Comparative Studies between Different Types Of Live Infectious Bursal Disease (IBD) Vaccine Strains In Egypt

Susan S El-Mahdy[*1], Hayam Farouk[* 1], Abd El-Wanis NA[*], [2] Hamouda MM[* 3]

1- Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), El-Seka El-Baida St. Abbasia 131, Cairo, Egypt

2- Department of Newcastle Disease, Vet. Serum and Vacc. Res. Ins. (VSVRI) El-Seka El-Baida St., Abbasia 131, Cairo, Egypt

3- Department of Poultry Diseases Faculty of Vet. Medicine, Cairo University, Giza, Egypt

Corresponding author: Susan, S. El-Mahdy, email: prof.s.elmahdy@gmail.com

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

ABSTRACT

The efficacy of different living attenuated commercial vaccines against IBDV was tested in ten groups of (20) Specific Pathogen Free chicks (SPF) for monitoring the immunosuppression effect. The immune responses were determined in nine groups of (25) "for each group", two weeks old SPF chicks in-vitro through application of Enzyme Linked Immunosorbent Assay (ELISA) and Serum Neutralization Test (SNT) titers post vaccination with evaluation of bursa/body weight ratio and histopathological examination of bursa of Fabricious; then in-vivo by challenging of birds with $10^{3.5}$ EID_{50}/dose challenge IBD virus strains (variant; classical and very virulent strains).The obtained 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 showed protection of 95%-100% with highest ELISA and SNT mean titers as "11344 and 1024", respectively. This confirms that under field condition, poultry industry can be protected from IBDV disease using commercial IBD vaccine strains in correct time and condition according to status of flock and location of farm.

INTRODUCTION

Infectious bursal disease (Gumboro) has been a great concern in Egyptian poultry industry for a long time but particularly for the past decade. Infectious bursal disease virus strains are member of the genus Birnavirus 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 induce 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 plus 228E strain results in 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. Identification the causative agent of IBDv in Egypt was in 1976 for the first time *(6)*. Then many trials were done to determine the current status of IBDv and the antigenic diversity in Egypt till now **(7, 8)**. This study was planned to evaluate the efficacy of some available commercial IBD vaccine strains which currently are 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 IBD2 Batch No. (0335G), Intervet D78 Batch No. (12601LJ01) and INDOVAX-Georgia strain Batch No. (BG 2911). Three Intermediate plus: IBD Xtreme, Batch No. (B045611), Gumboro L. Batch No (3106Z341A) and Nobilis Gumboro 228E Batch No. (A065A1J01). One Invasive intermediate INDOVAX- Bursa B2K Batch No. (GP 3311) and.

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 was used in vaccination of experimental chicks for evaluation of immunosuppression effect of IBD vaccines.

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 the form of infectious allantoic fluid (isolated from field cases and identified by phylogenic analysis) were kindly provided by Central Lab for Evaluation of Veterinary Biologics (CLEVB) *(7)*. Classical IBD was kindly provided in form of allantoic fluid *(9)*. All challenge IBD viruses titrated *(10)* and ID_{50} was calculated *(11)*.

Challenge Newcastle disease virus (VVNDV)

Virulent Newcastle disease virus field isolate was supplied by the Newcastle Disease Research Dept., Veterinary Serum and Vaccine Research Insitute, Abbasia, Cairo (VSVRI) with in infectivity titer was $10^{6.0}$ EID_{50} / ml.

Chicken Embryo Fibroblast (CEF) adapted IBD Virus

It was obtained from (CLEVB) and used in serum neutralization test.

Newcastle disease Haemagglutinating antigen

Lasota strain has been propagated in embryonating chicken eggs for preparation of ND antigen. ND heamagglutinatinating antigen was adjusted at 4 HA unit *(12)*.

Experimental Hosts

Four hundred and twenty five (425) one day old SPF chicks free from maternal drive antibodies from SPF Poultry Farm at Koum Osheim El-Fayoum, Egypt. 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)

These 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 *(13)* and for estimation of the Embryo Infected Dose (EID).

