Comparative studies between different commercial types of live Infectious bursal disease [IBD] vaccine strains in Egypt

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Abstract

This study was carried out to test the efficacy of different live infectious bursal disease (IBD) vaccines (intermediate; classical or intermediate plus) for controlling IBD or as it is more commonly known in Egypt as gumboro disease problems in the Egyptian poultry field.

The efficacy of different living attenuated commercial vaccines were applied in ten groups of (20) Specific Pathogen Free chicks (SPF) for monitoring the immunosuppression effect. The immune response were determined in nine groups of (75) two weeks old SPF chicks "for each group" in vitro by measuring Enzyme Linked Immuno-Sorbent Assay (ELISA) and Serum Neutralization Test (SNT) titer post vaccination with estimation of bursal /body weight ratio and histopathological examination of bursa of fabricious; then in vivo by challenging birds with $10^{3.5}$ EID_{50} / dose challenge IBD virus strains (variant; classical and very virulent strains).

Results revealed that; protection percentages were ranged between 90%-100% in birds vaccinated with intermediate or intermediate plus IBD vaccine and between 90%-95% in birds vaccinated with invasive intermediate Bursa B2K. While birds vaccinated with classical D78 gave protection 95%-100% with highest antibody ELISA mean titer and SNT were "11344 and 1024"; respectively. This confirms that under field condition, poultry industry can be protect from gumboro disease if using commercial IBD vaccine

strains in correct time and condition according to status of flock and location of farm to reduce the economic losses caused by IBD infection viruses in Egypt.

Key words: Infectious bursal disease; Live Gumboro (IBD) vaccines; different vaccine strains.

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Introduction

Gumboro viral disease has been a great concern in Egyptian poultry industry for a long time but particularly for the past decade. Infectious bursal disease strains virus are member of the Birnavirus genus of the family Birnaviridae have the potential of immunizing the chicks even in the presence of moderately higher levels of maternally derived antibodies (MDA) [1]. The first reported as severe kidney lesions; later it was termed as Infectious Bursal Disease virus (IBDv) referring to the specific lesions caused by the disease in the bursa of fabricious, and severe renal damages [2]. Immunization of chickens is the principle method used for control of IBD in chickens. The vaccine must be safe, pure and efficient [3]. There are many choices of available live vaccine based on virulence such as classical vaccine (D78) that gave protection against mortality ranging between 30-40% during the first 48 house post vaccination but the acute problem for disease control is still due to interference of maternally antibodies in the establishment of the vaccination schedule [4]. Maternal antibodies interfered with the development of satisfactory protection in commercial broiler chicks and vaccination at 2 weeks of age resulted in better immune response in vaccinated group with intermediate 228E strain and gave 90% protection [5]. In spite of vaccinations against IBD, some flocks suffered from immunosuppression due to IBD. As well as some flocks up to 3 weeks (unsusceptible age of classical IBD) were immunosuppressed with atrophied bursa indicating the possibility of infection with the variant form of IBDV.

In Egypt, the disease was reported by at early seventies for the first time in commercial broiler chickens; while [6] were the first to identify the causative agent of IBDv in Egypt. Since then many trials were done to determine the current status of IBDv and the antigenic diversity in Egypt till now [7, 8]. The aim of this study was planned to study the efficacy of some available commercial IBD vaccine strains which currently used in Egyptian commercial poultry farms.

Material and Methods

Vaccines:

Living Infectious bursal disease (IBD) vaccines: Seven IBD commercial imported live attenuated vaccines were used: Three Intermediate: IZO IBD_2 .Batch No. (0335G); Nobilis Gumboro 228E.Batch No (A065A1J01) &INDOVAX-Georgia strain Batch No (BG 2911).Two Intermediate plus: IBD Xtreme. Batch No (B045611); & Gumboro L. Batch No (3106Z341A) .One Invasive intermediate INDOVAX- Bursa B2K Batch No (GP 3311) and Classical Intervet D78 Batch No (12601LJ01).

