), (16), (15)** and **(18)** who reported that IBDV lost its virulence by progressive propagation on cell cultures.

Tables (4 and 5) show that IBD virus from passage 18 to passage 21 were used for determining the most safe passage that did not induce any morbidity or mortality when used in susceptible chicks. Passage 18 induces 10% mortality, while neither morbidity nor mortality was noticed in passages 19, 20 and 21. Therefore the best safe passage of choice was passage 20. This result in agreement with **(17).**

The prepared vaccine was tested for sterility and proved to be free from any contaminants (Table 6).

Table (7) show evaluation of the humoral immune response of chicken vaccinated with tissue culture vaccine in comparison with other commercial vaccines (mild, intermediate and intermediate plus), it was clear that the mean neutralizing antibody titer $\log_2$ in sera of vaccinated chicks with the prepared vaccine was more higher than others from the 1st week post vaccination till 16th week. These results agree with **(4), (16)** and **(13).**

Table (8) show that chicks vaccinated with prepared vaccines gave a high protection percentage reaching 100% when challenged with virulent IBD virus after 3 weeks, while control did not show any protection. This result agrees with **(7)** and **(19).**

Table (9) Discussing the keeping quality of the prepared live attenuated IBD virus vaccine when stored at -20 °C, it was clear that the vaccine was stable and potent for a period of 4 months where the protection reached 100%. This result agrees with **(9)** and **(2).**

Table (10) it is very clear that the locally prepared live attenuated vaccine is of mild type as there was no clear differences between the bursal body weight ratios of vaccinated and control chicks. This result agrees with **(11).**

We could conclude that the 20th attenuation passage of IBD virus on CB cells and the preparation of live attenuated vaccine gave safe, potent and immunogenic vaccine. So, we could use the cell culture system instead of embryonated chicken eggs.

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محاولة لتحضير لقاح زرع نسيجي لمرض الجمبورو باستخدام العترة المحلية على غدة فابريشيوس

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الملخص العربي

تم تحضير خلايا غدة فابرشيوس للزرع النسيجي من كتاكيت خالية من المسببات المرضية (CB) وإستخدمت لتمرير وتحضير لقاح حي مستضعف لفيروس مرض إلتهاب غدة فابرشيوس وتم تعيين التمريرة ذات أعلى قوة عيارية وأقل قدرة على العدوى بالمرض في الدجاج القابل للعدوى وقد تم حقنها في عدد من الكتاكيت وقياس المناعة باستخدام التجارب السيرولوجية (SNT) ومقارنتها باللقاحات الأخرى كما تم إختبار القدرة المناعية للدجاج المحصن باستخدام تجربة التحدي بالفيروس الضاري وقد تم حفظ اللقاح عند – ٢٠ ٥م لمدة ٤ شهور متتالية وإختبار قوة الصد في الكتاكيت المحصنة.