

Adaptation of Infectious Bursal Disease Virus (IBDV) on Vero Cell Line

M.M. Ahasan, K.M. Hossain and ¹M.R. Islam

Biotechnology Discipline, Khulna University, Khulna-9208, Bangladesh,

¹Animal Health Research Division, Bangladesh Livestock Research Institute,
Savar, Dhaka-1341, Bangladesh

Abstract: This experiment was conducted to study the adaptation of IBDV on vero cell line. Suspected IBDV isolates were collected from the bursa of dead chicken of a particular flock. For adaptation, the anchorage-dependant monolayer of vero cell was first subcultured to form semi-confluent monolayer in eagle's minimum essential medium (EMEM) with 5% fetal calf serum. This semi-confluent monolayer was then infected by local field IBDV isolates. The passage 1 (P1) viruses were harvested and used for the next passage. In this way, the viruses were given three serial passages on vero cell line where characteristic cytopathic effects (CPEs) were observed. During the first passage, no CPEs were found. The infectivity of IBDV on vero cells was observed by changes in the characteristics of vero cell monolayer. During the third passage, clear and consistent CPEs were observed. Vero cell monolayer was changed to form rounding cells. The P3 adapted viruses were confirmed to be IBDV by agar gel precipitation test. The P3 IBDV virus became well adapted to vero cell line.

Key words: Infectious bursal disease virus, vero cell line

Introduction

Livestock resource is a major portion of global resources. Among them poultry has a lot of contribution. Poultry industry is one of the rapidly developing areas in the livestock sector in Bangladesh. This industry is playing an important role in Bangladesh economy. In the poultry industry, the main loss is due to diseases. Among these diseases, infectious bursal disease (IBD) is a serious one, which is caused by a virus named infectious bursal disease virus (IBDV). IBDV causes significant losses to the poultry industries either by causing high mortality in an acute disease or as a consequence of immunosuppression in young chickens (Lasher and Shane, 1994; Van den Berg, 2000).

IBDV can infect and grow on various primary cell culture of avian origin and certain cell line of mammalian origin. Commonly used cell line to replicate IBDV are chicken embryo fibroblast (Sofei *et al.*, 1996), chicken embryo kidney, vero (Peilin *et al.*, 1997), baby hamster kidney (El-Ebriary *et al.*, 1997), ovine kidney (Kibenge and Mckenna, 1992), chicken embryo bursa (Lukert and Davis, 1974), normal chicken lymphocytes, B-cell lymphoblastoid, rabbit kidney (Rinaldi *et al.*, 1972), baby grivet monkey kidney and M4-104 cells (Jackwood *et al.*, 1987) etc. In addition to the above cell line, IBDV can also infect chicken embryo.

Among the above mentioned cell lines, vero cell line is previously established and frequently used cell line for virus propagation. Vero cells are fibroblast like cells. Its source is the kidney of a normal adult African green monkey. Due to greater suitability of *in vitro* growth, short generation time, availability, persistent cellular properties, easy to handle and maintenance, vero cells are used to cultivate, adapt and attenuate different viruses.

Most virological research is performed with strains of virus adapted to produce characteristic cytopathic effect (CPE) in cultures cell. During the isolation of viruses, there may emerge variants capable of multiplying more efficiently in the host cells used for this purpose than the original wild-type virus. This phenomenon is known as adaptation. Often such variants damage the original host less severely than the wild-type virus and are, therefore, said to be less virulent. Viruses are often purposely adapted to alter growth and virulence characteristics. An example is provided by the attenuated vaccine virus strains, which are obtained by repeated passaging of virus virulent for one host in some other host, until virus strains with decreased virulence for the original host have been selected. Following adaptation, the infectivity of virus to the adapted cells is increased but the virulence is decreased.

In Bangladesh, information in the area of adaptation of IBDV on vero cell line is not available. This is the first attempt to adapt IBDV on vero cell line. Therefore, this study was undertaken to adopt the IBDV on vero cell line and to identify the adapted virus by serological test.

Materials and Methods

The experiment was conducted at Virology Laboratory, Animal Health Research Division (AHRD), Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka-1341, Bangladesh during December 2001 to May 2002.

Collection of vero cell line: The source of vero cells is the kidney of a normal adult African green monkey. Vero cell line was collected from Gonashastha Vaccine Research Laboratory, Savar, Dhaka, Bangladesh and it was used for adaptation of IBDV.

Collection of suspected IBDV: Infectious bursal disease viruses were collected from IBDV suspected dead chickens to adapt them on vero cell line. Naturally occurring IBDV isolates were isolated from the IBDV suspected field samples of bursa of dead chickens of 33-37 days age group collected from the experimental flock of Bangladesh Livestock Research Institute (BLRI).

Preparation of inocula: Inocula were prepared from the collected field samples of bursa. Samples were triturated in a pestle and mortar. A required amount of phosphate buffer saline (PBS) was added to the tissue homogenate as to make a 20% w/v suspension. Suspension was centrifuged at 5,000 rpm for 15 min. Supernatant was collected from the suspension. Penicillin, streptomycin and neomycin solution was added to the collected supernatant @ 5ml/litre. The suspension was stored at -20 °C and was used as inoculum.