Newcastle disease (ND) vaccine: Hitchner B_1 vaccine strain obtained from Hipra-Hirpaviar- B_1 Batch No 27RG-4 with titer 7.5 $\log_{10} EID_{50}$ / dose it used in vaccination of experimental chicks for evaluation of immunosuppression effect of IBD vaccine.

Viruses**:**

Challenge IBD viruses**:** Three Challenge IBD viruses were used in this study: Field isolated variant viruses (Egy-IBD var 2009 Vp2 gene, partial cds submitted in gen bank at Accession No. : JN118617) and Very virulent (VVIBD) in form of infectious allantoic fluid(isolated from field cases and identified by phylogenic analysis) were kindly provided by [7] Central Lab for Evaluation of Veterinary Biologics (CLEVB).Classical IBD was kindly provided by[9] in form of allantoic fluid. All challenge IBD viruses titrated as described by [10] and calculated ID_{50} according to method of [11].

Challenge Newcastle disease virus (VVNDV): It is a virulent virus of Newcastle disease of field isolate, and obtained from the Newcastle disease Dep. Vet. Serum and Vacc. Res. Ins. Abb. Cairo (VSVRI) with in infectivity titer was $10^{6.0}$ EID_{50} / ml.

Newcastle disease Haemagglutinating antigen**:** Lasota strain has been propagated in embryonating chicken eggs for preparation of ND antigen.ND heamagglutinatinating antigen was adjusted 4HA unite according to [12].

Chicken Embryo Fibroblast (CEF) adapted IBD Virus: It was obtained from (CLEVB) and used in serum neutralization test.

Experimental Hosts**:**

One day old SPF chicks: Chicks free from maternal drive antibodies from SPF poultry farm at Koum Osheim El-Fayoum, 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.

Specific Pathogen free (SPF) embryonating chicken eggs (ECE)**:** Eggs were obtained from the SPF production farm Koum Osheim, El-Fayoum, Egypt.Eggs were kept in egg incubator at 37°c with humidity 40-60%.SPF eggs used for titration of egg adapted IBD vaccines according to [13] and estimated the Embryo Infected Dose (EID).

Tissue cultures (TC) and Cell culture media**:** Primary chicken embryo fibroblast cell (CEF) was obtained from (CLEVB); which prepared as describe [14]. Trypsin- version solution prepared according to Hanks balanced salt solution (HBSS).Minimum Essential Medium (MEM) was prepared according to the manufacturer's instructions, and Bovine Serum was Mycoplasma free and virus screened "Gibco Limited, Scotland and UK" The method used for inoculation in the microtitre plates was done according to [10].

Tissue culture used for titration of T.C. IBD strains vaccine according to [13]; Infected dose (ID_{50}) was calculated according to [11].

Polymerase chain reaction (PCR):

PCR used for detection the Identity of commercial vaccines under test according to [12] RNA extraction kit using Bio flux Simply total RNA extraction kit cat # (20111103). Amplification by using BIOER reverse transcription polymerase chain reaction (RT-PCR) kit one step cat # 20120603.

Serological tests**:**

Enzyme linked Immune, Sorbent Assay (ELISA): ELISA test was done according to [15].ELISA Kit was obtained from Biocheck serial No. F55351 for IBD.ELISA Reader: Micro plate reader USA, VERSA max with serial no was B02274.

Serum Neutralization Test (SNT)**:** A beta micro-neutralization procedure was carried out according to [16]. It was used for monitoring of IBDV antibodies.

Haemagglutination inhibition (HI) tests for NDV**:** The test was carried out according to the standard procedure described by [17] for the haemogglutnating activity (HA) of NDV antigen was an essential primary procedure using the HA test to determine HA unites used in HI test.

Evaluation of bursa lesions:

It was carried out according to [18]. Collected bursa was weighted and the bursa/ body weight ratio was determined also Bursa weight index were estimated.

Histopathological examination**:**

Autopsy samples were taken from the bursa of fabricious of birds; prepared according to [19]; bursa of fabricius were fixed, embedded in paraffin, sectioned and stained with hematoxylin and eosin for microscopic examination" and the severities of bursa lymphoid tissue lesions were scored on the basis of lymphoid necrosis and /or lymphocytic depletion according to [18].

