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Haemagglutination Antigen Preparation of Newcastle Disease Virus on Vero Cell Line

M. Mehedi, K.M. Hossain, M.J.F.A. Taimur, B.K. Sil and [1]M.R. Islam
Biotechnology Discipline, Khulna University, Khulna, Bangladesh
[1]Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh

Abstract: The research work was undertaken to prepare haemagglutination antigen of NDV on vero cell line as an alternative to the traditional chicken embryonated egg. Newcastle disease virus can grow within different animal cell line. Vero cell is an established cell line whose source is African green monkey's kidney. The anchorage-dependent vero cells were first subcultured in Eagle's minimum essential medium to form semi-confluent monolayer. This monolayer was then infected by collected passage 3 (P3) adapted NDV and maintained up to passage 7 (P7). The antigen was collected from this adapted NDV. Then the NDV antigen was assayed and tested for its purity by tissue culture infective dose ($TCID_{50}$) assay and haemagglutination test respectively. The titre of NDV was $10^{4.1}$ $TCID_{50}$. HA result showed NDV antigen agglutinate chicken red blood cells up to 1600 dilution, which is moderately higher titre than HA titre found for NDV, propagated in chicken embryos.

Key wards: Haemagglutination antigen, newcastle disease, vero cell line

Introduction

In Bangladesh newcastle disease is a big threat capable of destroying chickens of commercial farming and small scale one. Newcastle disease is caused by a virus named newcastle disease virus, which belongs to the family paramyxoviruses.

NDV can infect various primary cell line of avian origin and certain cell line of mammalian origin. Common used cell line as the hosts of NDV are monkey kidney's cell (vero), chicken embryo fibroblast (CEF), chicken embryo kidney (CEK), baby hamster kidney cell line (BHK-21), HeLa cell etc. Previously CEF were used to grow NDV through out the world for antigen preparation. Nevertheless, CEF cells are the source of many vertically transmitted disease like salmonella, reovirus, avian leukemia, Marek's disease etc. Vero cells are fibroblast like cells from African green monkey kidney, which is a previously established cell line. It is suitable to grow in laboratory using common medium and easy to handle and maintenance and therefore, used to cultivate, adapt and attenuate virus (Ahamed, 1999). The use of an immortalized cell line for virus cultivation offers many advantages, related to a better standardization of production technologies, lower costs and easier quality control, as well as a better evaluation of adventitious agents (Losio *et al.*, 1998).

Due to expense of specific pathogen free (SPF) chicken, ND antigen preparation from adapted virus on vero cell line is cheap and obtained more purified form in comparison to CEF. Purpose of this study was to prepare ND antigen from the adapted virus on vero cell line and characterization of ND antigen by serological test.

Materials and Methods

This study was conducted at the Virology Lab, Animal Health Research Division, Bangladesh Livestock Research Institute, Dhaka, Bangladesh, during was Nov. 2001-June 2002.

Cell line preparation: To maintain the cell line, vero cells were subcultured at plateau phase state when cells formed confluent monolayer.

Infection of vero cells by NDV: The previously adapted (P3 passaged) newcastle disease virus which $TCID_{50}$ was $10^{3.9}$ (Ahamed, 1999) collected from Virology Laboratory, Animal Health Research Division, Bangladesh Livestock Research Institute, Dhaka, Bangladesh.

One day following subculture, semi-confluent monolayer of vero cells developed in 25 cm^2 flasks. These cells were suitable to infect by P3 NDV for further adaptation. The cells were infected by 0.25 ml P3 passaged NDV inoculums of 10^5 pfu (plaque forming unit)/ml. The inoculum was spread uniformly over the monolayer and incubated at 37°C. Five milliliters of maintenance was added to each 25 cm^2 flask and the flask was capped. The flasks were incubated at 37°C and monolayer was examined twice per day under microscope for CPEs.

P4 passage (P4) NDV was infected again to vero cells using same technique to further adapts them perfectly on vero cell line. Similarly NDV passaged up to P7 passage.

NDV antigen preparation: P7 adapted ND virus was subculture in the 75 cm^2 culture flask in order to obtain large quantity of antigen. Same procedure of vero cell subculture was followed here. NDV adapted virus become ready to harvest when 80% cytopathic effects are observed. Within 20 h the virus have to collect in order to obtain virus in purified form. The flask were transferred to −20°C for overnight. The flasks were then thawed at normal temperature. The flask transferred to −20°C for 30 min. Again the flask thawed at normal temperature. The samples were poured into centrifuge tube. Then the samples were centrifuged at 5000 g for 5 min to pellet the cell debris. The viruses were present in the supernatant fluid due to its low molecular weight. Therefore, supernatant fluid was collected carefully by pipette and stored at −20°C freeze.

Assaying and testing of NDV antigen

Tissue culture infective dose 50 ($TCID_{50}$): Tissue culture infective dose 50 ($TCID_{50}$) was performed to determine the infectivity titre of vero cell passaged P7 NDV.