Tissue cultures (TC) and Cell culture media

Primary chicken embryo fibroblast cell (CEF) was obtained from (CLEVB) *(14)* using Minimum Essential Medium (MEM) was prepared according to the manufacturer's instructions and supplied with newborn calf

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

Groups / Type of vaccines	Strain	Antibody mean titer		Bursa Body Weight	Protection %		
		ELISA	SNT		VVIBD	Variant IBD	Classical IBD
G1 IZO IBD2	Intermediate	10705	1024	1.142	95	100	90
G2 D78		11344	1024	0.994	100	95	95
G3 INDOVAX-Georgia Str.		6146	512	1.503	90	95	90
G4 IBD Xtreme	Intermediate Plus	10927	1024	1.018	100	95	95
G5 Gumboro L		7077	512	1.112	90	90	95
G6 228E	Invasive Intermediate	10124	1024	1.310	100	95	95
G7 INDOVAX-Bursa B2K		7289	512	1.462	90	90	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 percent for IBD vaccine must be more than 90% *(12)*.

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

* Chicks with bursal index lower than 0.7 were considered to have bursal atrophy *(18)*. There are differences 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 are considered satisfactory potent. The results of potency and immunogenicity were done *(13, 25)*. Bursal indices in vaccinated SPF chicks were higher than in the challenge controls (Table 2). The commercial vaccines protected chicks against bursal damage as indicated by significantly lower bursal lesions in vaccinated birds as mentioned in previous work *(29)*. IBD vaccines including D78, 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 were found to be histologically normal and bursa/body weight ratio was calculated *(8)* who confirmed our results. Table (2) showed efficacy results of examined commercial live attenuated IBD vaccines as measuring in vitro by determination 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 *(12)*. Our results agree with this label and with or more that mentioned in previous study *(30)* that noticed that ELISA antibody titer was higher in chicken groups vaccinated with intermediate strain than those with mild strain vaccine. Intermediate 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 *(31)*. This report agrees with our results; where Intermediate IBD vaccine in group (2) gave highest ELISA antibody titer (11344). The SNT results were 512 and 1024 in vaccinated groups. Our results were in agreement with previous studies *(7, 28, 32)*. 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 agree with previous authors *(29, 32)* that reported the intermediate – plus vaccine provided better protection against IBD challenge virus. Vaccination of day 14 of age with intermediate strain of live attenuated IBD vaccine induced high and protective level of antibodies *(34)*. Our results for protection test and lesions agree with previous results *(19, 35)*. Results of some authors *(7, 9, 36,37)* agree with our results showing that different commercial vaccine strains give good protection against many challenge field isolated strains; and with another author *(38)* who reported that the very virulent IBDV (VVIBDV) strains have now spread allover 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 isolates "variant or classical" IBD strains were ranged between 90%-100% in groups (1-6) "birds vaccinated with intermediate and intermediate plus IBD vaccine" and between 90%-95% in birds vaccinated with Bursa B2K.

Based on the data presented in this study, it can be concluded that under experimental condition, the Intermediate and Intermediate plus, when administered in chicks at two weeks of age show protection % ranged from 95%-100% after 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) and ELISA antibody titers were 11344 and 10927 respectively. While, in case of invasive intermediate IBD vaccine, the protection % was ranged from 90-95% and ELISA antibody titer was 7289. Finally, this confirms that under field conditions, it could use vaccination programs based on present results to reduce the economic losses caused by IBD infection viruses in Egypt.

REFERENCES

1. ***Delmas B, Mundt E, Vakharia VN and WU JL (2011):*** Familybirnaviridae. In: King, A.M.Q., Lefkowitz, E., Adams, M.J., Carstens, E.B. (Eds) Virus Toxonomy Ninth Report of the International Committee on Taxonomy of Viruses. Academic press Inc., San Diego, California: 499-07.
2. ***Eterradossi N and Saif YM (2008):*** Infectious bursal disease. In: Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K., Swayne, D.E. (Eds), Disease of Poultry, 12th ed. Black well Publishing Ames Iowa, USA.
3. ***Mardassi H, Khabouchi N, Ghram A, Namouchi A and Karboul A (2004):*** A very virulent genotype of infectious bursal disease virus predominantly associated with recurrent infectioius bursal disease outbreaks in Tunisian vaccinated flocks. Avian Dis.48:829-40.
4. ***Hsieh MK, WU CC and Lin TL (2010):*** DNA-mediated vaccination conferring protection against infectious bursal disease in broiler chickens in the presence of maternal antibody.Vaccine.28:3936-43.
5. ***Azhar Abd El-Aziz (2000):*** Evaluation of different infectious bursal disease vaccines. Assiut. Vet. Med. J. 44(88), 242-54.
6. ***Ayoub NN and Malek G (1976):*** Identification of pathogen of Gumboro disease in Egypt. Mon. Vet. Med.J., 31,106-108.
7. ***Ahmed M H (2010):*** Preparation and evaluation of living Gumboro vaccine from isolated locally variant strain. M.V.SC. Thesis, Faculty of Vet. Med. Cairo Univ.
8. ***Helal AM, Susan S El-Mahdy and Manal A Afify (2012):*** Study the prevalence of

variant IBD strains in some Egyptian chicken farms. New York Science Journal 5(6):8-11.

