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BRIEF REPORT

Hefer valley virus: a novel ephemerovirus detected in the blood of a cow with severe clinical signs in Israel in 2022

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Abstract

A novel ephemerovirus was identified in a Holstein-Friesian cow in the Hefer Valley, Israel, that showed severe and fatal clinical signs resembling an arboviral infection. A sample taken during the acute phase tested negative for important endemic arboviral infectious cattle diseases. However, sequencing from blood revealed the full genome sequence of Hefer Valley virus, which is likely to represent a new species within the genus *Ephemerovirus*, family *Rhabdoviridae*. Archived samples from cattle with comparable clinical signs collected in Israel in 2021 and 2022 tested negative for the novel virus, and therefore, the actual distribution of the virus is unknown. As this is a recently identified new viral infection, the viral vector and the prevalence of the virus in the cattle population are still unknown but will be the subject of future investigations.

The genus *Ephemerovirus* of the family *Rhabdoviridae* comprises viruses that primarily infect ruminants and are transmitted by blood-sucking insects [1, 2], including Puchong virus (PUCV) from mosquitoes in Malaysia in 1965, Hayes Yard virus (HYV) from a bull in Australia in 2000, and Kotonkan virus (KOV) [3–5]. However, several ephemeroviruses have been detected in other animal species, including porcine ephemeroviruses 1 and 2, which were identified in porcine tissues in China [6], and New Kent County virus, which was isolated from ticks in North America (origin: GenBank).

Bovine ephemeral fever virus (BEFV, species *Ephemerovirus febris*) 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 biphasic fever, hypersalivation, ocular and nasal discharge, recumbency, muscle stiffness, lameness, and anorexia. The morbidity is high, but the mortality is usually low (< 1%) [7]. BEFV is apparently transmitted by two types of arthropod vectors: *Culicoides* and mosquitoes (culicine and anopheline) [8]. In addition to BEFV, other ephemeroviruses, such as HYV and KOV, have been reported to cause comparable clinical diseases in cattle [5, 8].

The genome of ephemeroviruses consists of negative-sense single-stranded RNA (ssRNA(-)) and is about 15 kb in length, containing 10 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, blood samples from two cows that showed clinical signs resembling arboviral infection were sent for laboratory diagnosis from Ein HaHoresh, a cooperative dairy farm (310 adult cows and 230 female calves

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from the age 0 to two years old) located in the Hefer Valley in central Israel (Fig. 1a). One of the cows died 14 days after the onset of clinical signs. A blood sample from this cow was collected two days after the onset of clinical signs, which developed during the first seven days after delivery of a healthy female calf. Clinical signs included fever and hypocalcemia, followed by ketonuria, milk reduction, and recumbency. Retrospective collection of data about this animal revealed that it was a six-year-old dairy cow that had been vaccinated several times with ULTRAVAC BEF VACCINE (Zoetis) to protect against BEFV. The blood samples were tested by RT-qPCR assays specific for frequently found important arboviral infections of cattle in the region, including bluetongue virus, epizootic hemorrhagic disease virus [9], and BEFV, targeting the G coding region [10] (Table 1). All of the PCR tests gave negative results. These samples were therefore also tested using a recently developed RT-qPCR assay specific for the N region of BEFV (Table 1). An AgPath-ID™ One-Step RT-PCR Kit (Applied Biosystems, Warrington, Cheshire, United Kingdom) was used for in-house RT-qPCR tests (Table 1, Supplementary Table S1). The real-time RT-PCR program for the thermocycler was performed as described previously [11], and the master mix preparation is shown in Supplementary Table S1. One of the blood samples (that of the animal that died) gave an equivocal result with a recently developed RT-qPCR assay specific for the N region of BEFV. The 100-bp RT-PCR product from that test was purified from a gel using a MEGAquick-spin Total Fragment DNA Purification Kit (iNtRON Biotechnology, Gyeonggi-do, South Korea) and subjected to Sanger sequencing. BLASTn analysis of the sequenced product revealed a high degree of sequence similarity to members of the genus *Ephemerovirus*. Attempts to isolate the virus from the blood sample on Vero (African green monkey kidney), BHK-BSR (baby hamster kidney-21 clone BSR), and C6/36 (*Aedes albopictus*) cells failed.

Due to their low melting temperatures, the probes were prepared as alternative MGB probes (https://www.biolegio.com/)

Metagenomic RNA sequencing and *de novo* assembly were performed on the blood sample from the cow that died (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 (Fig. 1a).

