VETERINARIA ITALIANA
RIVISTA DI SANITÀ PUBBLICA VETERINARIA

Paper

Detection and molecular characterization of the BEF virus in western Türkiye

Kemal Pekmez[1*], Murat Kaplan[1], Abdurrahman Anıl Çağırgan[1], Fatih Arslan[1]

[1]*Department of Virology, Izmir/Bornova Veterinary Control Institute, Izmir, Turkey - TR*

[*]*Corresponding author at: Department of Virology, Izmir/Bornova Veterinary Control Institute, Izmir, Turkey - TR*
E-mail: kemalpekmez07@hotmail.com

Veterinaria Italiana, Vol. 61 No. 4 (2025) DOI: 10.12834/VetIt.3810.35777.2

Abstract

Bovine ephemeral fever (BEF) is characterized by high fever, nasal and eye discharge, excessive salivation, muscle weakness, yield losses and or with high morbidity and low mortality. The first epidemic of BEFV in Turkey occurred in 1985 since then epidemics were reported every 2-4 years in the south and southeastern regions of Turkey. Since the first detection of the virus in Turkey, the BEF virus was reported from other parts of the country except the Aegean Region. In November 2020, the possible outbreaks of BEF were reported from two different locations of the Aegean Region in Turkey. In this study, it was aimed to determine the molecular characterization and possible origin of the virus that caused the epidemic in the Aegean Region. For this purpose, blood samples collected from clinically infected animals were tested by RT-qPCR, and complete G gene sequences were carried out of the positive sample using the primers designed in this study. According to the phylogenetic analysis, virus is located in the Middle East lineage. Based on field observations and the data obtained in the study, it was thought that the spread of the virus to the Aegean Region was caused by animal movements from other regions.

Keywords

Bovine ephemeral fever, Cattle, Epidemic, Molecular Characterization

Introduction

Bovine ephemeral fever (BEF) is characterized by high fever, nasal and eye discharge, excessive salivation, muscle weakness, and yield losses (Akakpo, 2015; Walker & Klement, 2015; Lee, 2019). This arthropod-borne viral disease of buffalo and domestic cattle is also known as three-day fever or three-day disease and is caused by bovine ephemeral fever virus (BEFV). BEFV, species Ephemerovirus febris, belongs to the Ephemerovirus genus of the Apharhabdovirinae subfamily of the *Rhabdoviridea* family (Dietzgen et al., 2012). The structure of BEFV virion is enveloped, bullet-shaped, 70-140 nm in size, and slightly tapers toward the rounded tip (Holmes & Doherty, 1970). The genome is a negative-sense, single-stranded RNA molecule, consisting of ~14.9kb nucleotides (Walker, 2005). Five structural proteins, namely the nucleoprotein (N), glycoprotein (G), RNA dependent RNA polymerase (L), matrix protein (M) and phospho-protein (P), are encoded from the genome (Walker et al., 1991). The G protein is a surface protein and contains target sites for neutralizing antibodies. Four antigenic epitope regions, designated as G1, G2, G3 and G4 were previously described for the G protein (Cybinski et al., 1990; Kongsuwan et al., 1998). Additional regions AA_{67-74}, $AA_{132-149}$, $AA_{196-225}$,and $AA_{315-546}$ that may also contain possible antigenic epitope regions have been described previously and they have been designated as site-I, II, III and IV, respectively in this study (Bakhshesh et al., 2018). Even though BEFV is considered to be serologically uniform, it is genetically divided into four lineages referred to as Australia, East Asia, Middle East, and South Africa, based on the phylogenetic characterization of the G protein sequence (Aziz-Boaron et al., 2012; Trinidad et al., 2014; Omar et al., 2020; Pyasi et al., 2020).

The disease is non-contagious but transmitted by various mosquito and Culicoides species, especially species belonging to the Culicidae family (Finlaison et al., 2010). It is widespread in East, South, and Southeast Asia, Australia as well as some parts of Africa (Lee, 2019). The first epidemic of BEFV in Türkiye occurred in 1985 (Girgin et al., 1986) and since then, epidemics have been reported to occur every 2–4 years in the south and southeastern regions of Türkiye (Aziz-Boaron et al., 2012). The occurring epidemics and subsequent serological studies indicate that the

How to cite: Pekmez et al. Detection and molecular characterization of the BEF virus in western Türkiye. Veterinaria Italiana, Vol. 61 No. 4 (2025) DOI: 10.12834/VetIt.3810.35777.2

virus is common around the middle, south, southeast, north, and northwestern parts of the country (Karaoğlu et al., 2007; Albayrak & Ozan, 2010; Aziz-Boaron et al., 2012; Tonbak et al., 2013; Karayel- Hacıoğlu et al., 2021; Özyörük et al., 2025). Despite the wide distribution of BEFV in Türkiye, the disease has never been reported in Aegean region. The Aegean region is located in the west of Türkiye and has suitable climatic conditions for vector activity.

The aim of this study was molecular detection, phylogenetic characterization, and investigation of the origin of BEFVs isolated during an epidemic within two different locations in the Aegean region in November, 2020.

Materials and methods

History of the epidemic

In the beginning of November 2020, cattle presented clinical signs of high fever, eye, and nasal discharge, loss of appetite, followed by a decrease in milk production in the Nazilli and Söke districts of Aydın. The symptoms continued for approximately 3-4 days, and the farmers reported these observations to the official veterinarian. The veterinarian suspected BEF, and whole blood samples were collected and submitted to the İzmir/Bornova Veterinary Control Institute for diagnosis. Real time reverse transcription polymerase chain reaction (RT-qPCR) identified the presence of BEFV nucleic acid in the samples. Subsequent field research was carried out by the regional and institute veterinarians to include additional examinations and sampling. The locations of BEF cases detected in other provinces and in this study in 2020 are shown in Figure 1.

