ISOLATION AND CHARACTERIZATION OF VIOLENT SYRINX OF INFECTIONOUS BURSA DISEASE VIRUS FROM BROILER BIRDS IN BANGLADESH

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ABSTRACT
The bursa Fabrics 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 birds was stored at -20 °C until processed for the isolation of virus in chicken embryo fibroblasts (CEF) cell culture. Of the 50 bursal tissues cultured in the chicken embryo fibroblasts (CEF) cells, 48 showed 80% to 100% tissue culture infectivity test (TCID50) and were positive for IBD virus. Each of the isolated IBD virus was characterized as IBD virus NR strain using type specific polyclonal antibody raised in chicken by Chai et al. (1982) and ADI test. These isolated IBD virus strains caused 100% mortality in 5 weeks old layer chickens on experimental infection through intramuscular route of inoculation. The results of this study indicate that the 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 strains, 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 (Cawgro, 1962). It is an acute, contagious viral disease of primarily young chickens caused by a virus belonging to the family Birnaviridae (Cathcart et al., 1997). This disease has been reported in all of the poultry producing countries of the world including Bangladesh. Although the outbreak of IBD have been encountered in 1992 for the first time in Bangladesh but the casual agents of this disease was first isolated and identified base on by Chowdhury et al. (1996) and Rahman et al. (1998). There are two serotypes of IBD virus exist in nature (McFerran et al., 1980), with only serotype 1 is pathogenic fow chickens, and within serotype 1, there are six unrelated to partially related strains have been classified as B and G (Browns and Green, 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 (Browns and Green, 1992). Recently, Islam et al. (2000) demonstrated the molecular and antigenic similarities of Bangladesh isolates of IBD virus with European, Asian and African virulent strains. This paper further describes the isolation and characterization of local isolates of IBD virus.

MATERIALS AND METHODS
The bursa Fabrics 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 °C until processed for the isolation of virus using chicken embryo fibroblasts (CEF) cell culture.
Each bursal sample was cut into small pieces and triturated by pestle and mortar. PBS was added to the tissue homogenate 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 shaken 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°C for 24 hours. Bacteriologically sterile inoculation was used as an inoculum for the isolation of virus.
Primary chicken embryo fibroblasts (CEF) cell culture was prepared using 6 to 10 days old chick embryo by warm trypsinization method (Freethy, 2000; Chat and Purchage, 1989). Confluent monolayer of CEF grown in 24 wells 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 cells with a pipette and 0.05 ml of each of the crude 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 unaffected controls. The plates were incubated at 37°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

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any cytotoxic effect (CPE). On day 5 of post-infection the cells in all plates were frozen at -20°C (except of the appearance of CPE). Two to more blind passages were conducted before the samples were considered to be negative for IPN 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 supernates, were pooled, and then reinoculated to fresh cell monolayers as before. For the preparation of working stock, the third tissue culture passaged 3-4 days stock virus (IBDV) Md was inoculated in CEF cell monolayer in 75 cm 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 at 20,000 rpm 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 AGID. This virus separation was divided into 1 ml aliquots in vials and stored in frozen condition at -20°C.

Determination of chick infective dose fifty (CID50)

Fifty-day-old layer chicks were raised in an isolated room. At 32 days, the chicks were divided into 10 groups, each group consisting of 5 chicks and housed in separate cages. Serial 10-fold dilutions of the stock virus (IBDV. Md) were made in PBS. Nine groups of chicks were infected orally with serial dilutions (10 to 105) of the virus suspensions, and the one group used as an unexposed control. Each bird received 0.5 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 (CID50) was determined as described by Reed and Muench (1938).

RESULTS AND DISCUSSION

The infectious bursal disease (IBDV) virus was isolated from 40 (60%) out of 67 isolated bursa samples in chicken embryo fibroblast (CEF) cell culture. IBDV virus positive samples in CEF cell culture was found negative for the isolation of viruses 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 Lecocq et al. (1971) and Frey and Maltais (1972). 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 genetically determined 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-Birgawi et al. (1971) and Kumal 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 AGID. The result of identification of the field virus of the present study agree with the identification result of IBDV of El-Birgawi et al. (1974), in their study they were able to identify field IBDV by AGID test within 48 to 72 hours of inoculation using type specific polyclonal sera against IBDV. The isolates of IBDV virus strain Md were tested for chicken infective dose fifty by inoculating four new dilutions (10 to 105) of the virus in chicks and 10 CID50 dose was found as chick infective dose fifty of the isolate.

REFERENCES


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