Hefer Valley virus – a novel ephemerovirus detected in blood of a cow with severe clinical signs, Israel, 2022

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Research Article

Keywords:

Posted Date: June 7th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2988991/v1

Abstract

A novel ephemerovirus was identified in a febrile cow from Hefer Valley, Israel. The animal showed severe and ultimately fatal clinical signs, that resembled those of an arboviral infection. Sequencing from blood revealed the full genome sequence of Hefer Valley virus, a likely new species within the genus *Ephemerovirus*, family *Rhabdoviridae*.

Full Text

The genus *Ephemerovirus*, which belongs to the family *Rhabdoviridae* comprises viruses that primarily infect ruminants and are transmitted by blood-sucking insects [1, 2], including e.g. Puchong virus (PUCV) from mosquitoes in Malaysia in 1965 and Hayes Yard virus (HYV) from a bull in Australia in 2000 [3,4]. However, several ephemeroviruses were detected in other origins, as porcine ephemeroviruses 1 and 2, which were identified in porcine tissues in China [5], and New Kent County virus was isolated from ticks in Northern America (origin:GenBank).

Bovine ephemeral fever virus (species: *Ephemerovirus febris*; BEFV) is the type member of the genus and an important pathogen of cattle and water buffalo. It causes a short-lasting disease characterised by a bi-phasic fever, salivation, ocular and nasal discharge, recumbency, muscle stiffness, lameness and anorexia. The morbidity is high, but the mortality is usually low (<1%) [6]. BEFV is apparently transmitted by two types of arthropod vectors: *Culicoides* and mosquitoes (culicine and anopheline mosquitoes) [7]. Besides BEFV, other ephemeroviruses have been reported to cause comparable clinical diseases in cattle [7,8].

The genome of ephemeroviruses consists of ssRNA(-) and is about 15 kb in length encoding ten open reading frames. The genes are flanked by conserved transcription initiation and transcription termination/polyadenylation (UGAAAAAAA) sequences, and are separated by intergenic regions [1,2].

In October 2022, a six-year old dairy cow from Ein HaHoresh, a cooperative farm in central Israel, located in the Hefer Valley (Figure 1a) manifested clinical signs resembling an arboviral infection. The cow was previously vaccinated several times with ULTRAVAC BEF VACCINE (Zoetis), to protect against BEFV. During the first seven days after delivery of a healthy female calf, the cow developed fever and hypocalcemia, followed by ketonuria, milk reduction and recumbency. The cow died 14 days after the onset of the clinical signs. A blood sample was collected and sent to the department of Virology, Kimron Veterinary Institute, Israel, for laboratory diagnosis at October 20th, 2022. The sample was tested by RT-qPCRs specific for arboviral viruses such as bluetongue virus, epizootic hemorrhagic disease viruses, and BEFV, targeting the G coding region [10] (Supplementary Table S1). These RT-qPCRs scored negative. The result of an RT-qPCR specific for the N-region of BEFV (Supplementary Table S1) was equivocal and BLASTn analysis of the sequenced BEFV N-region RT-qPCR product revealed highest identity to members of the genus *Ephemerovirus*. Virus isolation on Vero (African green

monkey kidney), BHK-BSR (baby hamster kidney - 21 clone BSR) and C6/36 (*Aedes albopictus*) cells failed.

Metagenomic RNA sequencing and *de novo* assembly was performed on a blood sample (the detailed methods are presented in Supplementary Material) and the resulting contigs were matched to protein references of ephemeroviruses using diamond BLASTx (version 2.0.14). A single contig was identified that resembled the viral genome of a potential novel ephemerovirus. We tentatively named it Hefer Valley virus (HVV), after the location of sample origin (Figure 1a).

The genome of the novel HVV was 15,033 nt in length. We predicted and characterized 10 open reading frames (ORF), that followed the typical genome structure of ephemeroviruses (3'-N-P-M-G-GNS-α1-α2-β-γ-L-5'; Figure 1b). Furthermore, we identified nine transcription termination sites (UGAAAAAAA) that were located adjacent to the ORFs.

For phylogenetic classification, individual amino acid sequence alignments from N and L proteins of 22 representative viruses from the genera *Ephemerovirus* and *Tibrovirus* along with HVV were generated using MUSCLE (version 3.8.425), then concatenated into a single alignment and a maximum-likelihood phylogenetic analysis was conducted using IQ-TREE2 (see Supplemental Material). The phylogenetic tree based on N and L proteins suggested that HVV as a novel species within the genus *Ephemerovirus* most closely related to HYV, PUCV and KOV (Figure 2). The amino acid identity of HVV to HYV, KOV and PUCV was between 87.7-89.4% and 77.3-78.4% for N and L proteins, respectively (Supplemental Figure S1a and S1b).

We submitted the annotated HVV genome sequence to the International Nucleotide Sequence Database Collaboration (https://www.insdc.org) under accession no. OQ679991.