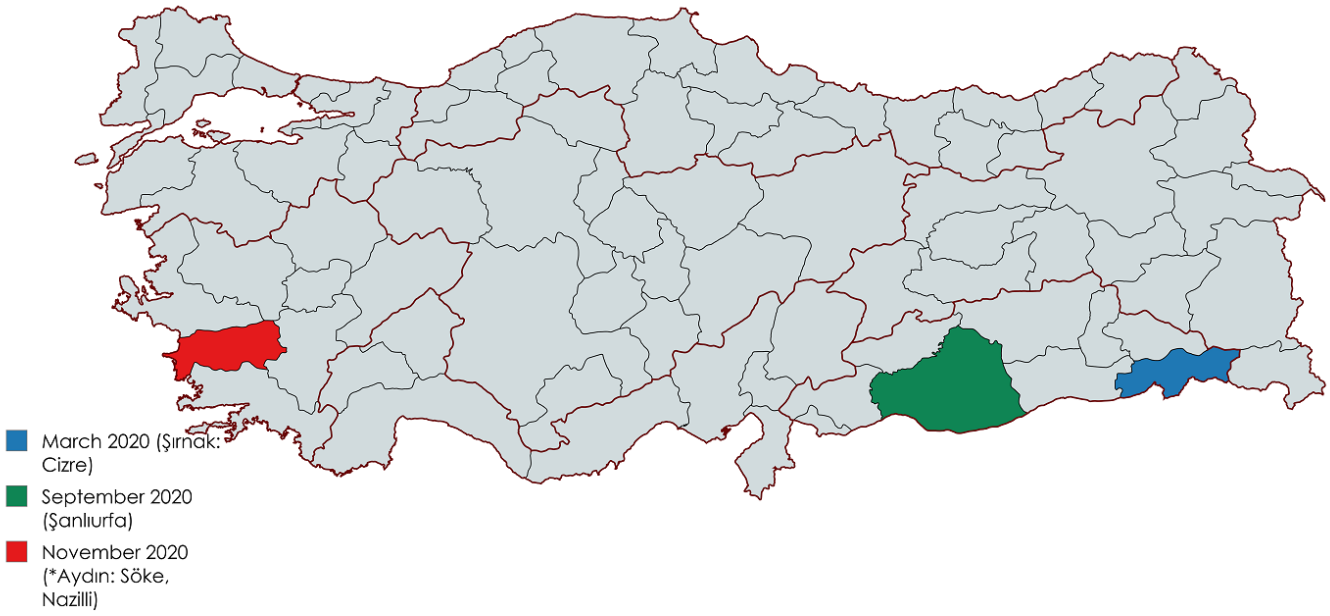

Figure 1. Locations of BEF cases reported from Türkiye in 2020.

RNA extraction Real Time RT-PCR and RT-PCR

Total nucleic acid extractions were performed using the Roche MagNA Pure LC device and MagNA Pure LC Total Nucleic Acid Isolation kit (Roche, Germany) in accordance with the manufacturer's instructions. RT-qPCR tests targeting the genome of BEFV were performed using the Real Time Ready RNA virus Master kit (Roche, Germany) and the primer probe set recommended by Erganis et al. (2014). The 20 µl reaction mix consisted of 0.5 µM primers, 0.5 µM probe, as well as 5 µl of sample and was performed under typical cycling conditions with annealing at 60 °C for 40 cycles.

How to cite: Pekmez et al. Detection and molecular characterization of the BEF virus in western Türkiye. Veterinaria Italiana, Vol. 61 No. 4 (2025) DOI: 10.12834/VetIt.3810.35777.2

Sequencing and phylogenetic analysis

The primers used in this study and encoding the complete G protein gene were designed by our laboratory using Oligo Analyzer Tool (https://www.idtdna.com/pages/tools/oligoanalyzer) and the reference sequence of BEFV/Ad12/TUR isolate obtained from GenBank, accession number: KY012742 (Table I). Xpert One Step RT-PCR kit (Grisp) was used for amplification. The 25 µl reactions contained 0.5 µM primers, Fast PCR master mix, and 5 µl of sample for each reaction as described by the manufacturer. cDNA synthesis was performed for 15 minutes at 45 °C, and then 3 minutes at 95 °C for hot start, followed by 40 cycles of 95 °C for 10 s, 55 °C for 10 s, and 72 °C for 15 s; and then 72 °C for 1 min for extension. The amplicons were visualized using agarose gel electrophoresis.

No	Name	Sequence	Position	Length (bp)	Reference
1	1F	5′-AAAGTTAGGTTAACAGGACCTCAC -3′	3035-3058	369	This study
	1R	5′- GCATCTGTGCAACCGGAATAC -3′	3383-3403		
2	2F	5′- TGTCACTTCAGGCTCATCATAA -3′	3196-3217	553	
	2R	5′- GCCTCCCATAATCTCTCTGTATC -3′	3726-3748		
3	3F	5′- GGAGGCTCCAGATATTGGATTAG -3′	3743-3765	683	
	3R	5′- GGTTGGTTCCACGAAGATCA -3′	4390-4408		
4	4F	5′- TGGTGTGAATACCGACCTTTC -3′	4233-4253	770	
	4R	5′- TGCCCTGTTGCCTCTTATTT -3′	4983-5002		

Table I. Primer combinations used for PCR and sequencing.

PCR products purification and Sanger sequencing of the two samples belonging to two different epidemiological units, were performed using both forward and reverse primer combinations at a commercial company (Microsynt, Switzerland). Sequencing reads were assembled and edited using DNADynamo software, and the resulting consensus sequences were used in subsequent phylogenetic analysis.

Multiple alignments incorporating the sequences obtained from this study and GenBank were performed using the ClustalW algorithm in MEGA-X software (Kumar et al., 2018). Maximum likelihood phylogenetic trees of both partial and complete G gene sequence datasets were constructed using the General Time Reversible model + gamma distribution (GTR+G) with 1000 bootstrap replicates in the MEGA-X software (Nei and Kumar, 2000; Kumar et al., 2018).

