Cytopathic effect of PPR vaccine virus strains in Vero cells

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Abstract

The present study describes the cytopathic effect of two different Peste des petits ruminants (PPR) vaccine virus strains presently being used in the country, in vero cells. The cytopathic effect (CPE) was visible from 4th day post infection in Sungri vaccine virus strain where as Arasur vaccine virus strain showed CPE, 36-48 hr post infection. With both vaccine virus strains the CPE in vero cells showed initial cell rounding, aggregation and syncytial development. The generalized CPE was noticed by 6th day in Sungri and by 96 hrs post infection in Arasur strain. However complete detachment of the cell monolayer was observed in Arasur strain by 120 hr, post infection. Infected coverslip cultures stained with H & E and May & Grunwald's Giemsa showed cell vaculation, cytoplasmic extension and syncytia comprising of five to six nuclei. Acidophilic intracytoplasmic and intranuclear inclusion bodies were also observed. Titers, HA activity and detection by s-ELISA of both the vaccine virus strains are also compared.

Keywords: Vaccine, Cytopathic Effect, Virus Strain, Vero Cells, Peste des Petits.

Introduction

Peste des petits ruminants (PPR) is an acute viral disease of small ruminants characterized by fever, occulonasal discharges, stomatitis, diarrhea and pneumonia (OIE, 2004). The disease is caused by PPR virus belonging to the genus morbillivirus in the family paramyxoviridae (Gibbs et al, 1979). Evidence of PPR in India was reported for the first time by Shaila et al., (1989). Since then several studies have been done to confirm the virus and successful vaccines have been developed against the disease. The present study describes the cytopathic effect of two PPR vaccine virus strains available in the country namely Sungri and Arasur strains in vero cells.

Materials and methods

Vero cells grown in Minimum Essential Medium (Gibco) with six percent foetal calf serum (Biological Industries, Israel) were infected with vaccine virus strains at a multiplicity of infection (m.o.i) of 0.01 per cell at a cell density of 1 X 10^5 cells/ml of the medium by co-cultivation method. The coverslip cultures were also infected. The infected cultures were stained with haematoxylin & eosin and May & Grunwald's Giemsa stain at periodic intervals and cytopathic effect of PPR virus was observed.

Quantitative estimation of the vaccine was carried out by microtiter plate method. Vaccines were titrated in 96 well cell culture plates with a vero cell system (3 X 10^5 cells/ml). Serial 10 fold dilutions from 10^{-1} to 10^{-8} of the vaccine were made in serum free medium. Five replicates were infected with 100 µl of each viral dilution. Five uninfected vero cell wells served as cell controls. Five wells with neat virus and cells served as virus control. 100 µl of vero cells were added to all the wells and the plates were incubated at 37^0 C with 5% carbon dioxide for a period of 10 days. The plates were examined daily for the appearance of cytopathic effect. The final results were recorded as positive for the presence of cytopathic effect and negative for the absence of cytopathic effect on day 10. $TCID_{50}$ was then calculated by Reed and Muench method (1938).

HA test was performed for both the vaccine strains using 0.5 per cent chicken erythrocyte in normal saline solution.

Both the vaccine virus strains were also subjected to sandwich ELISA. The test used a polyclonal antibody as capture antibody and Mab to 'N' protein of PPRV as detection antibody. A standard s-ELISA test as per the technique described by Singh (2004) was used to detect the virus in given vaccines.

Results and Discussion

With Sungri vaccine virus strain visible CPE was observed on fourth day post infection characterized by cell rounding and aggregation of cells. On day six post infection, there was generalization of CPE and

formation of syncytia concurred with earlier reports of Lefevre and Diallo (1990). Whereas Arasur strain of PPR vaccine virus strain showed CPE 36-48 hr post infection and was characterized by cell rounding and formation of syncytia. However by 96 hr post infection there was generalization of CPE and complete detachment of cell monolayer was observed by 120 hr. Similar observation was made by with Arasur vaccine virus strain in vero cells by Mohan (2004) and in John et al (2006) in BHK_{21} cells.

Infected coverslip cultures stained with H & E and May & Grunwald's Giemsa stain showed cell fusion cell vaculation which was extensive, cytoplasmic extension and syncytia comprising of five to six nuclei in both the vaccine strains. Acidophilic intracytoplasmic and intranuclear inclusions were also observed.

The infective dose of the vaccine virus strains were detected by microtiter plate titration. Sungri vaccine virus strain gave a Tissue culture infective dose 50% of 5.83/ml where as Arasur strain of vaccine virus gave $TCID^{50}$ of 7.37/ml.

When both the vaccine viruses were tested for their haemagglutinating property by haemagglutination (HA) test Arasur vaccine virus gave a HA titer of 1:64 whereas Sungri vaccine virus strain did not show any haemagglutinating activity. Low titer of Sungri virus compared to Arasur strain titer may be the reason for not showing HA activity as more number of viral particles are required per unit volume to show the HA (Nagendra *et al.* 1992) or haemagglutinating antigen of Sungri strain may be destroyed during culturing or it may be strongly cell associated (Wosu, 1985).

Sungri strain of vaccine virus was detected in sandwich ELISA whereas Arasur strain of vaccine virus was not detected The reason for not detecting of virus could be, the detecting antibody used in the sandwich ELISA kit was anti nucleoprotein (N) Mab raised against an Sungri isolate of PPR virus (Singh *et al*., 2004). The extensive sequence variation seen in a part of the "N" gene of different PPRV strains could result in differences in amino acid sequence, leading to change in the epitope (Dhinakar Raj *et al*., 2005).

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