NUCLEOTIDE SEQUENCE ANALYSIS OF THE L1 LOOP VARIABLE REGION OF HEXON GENE OF FOWL ADENOVIRUS 4 ISOLATES FROM INDIA

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Summary. – Three fowl adenovirus 4 (FAV4) isolates from chicken and one from quail, all from Tamil Nadu, India were analyzed. The L1 loop variable region of hexon gene of these isolates was amplified by PCR and sequenced. The nucleotide sequences (442 bp) and deduced amino acid sequences of the four isolates were compared with those of other isolates of FAV4. The nucleotide sequences of the four isolates had a 98% homology with other Indian isolates and a 96% homology with Belgian and Russian isolates. The amino acid sequences of the four Indian isolates had a more than 98% homology with other Indian isolates and a more than 92% homology with Belgian and Russian isolates. Hence, the variable of L1 loop region of hexon gene was found to be highly homologous in all the FAV4 isolates tested both at nucleotide and amino acid level.

Key words: Fowl adenovirus 4; hexon gene; hydropericardium syndrome; Indian isolates; L1 loop; PCR; nucleotide sequencing; phylogenetic analysis

Introduction

Hydropericardium syndrome (HPS) has been reported first from Pakistan (Khawaja *et al*., 1988). It was mainly seen in 3–5-weeks-old commercial broiler chicks and was characterized by a swollen pericardial sac filled with straw-coloured fluid with no clinical signs except sudden death and with mortality of up to 60–70%. (Ahmed *et al*., 1989). In northern India, HPS occurred with a mortality of 10–60% (Sreenivas Gowda and Satyanarayana, 1994). The disease has been reported from different parts of the world (Abdul-Aziz and A1-Attar, 1991; Borisov *et al*., 1997; Mazaheri *et al*., 1998). Based on serological evidence the HPS agent is fowl adenovirus 4 (FAV4) (the *Fowl adenovirus A* species, the *Aviadenovirus* genus). The so far published nucleotide sequences of various fowl adenoviruses showed that a serotypical variability is present in the hexon gene (Ganesh *et al*., 2001). Therefore, in order to characterize FAV4 at molecular level it is necessary to localize its variable gene region. Hence this study was aimed at comparing the nucleotide sequences of recent Indian FAV4 isolates with those of other FAV4 isolates.

Materials and Methods

Virus isolation. Liver samples from chicken or quails suspected for HPS infection were collected from the Namakkal and Trichy Districts in Tamil Nadu, India. A 20% homogenate was prepared by blending the liver in sterile saline and centrifuging at 4,000 x g for 15 mins. The supernatant was filtered through 0.45 µm disposable syringe filter. The filtrate was used as inoculum for experimental infection and in PCR as template. The liver from experimentally infected birds was tested by an agar gel immunodiffusion (AGID) test using a hyperimmune serum raised in chicken against the HPS vaccine strain (Ventri biologicals, India)

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Abbreviations: AGID = agar gel immunodiffusion test; FAV4 = Fowl adenovirus 4; HPS = Hydropericardium syndrome virus

Table 1. Indian FAV 4 isolates analyzed in this study

Isolate	Origin	Place	Year	GenBank Acc. No.
FAV4-1	Quail	Trichy, Tamil Nadu	2003	AY315183
FAV4-2	Chicken	Trichy, Tamil Nadu	2003	AY315184
FAV4-3	Chicken	Namakkal, Tamil Nadu	2003	AY315185
FAV4 4	Chicken	Namakkal Tamil Nadu	1998	AY315186

(Crowle, 1973). The FAV4 isolates obtained (Table 1) were identified at the Central Veterinary Laboratory, Weybridge, UK.

PCR. Twenty 20 µl of a supernatant from infected liver homogenate was boiled at 100°C for 10 mins. Uninfected liver homogenate served as control. The denatured samples were subjected to PCR (Raue and Hess, 1998; Parthiban *et al*., 2004) using a PCR kit (Biogene, USA).The reaction mixture (50 µl) consisted of 5 µl of sample DNA, 5 µl of the 10x buffer, 1 µl of dNTPs (10 mmol/l each), 1 µl (125 pmoles) of the forward primer H1 (5'-TGGA GATGGGGGCGACCTA-3'), 1 µl (125 pmoles) of the reverse primer H2 (5'-AAGGGATTGACGTTGTCCA-3'), 0.5 µl of Taq DNA polymerase (2.5 U), and 33.5 µl of nuclease-free water. The reaction proceeded in 30 cycles in a thermal cycler (Eppendorf). Each cycle consisted of 94°C/1 min (denaturation), 60°C/1 min (annealing), 72°C/90 secs (extension), and 72°C/5 mins (final extension). The PCR products were electrophoresed in 0.75% agarose gels at 70 V for 2 hrs and examined under UV light.

