EVALUATION OF PATHOGENICITY OF RECENT VARIANT INFECTIOUS BURSAL DISEASE VIRUS AND PROTECTION AFFORDED BY CLASSIC VACCINES

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Abstract

A recent Infectious bursal disease virus designated as V1 was detected in vaccinated broiler chickens suffering from severe bursal atrophy, immunosuppression and poor performance. V1 had monoclonal reactivity similar to that recorded for variant Delaware/E strain. In the pathogenicity study, V1 induced marked bursal inflammation and bursal atrophy at 7 days post-inoculation; therefore, it appears to differ in pathogenicity from Delaware/E virus. In the protection study, 4 Classic live (2 intermediate and 2 hot vaccines) did not prevent bursal atrophy following experimental challenge with the V1 virus in 25 day old broilers. The results of the experimental pathogenicity and protection studies in broiler chickens may confirm the possible role of antigenic variation of field IBDV in flocks had immunosuppression and bursal atrophy.

INTRODUCTION

Infectious bursal disease virus (IBDV), a member of genus *Avibirnavirus* within the family *Birnaviridae* (Pringle, 1998), causes a highly contagious immunosuppressive disease in chickens known as infectious bursal disease (IBD) (Cosgrove, 1962). Severe outbreaks of IBD were recorded in several European countries, Asia and Africa due to very virulent strains (vvIBDV), which are antigenically similar to the classic IBDV strains (Chettle *et al.*, 1989, Van den Berg *et al.*,1991, Lukert and Saif, 1997). In contrast, antigenically variant strains were recorded in USA (Rosenberger and Cloud,

1986, Lasher and Shane, 1994). These variants are different from classic serotype I strains in that they produced a very rapid bursal atrophy, but with minimal inflammatory response.

Several investigations were conducted to study the antigenicity of IBDV using Antigen Capture ELISA (AC-ELISA) (Snyder *et al.*, 1988), *in vitro* cross neutralization (Saif *et al.*, 1987) and polymerase chain reaction (PCR) (Jackwood & Jackwood, 1994).

Recently, a commercial available AC- ELISA kit, utilizing group and strain-specific monoclonal antibodies, became available and has been used in epidemiological investigations in vaccinated broiler flocks in Egypt (Metwally *et al.*, 2003). The increased incidence of subclinical IBD and poor performance in many broiler flocks along with the speculations about field isolates that may cause disease in vaccinated flocks have raised many questions from the poultry industry. In recent study by Aly *et al.* (in, press 2004), the immunogenecity of classic vaccines in SPF and commercial layers was investigated using classic challenge virus. Results revealed that the used vaccines provided partial protection. This situation directed our attention to the possible presence of antigenically variant strains that can escape neutralization induced by classic vaccines.

The aim of this study was to examine the pathogenicity of recent isolate of IBDV that showed monoclonal reactivity profile similar to that recorded for Del/E variant. Also, to evaluate protection induced by live intermediate and hot classic vaccines under experimental conditions.

MATERIALS AND METHODS

Flock history

Broiler chicken flocks suffering from uneven growth, respiratory symptoms and mortality rate higher than normal were examened . The post-mortem examination showed atrophy of bursa of Fabricius at age of 2 weeks, followed by pericarditis, perihepatits, air saculitis and hemorrhage in proventriculus.

IBD challenge virus

The IBDV designated V1 was obtained from two-week -old broilers that had severe bursal atrophy, immunosuppression and poor performance. The infected bursae were homogenized and used as a source of IBDV and titerated in SPF eggs. The dose of the challenge virus was 102 EID50/chick and was adminstered with the eye drop routes.

Antigenic typing using monoclonal antibody

The bursal homogenate containing the V1 virus was tested against 4 neutralizing monoclonal antibodies (Mabs) namely, #8, B69, R63 & #10 by commercial AC-ELISA according to the method described by the producer (Synbiotic Corp. San Diego, CA, USA). Mab #8 is directed to common group epitope and used for initial screening. Mabs B69 and R63 were prepared against the D78 strain, and Mab #10 was prepared against the varient Galaxo labortory strain (GLS) (Snyder *et al.,* 1988).