The genome of HVV is 15,033 nt in length. We predicted and characterized 10 open reading frames (ORF), with the typical arrangement found in ephemerovirus genomes (3′-N-P-M-G-GNS-α1-α2-β-γ-L-5′; Fig. 1b). Furthermore, we identified nine transcription termination sites (UGAAAAAAA) that were located adjacent to the ORFs.

For phylogenetic classification, individual amino acid sequence alignments of the N and L proteins of HVV and 22 representative viruses of the genera *Ephemerovirus* and *Tibrovirus* were made using MUSCLE (version 3.8.425) and then concatenated into a single alignment, and a maximum-likelihood phylogenetic analysis was performed using

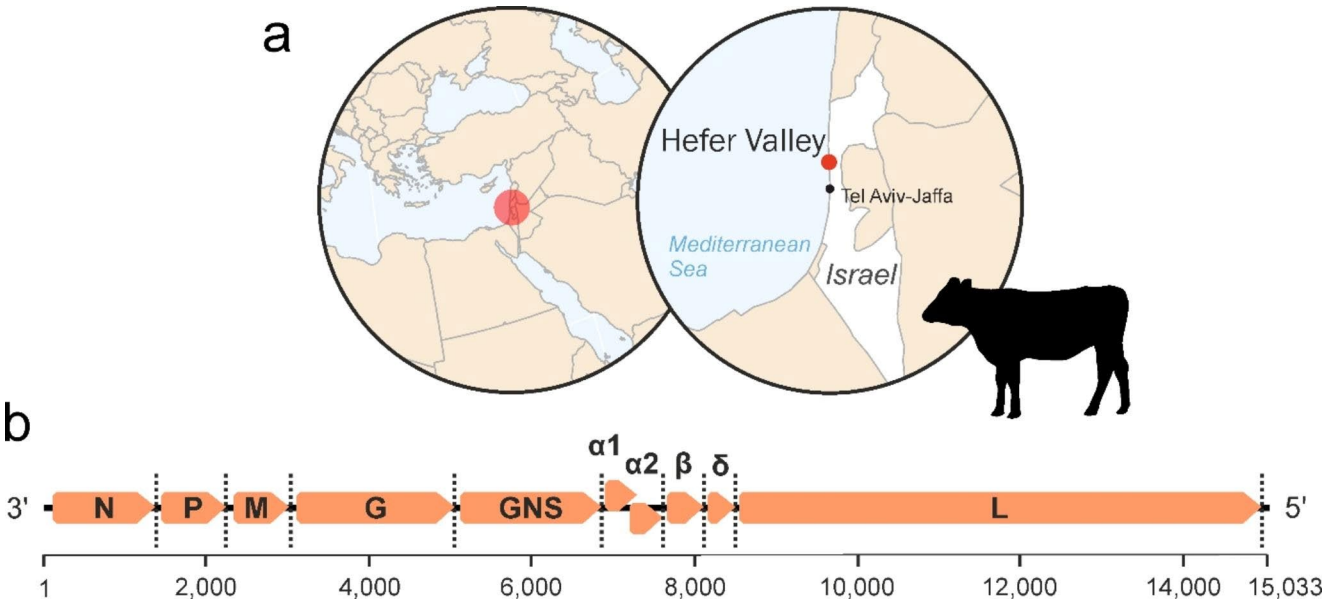

Fig. 1 Geographic origin of Hefer Valley virus and its genomic architecture. (**a**) Hefer valley virus was identified in blood from a febrile cow from the Hefer Valley, Israel (red dot). (**b**) The viral genome was sequenced, and 10 potential open reading frames (arrows) and nine transcription termination sites (UGAAAAAAA; dashed lines) were predicted. The overall genomic architecure was comparable to that of other members of the genus *Ephemerovirus*.