In order to check for the presence of HVV in cases of cattle with comparable clinical signs, we tested 249 available stored samples collected in 2021 and 218 samples collected in 2022 using a pan-ephemerovirus RT-qPCR (Supplementary Tables S1 and S2). All of the samples were collected from August until January, the usual arboviral season in the region. No additional positive samples were identified in 2022, while fifty samples collected in 2021 were positive. Identification of ephemerovirus species on these positive samples were performed using a BEFV-specific SYBR Green based RT-qPCR [9] in combination with the generic conventional RT-PCR for the N-coding region of ephemeroviruses (Supplemental Table S1 and S2). All of the fifty positive samples collected in 2021 were identified as BEFV only, but not HVV (data not shown).

In conclusion, we report identification of the HVV, a novel species within the genus *Ephemerovirus* most closely related to HYV, PUCV and KOV. The blood sample was collected in October, 2022 from an adult BEFV-vaccinated milking Israeli cow manifesting severe illness with consequent death. Future serological and molecular examinations are expected to reveal the extent of cattle exposure to HVV in Israel, and to determine its veterinary significance. Also, as the affected cattle had a history of vaccination against BEFV, the efficiency of these vaccines against HVV is up to debate.

As several well-known viruses of the genus *Ephemerovirus* are transmitted by blood-sucking insects, putative vector species should be collected in regions with affected cattle and investigated for the presence of HVV in order to identify its potential insect vector.

Declarations

Funding: This research was funded by Israeli Dairy Board, grant number 848-0351, received by NG and EK.

Data Availability: The annotated genome sequences generated during and/or analysed during the current study are available in the DDBJ/EMBL/GenBank databases under the accession number OQ679991.

Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

Author Contributions: Methodology: N.G., F.P.; material and data collection: L.O.; data curation: B.H., K.W., M.B., E.K.; writing: original draft preparation, N.G.; writing-review and editing: B.H., K.W., M.B., E.K, F.P.; visualization, F.P. All authors have read and agreed to the published version of the manuscript.

Ethical approval: This article does not contain any studies with human participants performed by any of the authors. The sample of the HVV infected cow was collected by the responsible farm veterinarian during health monitoring and the stored samples represent superfluous material of routine diagnostic submissions taken by the responsible veterinarians in the context of the health monitoring of the respective farm. No permissions were needed to collect these specimens.

Consent to participate and to publish: Not applicable.

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Figures

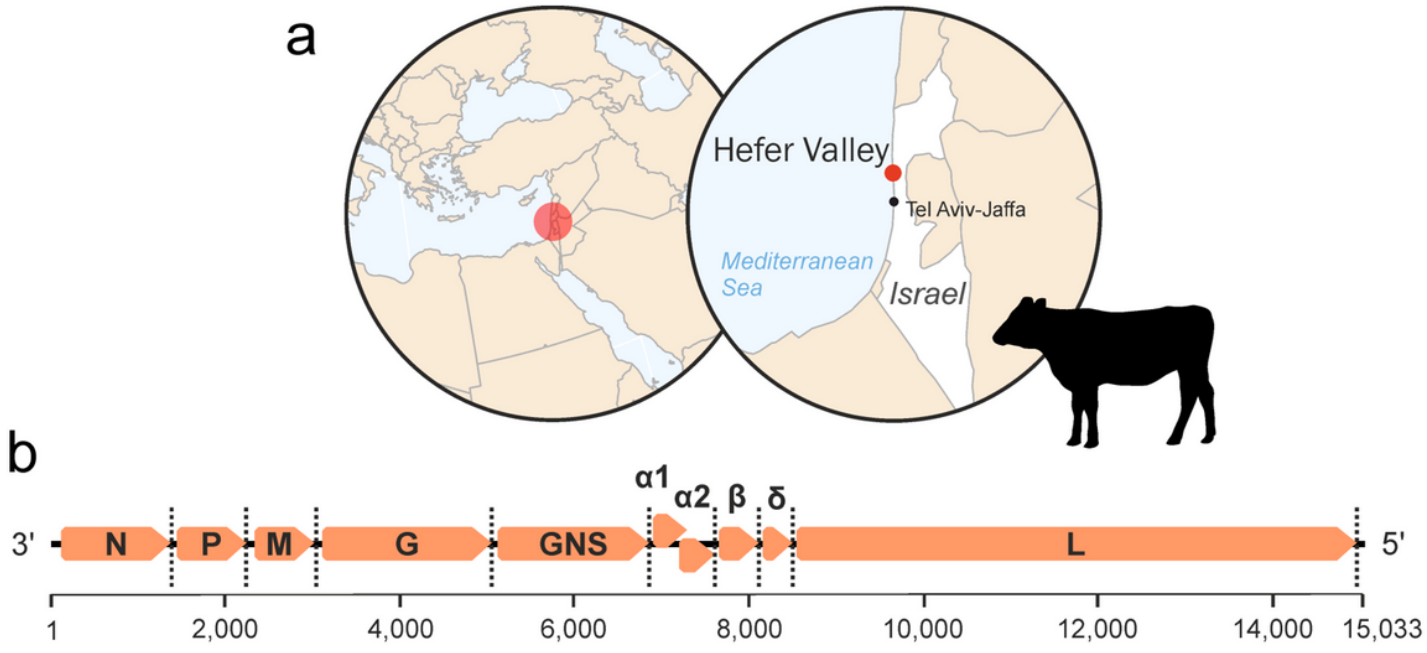

Figure 1

Geographic origin of Hefer Valley virus and its genomic architecture. (**a**) Hefer valley virus was identified in blood from a febrile cow from Hefer Valley, Israel (red dot). (**b**) The viral genome was sequenced and 10 potential open reading frames (arrows) and nine transcription termination sites (UGAAAAAAA; dashed lines) were predicted. The overall genomic architecure was comparable to that of other members of the genus *Ephemerovirus*.

