COMPARISON OF FIDELITY OF NUCLEIC ACID PROBE WITH CONVENTIONAL ANTIGEN DETECTION ASSAYS IN DIAGNOSIS OF INFECTIOUS BURSAL DISEASE VIRUS

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ABSTRACT

The efficiency of PCR generated probe coupled with enhanced chemiluminescent hybridization assay was compared with conventional antigen detection assays to detect the IBD virus infection in chicks in terms of sensitivity and specificity. The hybridization assay was found to be superior to conventional antigen detection assays in terms of sensitivity and specificity.

INTRODUCTION

Conventionally, antigen detection by agar gel precipitation test, dot-ELISA, immunoperoxidase test (or) immunofluorescence assay were employed to detect the IBD virus. More recently, cDNA probes were used as diagnostic tool for IBDV infection, in both radioactive and non-radioactive hybridization assays (Jackwood et al., 1989; Henderson and Jackwood, 1990; Jackwood et al., 1992). In the present study, PCR product was used as a probe to detect IBDV RNA in infected bursal tissue by the enhanced chemiluminescent (ECL) hybridization assay. The objective of the study was to compare the efficiency of PCR generated probe in ECL hybridization assay with conventional antigen detection assays to detect the IBDV infection in chicks in terms of sensitivity and specificity.

MATERIAL AND METHODS

Conventional antigen detection assays: One hundred and twenty bursal samples were collected from different outbreak areas of Tamil Nadu, India. The samples were processed by the method of Hirai et al., (1972). The clear aqueous phase was harvested and tested for the presence of antigen by AGPT and dot-ELISA. The 2% triton x 100 treated bursal samples were tested by AGPT (Nachimuthu et al., 1993) using hyperimmune serum raised in chicken. An improved dot-ELISA was performed as per the method of Maiti et al. (1993). IBDV hyperimmune serum raised in chicken and anti-chicken IgG HRP conjugate were used. IBDV Indovax vaccine strain was used as a positive control and uninfected bursal suspension as a negative control.

RNA extraction: Total RNA was extracted from Infectious Bronchitis virus (Massachusetts strain 41), New Castle disease virus (LaSota strain) and uninfected bursal homogenate by the guanidium acid phenol method described by Chomczynski and Sacchi (1987). The final RNA pellet was dissolved in 100 ml of diethyl pyrocarbonate treated RNase free water. Lithium chloride precipitation of dsRNA was done to eliminate the contaminating DNA and single stranded RNA as reported by Davis and Boyle. (1990). The purity and concentration of IBDV dsRNA was checked by the method of Wu et al. (1992) using the spectrophotometer.

Preparation of probe and ECL hybridization assay: For probe preparation, 5 μl of 90% DMSO was added as a denaturing agent to 10 μl of (1 μg) dsRNA sample of Indovax vaccine strain. Then, the mixture was heated in a boiling water bath for 10 min for efficient denaturation. Then, the denaturated RNA sample was added to 20 μl cDNA reaction mixture prepared as per the
protocol given by first strand cDNA synthesis kit (Boehringer mannheim, Germany). The cDNA was synthesized using random primers.

The resulting single stranded cDNA (10 μl) of Indovax vaccine strain was amplified by PCR utilizing the VP₂ region specific primers. The primer sequence and thermal cycle protocol given by Stram et al. (1994) were used in PCR assay.

The amplified product was detected on 1.5% agarose gel electrophoresis. The PCR product was eluted from agarose gel and purified using Qiagen gel extraction kit. 20 μl of purified PCR product denatured by boiling at 100°C for 5 min and immediately chilled on ice and used as a probe. The ECL hybridization assay was done by the method of Akin et al. (1993) using ECL labelling and detection kit (Amersham, UK).

RESULTS AND DISCUSSION

In the present study, out of one hundred and twenty samples tested, diagram 1 clearly indicated that sensitivity was high in case of hybridization in comparison with antigen detection assays like AGPT and dot-ELISA. The bursal homogenates treated with 2% triton x 100 so as to increase the sensitivity of AGPT as reported by Nachimuthu et al. (1993). Still, the sensitivity of AGPT was found to be less than that of dot-ELISA and hybridization assay in this study. The lower sensitivity of AGPT may be due to precipitins were sometimes undetectable (Marquardt et al., 1980). In dot-ELISA, 0.25% glutaraldehyde

Diagram 1. Percentage of positive samples demonstrated by AGPT, dot-ELISA and hybridization assay
Fig 1. 1.5 per cent agarose gel electrophoresis to demonstrate the amplified PCR product of VP1 gene region

Lane 1 and 2: PCR product of Indovax vaccine strain
Lane 3: pBR322 DNA Hinfl digest

Fig 2. ECL hybridization to detect IBD virus RNA extracted from suspected bursal samples

1-13: IBD virus RNA from field samples
14 and 15: Negative controls
was used a fixative to fix the antigen and antigen-antibody complex and 2% gelatin was used as a blocking agent has increased the sensitivity of the reaction as stated by Parthiban et al. (2000). Still, the sensitivity of dot-ELISA was lower than that of hybridization assay. It may be due to PCR generated probe not only detect viral genomic RNA, but also viral mRNA which was synthesized at very early stage of infection.

The part of the VP₂ gene region was amplified using IBDV specific primers yielded 365 bp amplicon size which was used as a probe (Fig. 1). cDNA probes prepared directly from viral dsRNA genome (Jackwood et al., 1982) was difficult and time consuming to obtain sufficient quantities of purified genomic IBDV RNA for the production of cDNA probes. To overcome this problem, PCR generated probe derived from part of VP₂ gene region was successfully used to detect the IBDV genome. The probe appeared to be highly specific for IBDV RNA, because they did not hybridize with cellular and other poultry virus nucleic acids (Fig. 2) whereas non-specific reactions are common in case of AGPT and dot-ELISA. Lithium chloride precipitation of dsRNA was to obtain high degree of purity of RNA in order to avoid non-specific binding during hybridization assay.

The utility of nucleic acid probes for routine diagnosis of pathogens has been limited by the use of radiolabels for their detection. However, this limitation has been overcome by the development of peroxidase labelled PCR generated probe followed by ECL labelling and detection system. The molecular diagnostic assay was superior than conventional antigen detection assays in terms of sensitivity and specificity. Rapid and accurate diagnosis of IBDV infection by the molecular detection assay was useful in controlling the disease effectively.

REFERENCES