



PREPARATION OF INACTIVATED INFECTIOUS BURSAL DISEASE VIRUS VACCINE FROM CLASSICAL STRAIN AND LOCAL VARIANT ISOLATES

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ABSTRACT

This study was conducted to assess an inactivated vaccine prepared from local field isolates of Infectious Bursal Disease (IBD) "local variant and virulent isolates" and a classical strain "bursa vacc" to improve the full control over the problem of IBD in Egypt. This experiment applied on 21 day old SPF chicks divided into six groups, the first group was vaccinated with inactivated vaccine containing (local variant isolate, classical bursa vacc strain and virulent isolate), the second group was vaccinated with inactivated vaccine containing (classical bursa vacc strain and local variant isolate), the third group was vaccinated with classical bursa vacc vaccine, and the group 4 vaccinated with local variant isolate vaccine while last two groups were left as a positive and negative control groups. The immune response of chicks was evaluated in vitro by measuring serum neutralization test (SNT) and enzyme linked immunosorbent assay (ELISA) and in vivo by challenging birds with $10^{3.5}$ EID₅₀/ dose of virulent virus of infectious bursal disease virus (vvIBDV). The highest average antibody level was obtained at the 4th week post vaccination in chicks in group 1 vaccinated with (local variant isolate, classical bursa vacc and local virulent isolate) vaccine, while the chicks in group 3 vaccinated with bursa vacc vaccine showed the lowest antibody level at the same week post vaccination using SNT and ELISA. The challenge of group 1, 2, 3 and 4 showed protection of 97.5%, 90%, 75% and 90% respectively using very virulent IBDV 28 day post vaccination with no clinical signs or lesions on examination. It was concluded that we can use an inactivated vaccine prepared from (classical strain "bursa vacc" and local variant and virulent isolates) of Infectious Bursal Disease (IBD) virus as a method for control IBD disease in Egypt.

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INTRODUCTION

Gumboro disease of chicken was first observed by (Cosgrove, 1962) in Gumboro district of Delaware, USA, hence the name "Gumboro disease. Other names of the disease entity based on the lesions produced included avian nephrosis- nephritis, avian infectious bursitis and Infectious bursal disease. Infectious bursal disease virus (IBDV) is the causative agent of infectious bursal disease (IBD), a highly contagious immunosuppressive disease in young chicken. Infectious bursal disease virus (IBDV) is a non-enveloped virus; it consists of the bi-segmented double-strand RNA genome and the genome-enclosing viral capsid that is mainly formed by the viral protein VP2 (Coulibaly *et al.*, 2005) which targets immature B lymphocytes of the bursa of Fabricius (BF), a primary lymphoid organ in avian species, and subsequently causes B-cell depletion in bursal follicles.

Macrophages may be susceptible to IBDV reviewed by (Khatri and Sharma, 2007). Differences in susceptibility may also occur between different SPF chicken lines. The highest susceptibility to acute IBD occurs in chicken between 3 and 6 weeks of age (Etteradossi and Saif, 2013).

Two serotypes of infectious bursal disease virus (IBDV) are known to exist. Serotype 1 viruses replicate in the bursa of Fabricius and some serotype 1 viruses cause clinical disease in chicken. Antibodies or virus are sometimes found in other avian species, but no signs of infection are seen. Serotype 2 viruses have been detected from the respiratory tract of turkeys, cloacal swabs of ducks or in the bursa of Fabricius of chicken. Antibodies against serotype 2 viruses are very widespread in turkeys and are sometimes found in chicken and ducks. There is no report of clinical disease caused by infection with serotype 2 virus (Etteradossi and Saif, 2013). Pathogenic serotype 1

(IBDV) is a pathogenic strains in chicken that classified as classical virulent (cvIBDV), very virulent (vvIBDV) and antigenic variant (avIBDV). (Van den Berg *et al.*, 2004).

