GROWTH CHARACTERISTICS OF LOW PATHOGENIC STRAIN OF AVIAN INFLUENZA VIRUS (H5N2) IN DIFFERENT CELL CULTURES


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ABSTRACT

A low pathogenic strain of avian influenza virus (H5N2) was successfully propagated in both primary chicken embryo fibroblast (CEF) cells and cell line of baby hamster kidney (BHK-21), African green monkey kidney (Vero) and Madine Darby canine kidney (MDCK) cells through ten successive passages; the virus induced cytopathic effect (CPE) from the 1st passage characterized by cell rounding, elongation and cell disintegration. The most suitable cell cultures for virus propagation were MDCK and BHK-21 cells that gave the highest titer 107.5 and 107 TCID50/ml respectively at the 10th passage while the highest titer for Vero and CEF cells were 105.5 and 106.5 TCID50/ml respectively at the 10th passage. H5N2 virus was cell free virus with higher titer comparing to the cell associated virus in both BHK-21 and MDCK cell line.

Key Words: avian influenza virus (H5N2), CEF, BHK-21, MDCK

1. INTRODUCTION

Influenza viruses are members of the family Orthomyxoviridae composing of 4 genera, A, B, C and Thogotovirus based on the basis of the nucleocapsid or matrix antigen [1]. However, only type A influenza viruses are able to infect and cause severe disease among variety of avian and mammalian species [2]. Influenza virus is an enveloped virus containing 8 segments of single stranded negative-sense RNA genomes. The envelope contains haemagglutinin (HA) and neuraminidase (NA) proteins. Seventeen serotypes of HA (H1–H17) and nine (N1–N10) of NA have been identified in both mammalian and avian influenza type A [3]. The viral particles are approximately 50-120 nm in diameter for spherical forms [1]. Most laboratory-adapted influenza viruses existing in the spherical morphology of approximately 100 nm in diameter are grown in the cell culture system. However, influenza viruses isolated from the clinical specimens are believed to be predominantly filamentous particles. In addition; the internalization of the filamentous influenza virus particles is delayed according to their spherical particles [4].

Isolation of avian influenza virus (AIV) with the use of embryonated chicken egg (ECE) tends to be costly and requires much forethought concerning scheduling because embryos must be incubated 9–11 days prior to use [5]. In addition; the persistent propagation of AIV in ECEs has been shown to lead to the emergence of mutations in the HA glycoprotein [6] and the harvested ECEs may contain various microbiological contamination and residual endotoxin [7]. Finally, the ability of diagnostic laboratories to maintain a large volume of high-quality avian embryos can