Amino acid and nucleotide distance

The genetic distances between all the aforementioned G gene sequences were calculated based on both nucleotide (nt) and predicted amino acid (aa) sequences. Comparisons between the sequences were calculated using isolate BB7721 from Australia in 1968 (AF234533) as reference sequence. Nucleotide distances of sequences were calculated in MEGA-X software using GTR+G method, while amino acid distances were calculated using the JJT+G method. Additional partial sequences, obtained from GenBank, were included in the predictions of amino acids as well as the putative changes involved in the previously described epitope regions: G1, G2, G3, G4, Site-I, Site-II, Site-III, and Site-IV.

How to cite: Pekmez et al. Detection and molecular characterization of the BEF virus in western Türkiye. Veterinaria Italiana, Vol. 61 No. 4 (2025) DOI: 10.12834/VetIt.3810.35777.2

Results

Clinical observation and laboratory confirmation

During field investigation, it was determined that 150 farms (Nazilli: 147, Söke: 3) and approximately 1782 (Nazilli:1624, Söke:158) cattle were affected from outbreak occurred villages of Nazilli and Söke, located in the Aegean Region of Türkiye, in November 2020. Cattle displayed clinical symptoms including high fever, salivation, and muscle weakness that lasted between 3-4 days. The general condition of the animals improved, however a noticeable decrease in the milk yield of the infected animals continued for 15-20 days following the clinical symptoms. A morbidity rate of 80% and no mortality were observed during this period. Blood samples from 11 animals, 9 clinically infected and 2 recovered, were submitted to the İzmir/Bornova Veterinary Control Institute for laboratory confirmation of suspected BEF, followed by additional field investigation performed by the veterinarians from the institute. The disease was confirmed as BEF through RT-qPCR and was suggested to have started nearly 20-25 days prior to the first sample arriving at the institute. All blood samples both clinically infected and recovered were found to be BEFV positive. The Ct values of the samples varied between 22 and 32.

Interviews with farmers and official veterinarians indicated that they had never witnessed similar symptoms in the cattle in this region prior to this outbreak. The interviews additionally indicated that no animals were recently introduced to the affected or neighboring villages. However, a slaughterhouse in another district, approximately 10 km away from the affected villages, was reported with continuous movement of animals in and out of the facility. Subsequent communications with the official veterinarians indicated that they struggled with insect vectors following a diagnosis of the disease, so no new spread of the disease to neighboring villages was observed.

RT-PCR, sequencing and phylogenetic analysis

Two of the samples (each district was determined as an epidemiological unit and 1 strongly positive sample representing each unit was sequenced: TR BVKE NZL 2020 and TR BVKE SK 2020) submitted during the outbreaks from two different locations were subjected to sequence analysis of the G gene region. The sequences obtained in this study were consisted of 1872 nucleotides in length and were deposited into GenBank under the accession numbers MZ936269 and MZ936270 respectively.

Maximum Likelihood phylogenetic trees were constructed using both partial and complete G gene sequences. The phylogenetic tree consisting of partial sequences is presented in Figure 2, whilst the tree containing the complete G gene is indicated in Figure 3. Irrespective of whether the tree contains partial or the complete G gene region, the strains obtained in this study clustered with sequences originating from the Middle East. This lineage contains additional sequences obtained from other studies in 2020.

The Middle East lineage was sub-divided into four clusters, irrespective of the partial or complete gene region was used for analysis. The first subgroup includes only EGY12, an isolate from Egypt in 2012 (KJ729108). The second subgroup contains strains causing epidemics in Türkiye between 2008-2012. The third subgroup contain strains isolated from Israel and Egypt. The fourth group includes both isolates from India (2018–2019) and all strains isolated from the 2020 outbreaks in Türkiye (Figures 2, 3).

How to cite: Pekmez et al. Detection and molecular characterization of the BEF virus in western Türkiye. Veterinaria Italiana, Vol. 61 No. 4 (2025) DOI: 10.12834/VetIt.3810.35777.2

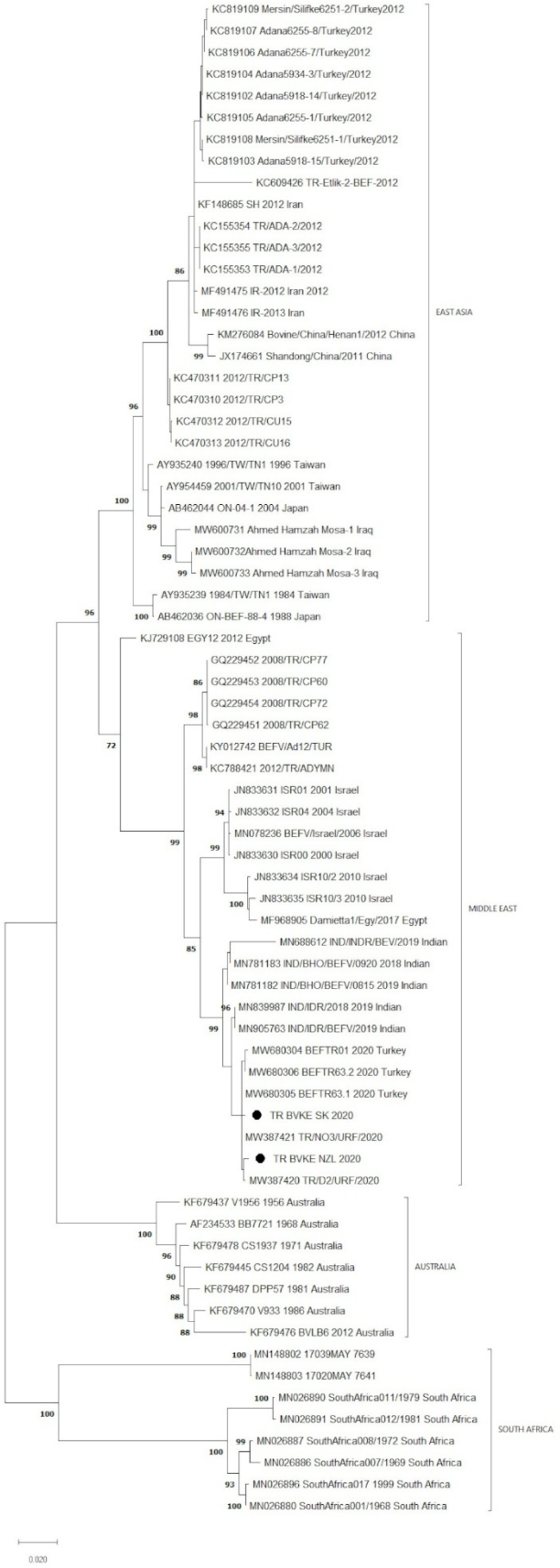

Figure 2. ML phylogenetic tree based on the partial nucleotide sequences of the G gene. The numbers at nodes represent bootstrap values (only values 70% are reported).

