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Rapid Communication

Molecular analysis of duck hepatitis virus type 1

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Abstract

The genome sequence of a duck hepatitis virus type 1 (DHV-1) strain was determined. Comparative sequence analysis showed that the genome possesses a typical picornarivus organization and also exhibits several unique features, such as the similarity of internal ribosome entry site to that of *Porcine teschovirus* 1 and *Hepatitis C virus*, the presence of a longest 3′ untranslated region and a shorter leader protein in the *Picornaviridae*, the absence of a predicted maturation cleavage of VP0, the association of an aphthovirus-like 2A1 and parechovirus-like 2A2, and the unprecedented presence of an AIG1 domain in the N-terminus of 2A2. It is concluded that DHV-1 belongs to a new group of the family *Picornaviridae* that may form a separate genus most closely related to the genus *Parechovirus*.

Keywords: Picornavirus; Duck hepatitis virus 1; Genome; AIG1 domain

Introduction

Duck hepatitis virus (DHV) is the causative agent of duck viral hepatitis, an acute and fatal disease of young ducklings. Three serotypes of DHV (DHV-1–3) have been described, and no antigenic relationships have been found between the three serotypes. DHV-1 is the most widely distributed and can cause mortality higher than 90% in ducklings under 3 weeks of age, while DHV-2 and DHV-3 have only been reported in the UK and the USA respectively. Although DHV-1 was first isolated 50 years ago and initial studies indicated that this virus had many characteristics similar to those of the genus *Enterovirus* (Woolcock, 2003), little was known about this virus in its molecular characteristics and its precise taxonomical position in the *Picornaviridae*. To date, nine genera of the family *Picornaviridae* are recognized (*Aphtho*-, *Cardio*-, *Entero*-, *Erbo*-, *Hepato*-, *Rhino*-, *Kobu*-, *Parecho*-, and *Teschoviruses*) (Stanway et al., 2005). Genera have been defined in the past largely on the basis of physiochemical properties, but molecular genetics is now playing a more significant role and members of different genera, although sharing the same basic structure, show some differences in genome organization which can aid assignment (Ghazi et al., 1998). In preparing this manuscript, Kim et al. (2006) and Tseng et al. (2007) reported their results of sequencing the full genomes of five DHV-1 strains and proposed that DHV-1 belong to a new group of the *Piconaviridae* due to its possession of several unique features. Here we report the complete nucleotide sequence of DHV-1 strain C80 (DHV-C80), a chicken-embryo-adapted vaccine strain in China. The further analysis of DHV-C80 genome is conducted by employing bioinformatic techniques. On the basis of results presented in this paper, it is also proposed that DHV-1 be classified into a separate genus of the family *Picornaviridae*.

Results

Genomic sequence of DHV-C80

The genome of DHV-C80 was found to be 7689 nt in length excluding the 18 nucleotide poly (A) tail at the 3′ end. The base composition of its genome is composed of 29% adenine, 23% guanine, 21% cytosine, and 28% uracil. The G+C content (44%) is similar to those of parechoviruses (42% for *Ljungan virus*) and teschoviruses (45% for *Porcine teschovirus* 1). Computer-assisted analysis of the nucleotide sequence of

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Table 1
Comparison of the genomic features of DHV-1 strains C80, O3D and DRL-62

Gene	Size [a]			Predicted N-terminal cleavage sites		
	C80	O3D	DRL-62	C80	O3D	DRL-62
5′UTR	625	623	626	–	–	–
Polyprotein	2249	2249	2249	–	–	–
L	30	–	–	–	–	–
P1	701	731	731	L/G	–	–
P2	757	757	757	E/S	E/S	E/S
P3	761	761	761	Q/S	Q/S	Q/S
VP0	226	256	256	L/G	–	–
VP3	237	231	237	Q/G	Q/G	Q/G
VP1	238	244	238	Q/G	Q/M [b]	Q/G
2A1	20	20	20	E/S	E/S	E/S
2A2	285	161 and 124 [c]	285	NPG/P	NPG/P and S/H [c]	NPG/P
2B	119	119	119	Q/S	Q/S	Q/S
2C	333	333	333	Q/S	Q/S	Q/S
3A	93	93	93	Q/S	Q/S	Q/S
3B	28	34	34	Q/S	Q/S	Q/S
3C	187	181	181	E/T	Q/S	Q/S
3D	453	453	453	Q/G	Q/G	Q/G
3′UTR	314	314	315	–	–	–

