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Pathogenicity study of different avipoxviruses in embryonated chicken eggs and cell cultures

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ABSTRACT

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Avipoxvirus infection, an economically important disease of domestic poultry and wild birds, is re-emerging in the recent past. Fowl poxvirus (FPV), quail poxvirus (QPV), turkey poxvirus (TPV) and pigeon poxvirus (PPV) could be adapted and propagated in chicken embryo fibroblast (CEF) cell culture, chorioallantoic membrane (CAM) of embryonated chicken eggs and QT-35 cell line with optimal titres. Studies were conducted on the pathogenicity of these viruses in these different host systems, which produced characteristic cytopathic effect (CPE) in CEF cell culture and QT-35 cell line viz. presence of intracytoplasmic inclusions in hypertrophied cells and plaque / syncytia formation and the lesions on CAM were distinct pocks, hyperplasia of ectoderm and intracytoplasmic inclusions.

Keywords: Avipoxvirus (APV), chorioallantoic membrane (CAM), chicken embryo fibroblast (CEF), fowl poxvirus (FPV), turkey poxvirus (TPV), quail poxvirus (QPV), pigeon poxvirus (PPV).

INTRODUCTION

Avian pox is an infectious, slow spreading viral disease that has been reported in numerous species of birds including poultry, wild and caged birds[2,5,19]. Avian pox viruses (APVs) are the members of genus *Avipoxvirus* under the subfamily *Chordopoxvirinae* of family *Poxviridae*. These can be grown in embryonated eggs, primary cell culture like chicken embryo fibroblast (CEF) as well as in cell line like QT-35 with optimal titres. The virus grows with high titer in these systems and produces characteristics cytopathic effects (CPE) 3-4 days after infection in cell cultures, and plaques in the chorioallantoic membrane (CAM) 5 days post inoculation[12].

MATERIALS AND METHODS

Viruses: Avian poxviruses (APVs) (Fowl poxvirus- FPV, turkey poxvirus - TPV, Quail poxvirus - QPV and Pigeon poxvirus - PPV) used in the present study were obtained from Virological Laboratory of the Division of Avian Diseases, IVRI. These were isolated separately from natural outbreaks of pox in fowls, turkeys, quails and pigeons as scabs and adapted in chorioallantoic membrane (CAM) of susceptible chicken embryos, chicken embryo fibroblast (CEF) cell culture as well as QT-35 cell line.

Embryonated chicken eggs, primary cell culture and cell line: Eleven-day-old susceptible embryonated chicken eggs were used for preparation of CEF cell cultures as well as for the inoculation of these APVs. Medium-199 (Life Technologies, USA) was used for growing the primary cell cultures. QT-35 cell line was also used for propagation of all the APVs. Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS) served as the growth medium, whereas the same medium with 2% FCS was used as maintenance medium.

Adaptation and propagation of APVs in different host system (CEF, QT-35 and CAM): All the APVs were adapted and propagated in the CEF cell culture[11]. The monolayers in Leighton's tubes were inoculated with 0.1 ml of the viruses. M-199 with 2% FCS was used. Monolayer showing 70-80% cytopathic effect (CPE) after 5-6 days was kept at -20^0C for further passaging. 0.2 ml of viral suspension of all APVs with 2000 units of benzyl penicillin and 200 µg of streptomycin were inoculated into the eggs. Eggs were observed for the presence of pock lesions up to 5 days post inoculation (DPI). The harvested CAM showing pock at highest dilution was passaged further. Confluent monolayers of QT-35 cell line were obtained after 72 hrs post seeding. The infected cells were stained with 1% crystal violet in 20% ethanol for characterization of different strains of poxviruses in cell line. A 10^{-1} dilution of CEF adapted isolates of APVs at their 5^{th} passage level was used for adaptation in QT-35 cell line. The method followed for the propagation of the APVs was same as described for CEF cultures. All the isolates of APVs were given five passages. Infected cell cultures were observed for the type of CPE produced both in unstained and stained preparations.