9. ***Khalid M El Bayoumi (2007):*** Studies on infectious bursal disease in chicken. Ph.D Thesis, Faculty of Vet. Med. Cairo Univ.

10. ***Villegas P (1990):*** Laboratory manual-avian virus disease. The Univ., College. of Vet. Med., Athens, Georgia, USA.

11. ***Reed LJ and Muench H (1938):*** A simple method of estimating fifty percent end point. Am. J. Hyg. 27, 493-97.

12. ***OIE (Office of International des Epizooties) (2012):*** Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Newcastle Disease: (Chap 2.3.14:576-89) Infectious Bursal Disease: (Chap.2.3.12: 549-65).

13. ***CFR (Code of Federal Regulation - 13CFR) USA (2012):*** Published by office of the federal register national archives records service, Animals 331-13: CFR.CH: 101-1-97 ED; CH.1.1-1-13 Ed. 11.30.

14. ***Schat KA and Purchase HG (1989):*** Cell culture methods. In: American Assoc. Avian Pahol. 3rd Ed., 167-75.

15. ***Brown MD and Skinner MA(1996):*** Coding sequences of both genome segments of an Euoropean vv infectious bursal disease virus.Virus Res., 40: 1-15.

16. ***Snyder DB, Marguardt WW, Mallinion ET, Russeteohen E, Svage PK and Allen DC (1986):*** Rapid serological profiling by enzyme linked imunosorbent assay. Avian Dis. 30, 139- 48.

17. ***Beard CW (1989):*** Serological procedures in a laboratory manual for the isolation and identification of avian pathogens. 3rd Ed. Published by the American Association of Avian Pathologists.

18. ***Majujabe KA and Hitchner SB (1977):*** Antibody response to strain combination of Newcastle disease virus as measured by haemagglutination inhibition. Avian Dis. 21,576- 84.

19. ***Sharma JM, Dohms JE, and Metz AL (1989):*** Comparative pathogenesis of serotype I and variant serotype II: Isolates of infectious bursal disease virus and their effect on humeral and cellular immune competence of specific –pathogen-free chickens. Avian Dis. 33, 112- 24.

20. ***Zhou X, Wang D, Xiong J, Zhang P, Li Y and She R (2010):*** Protection of chickens with or without maternal antibodies, against IBDV infection by a recombinant IBDV-VP2 protein vaccine. Vaccine.28 (23):3990-96.

21. ***Mahgoub HA (2010):*** An over view of infectious bursal disease. Arch. Virol. 157:2047- 57.

22. ***Ingrao F, Rauw F, Lambrecht B and Van den Berg T (2013):*** Infectious Bursal Disease: a complex host- pathogen interaction, Developmental and Comparative Immunology. Doi: http:// dx.doi.org/10.1016/j.dci.2013.03.017.

23. ***European Pharmacopoeia (2007):*** 2.6.1 (Bacterial and Fungal Sterility) 26.5. Test for extraneous virus using cell culture. 2.6.7 Mycoplasmas, culture method. V2.1.3.5. Using ELISA test for testing viral purity.

24. ***Alexander DJ (2003):*** Newcastle disease, Other Avian Paramyxoviruses and Pneumovirus infections: Newcastle disease. In: Diseases of Poultry. Saif, Y.M. (Ed in chief) Iowa state University Press USA .64-87.

25. ***Ismail N and Saif YM (1991):*** Immunogenicity of infectious bursal disease viruses in chickens. Avian Dis .35, 460- 69.

26. ***Khatri M and Sharma JM (2009):*** Response of embryonic chicken Lymphoid cells to infectious bursal disease virus.Vet.Immunol.Immunopathol.127 (3-4):316- 24.

27. ***Rosales AG, Villegas, A P, Lukert PD, Fletcher OJ and Brown J (1989):*** Immunosuppressive potential and pathologenicity of recent isolates of infectious bursal disease virus in

commercial broiler chickens. Avian Dis., 33: 724.

28.Singh, KV; Osman, OA, Ivon, FE and Thanaa, I (1967): Cloistral transfer of rinderpest neutralizing antibody to offspring of vaccinated dams. J. Comp. Med. Sci., 31: 295-298.