Outbreaks of IBD emerged among broilers and layers in Middle East, Africa and South America causing mortality (Muller *et al.*, 2003). IBD has been a serious problem in Egypt, vvIBDV strains were reported since its first introduction in 1989 (El-Batrawi, 1990), while variant viruses was first described as newly emergent in USA with increased mortality even in vaccinated flocks (Snyder *et al.*, 1988). These variant strains differed from classical ones in which they caused a rapid bursal atrophy with minimal inflammatory response. Circulating IBDV isolates was isolated from flocks vaccinated with classical IBDV vaccines (Abdel-Alim *et al.*, 2003; Hussein *et al.*, 2003, Metwally *et al.*, 2003; Metwally *et al.*, 2009; Helal *et al.*, 2012; Mohamed *et al.*, 2014; Sara *et al.*, 2014; and Nada, 2015).

The terms "variant", "classical" and "very virulent" have been used to describe IBDV strains that exhibit differences in pathogenicity. Based on the signs and lesions observed in two lines of White Leghorn SPF chicken during experimental IBD virus infection following a 10^5 50% embryo infective dose (EID₅₀) challenge, North American "variant" IBDVs induce little clinical signs and no mortality but marked bursal lesions, "classical" IBDVs induce approximately 10–50% mortality with typical signs and lesions whereas "very virulent" IBDVs induce approximately 50–100% mortality with typical signs and lesions (Etteradossi *et al.*, 1999 personal observation).

Important methods of IBD prevention and control in the poultry industry are disinfection, biosecurity and vaccination at the appropriate time (Chansiripornchai and Sasipreeyajan, 2009). Generally, IBD is effectively controlled by vaccination worldwide (Muller *et al.*, 2003). Different types of vaccines are mostly available for the controlling of IBD include Live attenuated vaccine (egg adapted or tissue culture one), inactivated oil-emulsion adjuvant vaccine and recombinant IBDV-vp2 protein vaccine (Schijns *et al.*, 2008). Inactivated oil adjuvanted vaccine used to boost and prolong immunity against IBD and these vaccines are most effective in chicken that have been primed with live virus either in the form of a vaccine or field exposure to the virus (Iukert and saif, 2003). In the United States of America, Australia and other countries, variant types of IBDV that induce severe atrophy of the bursa, but against which the classical type of IBD vaccine does not protect (Sapats and Ignjatovic, 2000), so that in this study we try to prepare an inactivated vaccine protect chicken against variant, virulent isolates and classical strain of IBD .

This study aims to prepare and evaluate an inactivated IBD vaccine prepared from a local field isolates and classical strain (bursa vacc) of IBDV propagated on SPF-ECE as a method of control and decrease the economic losses in chicken industry due to IBD.

MATERIALS AND METHODS

Viral strains

1. Local variant isolate of Infectious Bursal Disease Virus (IBDV)

Local Variant isolate was in form of infectious allantoic fluid; isolated from field broiler cases in Giza government Egypt (2013/2014) and identified by PCR and sequence analysis (Nada, 2015). This virus was propagated in SPF-ECE for several serial passages. Its infectivity titer was $10^{8.5}$ EID₅₀/ml and it was used in preparation of vaccine under test. The propagation of viruses and titration in SPF-ECE were carried out as described by (Villegas, 1990) and EID₅₀ is calculated according to method (Reed and Muench, 1938).

2. Very virulent isolate of IBD (vvIBD) virus

Virulent virus of IBD of field isolate was obtained from Central Lab for Evaluation of Veterinary Biologics (CLEVB). Its infectivity titer of $10^{8.6}$ EID₅₀/ml was used for preparation of vaccine under test and of $10^{3.5}$ EID₅₀/dose was used for challenge of all experimental vaccinated chicks 28 day post vaccination.

3. A classical (Bursa vacc) strain

The virus was supplied by the Department of Newcastle vaccine research belongs to Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The virus has an infectivity titer of $10^{6.8}$ EID₅₀/ml and used for preparation of the inactivated IBDV vaccine under test.

Specific Anti- IBD serum for different strains

It was kindly supplied by the Department of Newcastle disease vaccine research belongs to Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo which prepared according to (Mc Ferran *et al.*, 1980) used as positive control in SNT.

Primary chicken embryo fibroblast (CEF) cell culture

Primary CEF cell culture was obtained from Central Lab for Evaluation of Veterinary Biologics (CLEVB). It was prepared according to (Villegas, 1990) and used for propagation, adaptation and titration of all IBDV strains and in serum neutralization test.