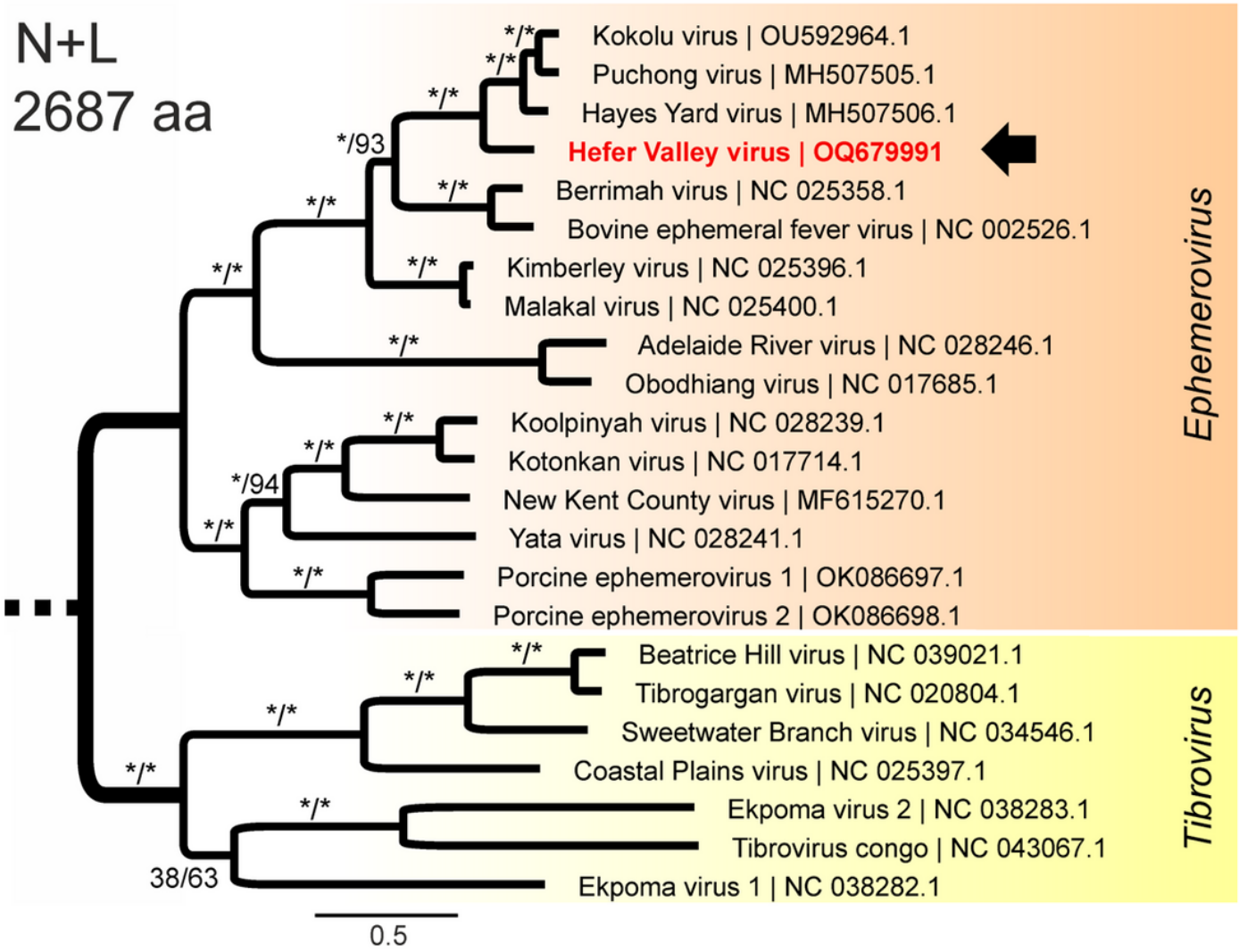

Figure 2

Phylogenetic classification of Hefer Valley virus within the genus *Ephemerovirus* based on Maximum-Likelihood analsis of L and N proteins. A concatenation of individual N and L amino acid sequence alignments of representative sequences from the genera *Ephemerovirus* (orange) and *Tibrovirus* (yellow; outgroup) was analysed using IQ-TREE2 (100,000 SH-aLRT and ultrafast bootstrap replicates). The position of Hefer Valley virus (red) is indicated with a black arrow. Branch support values are: SH-aLRT support (%) / ultrafast bootstrap support (%). Support by SH-aLRT and UFboot is indicated using asteriks if greater then 80% and 95%, respectively.

Supplementary Files

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- Submission2686012.txt
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