MOLECULAR DETECTION AND TYPING OF INFECTIOUS BURSAL DISEASE VIRUS IN BROILER FLOCKS

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SUMMARY

Infectious bursal disease virus (IBDV) was detected in bursal samples collected from broiler flocks using reverse transcriptase / polymerase chain reaction (RT/PCR). Results of detection of IBDV in bursal homogenates by RT/PCR indicated that 27 out of 38 (71.1 %) examined samples from 15 20-day-old chickens and 15 out of 24 (62.5 %) from 28 35-day-old chickens were positive for IBDV. IBDV-positive samples were examined for restriction fragment length polymorphisms (RFLPs).

The restriction enzymes BstNI and MboI were used to obtain RFLPs results. IBD viruses were placed into four molecular groups. The first and second groups contain classical IBDV. While third and fourth groups include the variant IBDV. 6 out of 27 (22.2 %) and 21 out of 27 (77.8 %) IBDV-positive samples, from 15 20-day-old chickens, were typed as classical and variant IBDV, respectively. On the other hand, 9 out of 15 (60%) and 6 out of 15 (40%)

IBDV-positive samples, from 28 35-day-old chickens, were typed as classical and variant IBDV, respectively. It could be concluded that RT/PCR-RFLPs represents a new method to differentiate IBDV. Variant IBD viruses are circulating in broiler flocks instead of vaccination with classical IBDV vaccines. Therefore, Variant IBDV strains should be included in IBDV vaccines.

INTRODUCTION

Infectious bursal disease virus (IBDV) is a member of the family Birnaviridae (Murphy et al.1999). IBDV becomes important in poultry industry worldwide because of the significant economic losses resulting from high mortality, impaired growth, carcass condemnation and immunosuppression (Phong et al. 20003 and Rojs et al, 2003).

IBDV is a double-stranded RNA virus that consists of two segments, A and B. The larger segment, segment A, about 3.2 kb in length. It encodes VP2, VP3, VP4 and VP5 (Azad et al 1985 and Hudson et al 1986). The smaller segment, segment B, about 2.8 kb in length. It encodes VP1, a multifunctional protein with polymerase activity (Cao et al. 1998). VP2 and VP3 are the major structural proteins of the virion, of which VP2 of IBDV displays the greatest amount of amino acid sequence variation between strains, particularly in the hypervariable region (Bayliss et al. 1990, Kibenge et al. 1990 and Vakharia et al. 1994).

Despite recent advance in vaccination programs, outbreaks of this disease still occur. Virus isolation, electron microscopy, immunofluorescence, immunodiffusion, virus neutralization and enzyme linked immunosorbent assay, all have been used for the diagnosis of IBD (Lukert and Saif, 1997 and Rosenberger and Cloud, 1986), however, they all suffer several disadvantages, such as being time consuming, labor-intensive, expensive, non-specific or insensitive. More importantly, these methods lack the ability to detect low levels of IBDV antigens in tissues (Wu et al. 1992). Thus, in order to control the disease, a specific and sensitive method for identifying IBDV infections is very important.

Antigenic variant was first reported in the United State (Rosenberger et al, 1985 and Rosenberger and Cloud, 1986). Variant strains of IBDV are usually isolated from IBDV-vaccinated flocks. These IBDV variants are antigenically different from classic strains of IBDV. They lack classical epitope(s) defined by neutralizing monoclonal antibodies (Snyder et al.1988a; Snyder, 1990 and Snyder et al. 1992). Most of these epitopes are located in the VP2 hypervariable region (Snyder et al. 1988b and Chen et al. 1998).

