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Efficacy of locally prepared inactivated infectious bursal disease (IBD) vaccine isolated from kalyobia governorate in Egypt

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This study was carried out to test the efficacy of locally prepared inactivated Infectious bursal disease virus (IBDV) vaccine (Kalyobia isolated strain) for controlling IBD or as it is more commonly known in Egypt as Gumboro disease which cause problems in the Egyptian poultry field. The efficacy was applied in four groups of specific pathogen free chicks (SPF). 1stgroup was used for monitoring the safety of locally prepared inactivated tissue culture vaccine. 2nd and 3rd groups for monitoring the immune response for prepared and imported IBD vaccine; respectively by measuring (ELISA) and serum neutralization test (SNT) titer three weeks post vaccination. Last group for monitoring the change occurs after challenge with virulent IBD strains. Results revealed that Chicks vaccinated with either prepared or imported vaccines showed high serum antibody titers from the 3rd week post vaccination and reached the highest titer at the 4th week post vaccination using SNT and ELISA. Duration of suitable immune response prolonged to 8 weeks post vaccination for the prepared vaccine and 7 weeks post vaccination for the imported vaccine. Both prepared and imported vaccines showed (96%; 93%) protection; respectively in vaccinated chicks challenged with the very virulent IBDV 28 days post vaccination with no clinical signs or lesions on examination. It was concluded that inactivated vaccine prepared from local isolated IBDV strain was safe, potent and immunogenic in young chicks.
Keywords: IBDV, local, inactivated, vaccine, SNT, ELISA.

INTRODUCTION

Infectious bursal disease virus (IBDV) is a member of the family Birnaviridae (Delmas et al., 2005). It is an acute, highly contagious viral disease of young chickens. This disease continues to pose an important threat to the commercial poultry industry. The emergence of antigenic variant as well as very virulent strains in vaccinated flocks considerably stimulated research efforts on both, IBD and IBDV. Some of the recent advances are the understanding of the structure, morphogenesis and molecular biology of the virus as well as in development of new diagnostic approaches and new vaccination against IBD (Müller et al., 2003). IBD was first reported in Egyptian flocks in the early seventies (El-Sergany, 1974). Also in Egypt vvIBDV strains were reported (El-Batrawi and El-Kady, 1990).

Circulating IBDV strains was isolated from flocks vaccinated using classical IBDV vaccines (Abdel-Alim et al., 2003, Hussein et al., 2003, Metwally et al. 2003, Helal et al., 2012, Mohamed et al., 2014, Sara et al., 2014 and El- Bagoury et al., 2015). Difference in virulence and antigenic

characters associated with IBDV has been the greatest difficulty for successful control of IBD (Van den Berg, 2000). Different types of vaccines are mostly variable for the prevention of IBD. Live attenuated vaccine (egg adapted or tissue culture one), inactivated oil-emulsion adjuvant vaccine and recombinant IBDV-VP2 protein vaccine (Schijns et al., 2008).

So, the present study was executed for evaluation a prepared inactivated vaccine from local IBDV Kalyobia isolate compared with inactivated imported IBDV vaccine for controlling problem of Gumboro disease in Egypt.

MATERIALS AND METHODS

viral strain

Locally isolated Infectious Bursal Disease Virus (IBDV):

IBDV Bursal homogenate isolated from broilers in Kalyobia governorate, Egypt .The virus has a titer of $10^{5.5}$ Tissue culture Infective Dose 50% ($TCID_{50}$)/ml and was used as the seed virus for preparation of the inactivated IBDV vaccine.

B- Virulent strain of IBDV:

The virus used in challenge was in form of infectious allantoic fluid. The virus was supplied from reference strain bank (Central Laboratory for Evaluation of Veterinary Biologics (CLEVB).

Inactivated infectious bursal disease (IBD) vaccine:

An inactivated oil emulsion CEVAC® IBD K (CEVAC® G K) vaccine was used for the immunization of chickens against Infectious Bursal Disease in a dose of 0.5 ml by subcutaneous inoculation.

Specific Anti- IBD "local strain" serum:

It was kindly supplied by the Department of Newcastle Disease Vaccine Research, Veterinary Serum and Vaccine Research Institute (VSVRI), Cairo, according to (McFerran et al., 1980). It was used as positive control in SNT.