Virus titration: Titration of all the four APVs was carried out in 96 well microtitre plates using CEF cells[8]. The virus was diluted to 10 folds in MEM (Minimum

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essential media, Sigma Chemicals Co., Louis, USA) with 10% Hank's balanced Salt solution (HBSS). Thereafter, 50 µl of virus suspension was added to each well keeping three wells per replicates for each dilution. The CEF cells were subcultured and 100 µl of cell suspension was added to each well (10^6cells/ml). Three wells were kept as control per plate and the plates were incubated at 37^0C under 5% CO_2 atmosphere. The wells were observed daily for the development of CPE. The titres of the viruses ($TCID^{50}$) were calculated[14]. The virus titration for APVs was also performed on CAM of 11-12 days old embryonated chicken eggs. Virus dilutions were prepared from 10^{-1} to 10^{-6} dilutions in phosphate buffered saline (PBS, pH 7.2). Three eggs per virus dilution were taken and 0.1 ml per dilution was inoculated in each egg. The eggs were candled daily for 6 days to check mortality, if any. Finally, the eggs were opened after chilling and checked on 7th day for the presence of characteristic pock lesions on CAM and the titre of the viruses (EID^{50}) was calculated[14].

RESULTS AND DISCUSSION

The four strains of avipoxviruses (APVs) viz. FPV, TPV, QPV and PPV were passaged in susceptible embryonated chicken eggs, CEF cell culture and QT-35 cell line for 5 times. These showed edematous thickening and diffused pock lesions in CAM at higher dilutions ($10^{-4}/10^{-5}$) of second passage level, while clear and distinct pock lesions were observed at the lower dilutions ($10^{-1}/10^{-2}$) of the same passage level. Large sized diffused pocks having diameter of about 4-6 mm were observed in case of FPV, QPV and PPV, while TPV isolates produced small sized (2-3 mm) distinct thickened and oedematous pocks (Fig. 1). These observations are in congruence with the earlier reports[7,16]. Different APVs (FPV, TPV, QPV and PPV) strains were found to have titre of $10^{6.4}$ EID_{50}/ml, $10^{5.0}$ EID_{50}/ml, $10^{4.5}$ EID_{50}/ml and $10^{5.5}$ EID_{50}/ml, respectively, at 5th passage level in CAM.

On histopathological examination, all the four strains of APVs revealed hyperplasia, hypertrophy and vacuolation of ectodermal cells with congestion of blood vessels. Presence of intracytoplasmic inclusion bodies and infiltration of large number of lymphocytes in the ectodermal cells was seen by 48-72 hrs PI. Marked vacuolation along with hyperplasia and hypertrophy of ectodermal cells was observed at 4th day while proliferation of mesodermal cells followed by nets of large epithelial cells containing inclusions were seen on 5-6th day of infection in the interstitial cells of CAMs (Fig. 2, 3). For the FPV vaccine strain the similar changes have been reported but the quail poxvirus produced clear pock lesions at 5th passage level[13]. Our finding is also in agreement with the earlier findings[16,18]. In case of QPV, diffused pock lesions (8-10 mm) with extensive thickening along with edema were observed in the center of CAMs of embryonated chicken eggs while the periphery of CAMs showed clear and distinct foci. The PPV also showed the similar type of oedematous thickening but was less diffused (4-6 mm). This finding corroborated with those of earlier workers[9,12,16]. However, plaque morphology and lesions produced on CAM have not been reported to be suitable and reliable methods for differentiation of APV strains[18].

