

HIGH TITRE PESTE DES PETITS RUMINANTS (PPR) VACCINE VIRUS PRODUCTION ON CYTODEX 1 MICROCARRIER CULTURE

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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 stationary and microcarrier cultures. Routine stationary cultures were performed in 175 cm² Roux flask. Cytodex 1 microcarrier (1g/L) was used to perform the microcarrier culture in bench top fermenter. Both the cultures were propagated under same cultural conditions and infected with same multiplicity of infection of PPR vaccine virus. Assessment of infectivity titres determined at periodic intervals in both cultures revealed that the microcarrier culture produced two log higher amount of virus than the stationary culture at 72 hour post infection. This preliminary study paves the way to utilize microcarrier as an alternative way for PPR vaccine production and the possibility of scaling up should be analyzed.

Keywords : Cytodex 1 microcarrier, Infectivity titre, Microcarrier culture, PPR vaccine virus, Stationary culture

INTRODUCTION

Peste des petits ruminants (PPR) is an acute contagious disease caused by a *morbilli virus* in the family Paramyxoviridae. It affects small ruminants, especially goats, which are highly susceptible and occasionally wild animals. Control of PPR outbreaks involves control of movement (Quarantine) combined with the use of focused (“ring”) vaccination and prophylactic immunization in high risk populations. Recently, a homologous PPR vaccine has been developed by attenuation of PPR virus in Vero cells at Tamilnadu Veterinary and Animal Sciences University (TANUVAS) as part of

National Project for Rinderpest Eradication (NPRE). As per the FAO (1997) recommendation, the developing countries should make provisions for producing large quantities of vaccine in a short period of time to meet the demands of animal immunization campaigns. To fulfill this requirement the scale up methods viz., roller and microcarrier culture should be exploited in large scale PPR vaccine production. The roller culture system was employed in the development of thermostable Vero cell adapted rinderpest virus vaccine (Mariner *et al.* 1991).

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The development of microcarriers facilitated adherent culture in a large single unit bioreactors (Van Wezel, 1967) allowing easier manufacturing. Microcarriers have been extensively used in culture systems to grow anchorage dependent mammalian cells to a high density in simple stirred flasks. Such a culture system has the advantage that it can easily be scaled to higher volumes in a stirred tank reactor for large scale viral vaccine production.

There is an extensive range of microcarrier types available that differ in terms of the basic matrix or the surface coating material. But Cytodex 1 microcarrier closely fulfilled the requirements for an optimal microcarrier (Van Wezel, 1977) and microcarriers were successfully used for a variety of viruses on laboratory as well as on industrial scale. Vero cells grown on Cytodex 1 microcarrier was used for propagation of poliovirus (Duchene *et al.* 1990), reovirus (Butler *et al.* 2000), rabies virus (Rourou *et al.* 2007) and rinderpest virus (Uma and Bandyopadhyay, 1994).

In the limited study conducted here, Vero cells grown on microcarrier beads were used to infect the PPR vaccine virus to increase the yield of virus.

MATERIALS AND METHODS

Stock cultures

A cell bank prepared with the Vero cells at the 142nd passage was used in all experiments. The cells were grown in Minimum Essential Medium (MEM)(GIBCO BRL) supplemented with 100 ig / ml streptomycin, 100 IU / ml penicillin and 10 per cent V/V foetal calf serum (Biological Industries, Israel). Routine stationary cultures were maintained under 5 per cent CO₂ as monolayers in 175 cm² Roux flask. Cells were subcultured by trypsinization.

Microcarriers

Cytodex-1 (Sigma, USA) microcarrier was prepared as per the manufacturers instructions. Briefly the dry microcarries were swollen and hydrated in Calcium and Magnesium free PBS (50-100 ml / g Cytodex) for at least 3 hr at room temperature. The microcarrier beads were washed twice with PBS and autoclaved. The number of microcarriers per gram was determined by counting beads in a standard volume of PBS on a haemocytometer grid. Prior to use, the microcarriers were briefly rinsed in warm culture medium and then transferred to the culture vessel.

Microcarrier cultures were established by inoculating cells (2 x 10⁵ cells / ml) into growth medium containing 1 g Cytodex / L. The cultures (1000 ml) were maintained in a 5 litre bench top fermenter (Braun B Biostat, Germany). The cultures were stirred continuously at 40 revolutions per minute (rpm) from the point of inoculation.

Culture sampling

Samples (1 ml) were removed daily from cultures into Eppendorf tubes to which 1 ml of Rappaport stain was added. It was incubated for at least 1 hr at 37°C and a sample of fluid was withdrawn, to load the haemocytometer grid. The purple coloured nuclei were counted using a hemocytometer.

Virus stock

The Vero cell adapted Peste des petits ruminants vaccine virus (P# 75), employed in the development of homologous PPR vaccine was used in this study.

