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ANNOTATED SEQUENCE RECORD

Complete genome sequence of an isolate of duck enteritis virus from China

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Abstract

Here, we present the complete genomic sequence of duck enteritis virus (DEV) strain SD, isolated in China in 2012. The virus was virulent in experimentally infected 2-month-old ducks. The DEV SD genome is 160,945 base pairs (bp) in length. The viral genome sequence, when compared to that of strain DEV CSC, which was isolated in 1962, showed three discontinuous deletions of 101 bp, 48 bp and 417 bp within the inverted repeats. A comparison of the amino acid (aa) sequences of all ORFs of the CSC and SD isolates demonstrated an11-aa deletion, two single-aa deletions, and one single-aa deletion in LORF3, UL47, UL4, respectively. Moreover, 38 single aa variations were also detected in 24 different ORFs. These results will further advance our understanding of the genetic variations involved in evolution.

Duck enteritis virus (DEV) infects ducks, geese, and swans,causing heavy economic loss [5]. The virus belongs to the species *Anatid alphaherpesvirus 1*, in the genus *Mardivirus*, subfamily *Alphaherpesvirinae*, family *Herpesviridae* (https://www.ictvonline.org). The DEV genome is approximately 160 kb long, composed of a unique long (UL) region and a unique short (US) region, each flanked by an inverted repeat (IR) and a terminal repeat (TR) [4, 8, 9, 11, 12]. In previous studies, we sequenced the complete genome of the Chinese standard challenge strain of DEV (DEV CSC) isolated in 1962, its passaged variant DEV C20E85, and a live vaccine strain, DEV K p63 (Table 1) [9–11, 13]. DEV is currently circulating in ducks in China, but no genomic information is available for the circulating strains. In this report, we describe the complete genome sequence of a DEV strain isolated recently in China.

DEV SD was isolated in 2012 from a duck farm in Shandong Province, China. The liver of a dead duck was collected, homogenized, and inoculated into duck embryos. After two passages, all inoculated duck embryos died within 5 days.

Four susceptible 2-month-old ducks were inoculated intramuscularly with 10^5 EID_{50} of DEV SD, and as a control, two 2-month old ducks were inoculated with M199 medium. They were housed in two isolators. The ducks were observed for signs of disease for 14 days after inoculation. All ducks inoculated with DEV SD developed pyrexia and died at 5–7 days post-inoculation (dpi) with typical symptoms of infection, whilst the control ducks remained healthy.

To identify genetic mutations, whole-genome sequencing was done. DEV SD genomic DNA was extracted using a TaKaRa MiniBEST Viral RNA/DNA Extraction Kit Ver. 5.0 (Dalian, China). Primers were designed based on the DEV CSC genome sequence (GenBank no. JQ673560) and synthesized by BGI (Beijing, China). The PCR amplicons were purified and sequenced by the Sanger method (BGI, Beijing, China). All of the sequences were assembled and compared using Seqman and MegAlign of DNASTAR (V5.01). The DEV CSC sequence was used as the reference for mapping and annotation. The DEV SD sequence was deposited in the GenBank database under accession number MN518864.

The assembled complete genome sequence of DEV SD was 160,945 bp in length, which is shorter than that of the DEV CSC (Table 1), due to three discontinuous deletions of 101 bp, 48 bp, and 417 bp, within the noncoding inverted repeats. Whether these deletions influence the pathogenicity of the virus has yet to be established.

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Table 1 Lengths of various regions in the DEV genomes

Strain	UL[a] (bp)	IR[b] (bp)	US[c] (bp)	TR[d] (bp)	Total (bp)
DEV CSC	123,350	13,028	12,725	13,028	162,131
DEV VAC	119,306	13,029	12,727	13,029	158,091
DEV K p63	119,307	13,028	12,726	13,028	158,089
2085	122,141	12,892	12,724	12,892	160,649
SD	123,296	12,462	12,725	12,462	160,945

[a]*UL* long sequence, [b]*IR* inverted repeat, [c]*US* short sequence, [d]*TR* inverted tandem repeat

Similar deletions in the non-coding inverted repeats have also been observed in other alphaherpesviruses. A German isolate (DEV 2085) also had two deletions (48 bp and 104 bp) in the inverted repeats [7] (Fig. 1). Equine herpesvirus 1 strain RacL11, isolated from an aborted foal, had 354-bp, 25-bp and 119-bp deletions in the inverted repeats compared with strain Ab4 [6]. Notable nucleotide insertions and deletions have also been reported in the inverted repeats of different bovine herpesvirus type 1.1 isolates [1]. The length of the inverted repeats has been shown to vary significantly among different infectious laryngotracheitis virus strains [2, 3].