Experimental Design**:**

Two experiments were design for studying the efficacy of different commercial live IBD vaccines:

Ten groups of (20) SPF chicks each were used in monitoring the immunosuppression of tested IBD vaccines by measuring HI titer against ND vaccine compared with control one. Chicks from groups (1 to 7) were vaccinated via eye drop route with recommended dose of different examined commercial vaccines at one day old .At two weeks old birds in groups (1 to 8) were given one field dose of live Newcastle disease vaccine by eye – drop. Birds of group (8) were kept as indicator for NDV and groups (9 and10) IBDV challenge control (+ve and–ve); respectively. The immune response for all groups was measured by HI test at 4 weeks old; and then birds of groups (1 to 9) were challenged with VVNDV with titer $10^{6.0}$ EID/dose. Group 10 was left as an unchallenged control. Before challenge a blood sample was collected from each bird in order to determine the HI antibody titer. Following challenge, all birds were observed daily for clinical signs attributable to ND infection.

Nine groups of (75) two weeks old SPF chickens were used for studying the efficacy of some commercial IBD vaccines used in Egyptian poultry field .Groups (1 to 7) vaccinated orally with one field dose with (IBD different vaccines); respectively while group (8) was kept as positive IBDV challenge control and group (9) non-challenged control for experiment. Chickens were kept under observation for 3 works post vaccination and serum samples were collected from all groups. The immune response was determined in vitro by measuring ELISA and SNT titer post vaccination with estimation of bursa / body weight ratio and histopathological examination for bursa of fabricious; then in vivo by challenging birds with $10^{3.5}$ EID_{50} / dose challenge IBD virus strains (20 birds from each groups for each IBD challenge:(variant; classical and very virulent strains).

Results and Discussion

This study was designed for compare efficacies between different strains of live IBD vaccine which using in the poultry farms as method of controlling the gumboro disease problem in Egypt and allover world [19]. Live IBD vaccines are produced from fully or partially attenuated strains of virus which known as (mild, intermediate or intermediate plus); respectively. Intermediate or intermediate plus vaccines are used to protect broiler chickens and commercial layer replacements. Some of these vaccines are also used in young parent chickens, if there is a high risk of natural infection with virulent IBD. They are sometimes administered at 1 day old as a coarse spray to protect any chickens in the flocks that may have no or minimum levels of maternal derived antibodies (MDA) [20] A clinical picture of IBD has dominated the field in different parts of the world since more than two decades, with high mortality rates and considerable economic losses. So that control and vaccination in order to achieve good protection [21]. Five parameters were used for the evaluation the efficacy of different IBD commercial vaccines including immunosuppression effect based on mean of HI titer for NDV with protection %; antibody level against IBD which monitoring by ELISA and SNT; Bursa body weight and histopathological change for bursa of fabricius. The last important parameter was protection%.

Detection of viral identity and titration: All the seven IBD vaccines used in this experiment identified by using: Reverse Transcriptase Polymerase Chain Reaction (RT-PCR): primer specific to IBD virus gives positive at 300bp amplification band which indicated that IBD viral DNA was present (figure-1) and titrated in ECE or T.C according to type of vaccines. The results indicated that vaccines, tested were $10^{5.5}$ EID_{50} / dose for all egg adapted gumboro vaccine strain; $10^{5.0}$ $TCID_{50}$ / dose; 2600 PFU/ Dose for Georgia strain Gumboro vaccine, Intermediate strain, Live, B.P. (Vet) and $10^{5.2}$ $TCID_{50}$ for another tissue culture adapted vaccine strains. So the results of titration were judged according to the parameters of [13], in which IBDV titers must be not less than $10^{3.5}$ $TCID_{50}$/dose in CEF and $10^{3.5}$ EID_{50}/dose in ECE for IBD vaccines.

PCR used for detect of identity of IBD vaccines as described in [12].These results were judged according to [13, 22].

