

DETECTION OF VARIANT STRAINS OF INFECTIOUS BURSAL DISEASE VIRUS IN BROILER FLOCKS IN SAUDI ARABIA USING ANTIGEN CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY

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ABSTRACT

Infectious bursal disease conditions were observed in 15 commercial and 9 backyard broiler flocks in central area of Saudi Arabia during 2007-2008. The age of birds ranged from 2 to 8 weeks. The size of commercial flocks ranged from 5000 to 15000 birds and these flocks were vaccinated with classical strain of infectious bursal disease (IBD) vaccine at 14 days of age through drinking water. Number of birds in backyard flocks ranged from 200 to 300 and the vaccination programme of these birds was not known. High mortalities, respiratory symptoms, stunting and enlargement of bursa were seen in diseased birds of commercial flocks. Infectious bursal disease was suspected based on these clinical symptoms and postmortem findings, although these birds had been vaccinated against IBD virus. In order to confirm our diagnosis and to identify the causative agent, antigen capture-enzyme linked immunosorbent assay (AC-ELISA) was carried out on 142 bursal samples collected from diseased birds using kits containing monoclonal antibodies against variant strains of IBDV and 61.23% samples were found positive. It was observed that traditional vaccinal strains (54.02%) were significantly higher than less pathogenic strains not used in vaccine preparation (29.89%) and non traditional highly pathogenic strains of IBDV (16.09%). It was concluded that new variant strains of IBDV were detected in the samples in Saudi Arabia and to our knowledge this is the first report about the existence of these virus strains in commercial and backyard broiler flocks in this country.

Key words: IBD, IBDV, vvIBDV, antigen capture-ELISA.

INTRODUCTION

Infectious bursal disease (IBD) has been of great concern to the poultry industry for a long time, particularly in the past decade. Indeed, its re-emergence in variant or highly virulent forms has resulted in significant economic losses (Lukert and Saif, 2003). The etiological agent of IBD, infectious bursal disease virus (IBDV), is a non-enveloped virus, belonging to the family Birnaviridae, with a bisegmented dsRNA genome (Kibenge *et al.*, 1988). In fully susceptible chicken flocks (between 3 and 6 weeks of age), the disease is responsible for severe losses due to impaired growth and death, and from excessive condemnation of carcasses because of skeletal muscular haemorrhages (Lukert and Saif, 2003). Susceptible chickens less than three weeks of age do not exhibit clinical signs (Hitchner, 1971) but have a subclinical infection characterized by microscopic lesions in the Bursa of Fabricius (Kibenge *et al.*, 1988) and immunosuppression (Toro *et al.*, 2009). Two antigenically distinct serotypes designated as serotype 1 and serotype 2 have been recognized in the Europe (McFerran *et al.*, 1980) and USA (Jackwood *et al.*, 1982). Several antigenically variant strains of serotype 1 IBDV have been described in the USA (Ismail *et al.*, 1990; Jackwood and

Jockwood, 1994). These strains differ from classical serotype 1 strain in that they produce a very rapid bursal atrophy, but with minimal inflammatory response in 3-4 week-old specific-pathogen-free (SPF) chickens. The virus was identified by reacting with a known anti-IBDV serum using any number of antigen-antibody tests (Müller *et al.*, 2003; Juneja *et al.*, 2008). The antigen-capture-enzyme-linked immunosorbent assay (AC-ELISA) was used to differentiate IBDV strains (Snyder *et al.*, 1988; Wang *et al.*, 2008).

It has been reported that neutralizing monoclonal antibodies (MAbs) R63 and B69 can be used to differentiate IBDV strains into three groups. Field isolates of IBDV were primarily placed in group III, whereas the vaccine viruses tested were placed in groups I and II. The antigenicity of the viruses in these three groups did not correlate with cross-virus neutralization test (Rosenberger and Cloud 1986; Jackwood and Saif, 1987). In another study, Snyder *et al.* (1992) used a panel of two non-neutralizing and six neutralizing MAbs in AC-ELISA to examine the antigenicity of 1301 wild types of IBDVs isolated from different poultry flocks throughout the USA.

In Saudi Arabia and many other countries, the disease picture of IBD is still unclear and requires further investigations (Müller *et al.*, 2003). The present

study was conducted to investigate suspected infectious bursal disease virus infection among broilers in the central part of Saudi Arabia, using AC-ELISA on bursal samples collected from diseased broiler flocks.

