

# COMPARISON OF ROLLER CULTURE AND BHK 21 SUSPENSION CELL CULTURE SYSTEM FOR PROPAGATION OF PESTE DES PETITS RUMINANTS (PPR) VACCINE VIRUS \*

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## ABSTRACT

*The present study deals with the analysis of infectivity titres of peste des petits ruminants (PPR) vaccine virus produced in roller culture and BHK 21 suspension cell culture system. Roller bottles having a surface area of 603 cm<sup>2</sup> were used for roller culture. BHK 21 cells were routinely cultivated in 5 ml volumes in 25 cm<sup>2</sup> tissue culture flask. Both the cultures were infected with same multiplicity of infection of PPR vaccine virus. Infecting roller culture of Vero cells with peste des petits ruminants vaccine virus increased the yield of virus hundred fold compared with BHK 21 suspension cell culture system. This preliminary study paves the way to utilize roller culture as an alternative to stationary culture for PPR vaccine production or the possibility of increasing the infectivity titre of virus in BHK 21 suspension cell culture system should be analyzed.*

**Key Words:** Peste des petits ruminants virus, Roller culture, BHK 21 suspension cell culture

## INTRODUCTION

Peste des petits ruminants (PPR) is a contagious disease affecting sheep and goats. It was first reported in India in 1981 (Shaila *et al.*, 1989). Since then it has been reported from all over the country and is a serious threat to the growing sheep and goat industry in India. The principal means of controlling the disease is by vaccination. A homologous PPR vaccine has been developed by attenuation of PPR virus in Vero cells at Tamil Nadu Veterinary and Animal Sciences University as part of National Project for Rinderpest Eradication (NPRE). In many developing countries animal immunization is conducted on a campaign basis and

planning of vaccine production must make provision for producing large quantities of vaccine in a short period of time (FAO, 1997). But the disadvantage of the culture system employed in vaccine production is that if the production is to be increased, the number of Roux flasks has to be increased often to thousands for a large single batch of vaccine production. This is extremely labour intensive, laborious and expensive. It may be more practical to use a scale up monolayer system like roller culture or BHK 21 suspension cell culture system for large-scale vaccine production. Hence the following report describes the application of roller culture and BHK 21 suspension culture system for the cultivation of PPR vaccine virus.

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## MATERIALS AND METHODS

### Roller culture system

Roller bottles (Wheaton, USA) having a surface area of 603 cm<sup>2</sup> and roller apparatus (Wheaton, USA) was used for Vero cell production. Vero cell adapted peste des petits ruminants vaccine virus (AR 87, P# 75) maintained in the Department of Veterinary Microbiology, Madras Veterinary College, TANUVAS was used. The growth medium used was minimum essential medium (Gibco Brl, USA) with 10% fetal calf serum (Biological Industries, Israel) supplemented with Penicillin (100 IU/ml) and Streptomycin (100 mg/ml). The maintenance medium was devoid of fetal calf serum.

Roller bottles were seeded at approximately  $3 \times 10^5$  Vero cells/ml in a 100 ml volume and cells were allowed to achieve confluence and all cultures were grown at 37°C in 5% CO<sub>2</sub> atmosphere. A multiplicity of infection 0.005 TCID<sub>50</sub>/cell was used for roller culture and an adsorption period of 1 hr was allowed (10 ml of virus suspension per roller bottle) after which the virus inoculum is discarded and the volume in each vessel was brought up to 100 ml. Cultures were incubated at 37°C. Roller bottles were rotated at a rate of 8 revolutions per hour. At regular intervals after infection (24, 48, 72, 96 and 120 hr) two roller bottles were removed and virus fluid obtained after freeze thawing were subjected to titration of virus by microtitre technique.

The basic microtitre technique described by Rossiter *et al* (1985) was followed with slight modifications. Virus fluids obtained at periodic intervals following infection of Vero cells with PPR virus was used for titration. Serial ten fold dilutions were made with each virus sample, and dilutions ranging from 10<sup>-3</sup> to 10<sup>-9</sup> were considered for infection. Hundred microlitre was inoculated in monolayers of cells grown in 96 well tissue culture plates (TPP Ltd, Switzerland) with the condition that one row is allotted

for each dilution along with controls. The plates were incubated at 37°C in 5 per cent CO<sub>2</sub> atmosphere and read from 48 h post infection for the development of cytopathic effect. Fifty per cent end point (TCID<sub>50</sub>) was calculated as per Reed and Muench (1938).

Vero cell counts in roller bottle were made by trypan blue exclusion technique. Briefly the cells in each roller bottle collected at periodic intervals after subcultures were rinsed with MEM, dispersed with 0.2% trypsin and counted in hemocytometer.