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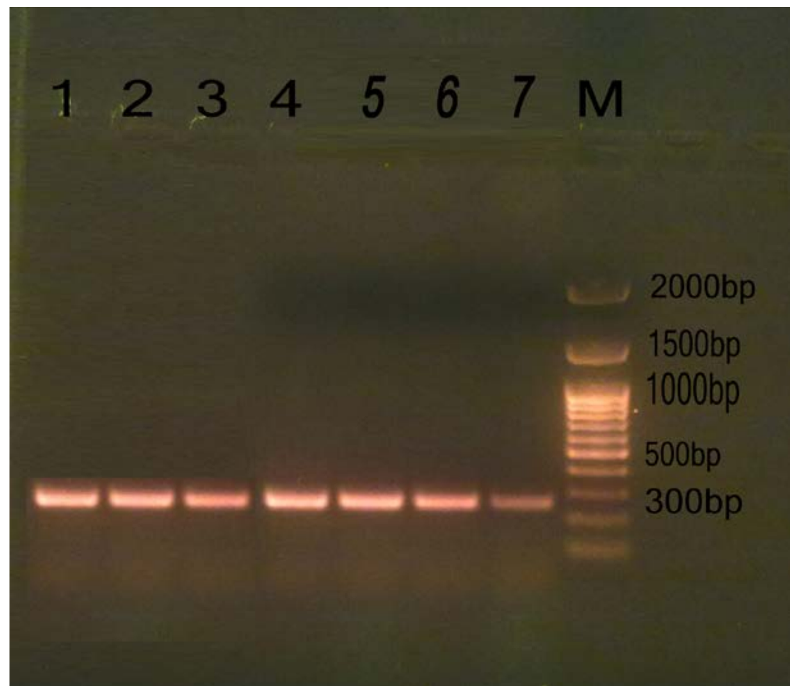

Figure- 1: PCR amplification of the spike gene of seven IBD polymerase gene vaccines under test.

The amplification of the 300bp fragment of the vp gene of IBD virus of seven vaccine batches under test indicated that IBD viral DNA was present according to [12].

Detection of immunosuppression effect: Chickens of groups (1-7) which vaccinated with different types of IBDv at one day old and at two weeks were given one challenge field dose of live Newcastle disease .The HI log_2 Mean titer for NDV ranging from 6.9 to 7.8 and 7.6 for indicator group (8) which was vaccinated with NDV only as showed in (Table-1).

Table -1: Results of immunosuppression test using the Newcastle disease virus challenge model

Groups No	Types of Vacc.	No of dead birds /total No	Protection %	Mean of HI log 2 titer for NDV
1	IZO IBD2	2/20	90%	6.2
2	228E	2/20	90%	6.5
3	INDOVAX Georgia strain	2/20	90%	7.0
4	IBD Xtreme	1/20	95%	6.8
5	Gumboro L	1/20	95%	7.02
6	INDOVAX Bursa B2K	1/20	95%	7.4
7	D78	1/20	95%	7.2
8	G_8 Indicator	1/20	95%	7.5
9	Centrol +ve (not vaccinated & NDV challenged)	20/20	0%	1.9
10	Control –ve (not vacc. non NDV challenged	0/20	100%	1.9

*As shown in table -1 there was no significance difference (<0.01) between HI titer in the vaccinated groups in compare with indicator one so all vaccines considered as non-immunosuppressive vaccine according to [12].
*Mean of HI log_2 titer for NDV must be not less than 6.0 according to [23].

The immunosuppression has been most often evidenced using experimental models based the measurement of humeral responses induced by Newcastle disease (ND) vaccines. The

best assessment is clearly the measurement of vaccine protection against challenge infection by (ND) virus as described in [12]. Our results agreed with [24, 25, 26] they studied the effect of pathogenesis of commercially available IBD vaccines and immune-suppressive effect.

Histopathological examination**:**

Following histopatholgical protocol, it was demonstrated as in shown in figure-2 (photos (1-7)) that bursa of fabricious of the chicks after 3 weeks post vaccination with different vaccine strains (G1-7) showing intact lining mucosal epithelium with mild depletion in the Lymphoid Follicles .However in photo(8) control –ve (G9) revealed no histopathological change. Our results agreed with [19] that reported the histopathological examination of the bursa of fabricius from chickens vaccinated with D78 and challenged with VVIBDV showed minimal necrosis.