The amino acid (aa) sequences of all ORFs of the CSC and SD isolates were compared, and the results showed that there was an 11-aa deletion, two single-aa deletions, and one single-aa deletion in LORF3, UL47, UL4 of DEV SD, respectively. In addition, 38 single-aa variations were detected in 24 different ORFs (Table 2). The other ORFs were completely conserved among the sequences of SD and CSC.

In summary, the complete DEV SD genome sequence was determined and found to have discontinuous deletions (101 bp, 48 bp and 417 bp) within the inverted repeats. These results will further advance our understanding of the genes involved in evolution.

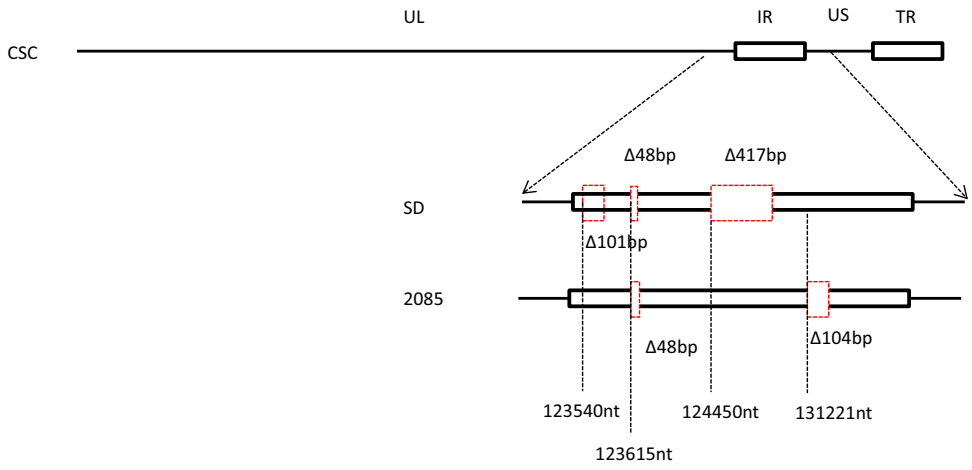

Fig. 1 Schematic illustration of deletions in the internal repeat sequence of the genomes of DEV strains SD and 2085. The DEV CSC sequence was used as the reference for mapping and annotation. The deletions are shown as red dashed boxes. Numbers under boxes represent fragment length. Vertical dashed lines represent positions in the DEV CSC genome

Table 2 Amino acid sequence differences in the proteins encoded by DEV strains CSC and SD

ORF	Amino acid position(s)	DEV CSC	SD
LORF5	221	I	V
LORF5	265	R	I
LORF5	370	T	A
UL53	84	A	V
UL48	203	W	G
UL48	469	L	S
UL47	113	R	H
UL47	148	D	–*
UL47	419	S	–
UL46	692	F	L
UL44.5	241	V	I
UL44.5	250	N	D
UL43	301	T	M
UL43	484	K	M
UL43	499	S	G
UL39	204	V	I
UL39	243	T	S
UL36	459	H	Y
UL36	1982	Q	H
UL29	887	P	S
UL26	598	S	T
UL16	40	R	L
UL15	694	A	P
UL13	21	I	V
UL8	10	M	I
UL7	258	L	I
UL5	324	N	K
UL5	717	T	I
UL4	176	E	–
LORF3	329	D	E
LORF3	291–301	NNIDADVGEED	–
LORF2	472	S	T
LORF2	509	K	E
ICP4	448	V	A
ICP4	508	A	S
ICP4	1073	T	S
ICP4	1119	E	G
SORF3	269	I	V
US5	79	L	F
US5	208	N	S
US8	451	P	S
US9	49	E	D

*Deletion

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval The animal experimental protocols were approved by the Animal Care and Protection Committee, China Institute of Veterinary Drug Control. All animal experiments were carried out in accordance with the requirements of the *Regulations of Experimental Animal Administration* of the P. R. China. All efforts were made to minimize suffering.

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