


## ORIGINAL ARTICLE OPEN ACCESS

Ruminants

# Environmental and Breed-Related Determinants of Bovine Ephemeral Fever Outbreaks in Southeastern Türkiye

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## ABSTRACT

**Background:** Bovine ephemeral fever (BEF) is a biphasic febrile, vector-borne viral disease affecting cattle and buffaloes, leading to significant reductions in milk production and economic losses. BEF's episodic nature often leads to its classification as non-notifiable disease.

**Objectives:** This study aimed to investigate the environmental and breed-related determinants of BEF in Şanlıurfa, a region in southeastern Türkiye notable for its vulnerability to vector-borne diseases.

**Methods:** We integrated temperature, precipitation and irrigation data with the occurrence of clinical BEF cases to identify critical environmental conditions associated with outbreaks. Additionally, we conducted an epidemiological analysis focused on a state-owned dairy farm to assess breed-specific morbidity, case fatality and mortality rates. A partial G-gene sequence from a positive sample was phylogenetically analysed to trace its origins.

**Results:** Temperatures above 15°C, excessive rainfall and increased agricultural irrigation were identified as key factors associated with the emergence and spread of BEF. The cropping pattern, including maize and cotton cultivation, significantly influences irrigation practices and, consequently, vector population dynamics. Holstein-Friesian cattle showed higher morbidity (58.4%), case fatality (12.9%) and mortality (7.5%) rates compared to Simmental cattle, which exhibited lower rates (morbidity 28.1%, case fatality 5.2%, mortality 1.5%). Phylogenetic analysis linked the virus strain to a Middle Eastern clade from the 2018 to 2020 period.

**Conclusions:** The study highlights the importance of considering environmental conditions, crop patterns, and breed-specific health risks in managing BEF. Tailored intervention strategies are crucial for mitigating the economic and health impacts of BEF on dairy farms.

## 1 | Introduction

Bovine ephemeral fever (BEF) is a viral disease of cattle and water buffaloes transmitted by hematophagous insects such as midges and mosquitoes. As complete recovery usually occurs within 3–5 days, it is also known as 3-day sickness. The disease is characterized by a sharp decrease in milk yield; biphasic fever; anorexia; tachypnea; ocular and nasal discharge; lameness and

stiffness; and a milk fever-like paralysis that is reversible with calcium borogluconate treatment. Losses in milk production, along with the impact on fattening calves and treatment costs, can be devastating for the livestock sector. BEF is either endemic or periodically epidemic in most tropical and subtropical regions of Africa, the Middle East (ME), Asia and Australia. It is endemic in tropical areas of central Africa, southern Egypt, eastern Australia, Indonesia and southern China, where it spreads to

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more temperate regions and causes seasonal outbreaks (St. George and Standfast 1988; Walker 2005).

*Bovine ephemeral fever virus* (BEFV) is an *Ephemerovirus* of the *Rhabdoviridae* family. It has a 14.9 kb single-stranded negative-sense RNA genome, which produces five structural proteins, resulting in a bullet-like shape of the virion. One of these, glycoprotein (G), coats the viral envelope and forms spikes that serve as targets for neutralizing antibody response and contain variable sites that are important for phylogenetic studies.

Phylogenetic analyses of the G gene sequence generated trees with four main clades: Africa, the ME, Asia and Australia. These branches are crucial for determining the origin of the sequence. In 2018, a new subtype of BEFV emerged from the ME clade, causing outbreaks first in Iran and India and later in 2020 in the Şanlıurfa province of Türkiye, which is located in the southeastern region of the country (Karayel-Hacıoglu et al. 2021; Pyasi et al. 2020; Rezatofghi et al. 2022).

Although BEF is not endemic in Türkiye, it has caused episodic epidemics at regular intervals since the 2000s, following the introduction of large-scale agricultural irrigation in southeastern Anatolia, where most of the initial cases were detected. Outbreaks occurred in 1985, 1999, 2003, 2008, 2012 and 2016 (Girgin et al. 1985; Oğuzoğlu et al. 2015; P. J. Walker and Klement 2015). Information on the 2020 outbreak—such as the timing and location of its onset, its spread and the extent of livestock losses—remains limited and has not been reported in detail. This study aimed to address this gap by providing critical data on irrigation practices, precipitation and temperature during the outbreak period, along with morbidity, mortality and case fatality rates from a large dairy farm. By incorporating geographically specific onset and end dates for BEF cases, this analysis also seeks to offer valuable insights to inform early warning and surveillance systems for managing future BEF outbreaks.

