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GENOME ANNOUNCEMENT

Complete Genome Sequence of a Novel Flavivirus, Duck Tembusu Virus, Isolated from Ducks and Geese in China

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Duck tembusu virus (DTMUV) is an emerging agent that causes a severe disease in ducks. We report herein the first complete genome sequences of duck tembusu virus strains YY5, ZJ-407, and GH-2, isolated from Shaoxing ducks, breeder ducks, and geese, respectively, in China. The genomes of YY5, ZJ-407, and GH-2 are all 10,990 nucleotides (nt) in length and encode a putative polyprotein of 3,426 amino acids. It is flanked by a 5′ and a 3′ noncoding region (NCR) of 94 and 618 nt, respectively. Knowledge of the whole sequence of DTMUV will be useful for further studies of the mechanisms of virus replication and pathogenesis.

In 2010, a novel infectious agent emerged in China and caused extensive epidemics in egg-laying and breeder ducks. The affected ducks showed a severe drop in egg production and paralysis. We isolated the causative agent, a virus, from affected ducks and determined the partial genome sequence. All of Koch's postulates were fulfilled, including duck trials. The isolated virus is considered to belong to a new genotype of tembusu virus (TMUV) and was designated duck TMUV (DTMUV) (1).

To date, the complete genome sequence of DTMUV has not been reported. To provide more information about DTMUV, we determined the entire genome sequences of two duck-origin isolates (YY5 and ZJ-407) and a goose-origin isolate (GH-2).

The 5′ and 3′ ends of the genomes were amplified using 5′ and 3′ rapid amplification of cDNA ends (RACE) strategies (5). Based on partial genomic sequences obtained (1), the remainder of the sequences were generated by five overlapping cDNA fragments covering each of the entire genomes and were sequenced using an Applied Biosystems (ABI) 3730xl DNA analyzer. Overlapping consensus sequences were assembled using the SeqMan II programs to generate contiguous full-genome sequences.

The complete genomes of the three DTMUV strains are 10,990 nucleotides (nt) in length, with an open reading frame (ORF) extending from nt 95 to 10,278 that encodes a putative polyprotein of 3,426 amino acids (aa). Aligning the sequence of the polyprotein with those of other mosquito-borne flaviviruses, we observed the highest aa similarity with those of Bagaza virus (BAGV; 82%), St. Louis encephalitis virus (SLEV; 66%), Ilheus virus (ILHV; 63.7%), West Nile virus (WNV; 63.4%), and Japanese encephalitis virus (JEV; 63.3%). Like that of other mosquito-borne flaviviruses, the polyprotein of DTMUV is processed into three structural proteins and seven nonstructural proteins by the viral or host cell proteases.

We analyzed the secondary structures and cyclization between the 5′ and 3′ noncoding regions (NCRs) of DTMUV the mfold program of Zuker et al. (6). The predicted results showed that the folding pattern of the three DTMUVs was similar to those of other mosquito-borne flaviviruses (2, 3, 4). The organization of conserved sequences (CS) of 3′ NCRs is RCS3-CS3-RCS2-CS2-CS1 for DTMUV, which is the same as that of the JEV subgroup but different from that of Ntaya subgroup members BAGV and ILHV, in which there is no RCS2 or RCS3, respectively.

Typical flavivirus cysteine residues were found in DTMUV, 6 in the PrM protein, 12 in the E protein, and 12 in the NS1 protein. Similarly, the potential N-linked glycosylation sites (NLGlyS) were also found in DTMUV, with a distribution of two in the PrM protein, one in the E protein, and three in the NS1 protein. Phylogenetic analyses of the whole polyprotein showed that the DTMUV isolates formed a distinct cluster within the clade of mosquito-borne flaviviruses and were more closely related to BAGV than to all other flaviviruses with completely determined genomes.

Taken together, our results show that the overall genome organization of DTMUV is similar to that of known flaviviruses. Knowledge of the whole-genome sequence of DTMUV is critical for further investigation of the mechanisms of virus replication and pathogenesis.

Nucleotide sequence accession numbers. The complete genome sequences of the three DTMUV isolates were submitted to GenBank under accession numbers JF270480, JQ314464. and JQ314465.

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