IBDV vaccines

Two different commercial live-intermediate IBD vaccines (vaccine A & B) and 2 more invasive "Hot" vaccines (C& D) were used. Chickens were inoculated with one vaccine dose via eye drop route.

Chickens

Commercial broiler type chickens (obtained from IBDV vaccinated parents) were used. Chickens were received as day old and kept in clean disinfected rooms.

Experimental design

Pathogenicity study

The chickens were allotted in 2 groups (30 birds per group) and placed in separate clean disinfected rooms. Each bird in the 1st group was inoculated via eye drop route, with 0.1 ml of bursal homogenate (containing 102 EID50/ chicken). The chickens in the 2nd group were left as uninoculated controls. On 3, 5, 7 and 9 days post-inoculation (PI) five birds per group were examined for PM lesions. Bursas were excised, and the bursa: body weight ratios (B: BW) were calculated (bursa weight in

grams X 1000/ total body weight in grams). Bursal sections were examined microscopically for lesions.

Immunogenicity of classic vaccines

Hundred and twenty 25-day-old broiler chickens (free from maternally derived IBDV antibody) were used. The chickens were allotted in 6 groups (20 birds per group). Each bird in the 1st and 2nd groups was inoculated with intermediate vaccines (A & B), respectively. The 3rd and 4th groups were inoculated with hot vaccines (C & D). The 5th & 6th groups served as positive (unvaccinated challenged) and negative (unvaccinated unchallenged) controls. Ten days post-vaccination (PV), chickens were challenged with 102 EID50 of the V1 virus via eye drop route. Ten birds per group were killed on 7 days post- challenge (PC). The bursa: body weight ratio (B: BW) was calculated. Criteria for evaluation of protection were conducted as described by Hassan and Saif (1996). B: BW ratios and bursal lesion scores were used to evaluate the results of challenge studies. The vaccines were classified according to the protection into these groups: 1) vaccines provided full protection = normal B: BW ratio and no bursal microscopic lesions, 2) vaccines provided partial protection = normal B: BW but bursa microscopic lesions were detected, 3) non-protective vaccines = low B:BW ratio and bursal microscopic lesions detected.

Serology

Serum samples were collected from individual blood samples (5 chicks/group), and the mean ELISA titer for each group was calculated using the IDEXX flock check software (IDEXX laboratory, main, USA).

Statistical analysis

The average B: BW ratios of the inoculated and control birds were compared using analysis of variance followed by Fisher least significant difference test .

Histopathology

Sections of bursal tissues from inoculated and control birds were stained with hematoxylin and eosin staining techniques. Bursal histological lesions were scored according to Rosales *et al.* (1989). Bursas were subjectively scored as: 1= no lesions;

2= focal mild cell depletion: 3= multifocal 1/3 to 1/2 of follicles show atrophy; and 4= diffuse atrophy of all follicles.

RESULTS

Antigenic typing using monoclonal antibodies

The bursal homogenates were examined and characterized with a panel of 4 neutralizing Mabs (Table 1). The V1 virus tested positive with monoclonal antibodies #8 and R63 and lack the neutralizing B69 and #10 epitopes. Therefore, on the basis of the reaction with the Mabs, the tested V1 virus was antigenically similar to the variant Del/E strain.

Pathogenicity study

Results of the pathogenicity study are illustrated in Table 2. No clinical signs of IBD were observed in the birds inoculated with V1 bursal homogenate and control birds. Also, no mortality was recorded in both groups throughout the experimental period. Macroscopically, bursal edema and congestion was marked on 3 days post-inoculation and eventually bursal atrophy was marked on 7 days post-inoculation. Histologically, acute inflammation with heterophilic infiltration, severe extensive necrosis of lymphocytic follicles, along with bursal atrophy was noticed in chickens inoculated with V1 bursal homogenate. On 9 days post-inoculation, bursas had abundant interlobular fibrous connective tissue separating lymphodepleted follicles (ghost follicles, cyst formation with little inflammation.) The bursas of uninoculated control birds had intact, large follicles with normal architecture.