Although several reports have classified the Egyptian IBDV isolates as classical IBDV (Khafagy et al. 1991; Bekhit, 1996 and Khaliel and Ellkany, 2000), only some reports have provided some evidence of the presence of antigenic variant IBDV strains in Egyptian flocks (El-Sanousi et al, 1994; Sultan, 1995 and Hussein et al. 2003). IBDV variant strains can be directly detected in bursal homogenates by Antigen-capture ELISA (AC-ELISA) using a panel of monoclonal antibodies reactive to a selected group of VP2 epitopes (El-Sanousi et al, 1994 and Metwally et al. 2003). IBDV variant strains were detected by reverse transcriptase/ polymerase chain reaction- restriction endonuclease assay (RT/PCR-RE) (Giambrone et al. 1994; Jackwood and Jackwood, 1994 ; Jackwood and Nielsen, 1997 and Liu et al. 1994). Recently, RT/PCR-restriction fragment length polymorphisms (RT/PCR-RFLPs) were used for molecular typing of IBDV strains (Jackwood and Sommer, 1999; Banda et al. 2001; Jackwood and Smily, 2001 and Banda et al. 2003).

IBDV variant strains escape the immune response induced by vaccination with classical standard IBDV vaccines (Rosenberger et al. 1985). Therefore, characterization of antigenic diversity of IBDV is important to develop an efficient vaccination program to control IBDV.

The objective of this study was to throw light on molecular detection and typing of IBDV in bursal samples collected from broiler flocks with history of IBDV-associated problems using RT/PCR-RFLPs assay.

MATERIAL AND METHODS

1. Bursal samples:

A total of 62 bursal samples were collected from commercially reared broiler chicken flocks suspected of having IBDV infection. 38 bursal samples were collected from 15 20-day-old chickens and 24 samples from flocks 28 35-day-old chickens. Five bursal samples were collected from each outbreak and were represented one sample. The samples were collected from October 2002 to January 2004 from different IBDV outbreaks at El-Bohera and Kafr El-Shiekh governorates.

2. IBDV vaccines:

Bursa Blen and IBD Blen, produced by Phylaxia Sanofi,Vet. Bio.Co. LTD, were used as classical IBDV in RT/PCCR-RFLPs.

3. Reverse transcription / polymerase chain reaction (RT/PCR) (Jackwood et al. 1996):

3.1. Preparation of samples:

A. Lyophilized IBDV vaccines (Jackwood et al. 1996):

Each vaccine vial was reconstituted in 5 ml TNE buffer (10 mM Tris-HCL , PH8.0, 100 mM NaCl and 1 mM ethylenediaminetetraacetic acid).

B. Bursal samples (Jackwood et al. 1996):

After freezing and thawing for three times, the bursas were homogenized in TNE buffer. The homogenates were centrifuged at 14,000 rpm for 20 min. at 4 C. The supernatants were collected for RNA extraction.

3.2. Extraction of RNA (Jackwood et al. 1996):

RNA was extracted from both vaccine viruses and bursal samples.A volume of each sample was extracted with an equal volume of chloroform and the aqueous layer was collected after centrifugation at 14,000 rpm for 20 minuntes. Sodium dodocycle sulphate (Sigma chemical co. St Louis, MO) and proteinase K (Sigma) were added to a final concentration of 0.5 % and 1.0 mg/ml, respectively. Following incubation at 37 C for 1 hour, the samples were extracted with an equal volume of phenol (PH, 4.3) (Amhersham Bioscince Co.) and then chloroform: isoamyl alchol (24:1). The viral RNA was precipitated with 2.5 volumes cold ethanol, RNA was collected by centrifugation at 14,000 rpm for 20 min. at 4 c. The RNA pellet was suspended in 100 ul of 90 % dimethyl sulfoxide solution at 98 C for 5 min.

3.3. Reverse transcription (RT):

2-ul volume of viral RNA was denatured at 95 C for 5 min. and then used in the RT reaction. A complementary DNA (cDNA) was synthesized by incubating the reaction mixture and a random hexamer primer at 42 C for 1 hour and then at 95 C for 5 min. for inactivating reverse transcriptase.

3.4. Polymerase chain reaction (PCR):

PCR was conducted using 2.5 units Taq DNA polymerase in a reaction buffer containing 50 mM KCl, 10 mM Tris-HCl (pH, 9.0), 2.5 mM MgCl2 and 200 uM each of dATP, dCTP, dGTP and dTTP.. The sequence of the primer pair were as follows: a) upstream primer: 5'TCAGGATTTGGGATCAGC-3' and b) downstream primer: 5' TCACCGTCCTCAGCTTAC (Synthesized