Table 1 Information on the PCR systems used for detection and identification of the ephemeroviruses from Israel

PCR type	Genome region	Oligonucleotide name	Oligonucleotide sequence (5' → 3')	Source
TaqMan RT-qPCR	BEFV	BEF-N-543F	TCA AAT TGA ATG CAC AGA TYA AAG G	This study
	N-coding region	BEF-probe-N571	ROX-AGA AAA GAT GCA CCC AAT ATT ATT GA-BHQ2	
		BEF-N-618R	CAG AAT TCA CTA TTT GTC ACC CAA C	
TaqMan RT-qPCR	pan-ephemerovirus	Eph-N-543F	TSA ARY TKA ATG CWC ARA TYA AAG G	This study
	N-coding region	Eph-probe-N571	ROX-AGR AAA GAT GCA CCC AAT ATT ATT GA-BHQ2	
		Eph-N-611R	GTA TTT GTC ACC CAR CTY CCA T	
conventional RT-PCR	Ephemeroviruses	Eph-N-451F	GAT GAA AMW GAT GAY KTA TGG TTA A	This study
	N coding region	Eph-N-727R	GCW GCT GCA TCY TTR TAT CTR GA	
SYBR Green RT-qPCR	BEFV	BEF-HRM-1140F	GAA TCA TTA TGG GAT MGG ATC	[10]
	G coding region	BEF-HRM-1273R	CCT CCT GCT GGT GCT GTT TC	

IQ-TREE2 (see Supplemental Material). The phylogenetic tree based on N and L proteins suggested that HVV is a member of a novel species within the genus *Ephemerovirus* and is most closely related to HYV, PUCV, and KOV (Fig. 2). The amino acid sequence identity of HVV to HYV, KOV, and PUCV was 87.7–89.4% and 77.3–78.4% for the N and L protein, respectively (Supplementary Fig. S1a and b).

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 assay (Table 1 and Supplementary Table S1). All of the samples were collected from August to January, the usual arbovirus season in the region. No additional positive samples were identified in 2022, while 50 samples collected in 2021 were positive. Identification of the specific ephemeroviruses in these positive samples was performed using a BEFV-specific SYBR Green–based RT-qPCR assay [10] in combination with the generic conventional RT-PCR for the N coding region of ephemeroviruses (Table 1 and Supplementary Table S1). For the generic conventional RT-PCR, a One-Step RT-PCR Kit (QIAGEN, Hilden, Germany) was used, and the conditions for thermocycling are shown in Supplementary Materials. All 50 positive samples collected in 2021 were identified as BEFV, and none were HVV (data not shown).

During the last decade, BEFV outbreaks were registered in Israel in 2014, 2015, 2018, and 2021, alternating with periods of "total silence" from the infection. The most serious, long-lasting (July-December) outbreak occurred in 2021, where cases were also reported in arid areas, which had not been observed before. However, due to the absence of systematic screening programs, there is no information available about whether members of the genus *Ephemerovirus* other than BEFV have circulated in the region and, if so, whether they are of veterinary importance.

In conclusion, we report the identification of HVV, a novel member of the genus *Ephemerovirus* that is most closely related to HYV, PUCV, and KOV. The blood sample was collected in October 2022 from an adult BEFV-vaccinated milking cow in Israel exhibiting severe illness that subsequently died. Future serological and molecular examinations are expected to reveal the extent of the exposure of cattle to HVV in Israel and to determine its veterinary significance. Also, as the affected cattle had a history of vaccination against BEFV, the efficacy of these vaccines against HVV needs to be evaluated.

As several well-known viruses of the genus *Ephemerovirus* are transmitted by blood-sucking insects, potential vectors should be collected in regions with affected cattle and investigated for the presence of HVV.