Virus infection

Cultures were infected by removing 90 per cent of the medium followed by addition of 100 ml of PPR vaccine virus at a multiplicity of infection

(MOI) of 0.005 TCID₅₀/cell which was found to be the optimum MOI used in the development of PPR vaccine by TANUVAS. During viral infection the culture was stirred intermittently at 40 rpm for 1 min every 12 min for 1 hr. Cultures were readjusted to their original volumes by the addition of free MEM and incubated at 37 °C. Culture samples were taken at regular intervals (24, 48, 72, 96 and 120 hr) for determination of viral titres. Each sample was freeze thawed (x3) to ensure complete release of intracellular virus.

RESULTS AND DISCUSSION

PPR virus production in stationary culture

Vero cells in Roux flask were infected with PPR vaccine virus at a MOI of 0.005 TCID₅₀/cell when a confluent monolayer was observed. The virus titre obtained at periodic intervals after infection was recorded (Table I). The titre of PPR virus reached its peak value of 7.68 log₁₀ TCID₅₀/0.1 ml by 72 hr post infection.

PPR virus production in microcarrier culture

The Vero cells were grown on Cytodex 1 to a cell density of 4.7 x 10⁵ cells / ml (Figure 1) and infected with PPR vaccine virus at a MOI of 0.005 TCID₅₀/cell using intermittent stirring régime. The virus titre obtained at periodic intervals (24, 48, 72, 96 and 120 hr) after infection was assessed (Table I). Peak infectivity titre of PPR virus in microcarrier culture was found to be 9.81 log₁₀ TCID₅₀/0.1 ml at 72 hr post infection.

Overall, in both the culture methods the peak infectivity titres were obtained by 72 hr post infection but a significant higher viral titre (P<0.05) was obtained in microcarrier culture when compared to stationary culture. Statistical analysis of titres of PPR vaccine virus obtained in both culture methods revealed that microcarrier culture differed significantly (P<0.05) from stationary culture at

various time periods after infection (Table I).

The present study was conceived to investigate the ability of the microcarrier Cytodex 1, to support both the Vero cell growth and the PPR virus production in a 5 litre bench top fermenter. The data presented show that, microcarrier culture method produced significantly higher PPR virus titre when compared to stationary culture. There was two log increase of PPR virus titre in microcarrier culture than the stationary culture. Uma and Bandyopadhyay (1994) reported that the yield of rinderpest virus was at least one log higher in microcarrier culture than the stationary culture. Furthermore they attributed that the causes for lower yield may be due to improper optimization of environmental conditions provided during growth of Vero cells in microcarrier. A regime of intermittent low speed stirring at reduced culture volume was followed to ensure adequate infection of the Vero cells attached to the microcarriers in the present study. This provided a balance between a stirring phase to ensure adequate distribution of the viral inoculum and a quiescent phase to ensure penetration of the virus into the cells. Although continuous stirring has been reported for infection of other viruses such as polio and rabies (Montagnon *et al.* 1981 and Rourou *et al.* 2007) such a protocol resulted in poor viral infection for reovirus (Berry *et al.* 1999).

The production of virus in cell culture system is dependent upon a number of parameters including the cell density at infection and the multiplicity of infection. In microcarrier culture the cell / bead ratio at infection is very critical and the ratio reflects the cell density on bead. But increased number of cells / bead may indicate a degree of multilayering of the anchorage dependent cells which may adversely affect the infectivity of the cells on the microcarriers (Berry *et al.* 1999). Uma and Bandyopadhyay (1994) used Cytodex 1 microcarrier at a concentration of 3 g / L and infected the Vero cells grown on microcarriers with rinderpest virus using spinner flask. Further in

their study, the yield of Vero cells was lower when compared to surface area provided by microcarriers and thus the yield of rinderpest virus. In the present study Cytodex is used at a concentration of 1g/L and the Vero cells grown on microcarriers were infected with PPR Virus. The yield of Vero cells obtained in microcarrier culture was better than that reported by Uma and Bandyopadhyay (1994) obtained in stationary culture.

Considering the multiplicity of infection (MOI), Vero cells grown on microcarriers were infected with MOI of 0.005 TCID₅₀ / cell. A MOI of at least 0.001 TCID₅₀ / cell should be used (OIE, 2004) and a MOI of 0.01 TCID₅₀ /cell was used by Uma and Bandyopadhyay (1994) in case of rinderpest virus. The MOI of PPR virus employed in the study was based on the findings explored in the development of homologous PPR vaccine by TANUVAS. At low MOI the virus productivity per cell was significantly higher at high cell / bead loading in microcarrier culture than at high MOI and high cell / bead loading (Berry *et al.* 1999).