How to cite: Pekmez et al. Detection and molecular characterization of the BEF virus in western Türkiye. Veterinaria Italiana, Vol. 61 No. 4 (2025) DOI: 10.12834/VetIt.3810.35777.2

Figure 3. ML phylogenetic tree based on the complete nucleotide sequences of the G gene. The numbers at nodes represent bootstrap values (only values 70% are reported).

Amino acid, nucleotide distance and comparing antigenic epitopes

The genetic distance between the isolates from Türkiye and other countries was given in table II. The highest percentage sequence identity to the isolates described in this study, was shared with TR/D2/URF/2020 and TR/NO3/URF/2020 from Türkiye in 2020 and India (MN839987 and MN905763). The sequences obtained in this study shared the second highest percentage sequence identity with samples from Türkiye between 2008 and 2012. It was interesting to note that isolate TR BVKE SK 2020 shared a higher percentage sequence identity with TR/D2/URF/2020 and TR/NO3/URF/2020 isolates from Türkiye than TR BVKE NZL 2020.

Totally eight BEF virus sequences have been reported from Türkiye in 2020. While four of these are complete, the other four are partial sequences of the G gene. Three of the partial sequence (MW680304, MW680305 and MW680306) have sequences between 75-1002 bases, while one (OR633345) contains nucleotide sequences between 985-1782 bases of G gene. Since most of the reported sequences in previous years belonged to the first 1000 bp region of the G gene, the strain with the accession number OR633345 could not be included in the phylogenetic tree. However, in order to avoid data loss, the nucleotide and amino acid similarities between the relevant sequence and the 2008, 2012 and 2020 isolates from Türkiye and the 2018 and 2019 isolates from India were calculated and shown in the table III.

A total of 38 aa substitutions were observed in the complete coding region of the G gene. Twenty substitutions were observed in antigenic epitopes and could be three of these in the G1 epitope (R486K, N499S, K503T), one in the G3 epitope (K224T), three in the G4 epitope (L459I, D465E, V480I), one in Site I (A72D), two in Site III (E198K, N206S) and ten in site IV. Seven of these were observed in both isolates N366S, V299I, Q410L, G419R, A429T, N435T and R436K, while (S362L) were unique to TR BVKE NZL 2020 and similarly (S418F) was unique to TR BVKE SK 2020. In contrast, (A427E) was predicted in both isolates.

How to cite: Pekmez et al. Detection and molecular characterization of the BEF virus in western Türkiye. Veterinaria Italiana, Vol. 61 No. 4 (2025) DOI: 10.12834/VetIt.3810.35777.2