Serum samples:

Serum samples were collected from all chicks (vaccinated and non- vaccinated) weekly till 8th week post vaccination. The sera were inactivated at 56°C for 30 minutes, and then stored at -20°C until used in ELISA and SNT.

Experimental Hosts:

A.Experimental specific pathogen free (SPF) chicks:

One day old SPF chicks were obtained from the SPF production farm, Koum Oshiem, El-Fayoum Governorate, Egypt. This farm is apart from ministry of Agriculture. All birds were housed in a separated negative pressure filtered air isolators and were provided with autoclaved commercial water and feed. At 3 weeks old chicks used for evaluation of prepared vaccine study.

Swiss mice:

Two groups of ten Swiss mice, each was used for monitoring safety in mammalian species.

Specific pathogen free- Embryonated chicken Egg (SPF-ECE):

SPF-ECE was purchased from the SPF egg project, Koum Oshiem, El-Fayoum Governorate. The eggs were used for propagation and titration of virus and ensuring of completion of virus inactivation.

Tissue culture and cell culture media:

Primary chicken embryo fibroblast cell (CEF) was obtained from Central Lab for Evaluation of Veterinary Biologics (CLEVB), which was prepared as (Schat and Purchase, 1989). Tissue culture was used for IBD virus seed propagation (attenuation) and titration. T.C was used also for detection of extraneous agents in prepared IBD seed before inactivation (AAAP, 2008).

Preparation of the inactivated IBDV vaccine:

Propagation of IBDV in chicken embryo fibroblast:

The locally isolated IBDV (Kalyobia isolated strain) used for vaccine preparation was propagated (6) times in CEF according to Villegas (1990) and the titer of the virus was calculated according to Reed and Meunch (1938). The aqueous phase used for vaccine formulation was adjusted to have a titer $10^{5.5}$ $TCID_{50}$ /ml of the seed virus.

Inactivation of the propagated IBDV:

Inactivation of the virus was done using formalin (37%), BDH that was used in a dilution 1:1000 according to Beard (1989).

Completion of the virus inactivation was tested by passage in 9-11 day old SPF embryonated eggs (0.1 ml /egg) via CAM and examined daily for 5 days. It was noticed that,

there were no any pathological lesions and / or deaths of inoculated embryos, compared with that of the control one.

Formulation of the vaccine:

It was prepared as water in oil emulsion (W/O) using Montanide ISA70 at a ratio of 3/7 (v/v) aqueous /oil ratio. Manufacturing process was carried out according to the standard protocol of SEPPIC and manufacture instruction.

Evaluation of the prepared inactivated IBD vaccine:

Sterility test:

It was applied according to the Federal Regulation (USA, 2017). A volume of the tested vaccine was inoculated into nutrient agar medium, thioglycolate and broth PPLO agar at 37°C for 72 hours and Saburaouds glucose agar that incubated at 25°C for 14 days.

Safety test in chicks:

Safety of the prepared inactivated IBDV oil emulsion vaccine was examined in a group of 3 weeks old chicks, inoculated with 1ml (double dose) of the vaccine subcutaneous at the neck. These chicks were observed for 2 weeks for any signs of local reaction or appearance of any clinical signs. After 5 days of inoculation, some birds were subjected to post mortem examinations to detect any pathological lesions.

C- Potency of the prepared vaccine in vitro by:

Studying humoral immune response using SNT:

Quantitative SNT (constant virus and variable serum) was carried out on sera of vaccinated chicks for titration of IBD neutralizing antibodies against 100 $TCID_{50}$/ml of the IBDV adapted on CEF cells using the micro titer technique according to Florence *et al.* (1992).

Studying humoral immune response using ELISA:

Serum samples were collected from experimental chicks and were preceded for measuring the humeral immune response using ELISA according to the ProFLOK® IBD PLUS ELISA (IBD antibody test kit), Synbiotics Corporation, Kansas, USA. Procedures were performed according to the test steps in the kit. It was used for detection and titration of IBD antibodies against VP2 of IBDV in sera from vaccinated birds. ELISA Reader Microplate reader USA, VERSA Max was used.