Enzyme linked immuno-sorbent assay (ELISA)

ELISA Kit was obtained from Symbiotic Corporation 11011 VIA Forntera San Digo; Infactionous bursal kit with Batch no FS5155 and used according to (Snyder *et al.*, 1986).

Serum neutralization test (SNT)

Quantitative SNT was carried out on sera of vaccinated chicks for titration of IBD neutralizing antibodies against 100 TCID₅₀/ml of the IBDV adapted on CEF cells using the micro titer technique according to (Florence *et al.*, 1992).

Preparation of the inactivated IBDV vaccine

Propagation of IBDV in SPF-ECE

The locally isolated IBDV isolates and bursa vacc strain used for vaccine preparation were propagated via chorioallantoic membrane (CAM) in 9-11 days old SPF-ECE (0.2 ml/egg) according to (Allan *et al.*, 1973). Harvested CAMs were homogenized and the virus was titrated on 9-11 day old SPF-ECE according to (Cho and Edgar, 1969) and the titer of the virus were calculated according to (Reed and Meunch, 1938). The aqueous phase used for vaccine formulation had a titer 10^{8.5} EID₅₀/ml for local variant isolate and had a titer 10^{8.6} EID₅₀/ml for local virulent isolate and had a titer 10^{6.8} EID₅₀/ml of the bursa vacc seed. The positive signs of inoculated eggs with IBDV were edema and hemorrhage of CAM and embryo with liver necrosis and death.

Completion of Inactivation of the propagated antigen

Inactivation of the viruses was done using formalin (37%) Analar, 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

Four vaccines were prepared as water in oil emulsion (W/O) using Montanide ISA71 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 as following:

Vaccine (1): inactivated IBD vaccine (local variant isolate, virulent isolate and classical bursa vacc strain). This vaccine has a final titer 10^{8.5} EID₅₀/ml.

Vaccine (2): inactivated IBD vaccine (local variant isolate and classical bursa vacc strain). This vaccine has a final titer 10^{8.0} EID₅₀/ml.

Vaccine (3): inactivated IBD vaccine (classical bursa vacc vaccine). This vaccine has a final titer 10^{6.8} EID₅₀/ml.

Vaccine (4): inactivated IBD vaccine (local variant isolate). This vaccine has a final titer 10^{8.5} EID₅₀/ml.

Experimental design

Three hundred of 21 day old SPF chicks were obtained from SPF poultry project, Kom Oshim, EL-Fayoum Governorate. This farm is a part from Ministry

of Agriculture in Egypt. All birds were housed in a separated negative pressure filtered air isolators provided with autoclaved commercial water and feed. These chicks used to study the efficacy of prepared vaccines. Chicks were divided into 6 groups (50 chicks /each group) as following: Forty chick for each group where they were vaccinated with vaccine 1, 2, 3 and 4 respectively using a dose of 0.5 ml / bird inoculated S/C while Group 5 left as unvaccinated challenged chicks (+ve control) and Group 6 left as unvaccinated unchallenged chicks (-ve control).

Ten chicks from each group have kept for safety test by inoculation double dose for each vaccine. Serum samples were collected from all chicks (vaccinated and non- vaccinated) weekly till 4th week post vaccination and before challenge. The sera were inactivated at 56°C for 30 minutes, and then stored at -20°C until used in ELISA and SNT.

Evaluation of the prepared inactivated IBDV oil emulsion vaccines

Testing the quality control of the prepared inactivated IBDV vaccines including sterility and safety was carried out according to (Code of American Federal Regulation, USA 2017).

RESULTS AND DISCUSSION

Two parameters were used for the evaluation the efficacy of different IBD prepared vaccines including antibody level monitoring by ELISA and SNT and the most important parameter which is protection percent (%) by challenge using virulent strain of IBDV. The propagation of the bursal homogenate of the isolated IBDV was carried out for five serial passages on SPF-ECE through chorioallantoic membrane. There was an increase in infectivity titer EID₅₀ from the first to the fifth passage. This result agreed with that obtained by (Nadia, 2011). Inactivation of the seed egg adapted IBDV using 0.1% formalin solution, showed complete virus inactivation after 24 hrs. The completion of virus inactivation was tested by its inoculation in 9-11 days old SPF-ECEs on CAM that showed no pathological lesions and / or deaths of inoculated embryos, compared with the control one. This result agreed with the studies used formalin 0.1% for IBDV inactivation showing complete virus inactivation after 24 hrs by (Habib *et al.*, 2006).