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	TR_BVKE_NZL_2020		0,0082	0,0033	0,0049	0,0183	0,0199	0,0200	0,0200	0,0098	0,0098	0,0115	0,0318	0,0389	0,0389	0,0407	0,0407	0,0371	0,0354	0,0475	0,0353	0,0617	0,0702	0,0719	0,0684	0,0686	0,0741	0,0723	0,0683	0,0667
2	TR_BVKE_SK_2020	0,0049		0,0049	0,0033	0,0166	0,0183	0,0183	0,0183	0,0082	0,0082	0,0098	0,0301	0,0371	0,0371	0,0389	0,0389	0,0371	0,0354	0,0336	0,0458	0,0335	0,0599	0,0683	0,0701	0,0666	0,0668	0,0723	0,0704	0,0665	0,0648
3	MW387420_TR/D2/URF/2020	0,0038	0,0043		0,0016	0,0149	0,0166	0,0166	0,0166	0,0065	0,0065	0,0082	0,0284	0,0353	0,0353	0,0371	0,0371	0,0336	0,0319	0,0440	0,0318	0,0581	0,0665	0,0682	0,0647	0,0650	0,0704	0,0686	0,0647	0,0650
4	MW387421_TR/NO3/URF/2020	0,0038	0,0021	0,0021		0,0132	0,0149	0,0149	0,0149	0,0049	0,0049	0,0065	0,0266	0,0336	0,0336	0,0354	0,0354	0,0319	0,0301	0,0422	0,0301	0,0563	0,0646	0,0664	0,0629	0,0631	0,0685	0,0667	0,0628	0,0611
5	GQ229452_2008/TR/CP77	0,0342	0,0349	0,0360	0,0336		0,0016	0,0082	0,0082	0,0115	0,0115	0,0166	0,0164	0,0315	0,0315	0,0333	0,0333	0,0282	0,0265	0,0351	0,0298	0,0473	0,0665	0,0682	0,0631	0,0631	0,0685	0,0667	0,0631	0,0612
6	GQ229451_2008/TR/CP62	0,0348	0,0355	0,0366	0,0343	0,0005		0,0098	0,0098	0,0132	0,0132	0,0183	0,0181	0,0332	0,0332	0,0350	0,0350	0,0299	0,0282	0,0368	0,0315	0,0491	0,0683	0,0700	0,0649	0,0649	0,0703	0,0685	0,0648	0,0629
7	KY012742_BEFV/Ad12/TUR	0,0336	0,0343	0,0354	0,0331	0,0043	0,0049		0,0000	0,0116	0,0116	0,0183	0,0214	0,0316	0,0316	0,0334	0,0334	0,0283	0,0266	0,0386	0,0299	0,0510	0,0705	0,0722	0,0635	0,0635	0,0689	0,0671	0,0634	0,0615
8	KC788421_2012/TR/ADYMN	0,0336	0,0343	0,0354	0,0331	0,0043	0,0049	0,0000		0,0116	0,0116	0,0183	0,0214	0,0316	0,0316	0,0334	0,0334	0,0283	0,0266	0,0386	0,0299	0,0510	0,0705	0,0722	0,0635	0,0635	0,0689	0,0671	0,0634	0,0615
9	MN839987_IND/HR/2018_2019_Indian	0,0109	0,0092	0,0092	0,0070	0,0337	0,0343	0,0331	0,0331		0,0000	0,0082	0,0249	0,0335	0,0335	0,0353	0,0353	0,0302	0,0284	0,0405	0,0318	0,0545	0,0664	0,0681	0,0647	0,0649	0,0703	0,0685	0,0646	0,0629
10	MN905763_IND/HR/BEFV/2019_Indian	0,0109	0,0092	0,0092	0,0070	0,0337	0,0343	0,0331	0,0331	0,0000		0,0082	0,0249	0,0335	0,0335	0,0353	0,0353	0,0302	0,0284	0,0405	0,0318	0,0545	0,0664	0,0681	0,0647	0,0649	0,0703	0,0685	0,0646	0,0629
11	MN078236_BEFV/Israel/2006_Israel	0,0342	0,0324	0,0335	0,0312	0,0316	0,0322	0,0323	0,0323	0,0300	0,0300		0,0301	0,0387	0,0387	0,0405	0,0405	0,0354	0,0336	0,0458	0,0335	0,0599	0,0719	0,0736	0,0666	0,0668	0,0723	0,0704	0,0665	0,0648
12	KJ729108_EGY12_2012_Egypt	0,0638	0,0631	0,0631	0,0618	0,0480	0,0486	0,0506	0,0506	0,0646	0,0646	0,0552		0,0349	0,0349	0,0367	0,0367	0,0316	0,0299	0,0418	0,0332	0,0473	0,0735	0,0752	0,0631	0,0630	0,0684	0,0666	0,0630	0,0611
13	KC470310_2012/TR/CP3	0,0946	0,0933	0,0923	0,0923	0,0823	0,0830	0,0823	0,0823	0,0955	0,0955	0,0888	0,0515		0,0000	0,0016	0,0016	0,0016	0,0065	0,0114	0,0197	0,0114	0,0580	0,0667	0,0684	0,0684	0,0666	0,0720	0,0685	0,0683	0,0629
14	KC470311_2012/TR/CP13	0,0946	0,0933	0,0923	0,0923	0,0823	0,0830	0,0823	0,0823	0,0955	0,0955	0,0888	0,0515	0,0000		0,0016	0,0016	0,0016	0,0065	0,0114	0,0197	0,0114	0,0580	0,0667	0,0684	0,0684	0,0666	0,0720	0,0685	0,0683	0,0629
15	KC470312_2012/TR/CU15	0,0961	0,0949	0,0939	0,0939	0,0837	0,0845	0,0838	0,0838	0,0970	0,0970	0,0903	0,0528	0,0011	0,0011		0,0000	0,0049	0,0131	0,0214	0,0131	0,0599	0,0686	0,0703	0,0703	0,0685	0,0740	0,0704	0,0702	0,0648
16	KC470313_2012/TR/CU16	0,0954	0,0941	0,0932	0,0931	0,0830	0,0838	0,0831	0,0831	0,0963	0,0963	0,0896	0,0522	0,0005	0,0005	0,0005		0,0049	0,0131	0,0214	0,0131	0,0599	0,0686	0,0703	0,0703	0,0685	0,0740	0,0704	0,0702	0,0648
17	AY935240_1996/TW/TN1_1996_Taiwan	0,0906	0,0894	0,0884	0,0884	0,0756	0,0763	0,0771	0,0771	0,0915	0,0915	0,0836	0,0438	0,0160	0,0160	0,0160	0,0154		0,0082	0,0165	0,0114	0,0546	0,0684	0,0701	0,0668	0,0651	0,0705	0,0669	0,0667	0,0613
18	AY935239_1984/TW/TN1_1984_Taiwan	0,0865	0,0852	0,0857	0,0843	0,0733	0,0740	0,0754	0,0754	0,0873	0,0873	0,0810	0,0457	0,0281	0,0281	0,0293	0,0287	0,0212	0,0245		0,0181	0,0098	0,0510	0,0666	0,0683	0,0650	0,0632	0,0687	0,0635	0,0649	0,0595
19	AY935239_1984/TW/TN1_1984_Taiwan	0,0865	0,0852	0,0857	0,0843	0,0733	0,0740	0,0754	0,0754	0,0873	0,0873	0,0810	0,0457	0,0281	0,0281	0,0293	0,0287	0,0212	0,0245		0,0214	0,0600	0,0792	0,0809	0,0760	0,0743	0,0798	0,0762	0,0760	0,0705
20	KM276084_Bovine/China/Henan1/2012_China	0,0996	0,0983	0,0980	0,0973	0,0899	0,0907	0,0885	0,0885	0,1005	0,1005	0,0937	0,0617	0,0227	0,0227	0,0227	0,0233	0,0292	0,0327	0,0387		0,0562	0,0700	0,0717	0,0649	0,0631	0,0685	0,0650	0,0648	0,0594
21	AF234533_BB7721_1968_Australia	0,1198	0,1191	0,1174	0,1182	0,1070	0,1078	0,1117	0,1117	0,1183	0,1183	0,1164	0,0949	0,1128	0,1128	0,1128	0,1136	0,1064	0,1112	0,1054	0,1138		0,0852	0,0870	0,0909	0,0894	0,0951	0,0902	0,0908	0,0860
22	MN148802_17038/MAY_7639	0,1615	0,1615	0,1613	0,1597	0,1531	0,1541	0,1556	0,1556	0,1593	0,1593	0,1631	0,1624	0,1518	0,1518	0,1536	0,1527	0,1525	0,1487	0,1582	0,1628	0,1767		0,0016	0,0743	0,0778	0,0834	0,0815	0,0742	0,0758
23	MN148803_17020/MAY_7641	0,1624	0,1624	0,1622	0,1606	0,1540	0,1550	0,1565	0,1565	0,1602	0,1602	0,1640	0,1633	0,1526	0,1526	0,1545	0,1536	0,1534	0,1496	0,1591	0,1637	0,1776	0,0005		0,0760	0,0796	0,0852	0,0833	0,0759	0,0776
24	MN026890_South-Africa/1111/1979_South_Africa	0,1705	0,1684	0,1690	0,1666	0,1742	0,1753	0,1735	0,1735	0,1697	0,1697	0,1660	0,1653	0,1791	0,1791	0,1812	0,1802	0,1743	0,1732	0,1733	0,1825	0,1847	0,1733	0,1742		0,0131	0,0181	0,0148	0,0032	0,0098
25	MN026887_South-Africa/0081972_South_Africa	0,1744	0,1714	0,1720	0,1695	0,1812	0,1824	0,1805	0,1805	0,1727	0,1727	0,1677	0,1681	0,1726	0,1726	0,1746	0,1736	0,1685	0,1653	0,1658	0,1780	0,1733	0,1605	0,1614	0,0360		0,0098	0,0115	0,0131	0,0065
26	MN026886_South-Africa/0071969_South_Africa	0,1760	0,1739	0,1745	0,1720	0,1816	0,1827	0,1808	0,1808	0,1752	0,1752	0,1703	0,1684	0,1728	0,1728	0,1748	0,1739	0,1709	0,1676	0,1660	0,1783	0,1756	0,1676	0,1655	0,0411	0,0071		0,0165	0,0181	0,0115
27	MN026896_South-Africa/017_1999_South_Africa	0,1727	0,1706	0,1712	0,1688	0,1787	0,1798	0,1779	0,1779	0,1720	0,1720	0,1674	0,1679	0,1740	0,1740	0,1760	0,1751	0,1712	0,1669	0,1684	0,1795	0,1702	0,1700	0,1709	0,0347	0,0120	0,0154		0,0148	0,0049
28	MN026891_South-Africa/012/1981_South_Africa	0,1695	0,1675	0,1681	0,1657	0,1733	0,1744	0,1726	0,1726	0,1688	0,1688	0,1651	0,1644	0,1782	0,1782	0,1802	0,1792	0,1733	0,1722	0,1723	0,1815	0,1837	0,1744	0,1754	0,0027	0,0366	0,0418	0,0353		0,0098
29	MN026880_South-Africa/0011968_South_Africa	0,1705	0,1685	0,1691	0,1666	0,1765	0,1776	0,1758	0,1758	0,1698	0,1698	0,1648	0,1653	0,1718	0,1718	0,1738	0,1729	0,1686	0,1654	0,1658	0,1773	0,1687	0,1674	0,1684	0,0329	0,0103	0,0138	0,0016	0,0336	

