

SENSITIVITY OF BHK₂₁ AND VERO CELL LINES IN PRELIMINARY ISOLATION OF BUFFALO POXVIRUS

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Key words : BHK₂₁, Vero cells, isolation, Buffalo poxvirus

Buffalo pox (BP) is a disease of buffaloes (*Bubalis bubalus*) caused by an orthopox virus that is closely related to vaccinia virus. The disease is characterized by pustular lesions on the teats and udder of milch buffaloes and pustular lesions around the mouth of suckling buffalo calves. Adaptations of the causative virus in BHK₂₁ (Mathew and Mathew, 1986), Vero (Chandra *et al.*, 1986), HeLa (Lal, 1975) and chicken embryo fibroblast (Paul *et al.*, 1983) cells have been reported. Sensitivity of BHK₂₁ And Vero cells in the preliminary isolation of buffalo pox virus from scab materials



Some buffaloes from Karur district were collected after three washes in phosphate buffered saline, pH 7.6 (PBS with penicillin, streptomycin) were made into 10 % suspension in MEM + 10 % FCS + antibiotics). Serial ten fold dilutions were made for each sample. Virus titrations were conducted in individual micro titration plates as per the methods described in FAO 118 bulletin (1994) with Vero and BHK₂₁ cells (obtained from Indian Immunologicals Limited, Hyderabad). Final readings were taken on the 4th day post infection (PI). All or none law was used for arriving at the end point.

An outbreak of Buffalo pox was confirmed among buffaloes (Fig.1) at Krishnarayapuram taluk of Karur district in Tamil Nadu by agar gel immunodiffusion test and electron microscopy (Fig.2). Buffalo pox infects naturally buffaloes, white cattle, goats,

horses and human beings (Mathew and Mathew, 1986). Joint FAO/WHO Expert Committee on Zoonoses (1967) has listed buffalo pox as imported zoonoses. Isolation of the in natural host or laboratory animals requires bio-containment facilities. Therefore a cell culture system for the same purpose is more suitable. BHK₂₁ and Vero cells were the two cell lines commonly used in the veterinary laboratory for isolation of ruminant viruses.

Fig.1 Pox lesions observed in udder And Teat of a buffalo

As Vero (Chandra *et al.*, 1986) and BHK₂₁ (Mathew and Mathew, 1986) cell lines have already been used for adaptation of BPV, these two-cell systems were selected to assess their suitability for preliminary isolation of BPV. At 1 in 10 dilution both in Vero and BHK₂₁ cell lines CPE developed in 18 h post infection, which was completed within 72 h post infection for all five-scab materials (Fig.3-5). But when read on the 4th day of infection, the titers were found to be more in BHK₂₁ cells than in Vero cells (Table 1).

Fig. 2 Electron micrograph of buffalo pox virus

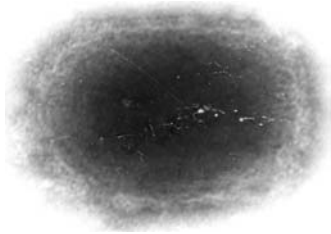


Fig. 4 H&E stained photomicrograph of vero cells infected with buffalo pox virus showing intracytoplasmic inclusions



Though BHK₂₁ and Vero cells have been employed for adaptation of BPV (Chandra *et al.*, 1986; Mathew and Mathew, 1986), their sensitivity in preliminary isolation of BPV has not been reported previously. The virus titer in BHK₂₁ cells was found to range between log 10^{2.5} and log 10^{4.5}, whereas the titer range of Vero was between log 10^{1.0} and log 10^{2.2} and the mean titers for BHK₂₁ and Vero were log 10^{3.4} and log 10^{1.76} respectively. From the results, it was evident that both Vero and BHK₂₁ cells could be used for isolation if scab suspension was used. However, BHK₂₁ cells were found to be more sensitive than Vero cells in the preliminary isolation.

Fig. 5 H&E stained photomicrograph of BHK21 cells infected with BPV showing intracytoplasmic inclusions

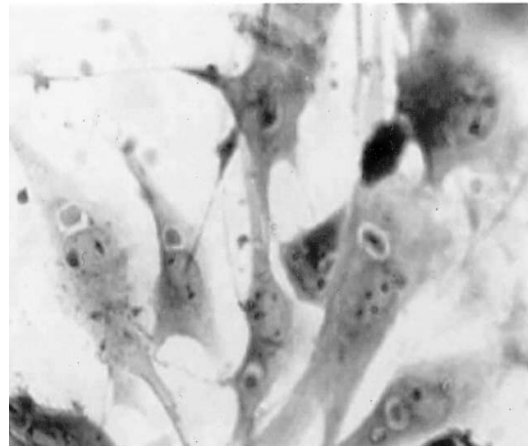


Table 1

Virus titers of BPV seed suspension in BHK₂₁ and Vero cells

Specimen description	BHK ₂₁	Vero
Scab 1	10 ^{4.5}	10 ^{2.1}
Scab 2	10 ^{4.0}	10 ^{2.2}
Scab 3	10 ^{2.5}	10 ^{2.0}
Scab 4	10 ^{3.5}	10 ^{1.5}
Scab 5	10 ^{2.5}	10 ^{1.0}
Titer range	10 ^{2.5-4.5}	10 ^{1.0-2.2}
Mean titer (Arithmetic mean)	10 ^{3.4}	10 ^{1.76}

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