Journal of Veterinary and Animal Sciences. 28 (1997) 61-63

Short Communication

PRIMARY ISOLATION OF NEWCASTLE DISEASE VIRUS IN MDBK CELLINE

Newcastle disease is a dreadful disease of poultry but accurate diagnosis of the disease almost always involves the isolation and characterisation of the virus (Alexander, 1988). For isolation of Newcastle disease virus (NDV) embryonated hens eggs (Beard and Hanson, 1981) and various cell lines like BHK-21 (French and George, 1965), Mouse L - 929 (Alexander *et al.*, 1973 a) MDBK (Alexander *et al.*, 1973 b; King, 1993) Pig kidney (Jully and Hipplito, 1973; Bansal and Kumar, 1975), sheep kidney (Ponkova *et al.*, 1972) and QT-35 (Reddy and Srinivasan, 1991) were used.

An attempt was made to isolate NDV from the intestinal contents of day old chicks belonging to six different commercial hatcheries of Tamilnadu in MDBK cell line and in emryonated hens eggs. Six materials which showed Haemagglutination (HA) with 1% chicken erythrocytes (CRBC) and the HA was inhibited by NDV specific antiserum, were used for this study. MDBK cell line was grown to confluence in milk dilution bottles with a growth medium Dulbecco's modification of MEM Eagle's (Sigma, USA) supplimented with 10% heat inactivated goat serum, 500 IU of pencillin/ml and 2.5 mg of streptomycin/ml. Growth medium with a reduced of serum (2%) served as maintenance medium.

Confluent monolayers were infected with one ml of sample (20% W/V suspension of intestinal contents in phosphate buffered saline were sterilized by filteration through membrance filter of pore size 0.45 μm).. Six different samples were used for infection and infected cells were incubated at 37° C for about one hour for virus absorption. Following that inoculum was decanted, fresh maintenance medium was added and cells were incubated at 37C. Cells were examined periodically for cytopathogenic effects (CPE).

Virus isolation in embryonated hens eggs was carried out as described by Alexander, (1988).

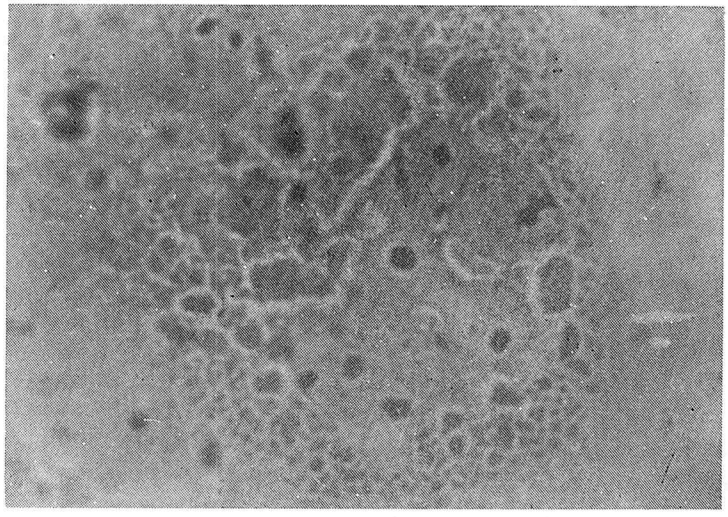
MDBK cell line started producing CPE by 16-20 hours after infection. CPE included multinucleated polykaryocytes (Plate - 1) and intracytoplasmic vacuolation. Cells were harvested by 32 hours after infection by repeated freezing and thawing. Clear cell culture fluids were tested for HA activity with 1% CRBC and HA titre ranged from $3_{log}2$ to $4_{log}2$ and the HA was inhibited by NDV specific antiserum. Inoculated chicken embryos died by 42 hours to 72 hours but clear allantoic fluid (AF) did not show HA activity. But HA activity was revealed after 3-4 serial passages in embryonated hens eggs and the HA was specifically inhibited by NDV antiserum.

Part of Ph.D. thesis of the first author approved by the Tamilnadu Veterinary and Animal Sciences University, Madras (1995).

* Associate Professor, Department of Animal Biotechnology, Madras Veterinary College, Madras - 600 007.

A similar observation was made by Alexander *et al.* (1973 b) in MDBK cell line after infection with Herts' 33 strain of NDV. Viral replication was indicated by the presence of CPE (Syncytia) (King, 1993). In the present study active multiplication of NDV in MDBK cell line was indicated by the consistant CPE induced by the virus. HA activity of the NDV isolates after first passage in MDBK cell line has been reported earlier (King, 1993). Our results correlate with the earlier reports.

In the present study it was seen that MDBK cell line posses an advantage in primary isolates of NDV and easier to maintain than the primary cell culture.



Acknowledgement

First author is thankful to the Council for Scientific and Industrial Research, New Delhi for the senior research fellowship.

Parimal Roy,
A.T. Venugopalan and
K. Kumanan*
Centre for Animal Health Studies
Tamilnadu Veterinary and
Animal Sciences University
Madhavram Milk Colony, Chennai-600 051

References

Alexander, D.J., Hewlett, G., Reeve, P. and Poste, G.(1973 a). Studies on the cytopathic effect of Newcastle disease virus. The cytopathogenicity of strain Herts 33 in five cell types. J. Gen. Virol **21** : 323-337

Alexander, D.J., Reeve, P. and Poste, G. (1973 b). Studies on the cytopathic effects of Newcastle disese virus. DNA synthesis in infected cells. J. Gen. Virol, **18** : 363-373

Alexander, D.J. (1988). Newcastle disease diagnosis in : Newcastle disease Ed. O.J. Alexander, Kluwer Academic pub; Boston pp. 147-160

Bansal, M.P. and Kumar, S. (1975). Effect of propagation in pig kidney cell culture on virulence of some stains or Ranikhet (ND) disease virus. Indian J. Anim. Sci. **45** : 470-473

Beard, C.W. and Hanson, R.P. (1981). Newcastle disease virus in: Diseases of poultry Ed. M.S. Hofstad *et al.* IOWA state University press, Amas, USA

French, E.L. and George, TD. St. (1965). The growth and neutralisation of Newcastle disease virus in BHK-21 tissue cultures. Australian J. Exp. Biol. Med. Sci., **43** : 371-380

Jully, J.R. and Hipplito, O. (1973). Newcastle disease vaccine prepared in kidney swine cells: a new possibility. Vet. Bull **43** : 2086

King, D.J. (1993). Newcastle disease virus passage in MBDK cells as an aid in detection of a virulent sub population. Avian Dis., **37** : 961-969

Ponkova, G.E., Oparain, V.N. and Kalimina, T.M. (1972). Susceptibility of sheep kidney cells of Newcastle disease virus. Cited in Vet. Bull **42** : 4611

Reddy, G.S. and Srinivasan, V.A. (1991). Immunisation of chickens with mesogenic strains of Newcastle disease virus grown in a Japanese quail fibroblast cell line (QT-35) Indian Vet. J., **68** : 907-910