Effect of Infectious Bursal Disease Virus on in vitro Propagation of Chicken Embryo Fibroblast Cells

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Abstract: The present research was undertaken to study the propagation and observation of cytopathic effect of IBDV in chicken embryo fibroblast cells. Chicken Embryo Fibroblast (CEF) is chicken embryo derived primary cell culture. Infectious bursal disease is an acute, highly contagious viral disease of young chickens caused by a double stranded RNA virus named Infectious Bursal Disease Virus (IBDV). For this purpose, suspected IBDV isolates were collected from the bursas of dead chicken of a particular flock. Local field IBDV isolates were then inoculated in chicken embryo for slight adaptation by several passages. CEF cell was prepared from 9-10 days chicken embryo and transferred in monolayer culture flasks containing maintenance media with 1-2% heat-inactivated fetal calf serum. Adapted IBDV was then inoculated in CEF cell culture for the purpose of propagation. After inoculation characteristics clear and consistent Cytopathic Effects (CPEs) were observed on CEF cell monolayer and after 72 h of infection, the cells were started to change its shape.

Keywords: Adaptation, propagation, cytopathic effect, IBDV

Introduction

Protein is the most important constituent of cells and its deficiency leads to mental as well as physical abnormalities in human especially among children. Animal protein is the only complete protein, which is required for brain development. Bangladesh is one of the many developing countries facing acute shortage of animal protein. Poultry meat and eggs are two major sources of animal protein.

In the recent years poultry raising has become a growing and prospective industry in Bangladesh. Despite the special emphasis of the Government of Bangladesh on this sector, the development of poultry industry is seriously threatened by the outbreaks of acute, contagious and fatal disease. Although some diseases like Newcastle disease, Mark’s disease, fowl cholera, coccidiosis etc. have been kept under control in most of the commercial poultry farms by vaccination and medication, new emerging diseases like Infectious Bursal Disease (IBD) has virtually brought the progress of poultry industry of Bangladesh to a halt.

Infectious bursal disease, popularly known as Gumboro disease, is a contagious viral disease of young chickens caused by a double stranded RNA virus belonging to the family Birnaviridae. Since the description of the disease by cosgrove, (1962), Gumboro disease has drawn the attention of avian virologists not only because of the high mortality from the disease proper but also due to the profound immunosuppression induced by the virus resulting in subsequent secondary infections and vaccination failure (Allan et al., 1772; Hiran and Shirmaluna, 1774). In the recent years emergence
of a very virulent pathotype of IBD virus with a potential of causing up to 100% mortality has stimulated the resurgence of interests in IBD among the avian virologists. The disease has been occurring in Bangladesh since March 1992 with very high morbidity and mortality (Islam et al., 1994a; Islam et al., 1994b; Rahman, 1994). Viruses are obligatory dependent on host systems. Without the host system, it cannot replicate, propagate or multiply. Host may be bacteria, lower animal or plant, higher animal or plant, insects or even animal cells. Among the host, animal cells are excellent host for viruses and being used for propagation or cultivation for virus.

IBDV can infect and grow on various primary cell culture of avian origin and certain cell line of mammalian origin. Commonly used cell lines to replicate IBDV are Chicken Embryo Fibroblast (CEF) (Sofe et al., 1996). Chicken Embryonic Kidney (CEK), Vero (Puellin et al., 1999), Baby Hamster Kidney (BHK) (El-Enany et al., 1999), chicken embryo bursa (Lukart and Davis, 1774), normal chicken lymphocytes, B-cell lymphoblastoid, baby grivet monkey kidney (BGM-70) and M4-104 cells (Jackwood et al., 1997) etc. In addition to the above cell line, IBDV can also infect chicken embryo (Sofe et al., 1996).

The present research was undertaken to study the propagation and observation of cytopathic effect of IBDV in chicken embryo fibroblast cells. Considering the economic importance and severity of infectious bursal disease in chickens of Bangladesh. The present study was designed to isolate, propagate and to confirm of the local field strains of IBDV in primary cell culture and to study the cytopathology of the local isolate in chicken embryo fibroblast cell culture.

Materials and Methods

The work was carried on Animal Biotechnology Laboratory of Bangladesh Livestock Research Institute (BLRI), Savar, Bangladesh at early of 2004.