Sterility and safety test

Testing quality of the prepared inactivated IBDV vaccines, as Sterility test was applied to confirm that the prepared vaccines were free from bacterial, mycoplasma and fungal contamination by inoculation into nutrient agar, thioglycolate broth, PPLO that were incubated at 37°C for 72

hours and Saburaoud glucose agar that was incubated at 25°C for 14 days showed that all prepared experimental vaccines were free from any bacterial and fungal contaminants. Safety of The prepared inactivated IBDV vaccines was tested in 60 chicks of 3 weeks old chicks (40 chicks inoculated with "double dose" of prepared vaccines under test subcutaneous at the neck and 20 chicks kept as a control +ve and control -ve). After 5 days of inoculation, some birds were subjected to post mortem examinations to detect any pathological lesions. It was observed that, there were no local or systemic reactions and also, no mortality in inoculated chicks till the end of observation period. These result in agreement with the directions of (Code of Federal Regulations USA, 2017).

Potency of the prepared vaccine

Humoral immunity plays an important role in poultry against IBDV infection. Current available live and inactivated IBD vaccines are usually evaluated based on their capacity of inducing antibody response, which contributes to the protection elicited by the vaccine (Tsukamoto *et al.*, 1995; and Rautenschlein *et al.*, 2002). The ELISA and SNT procedure are usually accepted as a serological test for antibody level to IBDV in poultry flocks (Briggs *et al.*, 1986).

As serum neutralization test (SNT), the results in table (1) revealed that the mean log₂ serum neutralizing antibody titer of chicks vaccinated with local field isolates (virulent and variant isolates) and classical bursa vacc strain in group 1 started to increase from the first week post vaccination (8 log₂), reached the suitable high level at 3rd week post vaccination (128 log₂), reached the highest level at 4th week post vaccination (256 log₂). Group 2 vaccinated with inactivated IBDV vaccine consist of a classical bursa vacc strain and a local variant isolate showed increased mean log₂ serum neutralizing antibody titer started from the first week post vaccination (8 log₂), reached the suitable high level at 3rd week post vaccination (64 log₂), reached high level at 4th week post vaccination (128 log₂).

The group 3 vaccinated with the prepared inactivated classical IBDV vaccine started to slightly increased from the first week post vaccination (4 log₂), reached the high level at 2nd week post vaccination (8 log₂), then returned to the

low level at 3rd and 4th weeks post vaccination (4 log₂) these results agreed with (Susan *et al.*, 2013) who reported that chicken vaccinated with intermediate and intermediate plus strains gave the lowest SNT antibody mean titer. Also agreed with (Magda *et al.*, 2013) who clarified that the experimental birds vaccinated with two doses of IBD vaccine (Intermediate and Intermediate plus strains) produce higher immune response than that received one dose of Intermediate vaccine "classical strain".

The group Four vaccinated with inactivated local variant IBDV vaccine showed increased mean log₂ serum neutralizing antibody titer started from the first week post vaccination (8 log₂), reached the suitable high level at 2nd and 3rd week post vaccination (64 log₂), then recording the highest level at 4th week post vaccination (128 log₂). These results agreed with (Amal, 2001) who reported that the variant isolate vaccine prepared from ISA-70 oil adjuvant induced the highest antibody titer after 4 weeks post vaccination and remained high up to 12 weeks post vaccination.

Table 1: Mean IBD serum neutralizing antibody titers in vaccinated chicks with the four prepared inactivated IBDV vaccines.