Considering the cell / bead ratio at infection, the mean diameter of the microcarrier is 180 μ m and this offers a surface area for the attachment of 200 Vero cells as a monolayer (Butler *et al.* 1983). In this study, the yield of Vero cells at the time of infection of PPR vaccine virus in microcarrier was 4.7 x 10⁵ cells / ml. The number of microcarriers present in one ml of medium was 4500. While calculating the number of cells present in a single bead the cell bead ratio corresponds to 120:1.

Several other authors had also reported that the yield of virus in microcarrier culture seems to be the best and the yield of those viruses in microcarrier culture was more than ten times higher than the stationary culture (Montagnon *et al.* 1981 and Lesko *et al.* 1993).

The development of microcarrier culture has opened up new prospects in the large-scale utilization of anchorage dependent cell lines to

produce biologicals, including human viral vaccines. Today, several large-scale applications have been developed upto 1,000 litres, especially for the production of inactivated polio vaccine. In the development of such vaccines cells are expanded upto large scale in batch process by increasing the volume of the culture vessel while maintaining the same concentration of microcarrier. Alternatively, large amounts of cells could also be obtained in a smaller bioreactor if the surface area available for cell growth could be simplified by increasing the microcarrier concentration and by introducing perfusion procedure.

In conclusion we report the feasibility of a culture process for PPR virus production based on the infection of Vero cells grown on Cytodex – 1 microcarrier. This culture system may be used as a basis for further development of a large-scale process for vaccine production. This has enormous implications with regard to cost effectiveness in vaccine production.

REFERENCES

- Berry, J.M., Barbabe, N., Coombs, K.M. and Butler, M. (1999). Production of reovirus type 1 and type 3 from Vero cells grown on solid and macroporous microcarriers. *Biotechnology and Bioengineering*, 62 (1): 12-19.
- Butler, M., Inamur, T., Thomas, J. and Thilly, WG. (1983). High yield from microcarrier cultures by perfusion. *Journal of Cell Science*, 61: 351-363.
- Butler, M., Burgener, A., Patrick, M., Berry, M., Moffatt, D. and Huzel, N. (2000). Application of a serum free medium for the growth of Vero cells and the production of reovirus. *Biotechnology Progress*, 16: 854-858.
- Duchene, M., Peetermans, J., D'Hondt, E., Harford, N., Fabry, L. and Stephenne, J. (1990). Production of polio vaccines: past, present

- and future. *Viral Immunology*, 3: 243-272.
- FAO (Food and Agriculture Organization of the United Nations). (1997). Vaccine Manual. The production and Quality control of Veterinary Vaccines for use in developing countries, Rome, FAO. pp. 125.
- Lesko, J., Veber, P. and Dusekova, E. (1993). Canine distemper virus replication in cells on microcarriers. *Acta Virologica*, 37: 412-416.
- Mariner, J.C., House, J.A., Sollod, A. and Stem, C. (1991). Production of a thermostable Vero cell adapted Rinderpest vaccine. *Journal of Tissue Culture Methods*, 13: 255 -256.
- Montagnon, B.J., Fanget, B. and Nicolas, A.J. (1981). The large-scale cultivation of Vero cells in microcarrier culture for virus vaccine production. Preliminary results for killed poliovirus vaccine. *Developments in Biological Standardization*, 47: 55-64.
- OIE(Office International Des epizooties). (2004). Manual of standards for diagnostic tests and vaccines for List A and B diseases of mammals, birds and bees, France.
- Rourou, S., Ark, A.V., Velden, T.V. and Kallel, H. (2007). A microcarrier cell culture process for propagating rabies virus in Vero cells grown in a stirred bioreactor under fully animal component free conditions. *Vaccine*, 25: 3879-3889.
- Uma V. and Bandyopadhyay, S.K. (1994). Suitability of microcarrier cell culture technique for propagation of Rinderpest virus. *Indian Journal of Animal Sciences*, 64(3): 253-255.
- Van Wezel, A.L. (1967). Growth of cell strains and primary cells on microcarriers in homogenous culture. *Nature*, 216: 64-65.
- Van Wezel, A.L. (1977). The large-scale cultivation of diploid cell strains in microcarrier culture. Improvement of microcarriers. *Developments in Biological Standardization*, 37: 143-147.

Table I.
Infectivity titre of PPR vaccine virus obtained at periodic intervals in stationary and microcarrier culture

Hours infection	Virus titre (\log_{10} TCID ₅₀ / 0.1 ml)*	
	Stationary culture	Microcarrier culture
24	4.27±0.06 ^a	5.12±0.02 ^b
48	6.35±0.06 ^a	7.73±0.02 ^b
72	7.68±0.03 ^a	9.81±0.03 ^b
96	7.27±0.02	9.30±0.02
120	6.42±0.08	8.75±0.02

* = Mean + Standard Error (SE) of four values

a, b = means bearing different superscripts differ significantly (P<0.05).

Fig. 1. Comparative growth pattern of Vero cells in stationary monolayer and microcarrier culture