Nucleotide sequencing. The PCR products were purified using a column purification kit (Life Technologies, USA) and after checking by electrophoresis in 0.75% agarose gels, they were subjected to nucleotide sequencing at Bangalore Genei Pvt Ltd, India. The nucleotide sequence data were submitted to GenBank. Their Acc. Nos are shown in Table 1. The homology search against all the known FAV4 sequences was carried out using the BlastN and FastA Programme (National Centre for Biotechnology Information, USA). The multiple nucleotide sequence alignment of the 442 bp region of the four local FAV 4 isolates with those of other FAV4 isolates were done using the MEGA software package. The phylogenetic analysis of all the so far sequenced FAV4 isolates was done using the Clustal 1.8 and Phylip software packages.

Results and Discussion

The hexon gene based PCR produced from the four FAV4 isolates an amplicon of expected size (1219 bp) but none was obtained with the uninfected control. The nucleotide sequence homology between the four FAV4 isolates with other isolates is presented in Table 2. The respective deduced amino acid sequences of the four Indian and other FAV4 isolates were compared (Fig. 1). The phylogenetic relationships of all so far known FAV4 isolates is presented in Fig. 2.

In PCR analysis, the hexon gene has been used for majority of avian adenoviruses (Hess, 2000). The hexon gene consists of conserved pedestal regions P1 and P2 and variable loop regions L1, L2 and L4 (Toogood *et al*., 1989). Major amino acid changes have been observed frequently

Table 2. The variable hexon gene L1 loop region homology between the four Indian and other FAV4 isolates

Isolate	India 1	India 2	KR95 Russia	506 Belgium	KR5 Belgium	ATCC829 Belgium
FAV4-1	99.80%	98.30%	98.30%	97.20%	96.97%	96.97%
FAV4-2	99.60%	98.00%	98.00%	97.40%	96.80%	96.80%
FAV4-3	99.80%	98.30%	98.30%	97.20%	96.97%	96.97%
FAV4-4	100%	98.50%	98.50%	97.83%	97.20%	97.20%

in the variable loop region of the hexon gene (Roberts *et al*., 1986). The exposed capsid surface is formed by a variable loop region of the hexon gene. Various fowl adenoviruses may exist due to a different capsid structure formed by the hexon gene (Toogood and Hay, 1988). PCR amplification of the hexon gene combined with restriction analysis has been employed for differentiation of 12 fowl adenoviruses (Meulemans *et al*., 2001). Similarly, PCR amplification of the hexon gene followed by restriction analysis has been used to find out the variation between the reference strain and field isolates of FAV4 (Toro *et al*., 1999).

A complete nucleotide sequences of the hexon gene of FAV1, FAV8 and FAV9 have been obtained (Chiocca *et al*., 1996; Sheppard *et al*., 1995; Cao *et al*., 1998). In the present study, a variable region of the L1 loop of the hexon gene of four recent Indian FAV4 isolates was amplified by PCR and sequenced to assess its variability. Among these isolates, only a few silent nucleotide changes were found. At the nucleotide level, these isolates had a more than 98% homology with other Indian isolates, while they had a more than 96% homology with other isolates. Hence, the present study revealed a high level of homology in the variable region of the hexon gene within FAV4. On the other hand, FAV4 was homologous in 91% to FAV9, in 51% to FAV1, and in 46–48% to other fowl adenoviruses at amino acid level (Meulemans *et al*., 2001).

The amino acid sequences of the four Indian FAV4 isolates possessed a more than 98% homology with other Indian isolates and a more than 92% homology with Belgian and Russian isolates. By phylogenetic analysis, the Indian isolates from 2003 (FAV4-1, FAV4-2 and FAV4-3) formed one cluster and differed from FAV4-4 isolate from 1998 which was close to other Indian isolates obtained before 2001. As recent Indian isolates did not differ in amino acid sequences from those obtained earlier, their biological properties such as antigenicity and pathogenicity were not altered. As only the variable region of the hexon gene loop L1 was analyzed in this study, other parts of the variable region of hexon gene should be analyzed to identify a suitable region for characterizing FAV4.