Weeks post vaccination	Mean log ₂ serum neutralizing antibody titers			
	Gp 1	Gp 2	Gp 3	Gp 4
1	8	8	4	8
2	32	16	8	64
3	128	64	4	64
4	256	128	4	128

Group 1: vaccinated with inactivated IBD vaccine consisted of (variant isolate+virulent isolate+bursa vacc)

Group2: vaccinated with inactivated IBD vaccine consisted of (variant isolate +bursa vacc)

Group 3: vaccinated with inactivated IBD vaccine (bursa vacc strain)

Group 4: vaccinated with inactivated IBD vaccine (variant isolate)

**group (5): unvaccinated challenged (+ve) control group did show 2 log₂ antibody response against IBDV.

**group (6): unvaccinated unchallenged (-ve) control group did show 2 log₂ antibody response against IBDV

Table (2) shown, The mean ELISA serum antibody titers of chicks in group 1 vaccinated with local field isolates (virulent and variant isolates) and classical bursa vacc strain showed increase the antibody level from 1st week post vaccination (568), reached the highest level at 4th week post vaccination (2814). These results agreed with (Silke *et al.*, 2003) who reported that virulent

strain of IBDV induced the highest enzyme-linked immunosorbent assay (ELISA) antibody levels detected at days 8–29 Post inoculation and the best protection against challenge virus replication in comparison with mild and intermediate strain of classical IBDV.

Table 2: Mean IBD ELISA serum antibody titers in vaccinated chicks with the four prepared inactivated IBDV vaccines.

Weeks post vaccination	Mean ELISA serum antibody titers			
	Gp 1	Gp 2	Gp 3	Gp 4
1	568	324	216	584
2	616	459	459	855
3	855	616	201	888
4	2814	855	126	846

Group 1: vaccinated with inactivated IBD vaccine consisted of (variant isolate+virulent isolate+bursa vacc)

Group2: vaccinated with inactivated IBD vaccine consisted of (variant isolate +bursa vacc)

Group 3: vaccinated with inactivated IBD vaccine (bursa vacc strain)

Group 4: vaccinated with inactivated IBD vaccine (variant isolate)

**group (5): unvaccinated challenged (+ve) control group did show 300 mean ELISA serum antibody titer against IBDV.

**group (6): unvaccinated unchallenged (-ve) control group did show 300 mean ELISA serum antibody titer against IBDV

*Positive ELISA titers are over 450 according to kind of used kits.

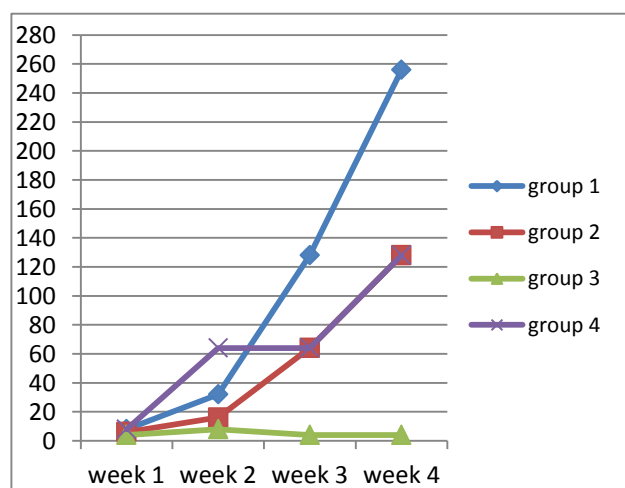
The humoral immune response of chicks in group 2 showed increased mean ELISA serum antibody titer started from the first week post vaccination (324), reached the high level at 4th week post vaccination (855). While chicks in group 3 that showed low mean ELISA serum antibody titer started from the first week post vaccination (216), then slightly increased at 2nd week post vaccination as (459) and reached the lowest level at 3rd and 4th week post vaccination as (201) and (126) respectively. These results agreed with (Mahgoub, 2010) who mentioned that intermediate and intermediate plus strains are sometimes administered at 1 day old to protect any chicken in the flocks that may have no or minimum levels of maternal derived antibodies and (Magda *et al.*, 2013) who clarified that the experimental birds vaccinated with two doses of IBD vaccine (Intermediate and Intermediate plus strains) exhibited higher immune response than that received one dose of Intermediate vaccine classical strain.