Potency of the prepared vaccine in vivo by:

Challenge test:

Chick groups (vaccinated and un vaccinated control) were challenged 28days post vaccination by 0.1 ml/bird of virulent IBDV containing $10^{3.5}$ EID_{50}/ml, by the eye drop route. The challenged birds were observed for 15 days and collect serum samples during challenge period, dead birds through this time were recorded and examined for post-mortem lesions.

RESULTS

The results of the titration of the IBDv in chicken embryo fibroblast (CEF) were illustrated in table (1) where the titer of prepared vaccine was measured by Kärber method after five days and it was $10^{5.5}$TCID50/dose.

Table (2) shows the results of the sterility test of the prepared inactivated IBDv vaccine and reveals the vaccine was sterile as it was free from any bacterial and fungal contaminants.

Regarding to the results of the safety test of the prepared inactivated IBDv vaccine, which is presented in table (3) it was found that there were no local or systemic reactions and also, no mortality in inoculated chicks, as shown in.

The prepared inactivated IBD vaccine was evaluated by using serum neutralization test (SNT) (Table 4) and the titer of the prepared inactivated IBDv vaccine was (7.9 log_2) 4 weeks post vaccination while the titer of imported inactivated IBDv vaccine was (7.7 log_2) after the same period.

Vaccinated chicks were challenged 28 days post vaccination and mean serum neutralizing antibody titers were measured and found that titer of prepared inactivated IBDv vaccine was (6.8 log_2) while titer of imported inactivated IBDv vaccine was (6.5 log_2) as shown in table (5).

On evaluation of the humoral immune response of chicks vaccinated with the prepared inactivated IBD vaccine using ELISA, it was noticed that mean serum antibody titer started to increase from the first week post vaccination (3350), reached the highest level at 4th week post vaccination (8956.9), then declined to (5400.9) at 8th week post vaccination. The humoral immune response was compared to that of chick group vaccinated with the imported inactivated IBDv vaccine that showed increased mean ELISA serum antibody titer started from the first week post vaccination (3200), reached the highest level at 4th week post vaccination (7560) then declined to (3750.1) at 8th week post vaccination.

Table (1): Titration of the IBDv in chicken embryo fibroblast (CEF)

Dilutions	Time onset for CPE (Days after inoculation)					Mean of titer (Kärber method) $Log_{10}TCID_{50}$/dose
	1	2	3	4	5	
10^{-4}	0/5	0/5	0/5	0/5	5/5	5.5
10^{-5}	0/5	0/5	0/5	0/5	5/5	
10^{-6}	0/5	0/5	0/5	0/5	5/5	
10^{-7}	0/5	0/5	0/5	0/5	5/5	
Control	0/5	0/5	0/5	0/5	0/5	-ve

Note: Preparation of the vaccine began with propagation of the bursal homogenate the isolate IBDV for (6) serial passage on embryo chicken fibroblast (CEF) there was an increase in infectivity titer TCID50 from the first to sixth passage as follow (3.5, 3.9, 4.3, 4.9, 5.1, 5.5), respectively.

Table (2): Sterility test of the prepared inactivated IBDv vaccine

Medium	Examined Microorganism	Result
Nutrient agar	Aerobic bacteria	No Growth
Thioglycolate broth	Anaerobic bacteria	No colonies
PPLO agar	Mycoplasma	No colonies
Sabarouds-agar	Fungus	No colonies

Table (3): Safety test of the prepared inactivated IBDv vaccine

Safety	Vaccinated	Control
Local reaction	Negative	Negative
Systemic reaction	Negative	Negative
Chick mortalities	No mortalities	No mortalities

Table (4): Mean serum neutralizing antibody titers of chicks vaccinated with the prepared inactivated IBDv vaccine in comparison to the imported inactivated IBDv vaccine

Weeks post –vaccination	Mean log_2 serum neutralizing antibody titers		
	* The prepared IBDv vaccine	** The imported IBDv vaccine	Control
1	1.6	1.8	0
2	2	2	0
3	7.7	7.2	0
4	7.9	7.7	0
5	7.9	7.7	0
6	7.7	7.5	0
7	7.5	7.5	0
8	7.5	7.2	0

* Inactivated IBDv vaccine titer = 7.9 log_2

** Imported inactivated IBDv vaccine = 7.7 log_2

Table (5): Mean serum antibody response titers in vaccinated chicks challenged 28 days post vaccination evaluated using SNT

Type of Vaccine	Mean log_2 serum neutralizing antibody titer	
	Pre-Challenge	Post-challenge
The prepared IBDv vaccine	7.9	6.8
The imported IBDv vaccine	7.7	6.5

The previous results were compared with that of the control group of SPF chicks that had negative results (ELISA serum antibody titers below 3000) against the virus as shown in table (6). Mean serum antibody titers by ELISA test in vaccinated chicks challenged 21 days post vaccination was measured and titer of prepared inactivated IBDv vaccine was 4000 while titer of imported vaccine was 3300 as shown in table (7).

