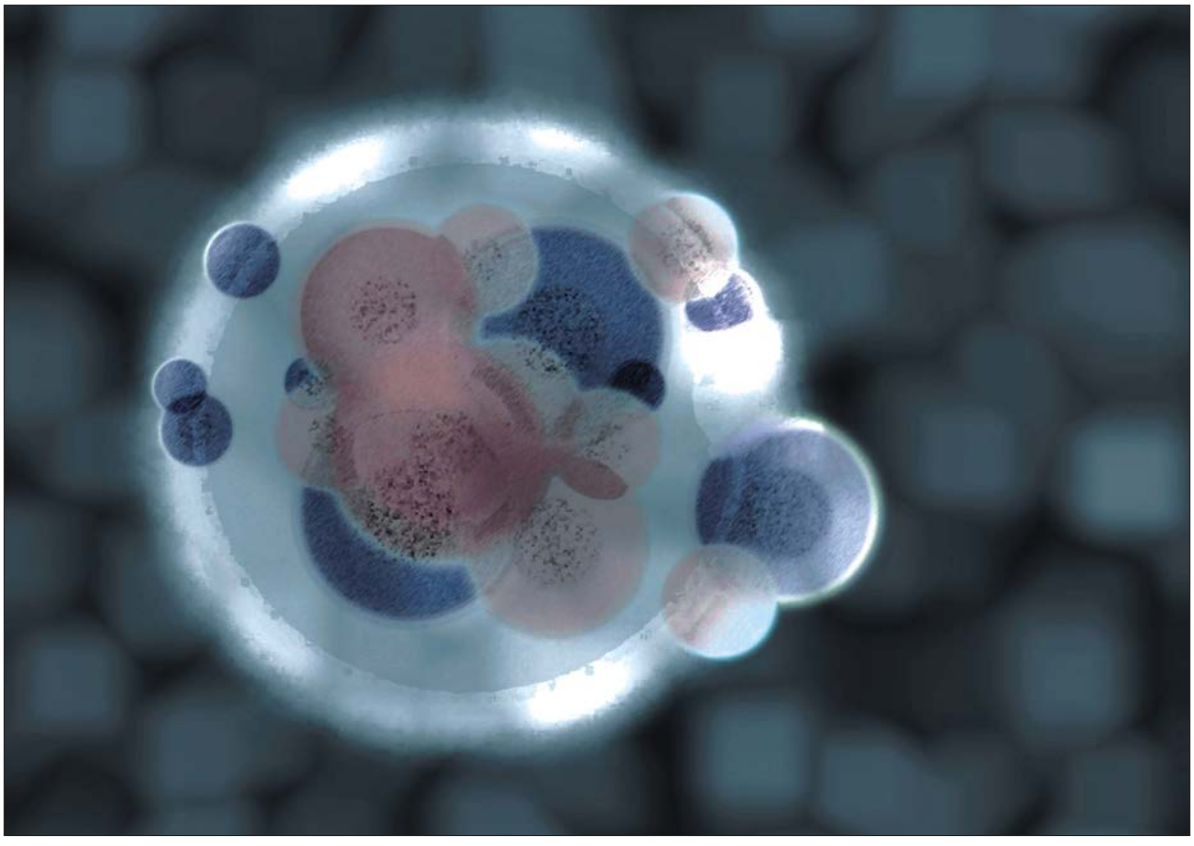

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Propagation of Infectious Bursal Disease Virus (IBDV) in Chicken Embryo Fibroblast Cells

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Abstract: 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). Chicken Embryo Fibroblast (CEF) is chicken embryo derived primary cell culture. 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. For this purpose, suspected IBDV isolates were collected from the bursas of dead chicken of a particular flock. Local field IBDV isolates was then inoculated in chicken embryo for slight adaptation by several passages. CEF cells 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. Characteristics clear and consistent Cytopathic Effects (CPEs) were observed on CEF cell culture after 144 h of incubation following infection of IBDV.

Key words: Cytopathic effect, adaptation of virus, propagation of virus, infectious bursal disease virus

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 such 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 rising 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 Brusal 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 is subsequent secondary infections and vaccination failure[1,2]. In the recent years emergence of a very virulent pathotype of IBD virus with a potential of causing upto 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[3-5].

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)[6]. Chicken Embryo Kidney (CEK), Vero[7], Baby Hamster Kidney (BHK)[8], chicken embryo bursa[9], normal chicken lymphocytes,

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B-cell lymphoblastoid, baby grivet monkey kidney (BGM-70) and M4-104 cells[10] etc. In addition to the above cell line, IBDV can also infect chicken embryo.

Some of nine avain families tested (Birnaviridae, Coronaviridae, Herpesviridae, Paramyxoviridae, Poxviridae, Reoviridae and Retroviridae) were found to replicate in a quail fibroblast cell line, designated QT35, resulting in a Cytopathic Effects (CPEs) visible with the naked eye or by low-power microscopy. In comparison, only one (Paramyxoviridae) of seven mammalian virus families tested produced observable CPEs. Cytopathic changes induced by examined viruses were round cell, syncytial and focus formation[11].