MATERIALS AND METHODS

Sample collection and preparation

A total of 142 bursa samples were collected from 15 commercial and 9 backyard broiler flocks in central area of Saudi Arabia during 2007-2008. The age of birds ranged from 2 to 8 weeks. The size of commercial flocks ranged from 5000 to 15000 birds and these flocks were vaccinated with classical strain of IBD vaccine at 14 days of age through drinking water. Number of birds in backyard flocks ranged from 200 to 300 and the vaccination programme of these birds was not known. Eight freshly dead or severely-ill birds from commercial flocks and three birds from backyard flocks were examined for postmortem lesions. Bursal samples were collected, chilled as quickly as possible and stored in frozen state for further processing.

Each bursal sample was weighed and placed in 1.5 ml Eppendorf tube. Antigen dilution buffer (diluted 1/20 in deionized water) was added to the sample in a ratio of 1 ml buffer per gram of bursa. Enough sand was added to the bursa after being cut to small pieces and the sample was ground using a pestle into a semi-homogenous dense suspension. The tube was capped and the homogenate was frozen at -20°C. Before performing the assays, the homogenates were thawed, thoroughly mixed, and centrifuged at 1500 x g for 10 minutes. The thawed bursal supernatant of each sample was diluted 1:5 in antigen dilution buffer and used for virus detection and typing, using monoclonal antibodies (MAbs).

Elisa kit

The ProFLOK® IBD Ag capture test kit was used in the investigation. The kit was obtained from Synbiotics Europe (Lyon, France) and contained IBD screening plates pack (2 plates coated with monoclonal antibody "MAb" # 8), IBD typing plates pack (1-MAb R63 coated plate, 1 MAb B69 coated plate and 1 MAb #10 coated plate), laboratory sand, antigen dilution buffer (10X), ready to use dilution buffer, IBD positive antiserum, goat anti-chicken horseradish peroxidase (HRP) conjugate, plate wash solution (20X), ABTS substrate solution and stop solution (5X).

Screening and differentiating AC-ELISA

The method described by Wu *et al.* (2007) was followed for AC-ELISA. Briefly, required wells of the IBDV MAb-coated plates were charged with antigen. Then bursa samples were added to the wells of MAb-coated plates. Positive samples were confirmed with rest of the MAb-coated plates (R63, B69 and # 10

MAb-coated plates). The well strips were incubated overnight at 4°C, contents were discarded and washed 3 times with 1X wash buffer, followed by delivery of the IBDV positive serum and incubation of strips at room temperature. After 3 washings using the 1X wash buffer, HRP conjugate was added to each of the wells, followed by 30 minutes incubation at room temperature and 3 washings using the 1X wash buffer. Then ABTS peroxidase substrate was added, followed by 15 minutes incubation at room temperature and then diluted stop solution was added. The optical densities (OD) of the well strips were read at 405 nm in an ELISA reader (Flow Laboratories, England). Positive and negative control wells were considered, as described by the kit manufacturer.

The OD values obtained from the plate reader were interpreted according to the kit supplier as followings: OD values ≥ 0.6 suggested the presence of sufficient IBD viral antigen to cause bursal damage (+), OD values ≤ 0.3 suggested the absence of IBD viral antigen (-), while OD values >0.3 and <0.6 were invalid and process was repeated. Differentiation between variant strains of IBDV was based on the pattern of reaction against the panel of monoclonal antibodies. Data thus collected were analyzed by Chi square test using Minitab software.

RESULTS

Infectious bursal disease (IBD) in commercial and backyard poultry was suspected based on clinical symptoms and presence of pathological changes in the bursa of Fabricius at postmortem examination. Later, the IBD was confirmed using AC-ELISA. By using ELISA screening plates coated with MAbs, 61.23% (87 out of 142) bursal samples were found positive for IBD. Four different IBD typing plates (MAbs coated plates i.e., #8, B69, R63 and #10) were used for further differentiation of these positive bursal samples (Table 1). It was observed that Classic, GLS, E/Del and RS593 virus was detected by using MAbs #8. MAbs coated plates B69 were able to detect Classic virus type only. MAbs R63 coated plates were able to detect classic and E/Del virus types, whereas # 10 MAbs coated plates detected Classic and GLS virus types (Table 1). Variant strains of IBDV were detected in broiler and backyard poultry bursal samples collected from birds younger than 21 days, while Classic viruses were not detected until 4 weeks of age.

Data analysis revealed that significantly higher samples (54.02%) contained traditional vaccinal strains compared to less pathogenic strains not used in vaccine preparation (29.89%; $\chi^2 = 4.287$; $P = 0.038$) and non traditional and highly pathogenic strains of IBDV (16.09%; $\chi^2 = 13.484$; $P = 0.001$). However, non-significant difference was observed between less

pathogenic strains not used in vaccine preparation and non traditional highly pathogenic strains of IBDV (Table 2). The field isolates of IBDV were primarily placed in group III, whereas the vaccine viruses tested were placed in groups I and II.

