

# Complete Genomic Sequence of an Equine Herpesvirus Type 8 Wh Strain Isolated from China

Cuiyun Liu,<sup>a</sup> Wei Guo,<sup>a</sup> Gang Lu,<sup>a,b</sup> Wenhua Xiang,<sup>a</sup> and Xiaojun Wang<sup>a</sup>

State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, People's Republic of China,<sup>a</sup> and Heilongjiang Bayi Agricultural University, Daqing, People's Republic of China<sup>b</sup>

**A new strain of equine herpesvirus type 8 (EHV-8), Wh, has been isolated from horses in China, and its complete genome has been sequenced and analyzed. The result indicates that the new strain has the same constitution and arrangement of open read frames as EHV-1 and EHV-9. This work is the first announced complete genome sequence of EHV-8.**

Equine herpesvirus type 8 (EHV-8) is a member of the subfamily *Alphaherpesvirinae*, along with EHV-1, EHV-3, EHV-4, and EHV-9. EHV-8 was first isolated by reactivating the virus from latently infected donkeys from Australia in 1988, and it can cause afebrile rhinitis in experimentally infected donkeys (1, 3, 5). The glycoprotein G gene was the only one that has been sequenced in the case of EHV-8, the analysis of which has revealed that EHV-8 is much more closely evolutionarily related to EHV-1 than to EHV-4 (4), while the complete genomes of other *Alphaherpesvirinae* subfamily members have been sequenced, including EHV-1, EHV-4, and a new member, EHV-9. In this work, the genome of a new strain of EHV-8 was sequenced, and this is the first announcement of the EHV-8 genome.

A new strain of EHV, Wh, was isolated from horses with fever and nasal discharge in northeastern China in 2010. Through analysis of the gene for glycoprotein G, a type-specific molecule (2, 4, 6, 7), strain Wh is thought to be the first isolate of EHV-8 from horses and also the first strain of EHV-8 isolated in China.

The virus was propagated on rabbit kidney (RK13) cells, and the genomic DNA was extracted from the virus supernatant after fewer than 5 passages by following the Omega Bio-Tek viral DNA kit procedure. To sequence the genome, many pairs of primers were designed with reference to the EHV-1 and EHV-9 genomes. The terminus of the genome was amplified by using the genome walking kit (Takara). The amplification products were cloned into the pMD18-T vector and sequenced using an ABI 3730XL Sanger-based genetic analyzer by the Beijing Genomics Institute (Shenzhen, People's Republic of China). Finally, the sequences were assembled by the DNASTar software (version 7.2), and annotation of the genome was performed by the GATU software (<http://www.virology.ca/gatu>) with reference to that of EHV-9.

Strain Wh shows much higher identity to EHV-8 than to other alphaherpesviruses based on the amino acid sequence deduced from the glycoprotein G gene. This result indicated that strain Wh is a new strain of EHV-8, and compared with the other EHV-8 strain from donkeys, there are eight nucleotide and two amino acids substitutions in the glycoprotein G gene.

Further analysis has shown that the genome of strain Wh has the same composition and arrangement of open reading frames (ORFs). Seventy-six proteins are encoded by 80 ORFs of strain Wh. Because of the existence of higher-order structure in the region of ORF56, about 33 nucleotides failed to identify compared with EHV-1 and EHV-9, and the levels of identity between the complete genomic sequence of strain Wh and those

of EHV-1 and EHV-9 ranged from 80.6 to 99.0% and from 84.5 to 99.1%, respectively. Strain Wh still has a higher level of identity to EHV-9 than to EHV-1 in every single gene when ORF56 is not included. Therefore, strain Wh is genetically closer to EHV-9 than to EHV-1.

The complete sequence of strain Wh is meaningful in understanding the molecular characteristics of EHV-8 and also helpful in elucidating the phylogenetic relationships of EHV-8.

**Nucleotide sequence accession number.** The complete genomic sequence of strain Wh has been submitted to GenBank and assigned accession number [JQ343919](https://www.ncbi.nlm.nih.gov/nuccore/JQ343919).

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Address correspondence to Wenhua Xiang, [xiangwenhua0@yahoo.com](mailto:xiangwenhua0@yahoo.com), or Xiaojun Wang, [xjw@hvri.ac.jp](mailto:xjw@hvri.ac.jp).

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