

# Complete Genome Sequence of Avian Tembusu-Related Virus Strain WR Isolated from White Kaiya Ducks in Fujian, China

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**Avian tembusu-related virus, which was first identified in China, is an emerging virus causing serious economic loss to the Chinese poultry industry. We report here the complete genome sequences of avian tembusu-related virus strain WR, isolated from a White Kaiya duck with disease characterized by an abrupt decrease in egg laying with ovarian hemorrhage, which will help in further understanding the molecular and evolutionary characteristics and pathogenesis of avian tembusu-related virus, the new flavivirus affecting ducks in Southern China.**

Since April 2010, a large-scale outbreak of disease characterized by an abrupt decrease in egg laying with ovarian hemorrhage, with nearly 100% morbidity but 0 to 12% mortality in infected duck farms, has been observed in nearly 13 coastal provinces in China, such as Hebei, Shandong, Zhejiang, Fujian, Guangdong, and Guangxi, and in nearly all duck species and breeds, such as Beijing duck, muscovy duck, Cheery Valley duck, and some Chinese traditional breeds (1, 6, 9). The causative agent was first identified by us, using the same strategy described by Wang et al. (8), and deposited in GenBank under accession number HQ828066 (7). We also first identified the new tembusu-related flavivirus as infecting domestic layer hens in Fujian, China (2). Now it has been identified as a new flavivirus—duck tembusu-related (or tembusu-like) virus (4) (6), and research showing that the virus may be infecting chickens and geese indicates that this new flavivirus has adapted to domestic avian species (7). Therefore, we suggest naming this new flavivirus avian tembusu-related virus (ATrV). We determined the complete genome sequence of avian tembusu-related virus strain WR from White Kaiya ducks; the strain was isolated in this outbreak by using 10-day-old muscovy duck embryonated eggs.

Based on the sequences of flavivirus Bagaza virus (AY632545) (3) and duck tembusu-related flavivirus strains TA (5) and YY5 (10), 12 pairs of oligonucleotide primers were designed to amplify the different regions of the strain WR genome with overlapping genome fragments covering each fragment. The 5'-terminal and 3'-terminal sequences were determined by using a 5'-Full RACE kit and a 3'-Full RACE core set kit, version 2.0 (TaKaRa, Dalian, China), as described by Liu et al. (5). All fragments were cloned into pMD-18T vector (TaKaRa) and sequenced in both directions in triplicate. The cloned plasmids were sequenced with an ABI (Applied Biosystems) 3730 DNA analyzer. All of the sequences obtained were assembled using the SeqMan programs (DNASTAR; LaserGene) to generate the contiguous full-genome sequences. The complete genome sequence of strain WR is 10,990 nucleotides (nt) in length with a typical flavivirus genome organization. The predicted polyprotein, encoded by a single open reading frame (10,278 nt) flanked by a 94-nt-long 5' untranslated region (5' UTR) and a 618-nt-long 3' UTR, comprises a leader protein, three structural proteins (capsid, preM, and envelope), and five nonstructural proteins (NS1 to NS5).

Compared to previous avian tembusu-related virus strains isolated from different breeds of ducks, layer hens, and geese, a phylogenetic tree based on the polyprotein sequence demonstrated that strain WR was more closely related to layer flavivirus CJD05 (GenBank accession number JF926699), which was isolated from layer hens also in Fujian, with 99.3% identity at the nucleotide level. All avian tembusu-related virus

polyprotein sequences possessed identities higher than 99.1% at the nucleotide level. Also, we noticed that there was less than a 4-nucleotide variation in its 3' UTR and a 1-nucleotide variation in the 5' UTR. The role of the variation needs further epidemiologic investigation.

The knowledge of the full-genome sequence of strain WR will not only facilitate future investigations of the epidemiology and evolution of avian tembusu-related virus but also contribute to avian tembusu-related virus pathogenesis.

**Nucleotide sequence accession number.** The whole-genome sequence of avian tembusu-related virus strain WR has been deposited in GenBank under accession number JX196334.

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