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ASSESSMENT OF THE PESTE DES PETITS RUMINANTS (PPR) ATTENUATED VIRUS PRODUCED BY INOCULATING VERO CELL LINE AT DIFFERENT STAGES OF CELL GROWTH

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ABSTRACT

This work presents cultural characterization of Peste Des Petites Ruminants (PPR) virus in VERO-culture. The VERO cells are currently considered as an acceptable cell substrate to produce a wide range of viruses. This study evaluates the best time for inoculation of PPR virus on VERO cell cultures; the study proved that the optimum time was 24 hours after subculture of VERO cell line using MOI 2: 1. It was also found that the best time of harvstation of virus fluid of PPR was 9th day post inoculation to reach the best titre 6 log10 TCID50 /ml.

Keywords:VERO cell - peste des petits ruminants - cell growth

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INTRODUCTION

The pest des petites ruminants (PPR) virus has been classified under family Paramyxoviridae, order Mononegavirales; genus Morbillivirus **(Van Regenmortel *et al.*, 2000).** Pest des petites ruminant (PPR) is an acute viral disease of small ruminants characterized by fever, oculo-nasal discharges, stomatitis, diarrhea and pneumonia with foul offensive breath. PPRV is transmitted mainly by aerosols between animals living in close contact **(Lefevre and Diallo, 1990).**Virus replication in cultures suspension containing serum was reported and reviewed by **Westwood *et al.*, (I960).** Since Vero cells are currently considered as an acceptable cell substrate to produce a wide range of viruses, a virus production platform was developed using Vero cells adapted to grow in suspension in serum-free media.

After adapting anchorage-dependent Vero cells to grow as a free-cell suspension, vesicular stomatitis virus, herpes simplex virus-1 and polio virus-1 production rates were evaluated in batch cultures using spinner flasks and perfused cultures in a bioreactor. The achieved results constitute valuable information for the development of a low-cost high-productivity process using a suspension culture of Vero cells to produce viral vaccines **(Paillet *et al.*, 2009).** Higher virus titres were obtained on BHK21 rather than LLCMK2 cells when the viruses were added to cell suspension **(Mayling *et al.*, 2005).**Virulence and biological properties of the virus can be detected by titration method **(Mariner *et al.* 1990).** Vero cells have been the most commonly used host for isolation and cultivation of morbilliviruses including PPRV **(Diallo *et al.*, 1989a).**

VERO cells and some lines of primary bovine kidney are sensitive to PPR virus multiplication where the cytopathic effects develop after four days **(rossiter *et al.*, 1985; rossiter and wardley 1985). Asim *et al.*, (2009)** mentioned that VERO Cells were found to be healthy till day 3 post inoculation. On fourth day, initiation of cytopathic effects was observed in the form of rounding of the cells which progressed gradually to aggregation of cells leading to formation of syncytia. Infected cells were first harvested when CPE was 60% on day six post inoculation, while second harvest was taken when more than 80% CPE was present. This work was planned for assessment the best time of inoculation of PPR virus in Vero cell culture to obtain the highest virus titer providing intensive production of vaccine.

MATERIALS AND METHODS

Attenuated pest des petites ruminant's virus (PPRV)

Attenuated strain of PPR virus (Nigerian Strain 75/1) **(Diallo *et al.*, 1989b)** was obtained from Rinder Pest Research Department, Veterinary Serum and Vaccine Research Institute, Abbasia Cairo, and used for vaccine preparation as well as for carrying out viral titration test.

Tissue culture

Vero cells were seeded at a density of 2 ×10^4 cells/cm2 into prescription flasks. These cells were propagated and supplied by the same department using Dulbecc's minimum essential medium (MEM) prepared according to the manufacturer's instructions. The medium was supplemented with 10 % new born calf serum.

Determination of ideal time to virus infection in Vero cell culture

Six groups of cell culture prescription flasks were cultured with Vero cells where the 1st group was kept as cell control (control negative)and the other 5 groups were infected with PPRV using MOI of 2:1 (2 viruses/cell) at different 0 time, 12; 24; 36 and 48 hours post cell culturing respectively.

Virus titration

Titration of PPR virus was performed to determine the virus titer obtained in Vero cells at different stages of cell growth. Virus titration was carried out using the micro titer technique as described by **Mariner *et al.*, (1990).**

The best time to obtain the highest titer of PPR virus was determined by titration in Vero cell culture grown by the stationary method. The virus titer was calculated according to **Reed and Muench (1938).**

Growth kinetics curve of PPR virus in VERO cell culture

Growth kinetics curve of PPR virus were studied on the virus obtained from cell culture infected on 24 hours post culturing. Both of the cell free, cell associated and total virus yield titers were determined to detect the best time of virus harvstation to obtain the highest virus titer.

RESULTS

PPR virus titer as affected by the time of cell culturing

The obtained results showed that infection of Vero cells using MOI of 2:1 (2 viruses/cell) at 0 time resulted in a virus titer of 4 log 10 $TCID_{50}$ /ml while cell infection at 12; 24, 36 and 48 hours post culturing induced virus titer of 5; 6; 5 and 5log10 $TCID_{50}$ /ml respectively as shown in table (1).

Growth kinetics curve

The results of growth kinetics curve of PPR virus in Vero cell culture infected after 24 hours of culturing showed that the titer of cell free virus was 1.6 log 10 TC ID50 / ml in the 1st day reached its peak on the 10th day to be 3.3 log 10 $TCID_{50}$ / ml. The cell associated virus titer was 1.2 log 10 $TCID_{50}$ /ml in the 1st day then titer increased gradually reached its peak by the 7th day to become 3.4log 10 $TCID_{50}$/ ml. The total virus yield in the 1st day was 2.5 log 10 $TCID_{50}$/ml and increased gradually reached its peak on the 9th day as 6.7log 10 $TCID_{50}$/ml (table-2 &Fig.1).

