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

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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 B1 vaccine strain obtained from Hipra- Hippavir- B1 Batch No. 27RG-4 with titer 7.5 log 10 EID50 / 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 (VIVIBD) 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 Biologies (CLEVB) (7). Classical IBD was kindly provided in form of allantoic fluid (9). All challenge IBD viruses titrated (10) and ID50 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 Institute, Abbasisia, Cairo (VSVRI) with in infectivity titer was 10^6.0 EID50 / 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 hemagglutinating 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 Koun 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 Koun 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

<table>
<thead>
<tr>
<th>Groups / Type of vaccines</th>
<th>Strain</th>
<th>Antibody mean titer</th>
<th>Bursa Body Weight</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ELISA</td>
<td>SNT</td>
<td>VVIBD</td>
</tr>
<tr>
<td>G1</td>
<td></td>
<td>10705</td>
<td>1024</td>
<td>1.142</td>
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<tr>
<td>IZO IBD2</td>
<td></td>
<td>11344</td>
<td>1024</td>
<td>0.994</td>
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<tr>
<td>G2</td>
<td>Intermediate</td>
<td>11344</td>
<td>1024</td>
<td>0.994</td>
</tr>
<tr>
<td>D78</td>
<td>Intermediate</td>
<td>11344</td>
<td>1024</td>
<td>0.994</td>
</tr>
<tr>
<td>G3</td>
<td>Intermediate</td>
<td>11344</td>
<td>1024</td>
<td>0.994</td>
</tr>
<tr>
<td>INDOVAX-Georgia Str. G4</td>
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<td>1024</td>
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<tr>
<td>IBD Xtreme</td>
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</tr>
<tr>
<td>G5</td>
<td>Intermediate</td>
<td>11344</td>
<td>1024</td>
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<tr>
<td>Gumboro L</td>
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<td>1024</td>
<td>0.994</td>
</tr>
<tr>
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<td>11344</td>
<td>1024</td>
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</tr>
<tr>
<td>228E</td>
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<td>0.994</td>
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<tr>
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<td>1024</td>
<td>0.994</td>
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<tr>
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<td>1024</td>
<td>0.994</td>
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<tr>
<td>Control +ve not Vacc. &amp; Chall. G9</td>
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<td>1024</td>
<td>0.994</td>
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<tr>
<td>Control -ve not Vacc &amp; not chall</td>
<td>11344</td>
<td>1024</td>
<td>0.994</td>
<td>100</td>
</tr>
</tbody>
</table>

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_90 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 MAb's 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 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 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.

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الملخص العربي

دراسات مقارنة بين اللقاحات المختلفة لفirus الالتهاب غدة فيشرشيا في مصر

سوزان المهدي، هيا فاروق، نبيل عبد الوينس، محمد حمودة

قد تم اختبار فعالية اللقاحات الحية المستضفة ضد مرض فيروس التهاب غدة فيشرشيا و هي من مصادر
الانتاجية مختلفة في عشر مجموعات كل مجموعة تتكون من (20) فراخ خالية من مسببات الأمراض (SPF)
لرصد و تنتاب这两 التحصينات التثبيتية لمناعة الطائر المحصن. تم تقييم الاستجابات المناعية خارج
جسم الطائر في سبع مجموعات (5 طائر خالية من مسببات الأمراض لكل مجموعة) في المختبر
باستخدام اختبار الإلإيزا (ELISA) و التعادل المصلي (SNT) و ذلك مع حساب نسبة وزن غدة فيشرشيا
إلى وزن جسم الطائر المحصن ثم تم تقييم الاستجابات المناعية في نفس المجموعات في حجم الطائر الحي
IBD من خلال اختبار التحدي و ذلك بحقيق كل طائر بجرعة 0/81050 من سلالات فيروس
المتغيرة (الكلاسيكية الضار). كشفت النتائج أن نسبة الحماية تراوحت بين 90% - 100% في الطيور التي
يمهم بلقاح الجمبرورو الوسيط أو الوسيط الموجب إلى IBD في الطيور المحصنة
بالعطة الغازية الوسيطة D2K. بينما الطيور التي تم تطعيمها بالعطرة D78 الكلاسيكية أظهرت حماية
95%. 7 - 0 مع أعلى نتائج مناعية في اختبار الإلإيزا و التعادل المصلي الذي وصل إلى "114 و 124 و 134 و"
على التوالي. وهذا يؤكد أنه في ظل الظروف القاسية في صناعة الدواجن في مصر يمكن السيطرة على
مرض الجمبرورو باستخدام سلالات لقاح IBD التجارية في الوقت الصحيح و حسب الحالة المناعية للقطاع
وموقع المزرعة.