Protection efficacy of classic intermediate and hot vaccines

The results of the immunogenicity study of live classic intermediate and hot vaccines in commercial broilers that were challenged and sacrificed on 7 days post-challenge are shown in Table 3. Neither clinical signs of IBD, nor mortality was observed in vaccinated and control chickens. On post-mortem examinations, the B:BW ratios of vaccinated challenged and nonvaccinated challenged control chickens were significantly lower from those of the negative controls ($P<0.05$). Microscopically, severe bursal lesions were observed in vaccinated challenged birds on 7 days PC

indicating that the intermediate and hot vaccines did not prevent bursal lesions against the the V1 challange.

No signficant differences in antiboy titres against IBD were deteced between treated groups. The negative control group had no antibody response.

DISCUSSION

Since it was described by Cosgrove in 1962, IBD has been considered as one of the important viral diseases threatening the poultry industry worldwide. During 1980s, in Delmarva (USA), variants of serotype 1 IBD virus were emerged (Saif, 1984). These variants caused a very rapid bursal atrophy, but with minimal inflammatory response in chickens having antibodies to classic serotype 1 vaccine strains. The first of these variants to be isolated, designated Delaware E (Del/E), was clearly distinguished from classic serotype 1 strains in monoclonal antibody based AC-ELISA. Del/E lacked an epitope recognized by monoclonal antibody B69, which was present on all classical strains. There was speculation that the emergence of pathogenic and antigenic variants was enhanced by high selective pressure resulting from very high infection challenge and intensive use of vaccines based on classic strains. In this context, it is obvious that such pressure is occurring in the commercial chicken flocks in Egypt.

Differentiation of IBD strains was based mainly on virus neutralization assay (Saif, 1984). Recently, with the introduction of highly sensitive molecular techniques, as monoclonal antibody (Mab) and polymerase chain reaction (PCR), it became easier to differentiate IBDV strains and utilize such information in studying the molecular epidemiology of the disease (Jackwood & Jackwood, 1994). However, such techniques are not predictors of pathogenicity or protective potential of the available vaccines against challenge with the typed virus. In this study, AC-ELISA was used to type recent V1 virus utilizing 4 neutralizing monoclonals. The tested IBDV had monoclonal pattern similar to Del/E variant strain recorded in USA. Metwally *et al.*, (2003) recored that 58% of tested IBDVs had monoclonal reactivity pattern similar to Del/E variant strain in vaccinated Egyptian broiler flocks.

The result of AC-ELISA prompted us to conduct pathogenicity and immunogenicity studies. In the pathogenicity study, the V1 virus induced pathological changes not similar to those recorded in case of Del/E variant strains. The V1 caused marked bursal edema and inflammation that was noticed up to 5 days post- infection and the bursal atrophy was noticed on 7 and 10 days post-infection (PI). In an earlier study (Hassan *et al.*, 1996), it was shown that the pathology induced by both the variant and classic viruses was similar. However, variant virus indiana (IN) strain triggered very rapid bursal inflammation which subsided by 4 days PI, while, the classic strain did not elicit an overt inflammatory response until 4 days PI and subsided by 8 days PI.

Prophylaxis of IBD is mainly based on vaccination. In this regard, commercial vaccines contain classic serotype 1 virus provide partial protection against challenge with variant serotype 1 viruses, while, variant virus vaccines provide complete protection against variant and classic challenge viruses (Ismail and Saif, 1991). In the current study, both classic intermediate and hot vaccines did not prevent bursal atrophy and microscopic lesions were recorded in all vaccinated challenged chickens. This result may be attributed to the antigenic variation illustrated by the V1 virus.

In conclusion, the results of the antigenicity, experimental pathogenicity and protection studies in broiler chickens may confirm the possible role of antigenic variation of field IBDV in recent problems. However, more investigations are needed to examine these newly emerged IBD isolates with another panel of monoclonal antibodies, to study the molecular basis of antigenic variation and to formulate better vaccination programs under field conditions. Finally, It is of prime importance to emphasize the role of sanitation and biosecurity to reduce the losses due to IBD.

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Table 1. Antigenic characterization of V1 IBDV strain by its reactivity with a panel of 4 neutralizing Mabs.