Table I: PPR virus titer as affected by the time of all culturing

Item	PPRV titer (log10 $TCID_{50}$/ml) on different time of infection post cell culturing				
TIPCC*	0 time	12 hours	24 hours	36 hours	48 hours
VT**	4	5	6	5	5

*TIPCC= time of infection post cell culturing
**VT= virus titer

Table 2: Growth kinetics of PPR virus in Vero cell culture infected 24 hours post cell culturing

Days post infection	Virus titer (log 10 $TCID_{50}$/ml)		
	Cell free virus	Cell associated virus	Total virus yield
1	1.6	1.2	2.5
2	1.4	2	3.2
3	1.5	2.3	3.5
4	1.4	2.6	3.8
5	1.6	2.5	3.9
6	1.7	3	4.4
7	2.5	3.4	5.7
8	2.9	3.2	5.9
9	3.2	3.3	6.7
10	3.3	2.8	6.2
11	3.1	2.5	5.8

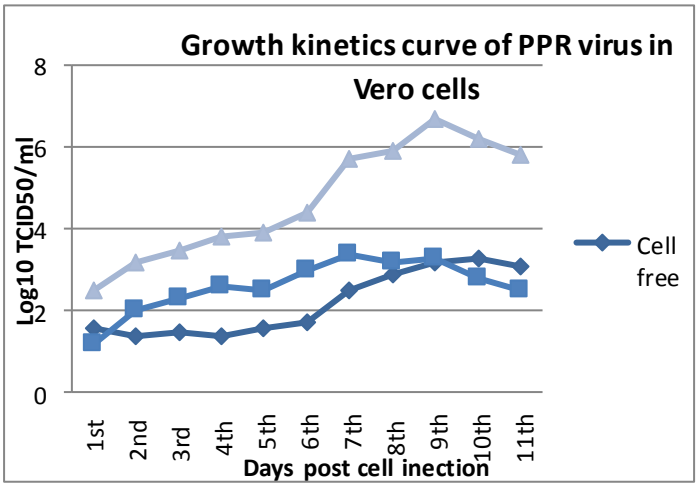

Fig 1: PPR virus growth kinetics in Vero cell culture

DISCUSSION

The replication cycle for different paramyxovirus is similar and the first step is the attachment of the virus on the cell surface and membrane fusion to release a genome into the cell cytoplasm **(Moss and Griffin, 2006).** The H protein is responsible for the attachment of the virus to the cell surface through recognition of and binding to vero cell receptor molecules **(Seki *et al.*, 2003, Adombi *et al.*, 2011).** Attachment of the H protein to receptors activates the fusion activity of the F protein, enabling a fusion of the viral envelope with the vero cell membrane and release of the viral genetic material into the cell cytoplasm. Morbilliviruses replicate solely in the cytoplasm of cells. The research documented in **OIE (2006)** stating that it is sometimes difficult to see the syncytia in Vero cells infected with PPR virus.

The production of virus in cell culture system is dependent upon a number of parameters including the cell density at infection, time of infection and the multiplicity of infection **(Berry *et al.*, 1999).** Parallel to this information we found that the pest time of PPR virus infection in Vero cell line was 24 hour after culturing using an MOI of 2:1 (2 viruses/cell) where the titer of PPR virus reached its peak value of 6 log 10 TC ID50 / ml. These results nearly agree with (**Mohan, 2004; John *et al.*, 2006 and Mohan *et al.*, 2009)**who mentioned that Arasur strain of PPR vaccine virus strain showed CPE after 36 hour post infection and was characterized by cell rounding and formation of syncytia in BHK 21 cells and vero cell.

The results of growth kinetics curve of PPR virus in Vero cell culture infected after 24 hours of culturing were 1.6 log10 $TCID_{50}$, 1.2log10 $TCID_{50}$ and 2.5 log10 $TCID_{50}$ to cell free, cell associated and total virus yield respectively on the1st day (table 2&Fig.1). These titers reached to their beak on the 9^{th} day to be 3.2 log10 TCID50, 3.3 log10 $TCID_{50}$ and 6.7 log10 $TCID_{50}$ to cell free, cell associated and Total virus yield respectively(table 2&Fig.1). In this respect, **Ahmed (1990)** documented the high susceptibility of Vero cells to PPR virus infection showing CPE by 48 post cell infections. In addition, **Osman *et al.*, (1994)** found that the highest PPR virus titer produced by Vero cells was represented by 5.6; 5.7 and 5.4 log10 $TCID_{50}$ /ml for the cell free, cell associated and total PPR virus yield by 72 hours post infection of Vero cell culture.

Moreover, they detected the presence of the virus 12 hours post cell infection through application of direct fluorescent antibody technique. Also the present results are similar to those reported by **Rashwan *et al.*, (2000)** who mentioned that the results of growth curve study of strain 75/1 in vero cell culture declared that the peak of the virus titre (6log10 $TCID_{50}$/ml) was obtained between the 8^{th} and 9^{th} days after inoculation. It was recommended that the minimal required PPR virus titer is 4.5log10 $TCID_{50}$/100 doses (**WHO, 1970).**

CONCLUSION

From this study, we can conclude that the best time of PPR virus inoculation must be applied at 24 hour after sub culturing VERO cell lines to obtain the highest virus titer (6 log10 TCID50 /ml) .which was reached at 9^{th} day post inoculation.

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