CEF adapted APVs showed titers of $10^{4.5}$ $TCID_{50}$/ml, $10^{5.0}$ $TCID_{50}$/ml, $10^{6.0}$ $TCID_{50}$/ml and $10^{6.5}$ $TCID_{50}$/ml with FPV, TPV, QPV and PPV, respectively. Characteristic changes consisting of aggregation of cells, syncytia formation and intracytoplasmic inclusions were seen on the 2nd - 3rd dayPI. APVs revealed CPE visible by 48-72 hrs PI, reaching with confluent plaque formation and intracytoplasmic inclusion bodies formation by 96 hrs and advancement of cell sheet degeneration could be seen by 5-6 days PI. The monolayer showed 70-80% CPE at about 5th days PI. CPE was observed earlier in case of FPV in comparison to other three strains of APVs. In case of FPV and TPV, the plaques which were formed within 96 and 120 hrs PI, respectively, were of large size and more distinct in the appearance than those of QPV and PPV but the FPV plaques were larger and fuzzy in appearance than those of TPV (Fig. 4, 5). Similar types of plaques have been observed by other workers[1,9] also.

With viruses at higher passage levels, the CPE was observed earlier than that of the lower passage levels, which is similar to that described earlier[16]. Similar findings were reported in case of FPV by many other workers[3,6,17]. TPV and QPV produced CPE somewhat late than FPV and PPV in CEF cell culture system. This might be attributed to strain variation and degree of adaptation or titre of respective viruses before inoculation[1].

A permanent cell line of avian origin QT-35 was also used for the propagation of avipoxviruses. In QT-35, the characteristics CPE consisting of rounding, vacuolation of cells, presence of intracytoplasmic inclusions and finally plaque formation was observed in all the four strains of APVs within 3-4 days post inoculation (Fig. 6). The changes were quite extensive in case of QPV. Similar findings have been observed earlier regarding FPV and QPV[13]. Due to inherent variability of these types of cells, the use of permanent cell line as the laboratory host was considered[15].

The above pathogenicity study indicated that all the four avipoxviruses (APVs) got easily adapted in different host systems viz. CAM, CEF and QT-35 and produced characteristic lesions and changes. The suitability of QT-35, an established cell line, is documented for the Indian isolates of APVs, which can be utilized for large scale production of avipoxviruses.

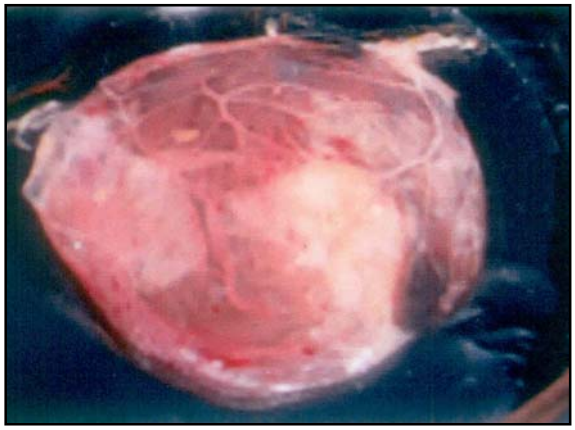

Fig. 1: PPV infected CAM of chicken embryo showing characteristic individual but less diffused pock lesions

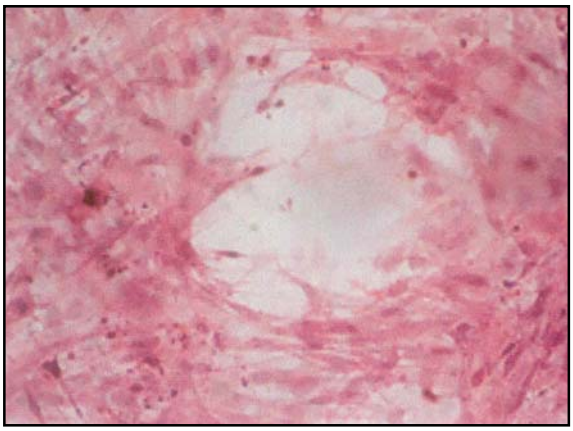

Fig. 4: CEF cell culture infected with TPV at 5th day PI showing extensive cytopathic effects (HE x 100)

