



Complete Coding Sequence of Western Equine Encephalitis Virus Strain Fleming, Isolated from a Human Case

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ABSTRACT We sequenced the complete coding genome of the western equine encephalitis virus (WEEV) strain Fleming. This strain was originally isolated in 1938 from a human WEEV case.

Western equine encephalitis virus (WEEV), a member of the *Alphavirus* genus, *Togaviridae* family, is a single-stranded, positive-sense RNA virus. WEEV clusters within the western equine encephalitis (WEE) antigenic complex, which includes five other virus species, namely, Sindbis, Aura, Fort Morgan, Highlands J, and Whataroa viruses (1, 2). Strains of WEEV are genetically and antigenically homogeneous, with >94% amino acid similarity between the structural glycoproteins.

Maintained through an enzootic transmission cycle between mosquitoes and birds (3), WEEV infects equids and humans naturally through a mosquito bite, and the incubation period is 5 to 10 days (4, 5). Infection with WEEV produces low-level, sporadic viremia in equids and humans, making these species dead-end hosts (6). Disease can progress from a febrile illness to meningismus, weakness, tremors, and altered mental status (<10% of symptomatic patients) (7–9). Juvenile and geriatric populations are more susceptible to severe clinical illness and neurological sequelae, with a 4 to 10% case fatality rate (7). The last recorded human cases of WEEV were in 2009 in Uruguay (10), and virus is rarely detected in mosquito pools (11). A trivalent alphavirus virus-like particle vaccine was recently developed (12) and is undergoing human safety testing. Due to the rarity of WEEV cases, the McMillan strain (GenBank accession number [GQ287640](https://www.ncbi.nlm.nih.gov/nuccore/GQ287640)) is the only complete human isolate sequence available.

WEEV strain Fleming was isolated from a human case in California in 1938 (13–15). The source material, WEEV Fleming B567 585 suckling mouse pass 5 (SM-5), lyophilized on 11 October 1967, was obtained from Robert Tesh (University of Texas Medical Branch, Galveston, TX). Reconstituted virus was passaged three times in Vero cells cultured in Leibovitz's L-15 medium plus 10% fetal bovine serum (SM-5, Vero-3; lot number 038). Material shipped to the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) was stored at –80°C. RNA was extracted using TRIzol LS reagent and converted to cDNA (16). Briefly, three primers, FR26RV-N (GCCGGAGCTCTGCAGATATCNNNNNN), FR40RV-T (GCCGGAGCTCTGCAGATATCT₂₀), and a template-switching oligonucleotide (TSO), Venter SISPA [GCCGGAGCTCTGCAGATATCGGCCATTATGGCC (Ribo-GGG)], were used in combination with reverse transcriptase with terminal transferase activity (Maxima H minus) to convert the entire genomic RNA to cDNA, including the 5' and 3' ends. Purified cDNA was amplified by sequence-independent single primer amplification (SISPA) (17, 18) with MyTaq DNA polymerase using primer FR20RV (GCCGGAGCTCTGCAGATATC). Purified PCR products were fragmented on a Covaris LE220 ultrasonicator. Libraries were prepared with the TruSeq DNA sample preparation

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kit. After quantification by real-time PCR with the KAPA library quantification kit, libraries diluted to 10 nM were sequenced on an Illumina MiSeq instrument with a 200-bp paired-end protocol.

Sequence reads were quality filtered with PRINSEQ lite v0.20.4 (19) and SISPA. Illumina adapter sequences were removed with Cutadapt v1.7 (20). A subset of the filtered sequence reads (~150,000) was assembled with Lasergene SeqMan NGen v15 to an average sequence depth of 1,268×.

Final assembly resulted in a genome length of 11,521 nucleotides with a GC content of 49%. It was determined to be complete by detection of a TSO sequence at the 5' end and poly(A) tail at the 3' end.

The complete genome sequence of WEEV strain Fleming shared 99% base identity with the McMillan strain (GenBank accession number [GQ287640](https://doi.org/10.1093/nuclemta/gqz287)) isolated in Canada in 1941 from a human brain sample.

Data availability. The GenBank accession number for WEEV strain Fleming is [MN477208](https://doi.org/10.1093/nuclemta/gzab028). Raw sequencing reads have been deposited in the NCBI SRA under BioProject accession number [PRJNA579577](https://doi.org/10.1093/bioinformatics/btr026).

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