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PROPAGATION OF DUCK PLAGUE VIRUS IN DESI DUCK EMBRYO FIBROBLAST CELLS*

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Duck plague or duck viral enteritis is a contagious virus disease of domestic and wild water fowls. The disease is caused by an alpha herpes virus which prefer duck embryos and duck embryofibroblasts for its propagation, particularly during primary isolation (Jensen, 1961; Leibovitz, 1991). During a recent outbreak of this disease in Kerala the virus was isolated in duck embryos. This virus was further passaged in duck embryo fibroblasts and the observations made are presented below.

Materials and methods

Virus

Virus isolated from the liver and spleen of ducks died during an outbreak of duck plague (DP) in Kerala during 1995, at its second passage level in duck embryos was used as the seed material for this study. The infected embryo liver, chorioallantoic membrane and allantoic fluid were pooled and processed as described by Kulkarni (1993) and was inoculated on to duck embryo fibroblast cells.

Duck embryo fibroblast cells

Duck embryo fibroblasts were prepared from 14-day old desi duck embryos received from the University Poultry Farm/purchased locally. The monolayer was prepared using the technique described by Nair (1978). Eagles minimum essential medium with 5% and 2% calf serum was used as the growth medium and maintenance medium respectively. When the monolayer was confluent it was inoculated with the processed material from the infected embryo adsorbed for 45 minutes at room temperature and then incubated at 37°C after refeeding the monolayer with maintenance medium. The monolayers were examined under an inverted microscope at 24 hr intervals. Two coverslips were taken at each time, washed gently in PBS and fixed in methanol/formol saline. These coverslips were subsequently stained with May Grunwald Geimsa or haematoxyline and eosin. Uninfected monolayers were also similarly processed to serve as controls. For infectivity titration, ten fold dilutions of the fifth cell culture passaged material was inoculated onto three tubes of DEF cultures per dilution. These infected monolayers were examined at every 12 hr. interval and the $TCID_{50}$ was calculated as per Reed Muench method.

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Results and discussion

A confluent monolayer of duck embryo fibroblasts was formed within 18-24 hr. The cells were spindle shaped and had the morphological features of fibroblasts (Fig. 1). Following infection, during the initial passages no appreciable changes were noticed. From the third passage onwards changes appeared initially in isolated foci which subsequently spread to the whole monolayer. In these areas the cells were rounded, refractile and had a tendency for clumping and finally the cell layer dropped off the glass surface. Stained cells revealed cytoplasmic balooning, granularity and syncytium formation. The infected cells showed margination of the nuclear chromatin with one or more eosinophilic inclusion bodies each surrounded by a clear halo (Fig. 2). In subsequent passages the CPE appeared much faster involving the whole monolayer within 24 to 36 hr.

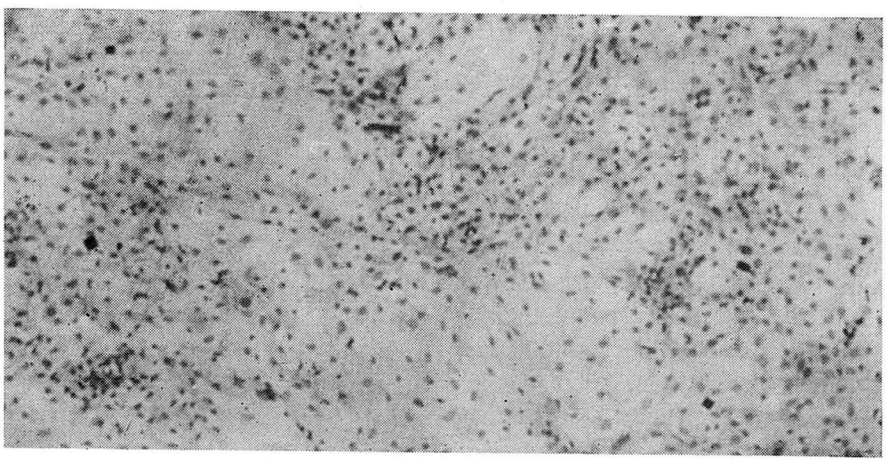

Fig. 1. Normal Duck embryo fibroblast cells x 100

The infectivity titer calculated at the fifth passage level by the Reed and Muench method was $10^{5.25}$/ml.

The cytopathic changes described above for DP virus was similar to the observations made by Kocan (1976) and Panisup *et al.* (1990). Wolf *et al.* (1976) reported that both duck embryo fibroblast cell line CC1-141 and primary cultures of DEF were equally accurate for quantifying the virus, attaining the peak infectivity at about 36 hr. They have also stated that primary cultures yeilded about 5-6 times as much virus as did the cell line. The suitability of primary DEF for the virus yield and sensitivity to infection was also studied and established by Kocan (1976).

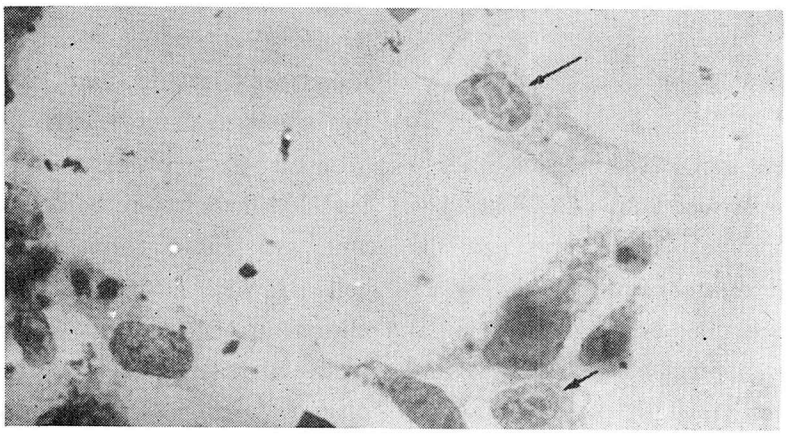

Fig. 2. Syncytium formation with cytoplasmic balooning x 100

Summary

A virulent strain of duck plague virus isolated from a field outbreak of the disease in Kerala was successfully cultivated in desi duck embryo fibroblast cultures. Though the CPE was not evident during the first two passages, characteristic changes were evident from the third passage onwards, with margination of the nuclear chromation and one or more eosinophilic intranuclear inclusion bodies. The $TCID_{50}$ calculated at the 5th passage was $10^{5.25}$/ml.

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