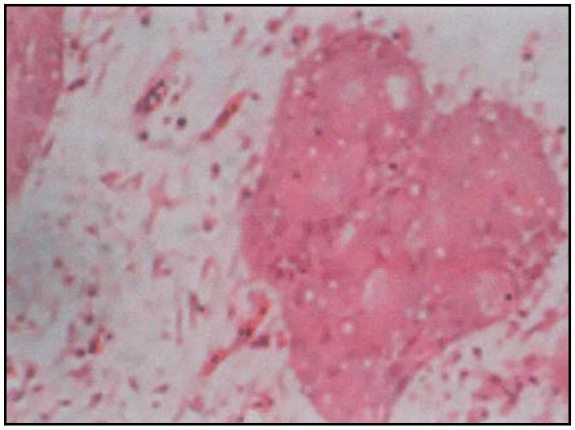

Fig. 2: CAM infected with QPV showing hyperplasia of ectoderm and presence of intracytoplasmic inclusions in the hypertrophied cells (HE x 100)

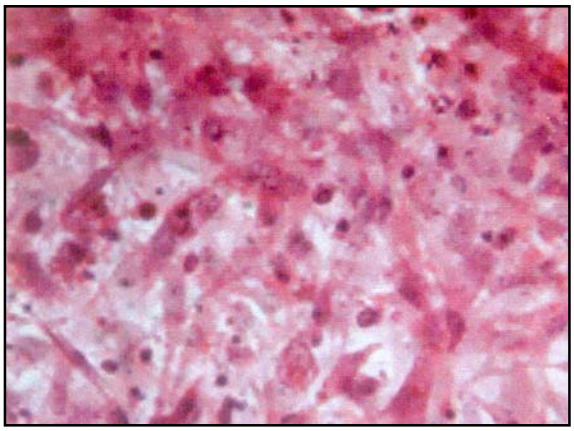

Fig. 5: CEF cell culture infected with PPV at 96 hr PI showing extensive cytopathic effects (HE x 100)

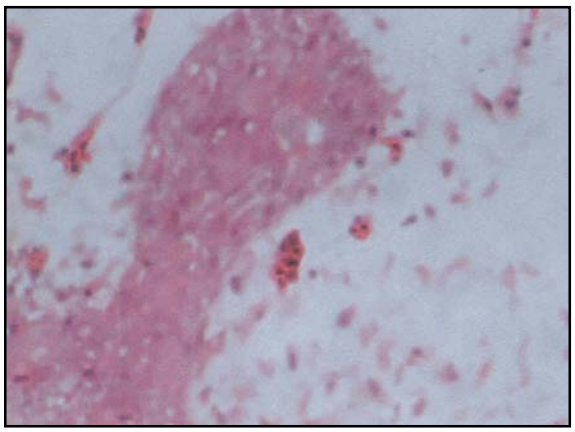

Fig. 3: CAM infected with PPV showing hyperplasia of ectoderm and presence of intracytoplasmic inclusions in the hypertrophied cells (HE x 100)

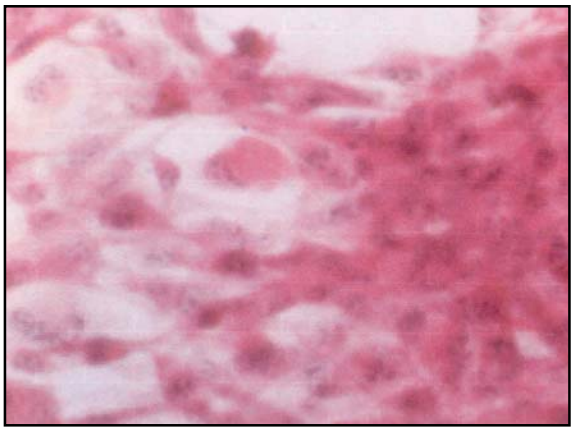

Fig. 6: QT-35 cell line infected with FPV showing plaque formation and intracytoplasmic inclusions at 96 hr PI (HE x 100)

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