Table 1: Screening and differentiation of infectious bursal disease virus variants in broiler bursal samples using monoclonal antibodies

Virus type	Monoclonal antibody and AC-ELISA reaction*			
	#8	B69	R63	#10
Classic	+	+	+	+
GLS	+	-	-	+
E/Del	+	-	+	-
RS593	+	-	-	-

*Monoclonal antibodies were supplied as ELISA-coated plate strips with the ProFLOK IBD Ag Capture test kit (Synbiotics)

Table 2: Identification and differentiation of infectious bursal disease viruses in bursal samples collected from diseased broiler farms (n=87) using AC-ELISA assay

Type of virus	Positive samples	
	Number	%
Traditional vaccinal viruses (same strains used in vaccine preparation)	47	54.02 ^a
Traditional non-vaccinal viruses (less pathogenic viruses not used in vaccine preparation)	26	29.89 ^b
Virulent viruses (non-traditional highly pathogenic viruses)	14	16.09 ^b

Values with different superscripts in a column differ significantly (P<0.05).

DISCUSSION

In this study, clinical investigation indicated that IBD was a major disease affecting broiler production in the investigated farms and localities in Saudi Arabia. Being the main target of the virus, bursa of Fabricius was selected as the tissue for antigen-capture ELISA (Müller *et al.*, 1979). Of the screened samples by AC-ELISA, 87(61.23%) were positive for the presence of IBD viral antigens. Though conventional ELISA adapted for IBDV serology is rapid, quantitative, sensitive and reproducible procedure (Ashraf *et al.*, 2006), yet it could not elucidate the real IBD viruses. The monoclonal antibody capture ELISA was developed by Lee and Lin (1992) to detect antibodies to IBDV in chicken sera and compared with conventional ELISA. It was found that the monoclonal ELISA assay

had lower non-specific reaction than conventional ELISA. In the present study, it was observed that AC-ELISA with MAbs was successful in differentiating the very virulent IBDV (vvIBDV) phenotype from less pathogenic types (Wu *et al.*, 2007).

In the present study, it was found that out of 87 ELISA-positive bursal samples, 54.02% represented traditional vaccinal viruses, 29.89% represented traditional non-vaccinal viruses, while 16.09% were identified as virulent and non traditional viruses (vvIBDVs) not previously identified in the region (Table 2). It was also noticed that variant strains of IBDV were detected in broiler bursal samples collected from birds younger than 21 days, while Classic viruses were not detected until 4 weeks of age. This agrees with the findings of Jackwood and Sommer (1999), who reported that new IBD viruses were detected in different places around the world using molecular technique or serology. This data also suggests that viruses continue to change and may circumvent the immune system of birds despite their vaccination against IBD (Müller *et al.*, 2003).

It has been reported that MAbs R63 and B69 can be used to differentiate the IBDV strains tested into three groups (Jackwood and Saif, 1987). In the present study, the field isolates of IBDV were primarily placed in group III, whereas the vaccine viruses tested were placed in groups I and II. The antigenicity of the viruses in these three groups did not correlate with cross-virus neutralization test (Jackwood and Saif, 1987). In another study (Snyder *et al.*, 1992), a panel of two non-neutralizing and six neutralizing MAbs were used in AC-ELISA to examine the antigenicity of 1301 wild types of IBDVs isolated from different poultry flocks throughout the USA. Examination of these isolates with protective, neutralizing MAbs directed against the VP2 structural protein of IBDV showed that four antigenically distinct groups of serotype 1 IBDV could be separated on the basis of the presence of one or more MAbs defined, conformation-dependent and multivalent neutralizing sites (Snyder *et al.*, 1992).

Conclusively, the AC-ELISA carried out in this study exhibited excellent specificity and sensitivity for the detection and differentiation of IBDV antigens in bursal samples, making it a powerful tool for epidemiological and vaccine efficacy studies. In conclusion, this study revealed that new variant strains of IBDV were detected in the samples that have been tested in Saudi Arabia and to our knowledge this is the first report about the existence of these virus strains in commercial and backyard broiler flocks in this country.

Acknowledgments

The author would like to thank the General Directorate of Research Grants Program, King Abdulaziz City for Science and Technology for funding the study through the project No. LGP-8-51. In

addition, the author thanks Mahmoud Hashad for laboratory assistance.

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