PHYSICO – CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF EGG DROP SYNDROME – 1976 (EDS –'76) VIRUS

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ABSTRACT

The Egg Drop Syndrome – 1976 (EDS –'76) virus isolate was characterized by physico-chemical and biological properties. The isolate withstood the chloroform (15 min), ethyl ether treatments (18 hr) and acidic pH (pH 3) for 1h. The isolate was resistant to 0.5 per cent trypsin treatment for 30 min and stable at 56°c for 40 min. The isolate lost its infectivity titer but not the HA activity when treated with 0.3 per cent formalin for 1h. The biological property was studied by infecting the isolate in duck embryo fibroblast monolayer and by appearance of cytopathogenicity like rounding and refractile cells. The peak CPE was noticed between 72 and 96 h post infection.

KEY WORDS : Egg Drop Syndrome – 1976 (EDS –'76) virus, Avian adenovirus, Physico- chemical characters, Duck embryo fibroblast culture.

INTRODUCTION

India has been experiencing a tremendous growth and development of poultry industry. The drop in egg production in layer birds has become a major concern in India. The losses incurred by poultry industry due to reduced productivity, culling and cost of medicine is often considered to be greater than loss due to mortality. Egg drop syndrome – 1976 (EDS –'76) is a major cause for loss of egg production (upto 40 per cent) and laying of thin shelled and shell less eggs by apparently healthy birds. Recently one EDS –'76 isolate was obtained in this area and it became prudent to characterize the same and confirm its presence.

MATERIALS AND METHODS

EDS - 76 virus isolate obtained at Namakkal (1.8 ml allantoic fluid) was mixed with 0.2 ml of chloroform and allowed to stand at room temperature for 15 minutes. Similarly, a mixture of 3 ml of the virus suspension and one ml of ethyl ether as well as untreated control was kept at 4° C for 18 h (Higashihara *et al*., 1983).

The isolate was diluted to 10^{-1} concentration in 1X maintenance medium (pH 3) and incubated at room temperature for 1h along with untreated control and treated with final concentration of 0.5% trypsin solution at 37° C for 30 minutes (Babjee and Spradbrow, 1974 ; Higashihara *et al*., 1983). Viral aliquots were placed at 56° c in water bath and samples were removed after 30, 40 and 60 minutes and exposed to a final concentration of 0.3 per cent formalin for one hour (Walker *et al*., 1982)

HA titre and infectivity in tissue culture before and after the treatments were assesed. The infectivity titer of the virus is expressed in terms of $TCID_{50}$ by the method described by Reed and Muench (1938). Primary cell cultures of Duck Embryo Fibroblast (DEF) were prepared as per the method described by Gulka *et al*. (1981) with slight modification.

The viral isolates at fifth passage level in duck embryos were propagated in DEF primary cell cultures. The tissue culture bottles were examined periodically at 24, 48, 72, 96 h for cytopathological changes.

RESULTS AND DISCUSSION

The chloroform and ethyl ether treatment to the isolate did not cause any reduction in infectivity titer as well as HA titer. This property suggests absence of envelope on the virus (Chetty *et al*., 1988). Though it is a naked virus, it is able to agglutinate RBC of chicken. This property was attributed to 25 nm single fiber projecting from each vertex (McFerran, 1991).

The acidic pH did not produce any effect on the infectivity and HA activity in tissue culture. The Stability to acid pH is one of the well known properties of avian adenoviruses (Adair, *et al*., 1979; Zanella *et al.,* 1980). There is a direct correlation between resistance to lipid solvent and stability to pH 3 among all naked viruses (Chandramohan, 1994).

The isolate was unaffected by 0.5 per cent trypsin treatment. Resistant to treatment of 0.5 % trypsin is a common property to all avian adenoviruses (Babjee and Spradbrow, 1974). However Todd and McNulty (1978) have reported contradictory finding and have reported the virus to be sensitive.

The isolate was stable upto 40 min at 56° C after that gradual reduction in infectivity and HA activity was noticed. This result concurs with the findings of Babjee and Spradbrow (1974). However, Walker *et al.*(1982) reported a rapid loss of infectivity even at 30 minutes.

The isolate was sensitive and lost its infectivity when subjected to 0.3 % formalin for 1 h treatment, however, the HA activity was not affected. This property of the virus could be best made use of while evolving an inactivated vaccine as suggested by Chandramohan (1994).

The resistances of the virus isolate to chloroform, ethyl ether, acidic pH, 0.5 % trypsin and heat inactivation pattern and characteristic CPE in DEF reveals that this is an avian adenovirus.

The isolate produced cytopathic effect (CPE) in duck embryo fibroblast cell culture. The CPE started to appear after 24 hours post infection and reached peak between 72 and 96 h post infection. The CPE was characterized by appearance of refractile round cells followed by their enlargement and eventual detachment from the surface, as also reported by Swain *et al.* (1993). The harvested tissue culture fluid showed a high HA titer of $\log_2$ 15 / 25 ml.

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