

Molecular Analysis of Duck Hepatitis Virus Type 1 Indicates That it Should be Assigned to a New Picornavirus Genus

Chun-Hsien Tseng^{1,2}, Nick J. Knowles³ and Hsiang-Jung Tsai¹

¹ Graduate Institute of Veterinary Medicine, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 106, Taiwan;

² Division of Bio-production, National Institute for Animal Health, Council of Agriculture, 376 Chung-Cheng Road, Tamsui, Taipei 251, Taiwan;

³ Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, UK.

ABSTRACT

The genome sequences of three duck hepatitis virus type 1 (DHV-1) strains were determined. Comparative sequence analyses showed that they possessed a typical picornavirus genome organization apart from the unique possession of three putative in-tandem 2A genes. The 2A1 protein of DHV-1 was related to the 2A protein of aphthoviruses, cardioviruses, erboviruses and teschoviruses. This protein is involved in an unusual proteolytic activity at the conserved NPG-I sequence motif at its own carboxy-terminus. The 2A2 protein is not related to any known picornavirus protein, but has sequence similarities with the A1G1 protein of plants and *gimaps* protein in mammals both of which share an NTPase motif with DHV-1. The 2A3 protein of DHV-1 is related to the 2A protein of an encephalomyelitis virus, Aichi virus, bovine kobovirus, human parechovirus (HPeV) and the 2A2 protein of Ljungun virus (LV) and contains a H-box/NC domain similar to the H-box family of proteins. The percentage identity of the polyprotein amino acid sequence of DHV-1 to all other picornavirus was less than 30%. The percentage identity of the amino acid sequence at 3D region of DHV-1 with LV and HPeV-1 is only 38.6% and 36.6%, respectively, and less than 30% with all other picornaviruses. Like parechoviruses and koboviruses, the DHV-1 capsid polypeptide VP0 is not proteolytically cleaved into VP4 and VP2. Phylogenetic and evolutionary analysis of DHV-1 reveals it to form a new branch of the Picornaviridae. It is therefore proposed that DHV-1 should be assigned to a new picornavirus genus.

INTRODUCTION

Duck virus hepatitis (DVH) is an acute and fatal disease of young ducklings characterized by its rapid transmission (Woolcock, 2003). It was first described on Long Island in 1949 (Levine and Fabricant, 1950). The major pathologic change in infected ducklings is hepatitis. Three distinct serotypes of duck hepatitis virus (DHV, types 1 to 3) have been described and all were originally classified as picornaviruses (Haider and Calnek, 1979; Toth, 1969), with DHV-1 considered most like viruses of the genus *Enterovirus* (Tausaro et al., 1989). Duck hepatitis virus type 2 (DHV-2) has since been re-classified within the *Astroviridae* and has been renamed duck astrovirus 1 (genus *Neustovirus*) (Gough et al., 1984, 1985; Monroe et al., 2005). No antigenic relationships have been found between DHV-1 and DHV-3 by the serum neutralization test (McNulty, 2001; Woolcock, 2003). Recently, DHV-3 was also found to be an astrovirus distinct from all other sequenced avian astroviruses, including DHV-2, by the partial sequencing of the polymerase-coding regions of each virus (N. J. Knowles, unpublished data). Among the three types of DHV, DHV-1 is the most widely distributed and most virulent and can cause mortality higher than 80% in ducklings under three weeks of age, while DHV-2 has only reported in the UK and DHV-3 has only found in the USA, both occur sporadically and cause a low mortality (McNulty, 2001; Woolcock, 2003).

To date, no molecular sequence data have been reported for DHV-1 and this has greatly hindered diagnosis and research. In the present study, we have determined the complete or nearly complete viral genomes of three DHV-1 strains, including one Taiwanese field isolate (03D), one American vaccine strain (5886), and one UK vaccine strain (H).

MATERIALS & METHODS

Virion RNA was extracted by the QIAamp® Viral RNA Mini Kit (QIAGEN Ltd., Hilden, Germany) and was used in the following study. The MegAlign program (Lasergene® expert sequence analysis software version 5, DNASTAR Inc., Madison, WI, USA) using the Clustal W method (Thompson et al., 1994) was used to align nucleotide and amino acid sequences of each gene region of representative virus strains from the nine genera in the Picornaviridae and the three candidate "sapeloviruses". Degenerate PCR primers were designed for each gene region based on conserved motifs in picornaviruses of different genera (Table 1). Prior to sequencing, PCR products were purified with either a QIAquick gel extraction kit (QIAGEN Ltd., Hilden, Germany) or High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany). Sequencing was performed according to the cycle sequencing protocol of ABI Perkin-Elmer. Sequencing products were run on an ABI Prism 3730 Genetic Analyzer. The nucleotide sequence was derived by assembly of contigs with the SeqMan program of the Lasergene system v5.0 (DNASTAR Inc., Madison, WI). The nucleotide sequences were then translated into amino acid sequences. The Blast (National Center for Biotechnology Information) and FastA3 (European Bioinformatics Institute) programs were used to search protein sequences database in order to confirm that the sequences of the amplified PCR products were picornavirus-like and also to confirm to which gene region they belonged. Primer sets were designed using the sequences obtained allowing intervening regions to be amplified by RT-PCR and sequenced. The above process of primer design, RT-PCR, sequencing, assembly of contigs, database search and bioinformatics analyses were repeated several times until the preliminary nearly complete polyprotein sequence of DHV-1 was obtained. The completeness and accuracy of the preliminary nucleotide sequence was confirmed by the process of primer design and primer walking. The 5' and 3' ends of the viral genomes were amplified using the 5'3'-Rapid Amplification of cDNA Ends (RACE) Kit (Roche Diagnostics, Mannheim, Germany).