Haemagglutination (HA) test: The test was conducted to determine the haemagglutinating titer of HA antigen according to the method designed by Anonymous (1971).

Results and Discussion

During the seventh passage, cytopathic effects were rapid and consistent. CPEs were characterized by granulation in cytoplasm, rounding of infected cells, development of micro plaque, clustering of infected cells, intracytoplasmic bridge connecting those clusters, vacuolization in the cell system and formation of syncytia. A large number of clear sincytia were observed after about 30-40 h of infection, which were altered by the formation of multinucleated giant cells and further changes on cell monolayer

Table 1: Tissue culture infective dose 50 ($TCID_{50}$) assay result

Log_{10} titre of virus dilution	Observed CPE percentage
0	100
-1	100
-2	100
-3	90
-4	50
-5	20
-6	0
No virus	0

Corresponding Author: Dr. Md. Rafiqul Islam, Scientific Officer, Animal Health Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh

Table 2: Observed HA test for NDV

Steps	No. of well	1	2	3	4	5	6	7	8
	Antigen dilution	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	Control
1	1:100	50}↗	50}↗	50}↗	50}↗	50}↗	50}↗	50}↗	No
	Antigen (μl)	50}	50}	50}	50}	50}	50}	50}↘	antigen
	Diluent (μl)							Discard 50μl	50
2	0.6% CRBC (μl)	50	50	50	50	50	50	50	50
3	Sensitization	At room temperature for 30-60 min.							
4	Observation	+	+	+	+	-	-	-	-

+ = indicates agglutination - = indicates no agglutination
↗ = indicates successive dilution ↘ = Sign indicates discard 50 μl

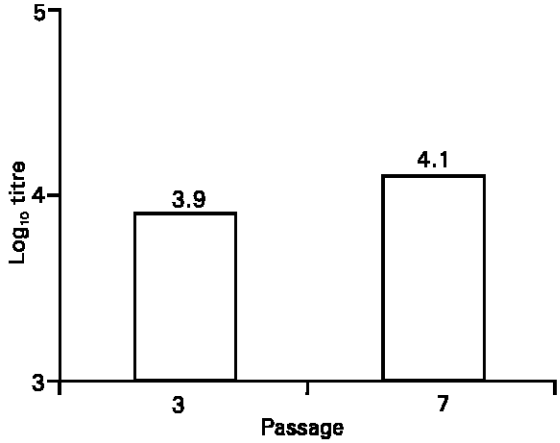

Fig. 1: The comparative infectivity titre between P3 and P7 adapted NDV on vero cell line

occurred to form dendritic shaped cells. During the terminal stage of cytopathic effects, the whole monolayer showed the maximum degeneration of cells and large gaps throughout the monolayer i.e. plaque.

About 54 h after infection NDV produced suitable CPEs on vero cells. Stained plate was observed under microscope for percentage of CPEs or cells damage obtained at each well (Table 1). The infectivity titre of P7 adapted virus was calculated by Garber method. The result showed that P7 NDV suspension was $10^{4.1}$ $TCID_{50}$. It indicates that, $10^{4.1}$ times diluted vero cells adapted P7 NDVs are able to produce 50% CPE of the inoculated cells.

HA was measured by the pattern aggregated cells form on plastic surface. NDV agglutinated the receptor substance on the red blood cell surface. In HA plate, well number one to four NDV antigens agglutinated chicken red blood cells, where well number four contained 1:1600 diluted antigens. At fifth, sixth and seventh number well of the HA plate, CRBC pellet formed at bottom of the well. NDV agglutinated the CRBC up to 1600 dilution (Table 2).

After 7th passages of NDV on vero cell line, the infectivity titre was $10^{4.1}$. This result showed that adapted NDV on vero cell increased its antigenicity (Fig. 1).

Sil (1996) adapted pesti des petits ruminants (PPR) virus on vero cell line after 5 serial passages. Rinderpest virus (Sukumer and Padmanaban, 1988), bovine diarrhea virus (Mukherjee *et al.*, 1989) adapted on vero cell line. Previously NDV adapted on chicken embryo fibroblast (CEF) cell line (Roy and Padmanan, 1990). Chicken RBC is used in haemagglutination test. Winslow *et al.* (1950) showed that human, mouse and guinea pig RBCs were agglutinated by all NDV strain tested, but the ability to agglutinate cattle, goat, sheep, swine and horses cells varied with the strain of NDV. The HA antigen titre found was 1:1600 which is reasonably high than HA titre found for NDV which is propagated in chicken embryos, 1:1024 (Losio *et al.*, 1998).

The demonstration of the maintenance of biological properties of the cell line adapted NDV strain previously reported (Losio *et al.*, 1995), together with the preservation of its immunological characteristics, suggest the use of vero cell culture for the production of NDV as an alternative to the traditional embryonated egg. Furthermore, the recent increasing concern over the use of laboratory animals for preparation of biological products makes it desirable to gradually decrease their use. Accordingly, vero cell culture (tissue culture) seems to be the most promising living laboratory system offering an alternative to the traditional ones, like chicken embryo.

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