### BHK 21 suspension cell culture system

BHK 21 cells were routinely cultivated in 5 ml volumes of minimum essential medium (GIBCO, BRL) with 10% foetal calf serum. Cell suspensions at initial concentration of  $5 \times 10^5$  /ml were incubated at 37°C in 5% CO<sub>2</sub> in 25 cm<sup>2</sup> tissue culture flask (TPP Ltd., Switzerland). After 48 hr of incubation, the cells were centrifuged at 500 x g for 10 min, supernatant was discarded and fresh medium was added to achieve the desired density. Appropriate dilutions of cell suspensions were made by counting the cell suspension using trypan blue. The PPR vaccine virus which was adapted in BHK 21 monolayer cells (Glasgow) was used to infect the BHK 21 suspension cell line. The cells were infected at a multiplicity of infection (MOI) of 0.002 TCID<sub>50</sub>/cell and the volume of virus inoculum added was approximately one percent of the total culture volume. An uninfected control flask was set up for comparison.

Infectivity titres and cell counts were made every 24 hr. When the full cytopathic effect (CPE) was observed, the contents of the flasks were harvested by freeze thaw and stored at -70°C. The infectivity titre of the virus was made in the BHK 21 (Glasgow) cells by a modified microtitre technique as originally described by Rossiter *et al.*, (1985).

## RESULTS AND DISCUSSION

### Roller culture system

The present study was conceived to investigate the ability of the roller culture to support both the Vero cell growth and the PPR virus production. Peste des petits ruminants vaccine virus was successfully grown in Vero cells in roller bottle. Table 1 gives comparative cell and virus yields from 100ml roller cultures. Cell densities ranged from approximately  $5 \times 10^{-5}$  cells / ml in 24 hrs to approximately  $2.0 \times 10^5$  cells / ml in 120 hrs in case of roller culture. It is obvious that a wide variation in both cell yield / day and maximum attainable cell density was observed. However, in all cases, cell attachment was good: Cells remained attached, appears healthy throughout the growth period and forms complete monolayers.

Mariner *et al* (1991) have used roller culture to propagate rinderpest virus in the preparation of thermostable Vero cell adapted rinderpest vaccine. Taylor (1979) used roller tube monolayers of Vero and calf kidney cells to perform neutralization studies with PPR virus. Rossiter *et al* (1985) used roller tube monolayers of Vero cells for titration of PPRV and Rinderpest virus.

The maximum infectivity titre of PPR vaccine virus ( $8.71 \log_{10} \text{TCID}_{50}/0.1 \text{ ml}$ ) was obtained at 72 hour post infection. It is attributed to the fact that higher surface area ( $603 \text{ cm}^2$ ) afforded by roller bottle permits maximum attainable cell density thus by higher viral yield. When the titre of PPR vaccine virus obtained in roller culture was compared with BHK 21 suspension cell culture system it was found that there was two log (hundred times) difference in virus titres at 72 hour post infection. The production of virus in cell culture systems depend upon a number of parameters including the cell density at infection and the multiplicity of infection. Considering the multiplicity of infection (MOI), Vero

cells grown on roller bottles were infected with PPR vaccine virus with a MOI of 0.005 TCID<sub>50</sub> / cell. A MOI of at least 0.001 TCID<sub>50</sub>/cell should be used for PPR vaccine development (OIE, 2004). The MOI of PPR virus employed in this study was based on the findings explored in the development of homologous PPR vaccine as part of National Project for Rinderpest Eradication by Tamil Nadu Veterinary and Animal Sciences University.

### BHK 21 suspension cell culture system

The PPR vaccine virus was found to get adapted to BHK 21 suspension cell line after 3 blind passages. The maximum infectivity titre of PPR vaccine virus was  $6.8 \log_{10} \text{TCID}_{50}/0.1 \text{ ml}$  at 72 hr post infection. The infectivity titre of PPR vaccine virus obtained in BHK 21 suspension was lower than roller culture. It is attributed to the fact that virus sensitivity to replicate in suspension, effect of multiplicity of infection and the improper cultural conditions provided during propagation of PPR virus in suspension cells. It may also be due to the fact that we have not yet determined the best method for using suspended cells in assay systems (Capstick *et al.*, 1962).

The immunogenicity of vaccines prepared from the BHK 21 suspension cells has to be assessed. The growth of virus in suspended cells can lead to the selection of antigenic variants that differ from those produced in monolayer cells (Brown *et al.*, 1998).

In conclusion, roller culture system may be used as a basis for further development of a large scale process for vaccine production or it is desirable that the PPR vaccine virus of low titres obtained in BHK 21 suspension cells should be exploited in vaccine development without any loss of immunogenicity of vaccines. It is based on the fact that suspension cell line has got enormous scaling up potential.

Table 1

Cell and Virus yields from roller cultures

Hours	Cell yield from 100 ml cultures after subculture (1 x 10 <sup>5</sup> cells / ml)	Virus yield from 100 ml cultures after infection (log TCID <sub>50</sub> / 0.1 ml) Mean ± standard error
24	5	5.42 ± 0.02
48	7.9	7.65 ± 0.02
72	11	8.71 ± 0.01
96	5.8	8.4 ± 0.02
120	2.0	7.68 ± 0.03

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