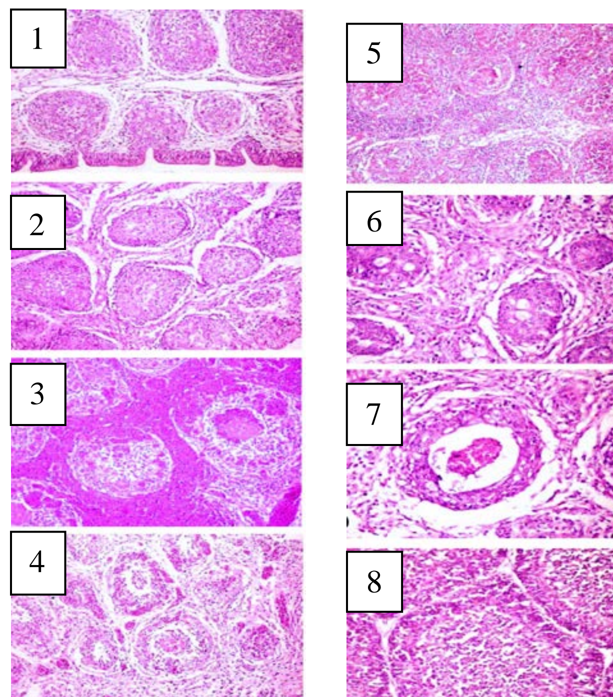

Figure- 2: Histopathological section in bursa of fabricious in chicks.

Histopathological section in bursa of fabricious in chicks vaccinated with IBD vaccine (H & E 10X).

Detection of immune response:

For studying the efficacy of some commercial IBD vaccines used in field; the immune response were determined in vitro by measuring ELISA and SNT titer post vaccination and in vivo by challenge as in Table -2.

The highest ELISA antibody mean titer can be detected in group (7) that equal 11344 which vaccinated with D_{78} (classical IBD strain) and the lowest one at group (3) that equal 6146 that vaccinated with INDOVAX-Georgia (intermediate IBD strain).In other hands SNT antibody mean titer high in all vaccinated groups 1024 except groups (3; 5 and 6) which vaccinated with intermediate Georgia strain; intermediate plus Gumboro L strain and invasive intermediate B2K strain; respectively which gave 512.

All birds were observed for two weeks post challenge and signs, lesions and mortalities were recorded; no clinical signs or lesions were recorded in all vaccinated groups. Dead birds in group (8) which was the positive challenge control group for three different challenged IBD viruses (VVIBD; variant IBD; and classical IBD) have different postmortem lesion .The bursa of chickens challenged with virulent IBDV appear yellowish; hemorrhagic and turgid with prominent striations, oedema and caseous material are found in some birds. Inflammation and hemorrhage in the proventriculus gland in between the tips in chickens challenged with variant strains. While in +ve challenged groups with classical strain showed petechial hemorrhages on the mucosal surface and dropping on bursa.

Protection percentages were ranged between 90%-100% in groups (1-5) “birds vaccinated with intermediate and intermediate plus IBD vaccine” and between 90%-95% in birds vaccinated with invasive intermediate Bursa B2K. While birds vaccinated with classical D78 gave protection 95%-100%.

Table -2 .Monitoring immune response in vitro & in vivo for different commercial imported live attenuated IBD vaccines

Groups/Type of vacc.	Antibody mean titer		Bursa Body Weight	Protection %		
	ELISA	SNT		VVIBD	Variant IBD	Classical IBD
G1 IZO IBD2	10705	1024	1.142	95	100	90
G2 228E	10124	1024	1.310	100	95	95
G3 INDOVAX-Georgia Str.	6146	512	1.503	90	95	90
G4 IBD Xtreme	10927	1024	1.018	100	95	95
G5 Gumboro L	7077	512	1.112	90	90	95
G6 INDOVAX-Bursa B2K	7289	512	1.462	90	90	95
G7 D78	11344	1024	0.994	100	95	95
G8 Control +ve not Vacc & Chall.	156	16	0.86	0	0	0
G9 Control –ve not Vacc & not chall	156	16	0.8	-	-	-

N.B: The protective dose for IBD vaccine must be more than 90% according to [12].

