Restriction Enzyme Analysis of Indian Isolates of Egg Drop Syndrome 1976 Virus Recovered from Chicken, Duck and Quail

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ABSTRACT

Egg drop syndrome 1976 (EDS-76) is caused by a haemagglutinating adenovirus belonging to group III of the genus Aviadenovirus in the family Adenoviridae. All isolates are serologically identical, but have been divided into three groups based on restriction endonuclease (RE) analysis. In this study the viral DNA of various Indian EDS-76 viral isolates (CEDS-A, CEDS-B, EDS-M, EDS-M1, EDS-1/AD/86, EDS-KC and QEDS) obtained from different avian species and different geographical regions were digested with restriction endonucleases viz., EcoRI, BamHI, HindIII and PstI. The results showed that one Indian isolate obtained from duck (EDDS-KC) was different from all other chicken and quail counterparts. All other isolates were identical to the reference viral strain BC-14, which belong to group I of EDS-76 viruses. The duck isolate EDS-KC could not be placed in any of the three groups reported earlier.

Keywords: egg drop syndrome 1976, restriction endonuclease analysis

Abbreviations: CEL, chicken embryo liver; CPE, cytopathic effect; EDS-76, egg drop syndrome 1976; RE, restriction endonuclease

INTRODUCTION

Egg drop syndrome 1976 (EDS-76) is an economically important disease characterized by a severe and sudden drop in egg production at the onset or peak egg production time, with a high percentage of shell defects (van Eck et al., 1976; McFerran et al., 1978). The disease is caused by EDS-76 virus belonging to group III avian adenoviruses, which has a dsDNA genome of 33.2 kb. The virus is not serologically related to the conventional group I and group II aviadenoviruses (McFerran, 1981; Dhinakar Raj et al., 2001). EDS-76 virus is the sole member of group III having haemagglutinating properties for fowl erythrocytes to high titres (Adair et al., 1979). It has been suggested that EDS-76 virus represents an intermediate between mammalian and avian adenoviruses (Hess et al., 1997).
Only one serotype has been recognized (Darbyshire and Peters, 1980; Yamaguchi et al., 1981, Dhinakar Raj et al., 2001). However, three different groups were assigned on the basis of restriction endonuclease analysis of the viral genome (Todd et al., 1988).

Group I included isolates obtained over an 11-year period from infected European chickens. Group II consisted of viruses isolated from ducks in the UK and group III comprised of one chicken isolate obtained from Australia.

This disease is a major problem in many intensive poultry rearing areas in spite of the practice of regular vaccination in India (Das and Pradhan, 1992; Das et al., 1995; Shaw et al., 1995). The virus was isolated from quails experiencing the disease condition as well as from chicken and ducks (Das and Pradhan, 1992). By partial sequencing of the hexon gene of five Indian EDS-76 virus isolates, Dhinakar Raj and colleagues (2001) found a maximum difference of 4.6% in one of the isolates and suggested the possibility of variation due to repeated passage in ducks. The present study was aimed to compare serologically identical Indian EDS-76 viral isolates obtained from chicken, duck and quail using restriction endonuclease analysis of the viral genome.

MATERIALS AND METHODS

Seven isolates of EDS-76 virus, CEDS-A, CEDS-B, EDS-M, EDS-ML, EDS-1/AD/86, EDS-KC and QEDS (Table I) were used in the study. They were maintained in the viral diseases laboratory of the Avian Disease Division of IVRI. The reference viral strain BC-14 was also used.

All the EDS-76 viral isolates were propagated in chicken embryo liver (CEL) cell culture. CEL cell culture was prepared following the method of Adair and colleagues (1979) with some modifications. Briefly, liver was removed aseptically from 15-day-old chicken embryos, minced with scissors, and trypsinized gently using trypsin versene (TV). After filtering through muslin cloth, the cells in the filtrate were pelleted by centrifugation (800g/10 min). The cell pellet was washed once and resuspended in growth medium (M-199 containing 15% calf serum). The cells were dispensed into culture flasks and incubated at 37°C. The monolayer was infected with virus and observed daily for the onset of cytopathic effect (CPE).

The viral DNA of EDS-76 virus propagated in CEL cell culture was extracted following the method of Shinagawa and colleagues (1983) with some modification. When the infected CEL cell monolayer showed 60% CPE, the medium was decanted and the monolayer cells were resuspended in TE buffer (10 mmol/L Tris; 1 mmol/L EDTA). NaCl (5 ml) and 10% sodium dodecyl sulphate (SDS) were added to final concentrations of 1 mol/L and 0.5%, respectively. The contents were mixed well and incubated at 4°C overnight. The lysate was extracted with equal volume of phenol (pH 8.0) and the aqueous phase was discarded. The phenolic phase was extracted twice with an equal volume of TE buffer. The protein interphase was precipitated by adding 1.5 vol absolute ethanol and kept at –20°C overnight. The protein was pelleted by centrifugation (1800g/10 min) and the pellet was washed twice using ethanol. After air drying, the pellet was resuspended in TE buffer containing 100 mmol/L NaCl and

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