

# MOLECULAR DETECTION OF CANINE ADENOVIRUS USING POLYMERASE CHAIN REACTION AND SEQUENCING

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## ABSTRACT

*A total of 32 faecal samples were initially screened for canine adenovirus using hemagglutination test. The field samples were adapted to MDCK cell line. The infected culture fluid was subjected to PCR using a common primers for rapid diagnosis and differentiation of CAV1 and CAV2. Three samples yielded the PCR amplicon size of 508 bp which indicates that these isolates belongs to CAV1. Sequencing of PCR products of these isolates were done which showed 99 per cent homology with other CAV type1 isolates.*

**Keywords:** canine adenovirus, polymerase chain reaction, sequencing

## MATERIALS AND METHODS

### INTRODUCTION

Two types of canine adenoviruses, canine adenovirus type1 and canine adenovirus type2 cause canine hepatitis and infectious laryngotracheitis in dogs. CAV1 replicates in vascular endothelium and cause generalized infection characterized by hepatitis. CAV2 replicates in respiratory epithelium and associated with respiratory disease. Diagnosis and identification of CAV1 and CAV2 are usually based on virus isolation which is time consuming and laborious. Likewise, CAV1 and CAV2 can be difficult to differentiate in the laboratory by haemagglutination and neutralization test, especially when the infection occurs in the digestive tract. Hence, a rapid diagnosis as well as differentiation was done using a polymerase chain reaction. Sequencing of amplified PCR product was done to confirm the type of canine adenovirus.

A total of 32 faecal samples were collected from out patient ward of clinics, Madras Veterinary College, Chennai. Faecal samples were dispersed in 0.5 ml of sterile PBS and centrifuged at 3000 rpm for 15 minutes. The supernatant were collected and tested for the presence of virus using haemagglutination test with Human O blood group (Kumanan *et al.*, 1997). The samples positive by HA test was used as inoculum for infecting MDCK cells (National Centre for Cell Sciences, Pune). The samples were passaged 5 times in MDCK cells.

DNA was extracted from CAV infected culture fluid, uninfected culture fluid, canine parvo virus and canine distemper virus (Linne, 1992) and subjected to PCR using one common primer for detecting both CAV1 and CAV2. The primer sequences and thermal cyclic conditions already described by Hu *et al.*, 2001 were used in this study.

The primer sequences were:

HA1-5' CGCGCTGAACATTACTACCTTGTC -3'

HA2- 5'-CCTAGAGCAC TTCGTGTCGGCTT - 3'

The cycle condition consisted of initial denaturation 95°C for 5min; 32 cycles of denaturation 96°C for 30 sec; annealing 58°C for 1min; extension 72°C 1min and final extension 72°C for 5min. The PCR products were electrophoresed in 1.5 % agarose gel at 80V 2hrs and examined under UV transilluminator.

The PCR products were purified using a column purification kit (Life Technologies, USA) and the purified PCR products were subjected to automatic sequencer (ABI, Switzerland ) available in our department. The nucleotide sequence data were submitted to GenBank (Accession No.EF-090910). The homology search against all the known canine adenovirus sequences was carried out using the Blast N/t Programme (National Centre for Biotechnology Information, USA).

## RESULTS AND DISCUSSION

The four samples showed HA titres above 1 in 16 were taken as positive as reported by Kumanan et al. (1997). The characteristic CPE were observed in MDCK cells only in 3 samples after 3<sup>rd</sup> passage onwards. The infected culture fluids of 3 isolates were found positive by PCR amplification of E3 gene. No amplification was observed in uninfected negative control as well as other canine virus DNA samples. This indicates that primers were found to be highly specific to canine adenovirus. All the three samples gave the amplicon size of 508 bp in agarose gel electrophoresis (Fig 1). The size of the PCR products clearly indicates that these isolates belongs to CAV1. Based on blast N analysis the nucleotide sequence data revealed that our local CAV isolates were possessed 99 percentage

of homology with other CAV-1 isolates.

The complete sequences of both CAV-1 and CAV-2 have been published by many workers (Morrison et al., 1997; Campbell and Zhao, 1996). There is about 75% homology between CAV-1 and CAV-2. The open reading frame of the E3 regions of CAV-1 and CAV-2 encodes a 22 kDa and a 40.7 kDa polypeptide, respectively and an additional region of about 510 nucleotides is present in CAV-2. This region was considered to contribute to the observed biological differences between CAV -1 and CAV- 2 (Linne, 1992). Pair of common primers in the E3 and flanking regions for both CAV-1 and CAV-2 used in polymerase chain reaction, so as to provide a relatively simple method for diagnosing and differentiating the two viruses. The optimum condition are needed to get accurate results in PCR assay, sequencing and blast analysis was done to confirm the PCR products which showed the high percentage of homology only with CAV1. In conclusion, only CAV-1 alone was found in the fecal samples.

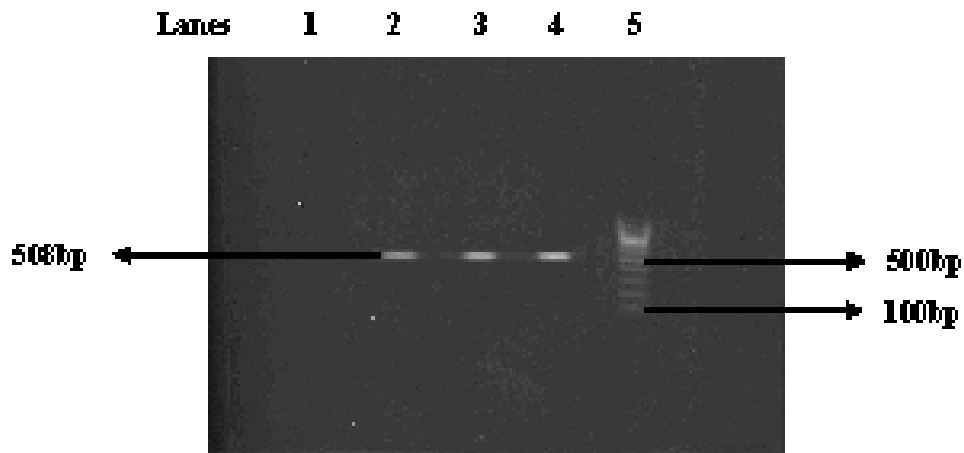
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**Fig.1 - PCR Amplification of E3-gene of Canine Type-1 Adenovirus**



**Lane 1 – Negative control**

**Lane 2, 3, 4 – Canine Adenovirus positive samples**

**Lane 5 - 100bp Marker.**