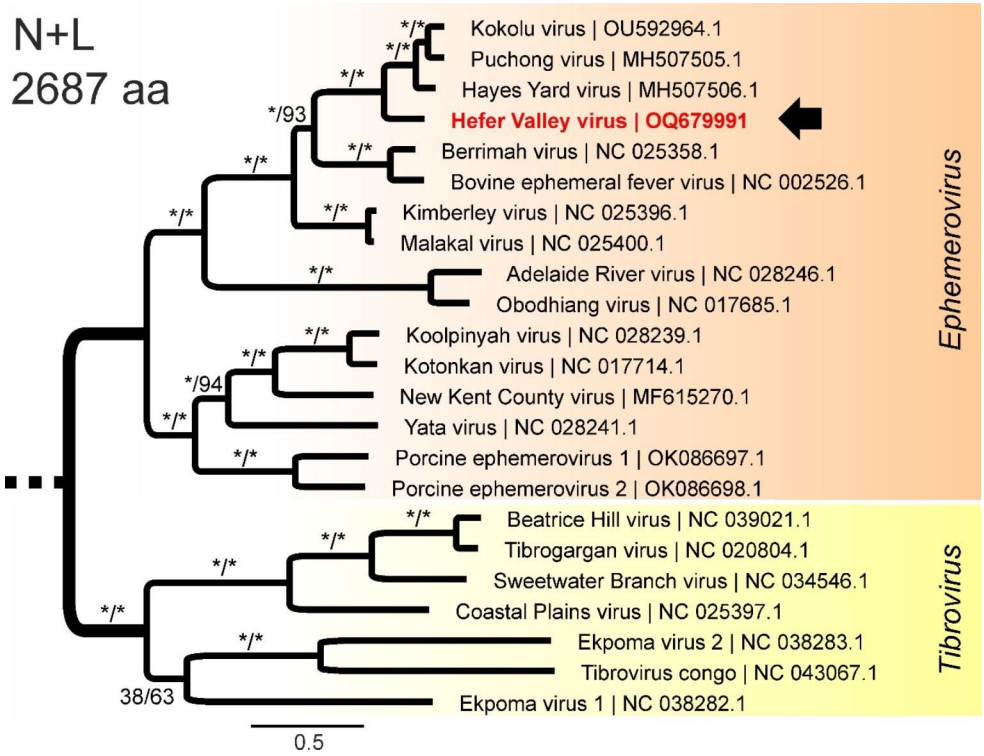

Fig. 2 Phylogenetic classification of Hefer Valley virus within the genus *Ephemerovirus* based on maximum-likelihood analysis of amino acid sequences of the L and N proteins. A concatenation of individual N and L amino acid sequence alignments of representative sequences from the genera *Ephemerovirus* (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 by a black arrow. Branch support values shown as SH-aLRT support (%) / ultrafast bootstrap support (%). Support by SH-aLRT and UFboot is indicated by asterisks if greater then 80% and 95%, respectively.

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

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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

Bibliography

1. Walker PJ, Blasdell KR, Calisher CH et al (2018) ICTV virus taxonomy profile: Rhabdoviridae. J Gen Virol 99:447–448
2. Walker PJ, Blasdell KR, Joubert DA (2012) Ephemeroviruses: arthropodborne rhabdoviruses of ruminants, with large and complex genomes. In: Dietzgen RG, Kuzman IV (eds) Rhabdoviruses: molecular taxonomy, evolution, genomics, ecology, cytopathology and control. Horizon Scientific Press, Norwich
3. Balinandi S, Hayer J, Cholleti H et al (2022) Identification and molecular characterization of highly divergent RNA viruses in cattle, Uganda. Virus Res 313:198739. https://doi.org/10.1016/j.virusres.2022.198739
4. Blasdell KR, Davis SS, Voysey R et al (2020) Hayes Yard virus: a novel ephemerovirus isolated from a bull with severe clinical signs of bovine ephemeral fever is most closely related to Puchong virus. Vet Res 51:58. https://doi.org/10.1186/s13567-020-00781-1
5. Kemp GE, Lee VH, Moore DL et al (1973) Kotonkan, a new rhabdovirus related to Mokola virus of the rabies serogroup. Am J Epidemiol 98:43–49
6. Wu Q, Yang Z, Lu Z, Mi S et al (2022) Identification of two novel ephemeroviruses in pigs infected by classical swine fever virus. Infect Genet Evol 100:105273. https://doi.org/10.1016/j.meegid.2022.105273
7. Walker PJ, Klement E (2015) Epidemiology and control of bovine ephemeral fever. Vet Res 46:124. https://doi.org/10.1186/s13567-015-0262-4
8. Fenner F, Bachmann PA, Gibbs EPJ, Murphy FA, Studdert MJ, White DO (1987) Chap. 30 – Rhabdoviridae. Veterinary Virology. Elsevier Inc., Amsterdam, pp 531–548
9. Golender N, Varsano JS, Nissimyan T et al (2022) Identification of Novel Reassortant Shuni Virus Strain in Clinical Cases of Israeli Ruminants, 2020–2021. Trop Med Infect Dis 7:297. https://doi.org/10.3390/tropicalmed7100297

10. Erster O, Stram R, Menasherow S (2017) al High-resolution melting (HRM) for genotyping bovine ephemeral fever virus (BEFV). Virus Res. 2;229:1–8. https://doi.org/10.1016/j.virusres.2016.11.030
11. Wernike K, Hoffmann B, Beer M et al (2015) Simultaneous detection of five notifiable viral diseases of cattle by single-tube multiplex real-time RT-PCR. J Virol Methods 217:28–35. https://doi.org/10.1016/j.jviromet.2015.02.023