Table II. The genetic distance between the isolates from Turkey and other countries. The distances of among to nucleotides and amino acids sequences (left panel: nucleotide, right panel amino acid).

How to cite: Pekmez et al. Detection and molecular characterization of the BEF virus in western Türkiye. Veterinaria Italiana, Vol. 61 No. 4 (2025) DOI: 10.12834/VetIt.3810.35777.2

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	OR633345_BEFV/HRU/1/2020_Turkey		98.98	98.98	99.32	99.66	98.98	98.98	97.62	97.96	98.3	98.3	97.96	98.3
2	TR_BVKE_SK_2020	99.43		98.64	99.66	99.32	99.32	99.32	97.96	98.3	98.64	98.64	98.3	98.64
3	TR_BVKE_NZL_2020	99.54	99.43		98.98	99.32	98.64	98.64	97.28	97.62	97.96	97.96	97.62	97.96
4	MW387421_TR/NO3/URF/2020	99.66	99.77	99.66		99.66	99.66	99.66	98.3	98.64	98.98	98.98	98.64	98.98
5	MW387420_TR/D2/URF/2020	99.54	99.43	99.54	99.66		99.32	99.32	97.96	98.3	98.64	98.64	98.3	98.64
6	MN905763_IND/IDR/BEFV/2019_Indian	99.32	99.21	99.09	99.43	99.09		100	98.64	98.98	99.32	99.32	98.98	99.32
7	MN839987_IND/IDR/2018_2019_Indian	99.32	99.21	99.09	99.43	99.09	100		98.64	98.98	99.32	99.32	98.98	99.32
8	MN781183_IND/BHO/BEFV/0920_2018_Indian	98.19	98.08	97.97	98.3	97.97	98.87	98.87		99.66	98.64	98.64	98.3	98.64
9	MN781182_IND/BHO/BEFV/0815_2019_Indian	98.42	98.3	98.19	98.53	98.19	99.09	99.09	99.77		98.98	98.98	98.64	98.98
10	KY012742_BEFV/Ad12/TUR	97.85	97.74	97.63	97.97	97.63	98.08	98.08	97.85	98.08		100	98.98	99.32
11	KC788421_2012/TR/ADYMN	97.85	97.74	97.63	97.97	97.63	98.08	98.08	97.85	98.08	100		98.98	99.32
12	GQ229451_2008/TR/CP62	97.63	97.51	97.4	97.74	97.4	97.85	97.85	97.63	97.85	99.54	99.54		99.66
13	GQ229452_2008/TR/CP77	97.74	97.63	97.51	97.85	97.51	97.97	97.97	97.74	97.97	99.66	99.66	99.88	

Table IIII. Nucleotide and amino acid similarities between OR633345 strain and 2008, 2012, 2020 Türkiye isolates and 2018, 2019 Indian isolates.