Evaluation of IBD vaccines in vivo by measuring the protection % (challenge test) (shown in table 8), it was clear that the protection percent of chicks vaccinated with inactivated IBDv vaccine after challenge using virulent IBDv was measured in prepared inactivated IBDV vaccine and resulted 96% while imported inactivated IBDV vaccine was 93% as shown in table (8) .The protection percent for IBD vaccine must be equal or more than 90% according to (OIE, 2017).

Table (6): Mean ELISA serum antibody titers of chicks vaccinated with the prepared inactivated IBDv vaccine in comparison to the imported inactivated IBDv vaccine

Weeks post - vaccination	GMT of ELISA serum antibody titers		
	*The prepared IBDv vaccine	**The imported IBDv vaccine	Control
1	3350	3200	1800
2	4800	3900	2000
3	6700.4	4900.3	2000
4	*8956.9	**7560	2000
5	7400	6301	2000
6	7200	5200	2100
7	5600.5	4300.9	2150
8	5400.9	3750.1	1900

NB: GMT: Geometric mean of ELISA antibody titer against IBDV equal or more than 3000 according to kit manufacture.

*GMT of inactivated IBDv prepared vaccine titer = 8956.9 at 28day post vaccination

**GMT of imported IBDv vaccine titer = 7560 at 28day post vaccination

Table (7): Mean serum antibody titers in vaccinated chicks challenged 21 days post vaccination evaluated using ELISA

Type of Vaccine	Mean ELISA Serum antibody titer	
	Pre-Challenge	Post-challenge
The prepared IBDv vaccine	8956.9	4000
The imported IBDv vaccine	7560	3300

Table (8): Protection percent of chicks vaccinated with inactivated IBDv vaccine after challenge using virulent IBDv

Challenged group	Number of chicks			Protection percent
	Challenged	Dead	Live	
The prepared IBDv vaccine	30	1	29	96%
The imported IBDv vaccine	30	2	28	93 %
Control non vaccinated group	10	10	0	0 %

DISCUSSION

This study was carried out with the aims to test the efficacy of locally prepared inactivated Infectious bursal disease virus (IBDV) vaccine for controlling Gumboro disease in the Egyptian poultry field in compared with the imported one. Preparation of the vaccine (Table -1) agreed with Duko et al., (1988) and Nadia (2011). Inactivation of the seed T.C adapted IBDV using 0.01% formalin solution, showed complete virus inactivation occurred after 33 hrs. This result disagreed with the studies used formalin 0.01% for IBDV inactivation that showed complete virus inactivation after 18 hrs and 24 hrs respectively by Amal (2001) and Habib et al., 2006) and agreed with El-Bagoury et al., (2015). Testing quality of the prepared inactivated IBDV vaccine, as Sterility test by culturing on different synthetic media for detection of bacterial and fungal growth showed that the vaccine was sterile as it was free from any bacterial and fungal contaminants. Safety of The prepared inactivated IBDV vaccine was tested by inoculation in 21 days old chicks showed that, there were no local or systemic reactions and also, no mortality in inoculated chicks. These results agreed with the Code of Federal Regulations USA (2017).