Infection of vero cells by suspected IBDV: Healthy and semi-confluent monolayer of vero cells were grown after 24 h following subculture in the 25 cm² flasks. These cells were suitable to infect by suspected IBDV. The working area under laminar flow cabinet was disinfected. PBS and maintenance media were warmed at 37 °C water bath. The growth medium of the flask was removed and the cell monolayer was washed twice by pre-warmed PBS. Then the vero cells were infected by 250 μ l of IBDV suspected inoculum by using 0.2 μ m pore size filter. The inoculum was spread uniformly over the monolayer and incubated at 37 °C for 45 min

with intermittent rotation to allow absorption. Five milliliters of maintenance media was added to each 25 cm² flask using a sterile pipette and the flask was capped. One flask of fresh cells was kept as control under the similar condition. The flasks were incubated at 37 °C and monolayer was examined twice per day under microscope for cytopathic effects (CPEs).

Harvesting of suspected IBDV: Any virus sample become ready for harvesting when 80 % CPEs are observed. Optimum CPEs of suspected IBDV on vero cell monolayer were found about 144 h after third passages. Then the suspected IBDV sample became ready for harvestings. The flasks were transferred to -20 °C for overnight and then thawed at normal temperature for two times. The virus suspension were poured into eppendorf tubes which were centrifuged at 5,000 rpm for 5 min to pellet the cell debris. The supernatant fluid was collected carefully by pipette and stored.

Adaptation of suspected IBDV: Viruses collected following first infection are called passage 1 (P1) viruses. Passage 1 (P1) suspected IBDV was infected again to vero cells using same techniques to adapt them perfectly on vero cell line. The viruses were harvested similarly after observation under microscope. The viruses obtained second infection are called passage 2 (P2) suspected IBDV. Similarly, P3 viruses were obtained through third infection. The P3 suspected IBDV were confirmed by agar gel precipitation test (AGPT).

Identification of suspected IBDV: Agar gel precipitation test (AGPT) was performed as described by Cullen and Wyeth (1975) to detect the precipitating antibodies in the known serum collected from the infectious bursal disease antibody test kit (IDEXX Laboratories, USA) against suspected IBDV (P3 adapted virus).

Results and Discussion

Cytopathic effects (CPEs) of IBDV on vero cell line: Cytopathic effects involved rounding, aggregation of vero cell monolayer due to infection by virus. The vero cells 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 cells were observed. The cells were gradually started to changes in shape 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 (Fig. 2).

Outcome of AGPT: AGPT was performed to detect the precipitating antibody in the positive serum. The white precipitation line, that is the binding of the antigen and antibody, were observed within 72 h. The antigen from virus and its corresponding antibody from the positive serum were present and diffused into the agar and formed a white precipitation line between two holes in which the antigen and the antibody were separately contained. This precipitation line clearly indicated that the resultant virus following adaptation on vero cell line was IBDV. To adapt IBDV on vero cell line, suspected IBDV were given three serial passages on vero cell line. The resultant CPEs were observed carefully under microscope. Viruses from each passage were harvested and clarified by centrifugation. Later, the P3 adapted viruses were confirmed to IBDV by AGPT. This test is also referred to as double immunodiffusion test or ouchterlony test. Because of easy procedure, with no special equipment or reagents, this method was used to test for viruses in this experiment. Detection and determination of antigens and antibodies, and antigen analysis was achieved by this method, however its sensitivity was not high.

During the first passage of the IBDV on vero cell line, the field virus did not produce clear evidence of CPEs. The virus just

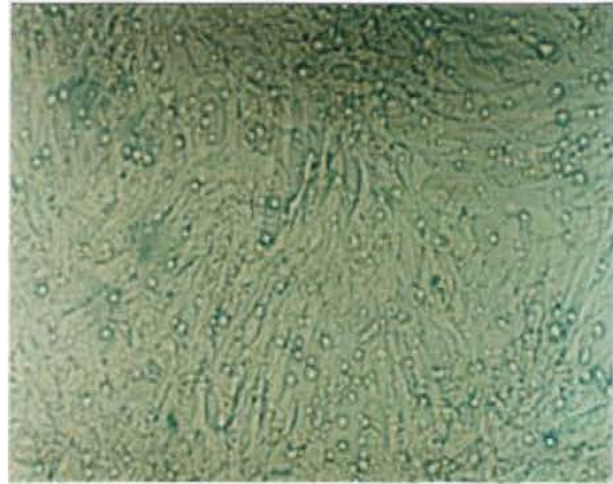


Fig. 1: Vero cell monolayer following 48 h infection by IBDV



Fig. 2: Formation of rounding cells on vero cell monolayer layer following infection by IBDV

started to adapt on vero cell line and their infectivity to vero cells were low. The P1 viruses were more virulent to vero cells. During second passage, some changes of vero cell monolayer began to develop after 72 h of incubation following infection. Monolayer showed rounding of infected cells. Nevertheless, complete CPEs of IBDV on vero cells were not found at this passage. During third passage, CPE was rapid and consistent. Following 144 h of infection, rounding of infected cells and aggregation of rounding cells were observed.

Ahmed (1999) observed CPEs of newcastle disease virus (NDV) on vero cell line following 36 to 40 h of infection during 3rd passage. CPEs of reovirus was also observed by Islam (1999) following 36 h of infection during 3rd passage. According to the findings of Peilin *et al.* (1997) complete CPEs of IBDV on vero cell line was stably produced in 65 to 72 h of inoculation during 4th passage. The present experiment showed that complete CPEs of IBDV was observed on vero cell line following 120 to 144 h of incubation during 3rd passage. This observation was found consistent with the findings of Ahmed (1999), Islam (1999) and Peilin *et al.* (1997). Following three serial passage of IBDV on vero cell line, P3 viruses became progressively more cytopathogenic or infective but less virulent to vero cell. Further study may help to determine the attenuation of virus for the production of live vaccine and mass production of test antigen.

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