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Detection of egg drop syndrome virus antigen or genome by enzyme-linked immunosorbent assay or polymerase chain reaction

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Mouse monoclonal antibodies (mAbs) were produced against an Indian isolate of egg drop syndrome (EDS) virus and characterized. Four hybridoma clones were secreting mAbs that bound to a 100 kDa protein, presumably the hexon protein. These mAbs were found to cross-react with two other Indian isolates of EDS virus and to the reference UK 127 strain. Three of these mAbs were mapped to the same epitope compared with the other mAb (F8), which bound to a different epitope. An antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) was developed using the F8 mAbs as capture antibody and polyclonal chicken serum against EDS virus as detection antibody. A polymerase chain reaction (PCR) was used to detect the EDS viral genome. Following experimental infection of oestrogen-treated chickens with EDS virus, cloacal swabs, oviduct, uterus and spleen were collected at different days post-infection and used in both AC-ELISA and PCR, directly and after a single passage in embryonated duck eggs. The sensitivity and specificity of antigen detection by AC-ELISA or PCR was 95% and 98%, respectively. For diagnosis of EDS viral infections, PCR is recommended due to its ease and the lack of requirement of prepared reagents such as mAbs or conjugates. We recommend that PCR be performed directly on boiled tissue homogenates. Any negative samples may be passaged in embryonated duck eggs and the allantoic fluids tested by PCR before a conclusive negative diagnosis is given.

Introduction

The family *Adenoviridae* is divided into two genera, the *Mastadenoviruses* infecting mammals and the *Aviadenoviruses* infecting birds. The egg drop syndrome (EDS) virus is the sole member of group III avian adenoviruses. It is serologically unrelated to group I viruses from chickens (i.e. the conventional fowl adenoviruses (FAV) serotypes 1 to 12) and group II avian adenoviruses such as the haemorrhagic enteritis virus of turkeys, the avian adenovirus spleenomegaly virus of chickens and marble spleen disease of pheasants. EDS, a condition resulting in a drastic reduction in egg production and deterioration in the quality of eggshells, emerged in the mid-1970s throughout the world (Baxendale, 1978). Although the disease affected broiler breeder chicken flocks almost exclusively, the adenovirus responsible was found to commonly infect ducks (Calnek, 1978), and the taxonomic name of EDS virus is duck adenovirus 1 (Benko *et al.*, 2000). Antibodies to EDS virus have been found in a number of wild and domestic bird species (Kaleta *et al.*, 1980; Bartha *et al.*, 1982), and this virus has recently been shown to be involved in acute respiratory disease of goslings (Ivanics *et al.*, 2001).

Serological evidence of EDS virus infections in India has been found in chickens and quail (Das *et al.*, 1995; Shaw *et al.*, 1995), and some of the Indian isolates have been characterized genotypi-

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