Reactivity with Mabs				Virus type
#8	B 69	R 63	#10	
+	-	+	-	Del/E
+	+	+	+	Classic
+	-	-	-	RS593
+	-	-	+	GLS
+	-	+	-	V1

The plus sign (+) indicates a relative titer level of 0.6 or greater and a negative sign (-) indicates relative titer of less than 0.6 (depend on the optical density reading at 405nm)

Table 2. Pathogenicity of the V1 (IBDV) in 25 day old broilers.

Group	Challenge [1]	Bursa/body weight ratio at days PI [2]			
		3	5	7	9
1	V1 (IBDV)	3.2[a]	2.34[a]	0.84[b]	0.91[b]
2	None	2.17[a]	2.81[a]	2.49[a]	2.49[a]

[1]Each bird was challenged, via eye drop route with 10^2 EID_{50}.

[2]Values represent the mean of 5 chickens. Values within a column followed by the same superscript letters were not significantly different ($P>0.05$).

Table 3. Evaluation of protection afforded by classic intermediate and hot vaccines in 25 day old commercial broiler chickens. Bursa index, histological bursal lesions and antibody response after 7 days post challenge with IBDV challenge virus.

3Group	Vaccine[1]	Challenge [2]	Bursa/body weight ratio [3]	Mean Bursal lesion score[4]	ELISA mean titer
1	Intermediate (A)	+	0.80[a]	3	6231
2	Intermediate (B)	+	0.85 [a]	3	14022
3	Hot (C)	+	0.77 [a]	4	7970
4	Hot (D)	+	0.86 [a]	4	8365
5	None	X	0.58 [a]	4	3800
6	None	-	2.14 [b]	1	0

[1]Each chicken received one dose of live vaccine, via eye drop route at 4 week old (20 bird/group).

[2]Each bird was challenged via eye drop routes with 10^2 EID_{50} at 10 days post vaccination. + = vaccinated challenged chickens, X = nonvaccinated challenged chickens, and - = nonvaccinated nonchallenged chickens.

[3]Values represent the mean of 5 chickens. Values within a column followed by the same superscript letters were not significantly different ($P>0.05$).

[4]Bursal lesion score: 1=no lesion, 2= focal, mild cell necrosis or depletion, 3= multi-focal 1/3 to ½ of the follicles show atrophy, and 4= diffuse, atrophy of all follicles.

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تقييم ضراوة عترة مغايرة من فيروس الجمبورو و الحماية الناتجة عن اللقاحات الكلاسيكية

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تم كشف و تصنيف عترة حديثة من فيروس الجمبورو (V1) في قطيع دجاج تسمين يعاني من ضمور شديد في كيس فابريشيا و نقص مناعي و ضعف في الأوزان.

ولهذه العترة تفاعل مع الأجسام المناعية وحيدة المصدر مشابهة للتفاعل المسجل للعترة المغايرة Delaware/E .

وقد تمت دراسة هذة العترة (V1) من ناحية التغيرات الباثولوجية التي تطرأ علي الدجاج بعد الإصابة بهذا الفيروس ومن الناحية المناعية.

ففي الدراسة الخاصة بالتغيرات الباثولوجية أدى استخدام العترة(V1) إلى حدوث التهاب واضح وضمور في كيس فابريشيا بعد مرور ٧ أيام من العدوي وهذا يوضح أن هذه العترة مختلفة عن العترةDelaware/E .

وفى الدراسة الخاصة بالناحية المناعية تم استخدام ٤ لقاحات حية ضد مرض الجمبورو (اثنين متوسطى الضراوة واثنين شديدى الضراوة) وهذة التحصينات لم تمنع حدوث ضمور في كيس فابريشيا بعد إجراء التحدى بالعترة (V1) فى دجاج التسمين عند عمر ٤ أسابيع.

هذة النتائج تشير إلى احتمال دور التحور الذى يحدث لفيروس الجمبورو تحت ظروف الحقل وربما كان سببا لإحداث نقص المناعة وضمور كيس فابريشيا فى قطعان دجاج التسمين التجارى.