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ISOLATION AND CHARACTERIZATION OF VIRULENT STRAIN OF INFECTIOUS BURSAL DISEASE VIRUS FROM BROILER BIRDS IN BANGLADESH

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ABSTRACT

The bursa Fabricius of 50 dead broiler birds aged between 2 to 5 weeks were collected from three different private and Bangladesh Agricultural University (BAU) poultry farms for isolation of infectious bursal disease (IBD) virus during the period from August to September 2002. Each of the collected bursa was stored at -20^{0}C until processed for the isolation of virus in chicken embryo fibroblast (CEF) cell culture. Of the 50 bursal samples cultured in the chicken embryo fibroblast (CEF) cells, of which 40 (80%) bursal samples were found positive for IBD virus. Each of the 40 isolated IBD virus was characterized as IBD virus M6 strain using type specific polyclonal serum raised in chickens by agar gel immunodiffusion test (AGIDT). These isolated IBD virus M6 strain caused 100% mortality in five weeks old layer chickens on experimental infection through intramuscular route of inoculation. The results of this study indicate that the local isolates of IBD virus are associated with high mortality in chickens under both the natural and experimental conditions in Bangladesh.

Key words : Isolation, characterization, virulent strain, IBDV, broiler birds

INTRODUCTION

The infectious bursal disease (IBD) is popularly known as Gumboro disease because it was first reported to be occurring on farms near Gumboro Delaware, USA (Cosgrove, 1962). It is an acute, contagious viral disease of primarily in young chickens caused by a virus belonging to the family *Birnaviridae* (Calnek *et al.*, 1997). This disease has been reported in all of the poultry producing countries of the world including Bangladesh. Although the outbreaks of IBD have been encountered in 1992 for the first time in Bangladesh but the causal agents of this disease was first isolated and identified later on by Chowdhury *et al.* (1996) and Rahman *et al.* (1996). There are two serotypes of IBD virus exist in nature (McFerran, *et al.*, 1980), with only serotype 1 is pathogenic for chickens, and within serotype 1, there are six unrelated or partially related strains have been classified (Brown and Grieve, 1992). Although the multiple subtypes or pathotypes of serotypes have been identified but the standard or classical serotype 1 is mainly associated with clinical disease throughout the world (Brown and Grieve, 1992). Recently, Islam *et al.* (2001) demonstrated the molecular and antigenic similarities of Bangladeshi isolates of IBD virus with European, Asia and African virulent strains. This paper further describes the isolation and characterization of local isolates of IBD virus.

MATERIALS AND METHODS

The bursa Fabricius of 50 dead broiler chickens aged between 2 to 5 weeks were collected aseptically from three private and BAU poultry farms, Mymensingh during the period from August to September 2002. All the collected 50 bursa were stored at -20^{0}C until processed for the isolation of virus using chicken embryo fibroblast (CEF) cell culture.

Each bursal sample was cut into small pieces and triturated by pestle and mortar. PBS was added to the tissue homogenate as to make 10% suspension of bursal tissue. The suspension was then centrifuged at 3000 rpm for 15 minutes. The supernatant were collected and treated with antibiotics (Penicillin @ 10,000 IU / ml and Streptomycin @ 10 mg / ml). After adding antibiotics, the suspension was kept at room temperature for 45 minutes and shaked gently for every 10 minutes. The suspension was then inoculated into sterile blood agar media for bacteriological sterility. The inoculated blood agar media was incubated at 37^{0}C for 24 hours. Bacteriologically sterile suspension was used as an inoculum for the isolation of virus.