Preparation of Chicken Embryo Fibroblast (CEF) Cells

Eight to ten-day incubated eggs are chosen for the preparation of chick embryo fibroblasts. The eggs are candled and the air space marked. After disinfection with alcohol 70% the shell over the air space is removed. The contents of the egg are poured into a petri dish and the embryo is taken out of the amnion sac. The chick embryos are decapitated and the liver and intestines are removed. Then chopping the rest part (excluding liver, intestines and fat) properly by scissors. Then put the carcasses into the syringe reservoir, and insert the plunger by pointing the syringe upwards. The carcasses are ground by pressing them through the syringe opening. The tissue pulp is washed gently before trypsinization. As the tissue is easily disintegrated, only a few cycles of trypsinization are required. Then 1-2 mL of trypsinized tissue sample solution was inoculated into a 25 cm² culture flask containing 10 mL of growth medium. Culture flask was placed into a CO₂ incubator for 20-25 min. After 20-25 min, culture flask was replaced by 10 mL of maintenance media. The Infectious bursal disease viruses were isolated from IBDV suspected dead chickens to propagate them on CEF cells.

Preparation of inoculum with the IBDV Suspected Bursal Samples

Each bursal sample was cut into small pieces and triturated by a pestle and mortar. PBS was added to the tissue homogenate as to make a 10% weight/volume (w/v) suspension of bursal tissue. The suspension was then centrifuged at 3000 rpm for 15 min. The supernatant of the centrifuged tube was collected from the suspension. Penicillin and Streptomycin in the dose rate of 10,000 IU mL⁻¹ and 10,000 µL mL⁻¹ were added to the collected supernatant respectively. After
adding antibiotics the suspension was kept at room temperature for 45 min and shaken gently for every 10 min. The suspension was then inoculated into sterile blood agar media for bacteriological sterility of the antibiotic treated suspension. The inoculated blood agar media was incubated at 37°C for 24 h. Bacteriologically sterile suspension was used as an inoculum for the isolation of virus from the bursal suspension.

**Inoculation of Virus in Cell Culture**

Confluent monolayer of CEF grown in 25 cm² culture flasks shown in the photograph 5. Within 48 h after seeding when the cells were fully confluent, the growth medium was removed from the culture flasks with a pipette and 0.3 to 0.5 mL field virus (adapted in chicken embryo) isolates were then inoculated in each culture flask for the purpose of propagation of field IBDV isolates. The flasks 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 flask and the flasks were taken back to the incubator. The cells were daily under an inverted microscope for the appearance of any Cytotoxic Effect (CPE). On day 5 infection (p.i) the cells in the flasks were frozen at -20°C irrespective of the appearance of CPE.

**Results and Discussion**

**Formation of Confluent Monolayer of Chicken Embryo Fibroblast (CEF) Cells**

Culture flask containing tissue culture sample were placed in a CO₂ incubator. During first day observation under inverted microscope, cells were shown to grow to form monolayer culture. Confluent monolayer of Chicken Embryo Fibroblast (CEF) cells (Fig. 1) was found during second day observation under inverted microscope.

**Propagation of IBDV on CEF Cell Culture**

Isolated slightly adapted IBDV is inoculated in confluent monolayer of chicken embryo fibroblast cells for the purpose to propagate. After 144 h of inoculation Cytopathic Effects (CPEs) were observed. Consistent and clear CPEs indicate optimum propagation of IBDV (Fig. 2).

The infectious bursal disease viruses were isolated from IBDV suspected dead chickens to propagate on Chicken Embryo Fibroblast (CEF) cells. Suspected IBDV was inoculated through injection into live birds of the experimental flock of Bangladesh Livestock Research Institute. Live birds became dead after 13-14 days following infection. The results indicated the confirm identity of IBDV, collected from suspected field samples of bursa of dead chickens of 33-37 days age group.

To propagate IBDV on Chicken Embryo Fibroblast (CEF) cells, suspected IBDV were inoculated in chicken embryo for the purpose of adaptation. Field isolates IBDV can never propagate directly after inoculation in primary chicken embryo fibroblast cell culture. Therefore, suspected field isolates IBDV need to be adapted in chicken embryo. For this purpose, field isolates IBDV were inoculated in chicken embryo by several passages. After several passages (7 passages) IBDV was slightly adapted in chicken embryo. These virus samples were used for the propagation in Chicken Embryo Fibroblast (CEF) cells.

Cytopathic effects involved rounding, aggregation of CEF cells monolayer due to infection by virus. The CEF cell monolayer was examined under inverted microscope twice a day for observing CPEs. Following 48 h of infection no CPEs was found, the cells were like as confluent monolayer (Fig. 1). After 72 h of infection, the cells were just started to change its shape. At
this stage, few rounding were observed. The cells gradually started to change its shape in order to produce CPEs following 96 to 120 h of infection. CPEs were characterized by formation of rounding cells. Aggregation of rounding cells was formed during 120 to 144 h of incubation following infection. Clear and optimum CPEs were formed after 144 h of incubation following infection (Fig. 2). Formation of clear and optimum CPEs on CEF cell monolayer shows optimum propagation of infectious bursal disease virus.

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References