The susceptibility of the five cell lines-IB-RS-2, RK-13, Vero, BHK-21, CER and CEF cell cultures to reovirus S1133 and infectious bursal disease virus was studied to better define satisfactory and sensitive cell culture systems. Cultures were compared for presence of cytopathic effects (CPEs)[12].

Considering the economic importance and severity of infectious bursal disease in chickens of Bangladesh, the present study was designed with the objectives; to isolate, propagate and to confirm of the local field strains of IBDV in primary cell culture to study the cytopathology of the local isolate in chicken embryo fibroblast cell culture.

MATERIALS AND METHODS

The experiment was conducted from October 2004 to January 2005 at Poultry Diagnostic Laboratory, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1341, Bangladesh.

Methods

Preparation of Chicken Embryo Fibroblast (CEF) Cells: Chicken embryo fibroblast (CEF) cultures were prepared from 9 to 10 day old embryos of Specific-pathogen Free (SPF) chicken eggs by the following standard procedures[12].

Chick embryo fibroblasts (1): Nine to ten-day incubated eggs are chosen for the preparation of chick embryo fibroblasts. The eggs are candled and the air space marked.

Chick embryo fibroblasts (2): After disinfection with alcohol 70% the shell over the air space is removed.

Chick embryo fibroblasts (3): The contents of the egg are poured into a petridish and the embryo is taken out of the amnion sac.

Chick embryo fibroblasts (4): The chick embryos are decapitated and the liver and intestines are removed.

Chick embryo fibroblasts (5): Chopping the rest part (excluding liver, intestines and fat) properly by scissors.

Chick embryo fibroblasts (6): Put the carcases into the syringe reservoir and insert the plunger by pointing the syringe upwards. The carcases are ground by pressing them through the syringe opening.

Chick embryo fibroblasts (7): The tissue pulp is washed gently before trypsinization. As the tissue is easily disintegrated, only a few cycles of trypsinization are required.

Chick embryo fibroblasts (8): One to two milliliter of trypsinized tissue sample solution was inoculated into a 25 cm^2 culture flax containing 10 mL of growth medium. Culture flax was placed into a CO_2 incubator for 20-25 min. After 20-25 min, culture flax was replaced by 10 mL of maintenance media.

Collection of suspected IBDV: 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 at 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 shacked 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[13].

Inoculation of virus in cell culture: Confluent monolayer of CEF grown in 25 cm^3 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[13]. The flasks were incubated at 37°C in a humidified incubator for 1 h to allow the virus to adsorb. After that, 1 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 Cytopathic 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

Formation of confluent monolayer of chicken embryo fibroblast (CEF) cells: Culture flax containing tissue culture sample were placed in a CO_2 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).

DISCUSSION

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.

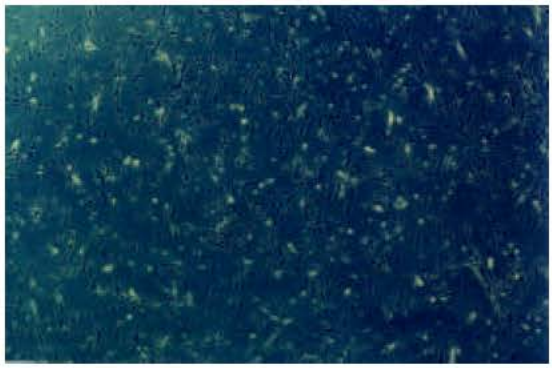

Fig. 1: Confluent monolayer of CEF cells

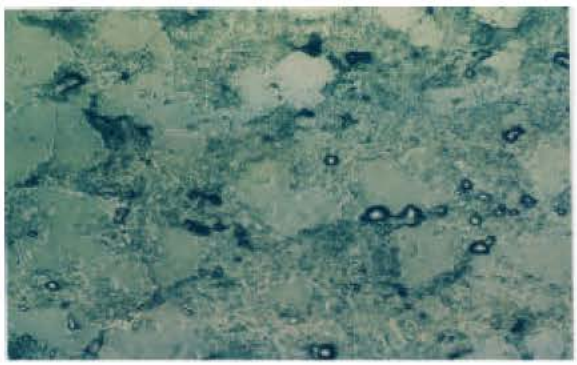

Fig. 2: Cytopathic Effect (CPEs) on CEF cell culture

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 (IBDV) (Fig. 2).

CPEs of an attenuated egg-adapted strain of IBDV in Chicken Embryo Fibroblast (CEF), vero cells and baby hamster kidney (BHK) cells was observed by Simony *et al.*[12] following 36 to 40 h of incection during 3rd blind passage. Ahasan[13] observed clear rapid and consistent CPEs of IBDV on vero cell line following 144 h of infection during 3rd passage. The present experiment showed that complete CPEs of slightly adapted IBDV was

observed in CEF cells following 120 to 144 h of incubation. This observation was found consistent with the findings of Ahasan[13] and Isbela *et al.*[12].

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