In conclusion, the variable region of hexon gene L1 loop was found to possess a high level of homology among FAV4 isolates both at nucleotide and amino acid level.

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                1                                                    50
India1          PRAVLQALLR HGLQPAGSQG VHVQLVGDGT RAERVRLRSA VQCLYQHEHL
KR95Russia      .......... .......... .......... .......... ........QH
506Belgium      .......... .......... .......... .......... ..........
KR5Belgium      .......... .......... .......... .......... .........H
ATCC829Belgium .......... .......... .......... .......... .........H
India2          .......S.. .......... .......... .......... ..........
FAV4-1          .......... .......... .......... .......... ..........
FAV4-2          .......... .......... .......... .......... ..........
FAV4-3          .......... .......... .......... .......... ..........
FAV4-4          .......... .......... .......... .......... ..........

                51                                                   100
India1          QRHDGGAGDE DFRRLPQSQP GTRKKSSATG RKRQHRRARS LRQVSVQLRL
KR95Russia      .......... .......... ...S..P... .......... ..........
506Belgium      ........Y. .......... ...N....A. .......... ..........
KR5Belgium      .......... .....S.P.. ...N....A. .......... ..........
ATCC829Belgium .......... .....S.P.. .......... .......... ..........
India2          .......... N......... .......... .......... ..........
FAV4-1          .......... .......... .......... .......... ..........
FAV4-2          .......... .......... .......... .......... ..........
FAV4-3          .......... .......... .......... .......... ..........
FAV4-4          .......... .......... .......... .......... ..........

                101                                                  150
India1          RCLRQARRRR RFPVPHADPL LDHGHGHQLP GSGGRRGLHQ QPLVPRYHSR
KR95Russia      .......... .......... ...ER..RI. .G.S...... ........D.
506Belgium      .......... .......... ...ER..RI. .G.S...... ........D.
KR5Belgium      .......... .......... ...ER..RI. .G.S...... ........D.
ATCC829Belgium .......... .......... ....R..... .G.S...... ........D.
India2          .......... ...I...... .......... .......... P.........
FAV4-1          .......... .......... .......... .......... ..........
FAV4-2          .......... .......... .......... .......... ..........
FAV4-3          .......... .......... .......... .......... ..........
FAV4-4          .......... .......... .......... .......... ..........

              151 152
India1          A A
KR95Russia      . .
506Belgium      . .
KR5Belgium      . .
ATCC829Belgium . .
India2          - .
FAV4-1          . .
FAV4-2          . .
FAV4-3          . .
FAV4-4          . .
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Fig. 1

Analysis of amino acid sequences of variable hexon gene L1 loop region of FAV4 isolates

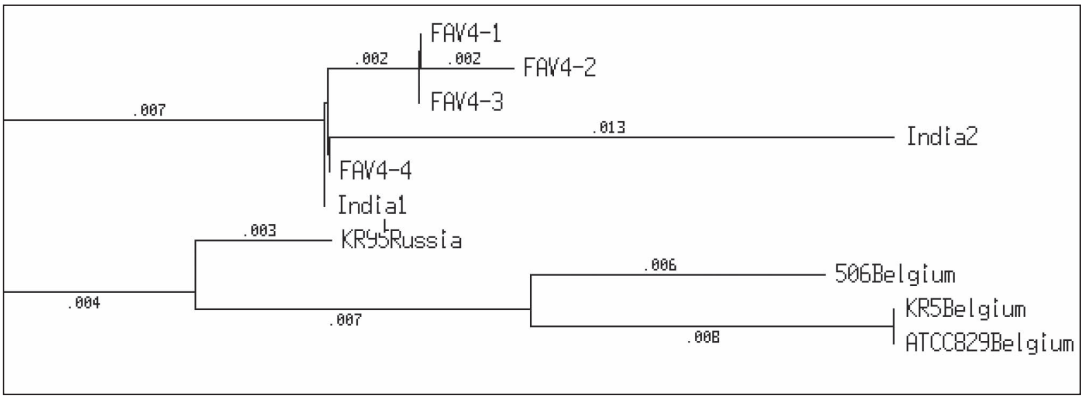

Fig. 2

Phylogenetic analysis of FAV4 isolates based on the amino acid sequence of variable hexon gene loop L1

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