Discussion

BEFV could cause epidemics at regular intervals in endemic regions. Recent BEF epidemics in Türkiye occurred in 2008, 2012 and 2020. The 2008 outbreak was limited to the south of the country while the 2012 outbreaks had a larger distribution towards the northwest of the country (Aziz-Boaron et al., 2012; Tonbak et al., 2013; Karayel-Hacıoğlu et al., 2021). Serological studies indicated that the virus appeared to be circulating in the north and European part of the country (Karaoğlu et al., 2007). Until this study, no reports of BEF have been from the Aegean region, located in the west of the country. This region has suitable climate conditions for the insect vectors involved in BEFV transmission. On the contrary, a serological study was performed in two provinces in Aegean region, but, researchers found that no seropositivity of BEFV (Erol et al., 2015). The first BEFV detection in 2020 was reported from the Cizre district of Şırnak province in Türkiye in March followed by from Şanlıurfa in September (Karayel-Hacıoğlu et al., 2021; Özyörük et al., 2025). Two small-scale epidemics that occurred concurrently in two different locations in the Aegean region, the subject to this study, occurred nearly eight months after abovementioned outbreaks. In addition to the long distances that the insect vectors could travel aided by air flow, animal transportation to this region could be responsible for the spread of BEFV (Aziz-Boaron et al., 2012). It is thought that the slaughterhouses located in close distance (10 kilometers) to two epidemic areas may play a role of spread of BEFV to the Aegean region. Animals could be transported from all part of country in slaughterhouses. According to interviews with official veterinarians, they reported that some animals were transported to slaughterhouse from southeast of Türkiye where BEFV was detected provinces. In addition, the onset of the epidemic coincided with the months when temperatures decreased resulting in a decline in vector activity, which may play a role in limiting the spread of the epidemic.

Phylogenetically, the BEFVs involved in the 2008 outbreak in Türkiye clustered within the Middle East lineage, whilst the isolates detected from 2012 outbreak clustering in both the East Asia and Middle East lineages (Aziz-Boaron et al., 2012; Tonbak et al., 2013). In contrast, all the isolates clustered within in the Middle East lineage in 2020. Totally eight isolates reported from Türkiye in 2020, two of them this study, two of reported by Karayel Hacıoğlu (2021) (MW387420, MW387421), one of reported by Özyürük (2025) (OR633345) and three of obtained from Genbank (MW680304, MW680305 and MW680306). Strains detected in this study shared a higher percentage sequence identity to the 2018-2019 India strains than the 2008-2012 Türkiye strains. These findings support the hypothesis of Özyörük (2025) that a BEFV strain was introduced from India in 2020 and subsequently spread from southeast to west of Türkiye.

Phylogenetic analysis both partial and complete G gene sequences sub-divided the Middle East lineage into four subgroups. These subgroups contain isolates from Egypt, Türkiye, Israel and India representing outbreaks in 2008, 2012 (Türkiye), 2000, 2006 and 2010 (Israel), 2017 (Egypt) and 2018, 2019 (India) as well as the 2020 (Türkiye). This indicates the necessity to study the evolutionary dynamics of the virus in association with its epidemiology and global distribution.

Investigation of the predicted amino acid exchanges in the previously described antigenic epitope areas (G1, G2, G3, G4, Site I, Site II, Site III, Site IV), G2, Site II areas and G3 area aa49-63 suggests sequence conservation. The other possible changes in antigenic epitope areas were residue substitution. The two isolates described in this study had three individual exchanges in Site IV region. Even though BEF virus is a single serotype, identifying novel differences

How to cite: Pekmez et al. Detection and molecular characterization of the BEF virus in western Türkiye. Veterinaria Italiana, Vol. 61 No. 4 (2025) DOI: 10.12834/VetIt.3810.35777.2

in the genotypes could provide useful information in the protectiveness of vaccines against different genotypes.

Conclusion

This study described the first detection and molecular characterization of BEFV isolated from the Aegean region. The origins of the viruses involved in this outbreak could be from the south-southeast of the Türkiye through animal movements. Additionally, in this study, new primer design was performed to be used in Sanger sequencing analysis.

Acknowledgments

We are grateful to Dr. Antoinette Van Schalkwyk from ARC-Onderstepoort Veterinary Research for kindly English editing of manuscript.

Ethical approval

Samples used in this study were sent and/or collected for diagnostic purposes. No ethical approval was required for this study.

Conflict of interest

The authors declare no competing interests.

Author Contributions

Conceptualization: K.P, M.K; Methodology: K.P, M.K, A.A.Ç; Formal analysis: K.P, M.K, A.A.Ç, F.A; Investigation: K.P, M.K, A.A.Ç, F.A; Writing original draft preparation: K.P; Visualization: K.P, M.K, A.A.Ç, F.A; Supervision: K.P, M.K; Project administration: K.P, M.K; Funding acquisition: K.P, M.K, A.A.Ç, F.A.

All authors have read and agreed to the published version of the manuscript.

Data availability

All data are available upon request to the corresponding author.

Fundings

This research received no external funding.

References

Akakpo, A. J. (2015). Three-day fever. Revue scientifique et technique (International Office of Epizootics), 34 (2), 525–538.

Albayrak, H., & Özan, E. (2010). Orta Karadeniz bölgesinde Ruminant ve Tek Tırnaklılarda Kan Emici Sineklerle Nakledilen Bazı Arboviral Enfeksiyonların Seroprevalansı. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 16, 33-36.

Aziz-Boaron, O., Klausner, Z., Hasoksuz, M., Shenkar, J., Gafni, O., Gelman, B., David, D., & Klement, E. (2012). Circulation of bovine ephemeral fever in the Middle East--strong evidence for transmission by winds and animal transport. Veterinary microbiology, 158 (3-4), 300–307. https://doi.org/10.1016/j.vetmic.2012.03.003.

