The Use of PCR Combined with Restriction Enzyme Analysis to Characterize Fowl Adenovirus Field Isolates from Northern India

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ABSTRACT

Ten fowl adenoviruses (FAVs), isolated from suspected cases of inclusion body hepatitis (IBH) in quails and broilers, were characterized by a hexon-based polymerase chain reaction (PCR) combined with restriction enzyme analysis (REA) of the amplified DNA fragments. All the isolates could be detected using H1/H2 and H3/H4 primer sets. Amplification of DNA with H1/H2 and H3/H4 primer sets resulted in fragments of approximately 1219 bp and 1319 bp, respectively. HpaII digestion of the H1/H2 PCR products and HpaII digestion of the H3/H4 PCR products characterized all the isolates in FAV groups, known from genomic typing using the whole DNA. For some of the isolates, neutralization tests were used to confirm these results. The results revealed that, as well as FAV serotype 1, which is the sole member of DNA group A, FAVs of DNA group E are also associated with IBH in poultry in northern India. The FAV specific PCR combined with REA was found to be very useful in investigating the epidemiological situation in the field. It was even possible to define mixed infections with more than one FAV.

Keywords: adenovirus, chickens, diagnosis, DNA groups, epidemiology, inclusion body hepatitis, quails, serotype

Abbreviations: CEL, chicken embryo liver; DNA, deoxyribonucleic acid; FAV, fowl adenovirus; IBH, inclusion body hepatitis; PCR, polymerase chain reaction; REA, restriction enzyme analysis

INTRODUCTION

Fowl adenoviruses are very widespread in chickens and can be isolated from both apparently healthy and diseased chickens (McFerran, 1997). Inclusion body hepatitis of chickens is a sporadic disease condition caused by several serotypes of fowl adenoviruses (Monreal, 1992; McFerran, 1997). In India, serotype 1 of FAV has been found to be associated with field outbreaks of IBH (Grewal et al., 1981; Nagal et al., 1990; Singh and Oberoi, 1994; Singh et al., 1996). Results of these studies are mainly based on available diagnostic methods for FAV, such as isolation in cell culture,
immunodiffusion, immunofluorescence techniques, enzyme-linked immunosorbent assay, and electron microscopy, none of which allows an isolate to be assigned to a precise serotype (Hess, 2000). This can only be done by neutralization assays in cell cultures, according to standard methods (Grimes et al., 1976; Monreal et al., 1980). Recently, Raue and Hess (1998) developed a more rapid and sensitive, FAV-specific PCR, which is combined with restriction enzyme analysis, for the detection and serotypic identification of FAV isolates. The present study was conducted to verify the ability of this method to detect FAV field isolates associated with suspected outbreaks of IBH in India.

MATERIALS AND METHODS

Source of virus material

The history of the FAV isolates used in this study was described earlier in connection with field outbreaks of IBH in quails (Grewal et al., 1994; Singh et al., 1995) and broiler chicks (Singh and Oberoi, 1994; Singh et al., 1996). All the isolates were grown in chicken embryo liver (CEL) cell cultures, prepared from 12- to 13-day-old chicken embryos (Northern Hatcheries Pvt Ltd, Ludhiana, India). Each isolate was given three passages in CEL cell culture, after which the culture fluid was tested by counter-immunoelectrophoresis (Oberoi et al., 1990) and dot enzyme-linked immunosorbent assay (Khanna et al., 1992) for the presence of FAV group antigens. The details of these isolates are given in Table I.

Serotyping

Serotyping was performed on isolates PL-2, PL-3 and PL-4, using the microneutralization test in a 96-well tissue culture plate, as described previously (Grimes et al., 1976). These isolates were then tested against standard rabbit serum against FAV-1, FAV-2, FAV-3 and FAV-4 (received from Dr Pei W. Chang, Department of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, RI, USA). The virus–serum mixture was allowed to react at 37°C for 2 h and titrated along with untreated virus in a 96-well tissue culture plate. A fourfold or greater decrease in the viral titre was taken as the criterion for virus neutralization.

PCR and restriction enzyme analysis

DNA was extracted from infected liver cell cultures for each isolate using a commercially available QIAamp Tissue Kit (Qiagen, Hilden, Germany) and following the manufacturer’s recommendations. The DNA concentration was determined fluorometrically (VersaFlour, BioRad, Munich, Germany) at 260 nm. For use in PCR, the volume containing 50 ng DNA was then calculated. The purified DNA