[a] No. of nucleotides for 5′UTR and 3′UTR and amino acids for proteins.

[b] There is an alternative possible cleavage site (Q/G) six amino acids downstream, resulting in a VP3 of 237 aa and a VP1 of 238 aa (Tseng et al., 2007).

[c] An extra cleavage site S|H was predicted to generate 161-aa-long 2A2 and 124-aa-long 2A3 suggested by Tseng et al. (2007).

DHV-C80 indicated that the genomic RNA contains one large open reading frame (ORF nt 626–7375), encoding a putative polyprotein of 2249 amino acids, which is preceded by 625 nt and followed by 314 nt and a poly (A) tail. The genome organization is analogous to that of a typical picornavirus with 5′ and 3′ untranslated regions (UTR) (Table 1). As shown in Table 2, the polyprotein of DHV-C80 has between 11 and 28% identity with representatives of the other nine genera in the family *Piconaviridae*, with parechoviruses having relatively higher homology (26 to 28%). A detailed domain comparison of DHV-C80 with selected viruses of all genera of the *Picornaviridae* confirmed the closest relationship with *Human parechovirus* (HPeV) and *Ljungan virus* (LV). The genome in length of DHV-C80 is similar to those (7664–7691 nt) of other DHV-1 strains sequenced by Kim et al. (2006) and Tseng et al. (2007). All these DHV-1 strains analyzed possess the same size in ORF and 3′UTR with the exception of DHV-1 strain DRL-62 which contains 3′UTR of 315 nt and give differences in length of 5′UTR (Table 1; Kim et al., 2006; Tseng et al., 2007).

5′UTR

The 5′UTR of DHV-C80 precedes the putative initiation codon, which is located in an optimal Kozak context (GxxAUGG) (Kozak, 1986) at position 626. The length of the 5′UTR is similar to that of other picornaviruses and must encode

Table 2
Comparisons of nucleic acid (5′UTR and 3′UTR) and amino acid sequences of DHV-C80 with selected picornaviruses [a]

Region	PV1M	HRV14	EMCV	FMDV	HAV	HPeV2	LV87-012	ERBV	AiV	PTV1
5′UTR	43	43	43	43	44	43	45	44	45	49
Polyprotein	14	14	14	11	15	26	28	14	14	14
L	– [b]	–	6	16	ND	–	–	13	6	16
VP0	13	13	17	7	14	30	32	15	10	18
VP3	14	6	13	9	14	24	28	10	16	11
VP1	7	6	7	4	12	25	23	8	11	8
2A1	ND	ND	33	31	ND	ND	28	35	ND	23
2A2	ND	ND	ND	ND	ND	12	11	ND	2	ND
2B	8	12	9	5	4	28	26	5	9	9
2C	22	20	20	22	21	31	33	20	21	17
3A	5	7	11	4	10	16	11	9	8	6
3B	18	17	15	13 [c]	13	16	16	23	32	12
3C	18	18	11	8	15	22	23	14	10	16
3D	25	25	20	16	23	34	37	17	19	22
3′UTR	54	52	40	50	56	46	48	48	43	54

[a] Values are percent amino acid identities and percent nucleotide identities (for the 5′ and 3′ UTRs) with selected picornaviruses. ND, not determined.

[b] –, no leader protein is encoded.

[c] FMDV protein 3B1 is used for comparison.