Bakhshesh, M., Ranjbar, M. M., & Almasi, S. (2018). Immunoinformatic analysis of glycoprotein from bovine ephemeral fever virus. Biomedical and Biotechnology Research Journal, 2, 208-212.

Cybinski, D. H., Walker, P. J., Byrne, K. A., & Zakrzewski, H. (1990). Mapping of antigenic sites on the bovine ephemeral fever virus glycoprotein using monoclonal antibodies. The Journal of general virology, 71 (9), 2065–2072. https://doi.org/10.1099/0022-1317-71-9-2065.

Dietzgen, R. G., Calisher, C. H., Kurath, G., Kuzman, I. V., Rodriguez, L. L., Stone, D. M., Tesh, R. B., Tordo, N., Walker, P. J., Wetzel, T., & Whitfeld, A. E. (2012). Family Rhabdoviridae In: A. M. Q. King, M. J. Adams, E. B. Carstens, & E. J. Lefkowitz (Eds.), Virus taxonomy: ninth report of the international committee on taxonomy of viruses (pp. 686–713). Academic Press.

Erganis, O., Yavru, S., Sayın, Z., Avcı, O., Sakmanoglu, A., Yanık, B., Ertürk, A., & Özmen, M. (2014). Vaccine preparation for Three Days Sickness: Safety and Efficacy Studies of Trivalent BEF Vaccine. Infovet, 142-163.

Erol, N., Koç, B., Gür, S., Çavlav, V., & Tan, M. (2015). A Serological Investigation for Bovine Ephemeral Virus Infection in Aydın and Muğla Provinces. Kocatepe Veterinary Journal, 8, 45-49.

Finlaison, D. S., Read, A. J., & Kirkland, P. D. (2010). An epizootic of bovine ephemeral fever in New South Wales in 2008 associated with long-distance dispersal of vectors. Australian veterinary journal, 88(8), 301–306. https://doi.org/10.1111/j.1751-0813.2010.00596.x.

Girgin, H., Yonguç, A. D., Akçora, A., & Aksak, E. (1986). First outbreak of bobine ephemeral feverin Turkey (Türkiye'de ilk bovine ephemeral fever salgını). Etlik Veteriner Mikrobiyoloji Enstitüsü Dergisi, 5, 5-12.

Holmes, I. H., & Doherty, R. L. (1970). Morphology and development of bovine ephemeral fever virus. Journal of virology, 5(1), 91–96. https://doi.org/10.1128/JVI.5.1.91-96.1970.

Karaoğlu, T., Özgünlük, İ., Demir, B., Özkul, A., & Burgu, İ. (2007). Seroprevalence of culicoides-borne disease in cattle in European Turkey. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 54,121-125.

Karayel-Hacioglu, I., Duran Yelken, S., Vezir, Y., Unal, N., & Alkan, F. (2021). Isolation and genetic characterization of bovine ephemeral fever virus from epidemic-2020 in Turkey. Tropical animal health and production, 53(2), 276. https://doi.org/10.1007/s11250-021-02715-1.

Kongsuwan, K., Cybinski, D. H., Cooper, J., & Walker, P. J. (1998). Location of neutralizing epitopes on the G protein of bovine ephemeral fever rhabdovirus. The Journal of general virology, 79 (11), 2573–2581. https://doi.org/10.1099/0022-1317-79-11-2573.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular biology and evolution, 35(6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Lee, F. (2019). Bovine Ephemeral Fever in Asia: Recent Status and Research Gaps. Viruses, 11(5), 412. https://doi.org/10.3390/v11050412.
Nei, M., & Kumar, S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press.

Omar, R., Van Schalkwyk, A., Carulei, O., Heath, L., Douglass, N., & Williamson, A. L. (2020). South African bovine ephemeral fever virus glycoprotein sequences are phylogenetically distinct from those from the rest of the world. Archives of virology, 165(5), 1207–1210. https://doi.org/10.1007/s00705-020-04568-9.

Özyörük, F., Özgünlük, İ., Turgut, S. İ., Şengül, Y., Yığman, A. A., & Gülyaz, V. (2025). Environmental and Breed-Related Determinants of Bovine Ephemeral Fever Outbreaks in Southeastern Türkiye. Veterinary medicine and science, 11(2), e70257. https://doi.org/10.1002/vms3.70257.

Pyasi, S., Sahu, B. P., Sahoo, P., Dubey, P. K., Sahoo, N., Byrareddy, S. N., & Nayak, D. (2020). Identification and phylogenetic characterization of bovine ephemeral fever virus (BEFV) of Middle Eastern lineage associated with 2018-2019 outbreaks in India. Transboundary and emerging diseases, 67(5), 2226–2232.

https://doi.org/10.1111/tbed.13531.

Tonbak, S., Berber, E., Yoruk, M. D., Azkur, A. K., Pestil, Z., & Bulut, H. (2013). A large-scale outbreak of bovine ephemeral fever in Turkey, 2012. The Journal of veterinary medical science, 75(11), 1511–1514. https://doi.org/10.1292/jvms.13-0085.

Trinidad, L., Blasdell, K. R., Joubert, D. A., Davis, S. S., Melville, L., Kirkland, P. D., Coulibaly, F., Holmes, E. C., & Walker, P. J. (2014). Evolution of bovine ephemeral fever virus in the Australian episystem. Journal of virology, 88(3), 1525–1535. https://doi.org/10.1128/JVI.02797-13.

Walker, P. J. (2005). Bovine ephemeral fever in Australia and the world. Current topics in microbiology and immunology, 292, 57–80. https://doi.org/10.1007/3-540-27485-5_4.

Walker, P. J., & Klement, E. (2015). Epidemiology and control of bovine ephemeral fever. Veterinary research, 46, 124. https://doi.org/10.1186/s13567-015-0262-4.

Walker, P. J., Byrne, K. A., Cybinski, D. H., Doolan, D. L., & Wang, Y. H. (1991). Proteins of bovine ephemeral fever virus. The Journal of general virology, 72 (1), 67–74. https://doi.org/10.1099/0022-1317-72-1-67.