Chicks in Group 4 showed increased mean ELISA serum antibody titer started from the first week post vaccination (584), and reached at 4th week post vaccination to level (846). The previous results were compared with that of the control group of chicks that had negative results (ELISA serum antibody titers below 450) against the virus. These results agreed with the results of (Amal, 2001) who found that evaluation of the chick’s immune response to the locally prepared inactivated IBDV vaccine adjuvant with Montanide oil ISA 70 showed the highest antibody titers at 4th week post vaccination and were found to be protective and also agreed 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 antibody titers in all inoculated groups two weeks post inoculation. In addition, these results agreed with the facts showed that the humoral immune response plays the principal role in defense against vvIBDV (Lukert and Saif, 1997). Inoculation of inactivated IBDV could give complete protection with no obvious IBD clinical signs, as reported previously by (Maas *et al.*, 2001).

Figure (1) and (2) showed, The chicken in group 3 that vaccinated with inactivated bursa vacc vaccine show the lowest mean log₂ serum neutralizing antibody titer and mean ELISA titer level and antibody titer. These results agreed with (Thayer *et al.*, 1983) who proved that IBDV (bursa vacc) in the monovalent form had a GMT of 55 at 1st day post vaccination. The GMTs declined at steady and comparable rates for all treatment groups, with detectable titers manifested through 21 days of age post vaccination as a GMT of 6. There were no detectable titers at 28 days of age.

On the other hand the chicken in group 1 that vaccinated with virulent, variant isolates and a classical strain show the highest mean log₂ serum neutralizing antibody titer and mean ELISA titer level and these results agreed with (Silke *et al.*, 2003) who reported that virulent strain of IBDV induced the highest antibody levels detected and the best protection against challenge virus replication in comparison with mild and intermediate strain of classical IBDV.

Figure 1: Mean IBD serum neutralizing antibody titers in vaccinated chicks with the four prepared inactivated IBDV vaccines.



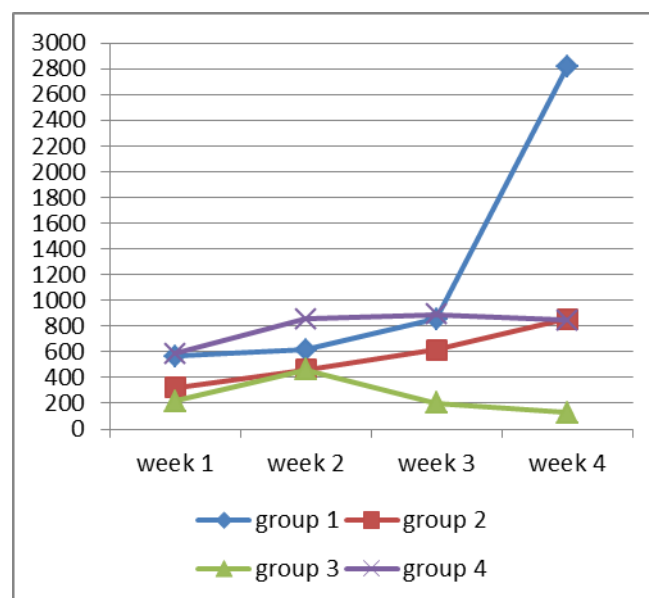
Group 1: vaccinated with inactivated IBD vaccine consisted of (variant isolate+virulent isolate+bursa vacc)
 Group2: vaccinated with inactivated IBD vaccine consisted of (variant isolate +bursa vacc)
 Group 3: vaccinated with inactivated IBD vaccine (bursa vacc strain)
 Group 4: vaccinated with inactivated IBD vaccine (variant isolate)

Challenge test

As shown in table (3), Protection percent in chicks vaccinated with the four prepared vaccines were 97.5%, 90%, 75%, 90% for group 1, 2, 3 and 4 respectively and 0% for challenged control unvaccinated (+ve) group. This result agreed with (Shaw and Davison, 2000) who mentioned that Protection against IBDV is highly dependent on the antigenic correlation between the vaccine and the circulating virus strains. Chicks in challenged control non vaccinated group showed atrophied yellowish bursa and slight hemorrhages on proventriculus 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 by examination for clinical signs and development of lesions in challenged birds which showed no clinical signs or lesions in all vaccinated groups of birds showing protection.. These results agreed with (Silke *et al.*, 2003) who reported that virulent strain of IBDV induced the best protection against challenge virus replication in comparison with mild and intermediate strain of classical IBDV, also agreed with (Susan *et al.*, 2013) who showed that the protection in challenged chicks which vaccinated