The mean log_2 serum neutralizing antibody titer started to increase from the first week post vaccination (1.6 -1.8 log_2),–reached the highest level at 4th week post vaccination (7.9- 7.2 log_2) and persisted in the suitable values till the 8th week post vaccination (7.5- 7.2 log_2)for chicks vaccinated with the prepared or imported inactivated IBDV vaccine; respectively as showed in (table -4 and fig -1). These results were confirmed by using ELISA for studying the humoral immune response in table (6), the mean ELISA serum antibody titers increase from the first week post vaccination (3350 or 3200), reached the highest level at 4th week post vaccination (8956.9 or 7560), then fluctuated and declined to (5400.9or 3450.1) at 8th week post vaccination for chicks vaccinated with the prepared or imported one; respectively. The previous results were compared with that of the control group of SPF chicks that had negative results (ELISA serum antibody titers below 4000) against the virus. These results agreed with the results of Amal (2001) which showed the highest antibody titers at 4th week post vaccination using SNT and ELISA. These results agree with that of Habib et al., (2006), who showed that on the basis of humoral immune response, the inactivated IBDV vaccines were immunogenic with increased in antibody titers in all inoculated groups 2 weeks post inoculation. These results agreed also with the facts showed that the humoral immune response plays the principal role in defense against vvIBDV (Lukert and Saif, 1997). Also agree with that reported by El-Bagoury et al., (2015) who showed the chicks vaccinated with either prepared or imported vaccines showed high serum antibody titers at the 4th week post vaccination using SNT and ELISA.

Inoculation of inactivated IBDV could give complete protection with no obvious IBD clinical signs, was reported previously (Maas et al., 2001). Protection percent in chicks vaccinated with both the prepared and the imported vaccine were 96%.

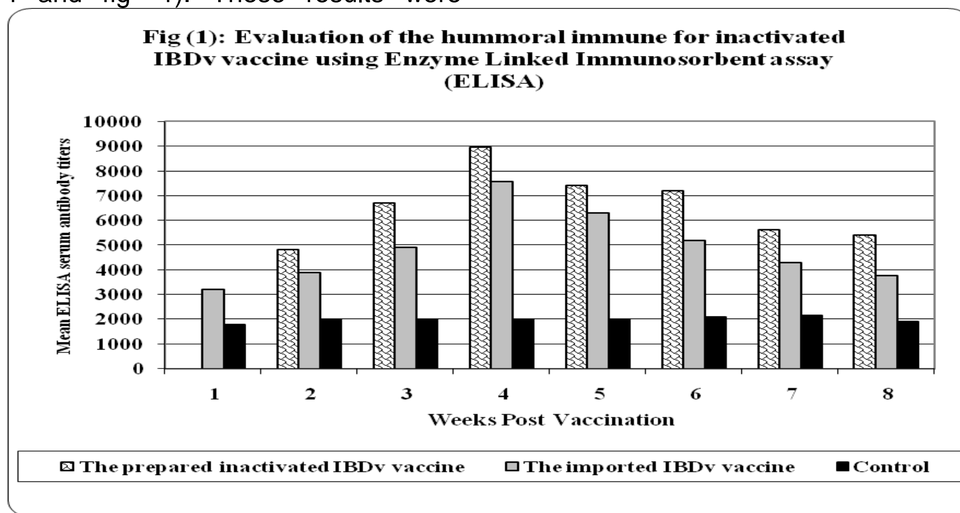

Fig (1): Evaluation of the hummoral immune for inactivated IBDv vaccine using Enzyme Linked Immunosorbent assay (ELISA)

This protection percent was confirmed by titration of the serum pre- challenge and one week post challenge using SNT and ELISA which indicated suitable IBD antibody titers and also confirmed also by examination for clinical signs and development of lesions in challenged birds which showed no clinical signs or lesions all vaccinated groups of birds showing (96%-93%) protection. Chicks in challenged control non vaccinated group induced 100% mortality, showed atrophied yellowish bursa and slight hemorrhages on proventriculus. The result of this study also showed that a single dose of the inactivated IBDV vaccine gave 96%-93% protection against vvIBDV challenged, which is in contrast with the report of 100 % protection obtained with the use of two doses of killed IBD vaccines at a week interval in 3 weeks SPF chickens (Hsieh et al., (2007) and agree with that reported by El-Bagoury et al., (2015) that use one dose of egg adapted inactivated IBD vaccine that give 100% protection.

CONCLUSION

Finally, it was concluded that using inactivated vaccine prepared from local isolated IBDV strain was safe, potent and immunogenic in young chicks that may had major advantage over imported vaccine for control Gumboro disease in Egypt being prepared from the local recently isolate.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

AAEM and SSAM performed the experiments and wrote the manuscript. MAEM, ABAM and SSAM designed the experiments and reviewed the manuscript. AAEM and SSAM designed the experiments and prepared the vaccine. All authors read and approved the final version.

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