Primary chicken embryo fibroblast (CEF) cell culture was prepared using 9 to 10 days old chicken embryos by warm trypsinization method (Freshney, 2000; Schat and Purchage, 1989). Confluent monolayer of CEF grown in 24 wells of tissue culture plates and were used for the isolation of virus. Within 24 hours after seeding when the cells were fully confluent, the growth medium was removed from the wells with a pipette and 0.05 ml of each of the field sample was inoculated on the cell sheet in the well. Two to three wells were used for each sample and at least two wells in each plate were left as uninfected controls. The plates were incubated at 37^{0}C in a humidified incubator for one hour to allow the virus to adsorb. After that, one ml of maintenance medium was added to each well and the plates were taken back to the incubator. The cells were examined daily under an inverted microscope for the appearance of

any cytopathic effect (CPE). On day 5 of post-infection the cells in the plates were frozen at -20^{0}C irrespective of the appearance of CPE. Two to more blind passages were conducted before the samples were considered to be negative for virus isolation in cell culture. For this purpose, the cells were disrupted by vigorous pipetting with sterile Pasteur pipettes. Then the tissue culture fluids from two wells, inoculated with the samples, were pooled, and then reinoculated to fresh cell monolayers as before. For the preparation of working stock, the third tissue culture passaged laboratory stock virus IBDV M6 was inoculated on CEF cell monolayer in 75 sq.cm. tissue culture flasks. When maximum CPE manifested, the tissue culture fluid was harvested after three cycles of freezing and thawing, and vigorous pipetting. The harvested tissue culture fluid was centrifuged for 20 minutes at 3000 rpm and the supernatant fluid was collected. The isolated virus from the 40 positive field samples of the present study was identified as IBDV using type specific polyclonal sera against IBDV by AGIDT. This virus preparation was divided into 1 ml aliquots in vials and stored in frozen condition at -20^{0}C.

Determination of chick infective dose fifty (CID_{50})

Fifty day-old layer chicks were raised in an isolated room. At 32 days, the chicks were divided into 10 groups, each group consisting 5 chicks and housed in separate cages. Serial 10 fold dilutions of the stock virus IBDV M6 were made in PBS. Nine groups of chicks were infected orally with 9 serial dilutions (10^{-1} to 10^{-9}) of the virus suspensions, and the rest one group used as uninfected control. Each bird received 0.3 ml of the inoculum through IM route. The chicks were observed closely for clinical signs and mortality, if any. Detailed postmortem examination was conducted on some of the dead birds.

The chick infective dose fifty (CID_{50}) was determined as described by Reed and Muench (1938).

RESULTS AND DISCUSSION

The infectious bursal disease (IBD) virus was isolated from 40 (80%) out of 50 infected bursal samples in chicken embryo fibroblast (CEF) cell culture. IBDV virus positive samples in CEF cell culture was found negative for the isolation of virus in embryonated hen eggs. The result of isolation of IBDV of the present study using CEF cell culture and avian embryo slightly differ with the IBDV isolation result of Lucio *et al.* (1971) and Jayaramaiah and Mallick (1974). In their study they were able to isolate IBDV from field samples easily by using chicken embryos. The failure of growth and propagation of the field IBDV in the chicken embryos may be due to presence of maternally derived antibody (MDA) in the embryonated hen eggs used in this study. The result of successful isolation of the IBDV from the field samples using CEF cell culture in the present study closely agree with the isolation result of IBDV of EL-Ebiary *et al.* (1997) and Kumar *et al.* (2000). In their study they found CEF cell culture system better for the isolation of IBDV from the field samples compare to other indicator host system. The isolated virus from the 40 positive field samples of the present study was identified as IBDV using type specific polyclonal sera against IBDV raised in rabbit by AGIDT. The result of identification of the field virus of the present study agrees with the identification result of IBDV of El-Zein *et al.* (1974). In their study they were able to identify field IBDV by AGID test within 48 to 72 hours of incubation using type specific polyclonal sera against IBDV. The isolates of IBD virus strain M6 were tested for chicken infective dose fifty by inoculating nine serial dilutions (10^{-1} to 10^{-9}) of the virus in chicks and $10^4 CID_{50}$/ dose was found as chick infective dose fifty of the isolate.

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