with TCIBD (variant isolate) was in high percent (96-100%) when they challenged by vvIBD virus and variant strain. Finally, the results of challenge test agreed with (OIE, 2016) who mentioned that The vaccine fails the challenge test unless at least 90% of the vaccinated chicken survives without showing either clinical signs or severe lesions in the bursae of Fabricius at the end of the observation period.

Figure 2: Mean IBD ELISA serum antibody titers in vaccinated chicks with the four prepared inactivated IBDV vaccines.



Group 1: vaccinated with inactivated IBD vaccine consisted of variant isolate+virulent isolate+bursa vacc)
 Group2: vaccinated with inactivated IBD vaccine consisted of (variant isolate +bursa vacc)
 Group 3: vaccinated with inactivated IBD vaccine (bursa vacc strain)
 Group 4: vaccinated with inactivated IBD vaccine (variant isolate)

As shown in figure (3), the chicks of group 3 vaccinated with bursa vacc strain vaccine showed the lowest parameters comparing with the other groups while the chicks of group 1 vaccinated with local isolates and classical strain show the highest parameters comparing with the other groups.

CONCLUSION

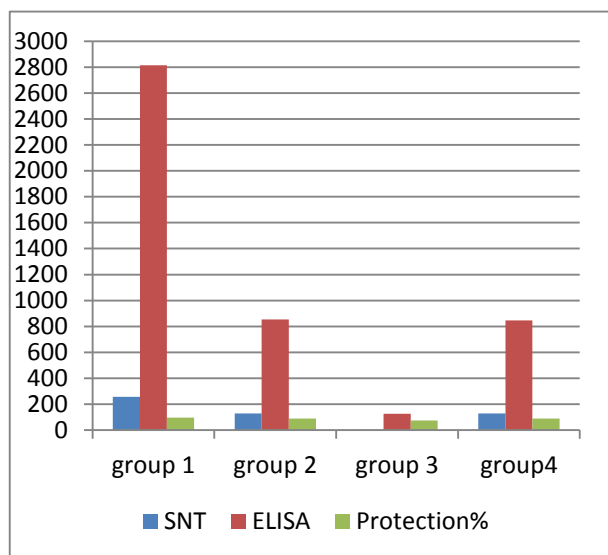
It was concluded that using inactivated vaccine prepared from local isolates and standard classical IBDV strain was safe, potent and immunogenic in young chicks and may had major advantage for control IBD disease in Egypt.

Table 3: Protection percent of chicks vaccinated with the four prepared inactivated IBDV vaccines after challenge using virulent IBDV

Challenged group	Number of chicks			Protection percent%
	Challenged	Dead	Live	
Group 1	40	1	39	97.5
Group 2	40	4	36	90
Group 3	40	10	30	75
Group 4	40	4	36	90
Group 5	40	40	0	0
Group 6	40	0	40	100

Group 1: vaccinated with inactivated IBD vaccine consisted of (variant isolate+virulent isolate+bursa vacc)
 Group2: vaccinated with inactivated IBD vaccine consisted of (variant isolate +bursa vacc)
 Group 3: vaccinated with inactivated IBD vaccine (bursa vacc strain)
 Group 4: vaccinated with inactivated IBD vaccine (variant isolate)
 **group (5): unvaccinated challenged (+ve) control group
 **group (6): unvaccinated unchallenged (-ve) control group

Figure 3: comparing the results of SNT, ELISA and protection% of the four prepared inactivated IBDV vaccines at the 4th week post vaccination



Group 1: vaccinated with inactivated IBD vaccine consisted of (variant isolate+virulent isolate+bursa vacc)
 Group2: vaccinated with inactivated IBD vaccine consisted of (variant isolate +bursa vacc)
 Group 3: vaccinated with inactivated IBD vaccine (bursa vacc strain)
 Group 4: vaccinated with inactivated IBD vaccine (variant isolate)

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