29.Perozo F, Villegas AP, Fernandez R, Cruz J and Pritchard N (2009): Efficacy of single dose recombinant herpes virus of turkey infectious bursal disease virus. (IBDV) vaccination against a variant IBDV strain. Avian Dis. 53 (4), 624- 28.

30.Singh NK; Dey S; Madhan Mohan C, Mohan Kataria J and Vakharia VN (2010): Evaluation of four enzyme linked immunosorbent assays for the detection of antibodies to infectious bursal disease in chickens.J.Virol.Methods.165(2):277- 82.

31.Van Den Berg TP (2000): Acute infectious bursal disease in poultry. Avian pathology.29, 175- 92.

32.Susan S El-Mahdy, Manal A Afify and A M Helal (2013): Evaluation of live Gumboro vaccine prepared from local variant strain for control of infectious bursal disease in Egypt. Vet World.6 (9):674- 80.

33.Khalil SA and El-Manakhly (1998): Pathologic, immune cytochemical and immunologic studies on a new Infectious bursal disease vaccine "Intermediate-plus" in chickens. Egypt. J. Comp. and clinical Pathol. 11(2), 185-198.

34. Hair-Bejo M, Ng MK and Ng HY (2004): Day old vaccination against infectious bursal disease in broiler chickens. International Jounrma of poultry science. 3 (2), 124-128.

35.Hassan MK, Al-Natour MQ, Ward LA, and Saif YM (1996): Pathogenicity, attenuation, and immunogenicity of infectious bursal disase virus Avian Dis. 40,567-71.

36.Salama SS and Amal I Abd El-Hady (2010): Studying the effect of simultaneous vaccination with Salmonella Typhimurium and infections bursal disease vaccines in chickens.SCVMJ, XV (1) 279-88.

37.Zorman Rojs O, Krapez V, Slavec B, Jursic-Cizerl R and Poljanec TA (2011): Field efficacy of different vaccines against infectious bursal disease in broiler flocks. Acta Vet Hung. 59(3):385-98.

38.Aricibasi M, Jung A, Heller E-D and Rautenschlein S (2010): Differences in genetic back ground influence the induction of innate and acquired immune responses in chickens depending on the virulence of the infecting infectious bursal disease virus (IBDV) strain. Vet. Immunol. Immunopathol.135:79-92

الملخص العربي

دراسات مقارنة بين اللقاحات المختلفة لفيرس التهاب غدة ثبريشيا فى مصر

سوزان المهدي ، هيام فاروق، نبيل عبد الونيس، محمد حمودة

قد تم اختبار فعالية اللقاحات الحية المستضعفة ضد مرض فيروس التهاب غدة فابريشيا و هى من مصادر انتاجية مختلفة في عشر مجموعات كل مجموعة تتكون من (٢٠) فراخ خالية من مسببات الأمراض (SPF) لرصد و متابعة اثر هذه التحصينات التثبيطى لمناعة الطائر المحصن. تم تقييم الاستجابات المناعية خارج جسم الطائر في تسع مجموعات (٢٥ طائر خالية من مسببات الأمراض لكل مجموعة) في المختبر باستخدام اختبارى الاليزا (ELISA) و التعادل المصلى (SNT) و ذلك مع حساب نسبة وزن غدة فبريشيا الى وزن جسم الطائر المحصن ثم تم تقييم الاستجابات المناعية فى نفس المجموعات في جسم الطائر الحي من خلال اختبار التحدي و ذلك بحقن كل طائر بجرعة EID50/١٠٣,٥ من سلالات فيروس IBD (المتغير ، الكلاسيكية الضارى). كشفت النتائج أن نسب الحماية تراوحت بين ٩٠٪ -١٠٠٪ في الطيور التى تم حقنها بلقاح الجمبورو الوسيط أو الوسيط الموجب إلى IBD وبين ٩٠٪ -٩٥٪ في الطيورالمحصنة بالعترة الغازية الوسيطة B2K. بينما الطيور التى تم تطعيمها بالعترة D78 الكلاسيكية أظهرت حماية ٩٥٪ - ١٠٠ ٪ مع أعلى نتائج مناعية فى اختبارى الاليزا و التعادل المصلى الذى وصل الى "١١٣٤٤ و ١٠٢٤"، على التوالي. وهذا يؤكد أنه في ظل الظروف الحقلية فى صناعة الدواجن فى مصر يمكن السيطرة على مرض الجمبورو باستخدام سلالات لقاح IBD التجارية في الوقت الصحيح و حسب الحالة المناعية للقطيع وموقع المزرعة.