*GMT of ELISA titer of control positive serum is equal or more than (3000) for IBD living vaccine according to (Kit manufacture).

*IBD Serum neutralizing antibody titer = the reciprocal of serum dilution which neutralized and inhibit the CPE of 100 $TCID_{50}$ of IBDV according to [27].

*Chicks with bursal index lower than 0.7 were considered to have bursal atrophy according to [18].There are significant difference ($P \leq 0.01$) between all seven vaccinated groups in bursa body weight and antibody mean titer which determined by ELISA and SNT.

From above mentioned results in Table -2 the IBD vaccines under test considered satisfactory and potent. The results of sterility, safety, potency and immunogenicity were done according to [13, 24]. Bursal indexes in vaccinated SPF chicks were significantly higher than the challenge controls (Table-2) .The commercial vaccines protected against bursal damage as indicated by significantly lower bursal lesions in vaccinated birds as mention by [28].IBD vaccines including D_{78}, 228E, IBD Blen and Burse Vac caused varied destructive effect on bursa [9] the bursae from chickens with bursa/ body weight index higher than 0.7 found to be histologically normal and bursa/body weight ratio was calculated according to [8] who revealed our results. Table -2 shown efficacy results of examined commercial live attenuated IBD vaccines as measuring in vitro by detection of antibody response and in vivo by monitoring the protection percentage against different types of challenge strains “VVIBD; variant and classical strains”. Antibody response evaluated by serological tests (ELISA and SNT).GMT of ELISA titer of control positive serum is equal or more than 3000 according to [12]. Our results agree with this label and with or more that mention in [29] who noticed that ELISA antibody titer was higher in chicken groups vaccinated with intermediate strain than those with mild strain vaccine. [30] Reported that classical serotype-1 vaccines still induce good protection but the actual problem for disease control is still due to interference of MAbs in the establishment of the vaccination schedule. This report agrees with our results; which classical IBD vaccine in group (7) gave highest ELISA antibody titer (11344). The SNT results were 512 or more in vaccinated groups according to [27]. Our results were accord with [7, 31]. Cross protection trial gave protection percentage more than 90% against many challenge field isolate “VVIBD; variant or classical” strains of IBD against living attenuated commercial vaccines. Our results accord with [29, 32] that reported the intermediate – plus vaccine provided better protection against IBD challenge virus. [33] Notice that vaccination of

day 14 of age with intermediate strain of live attenuated IBD vaccine induce high and protective level of antibodies. Our results for protection % and lesions agree with [18, 34]. Also [7, 9, 35,36] agree with our results that different commercial vaccine strains give good protection against many challenge field isolated strains; and with [37] that reported the very virulent IBDV (VVIBDV) strains have now spread all over the world. Immunization of chickens by vaccination is the principle method used for control of IBD in chickens [3]. Our results in table -2 clarified that: Protection percentages against vvIBD or field isolated" variant or classical" IBD strains were ranged between 90%-100% in groups (1-5) "birds vaccinated with intermediate and intermediate plus IBD vaccine" and between 90%-95% in birds vaccinated with Bursa B2K. While birds vaccinated with classical D78 gave protection 95%-100% with highest antibody ELISA mean titer and SNT were "11344 and 1024"; respectively.

Conclusion

Based on the data presented in this study it can be concluded that under experimental condition the"Intermediate (228E); Intermediate plus (Xtreme) and Classical (D78) when administered at two weeks protect chickens with (95%-100%) from infection and disease following challenge with different IBD strains (Field isolated variant viruses (Egy-IBD var 2009 Vp2 gene, partial cds submitted in gen bank at Accession No. : JN118617) or Very virulent (VVIBD) or Classical IBD) with ELISA antibody titers 10124; 10927 and 11344 respectively. This confirms that under field conditions we can use vaccination programs based on our results to reduce the economic losses caused by IBD infection viruses in Egypt.

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