## 2 | Materials and Methods

### 2.1 | Study Area

This study was conducted in the southeastern Anatolian region of Türkiye, centred on Şanlıurfa and bordered by the Euphrates in the west, the Tigris in the east, the Türkiye–Syria border in the south, and latitude 38 in the north. There are three provinces and 29 districts, with 12 districts marked by drop pins on the map (Figure 1). Climatically, this area is located in the hot-summer Mediterranean climate zone, where summers are dry and hot and winters are mild and rainy. The long-term (1991–2023) annual mean precipitation in the region is 533 mm.

The plains (polygonal areas) shown in Figure 1, which were traditionally used for rainfed agriculture, have been gradually transformed into irrigated areas since 1995. These converted lands are irrigated by Atatürk Dam, part of the Southeastern Anatolian Project, Türkiye's most comprehensive rural development initiative (GAP project). However, instead of using modern pressurized irrigation methods that conserve water, flood irrigation—which creates stagnant water—remains the primary practice (Yenmez 2005). Multiple reports indicate that the study area has histori-

cally served as a gateway for BEF and other vector-borne diseases in Türkiye (Erganiş et al. 2010; Ergunay et al. 2014; Girgin et al. 1985; Hakioglu 1963).

### 2.2 | Sample Collection

During the outbreak period (August–September 2020), locations shown on the map with blue and green drop pins were visited. Whole blood was collected in tubes containing EDTA from 22 cows exhibiting typical clinical signs, such as high fever, trembling, stiff joints, and laboured breathing. Blood samples were transported to the laboratory under a cold chain. Total RNA was extracted from blood samples using MagMAX-96 Viral RNA Isolation Kit (Applied Biosystems by Thermo Fisher Scientifics, Lithuania) according to the manufacturer's instructions and stored at  $-20^{\circ}\text{C}$  until analysis.

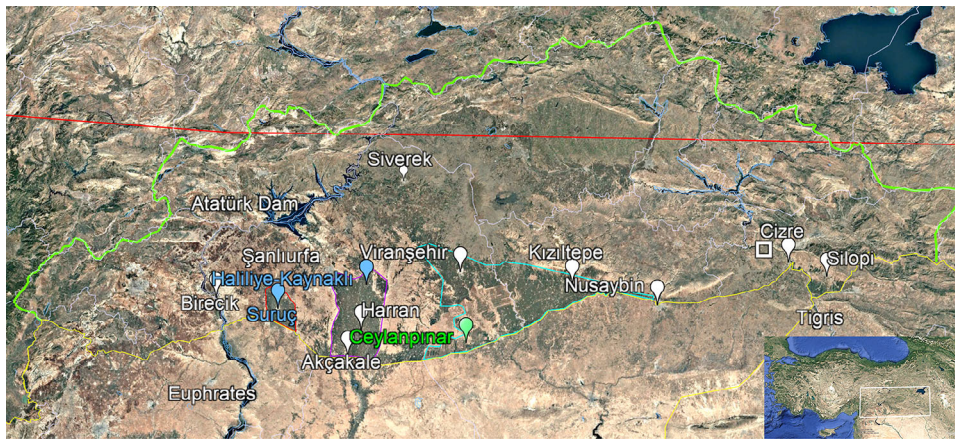
### 2.3 | Screening of Positive Samples by RT-PCR and Partial G Gene Sequencing

For the initial diagnosis of BEFV, RT-PCR was performed using the AgPath-ID One-Step RT-PCR Reagents (Applied Biosystems by Thermo Fisher Scientifics, Lithuania) as per the manufacturer's protocol. The screening was performed using primers (BEFV-AO-F 5'-GAATCATTATGGGATCGGATC-3' and BEFV-AO-R 5'-CCAACCTACAACAGCAGATAAAAC-3') and PCR cycling conditions, which included  $50^{\circ}\text{C}$  for 30 min for reverse transcription,  $95^{\circ}\text{C}$  for 15 min for the activation of Taq DNA polymerase, inactivation of the reverse transcriptase enzyme and denaturation of the template cDNA, followed by 35 cycles of  $94^{\circ}\text{C}$  for 30 s (denaturation),  $55^{\circ}\text{C}$  for 30 s (annealing) and  $72^{\circ}\text{C}$  for 1 min (extension), with a final extension step at  $72^{\circ}\text{C}$  for 10 min (Niwa et al. 2015).

One sample with a positive result was selected for partial sequence analysis of the G gene. In this step, the method described above was modified with primers developed in this study (BEFV-G-4048-F3 5'-GGCTCCAACAAGACCAGGAA-3' and BEFV-G-5018-R3 5'-TGCAGGAACATGATTGCCCT-3') targeting the 4042–4916 region of the BEFV reference genome (NC\_002526.1). The amplicons were electrophoresed in a 2% agarose gel stained with ethidium bromide to visualize the DNA bands. A positive PCR product was then purified and sequenced at BM Labosis Company (Ankara, Türkiye) using the same primers. The sequence identified in this study was submitted to the GenBank database and designated as accession number OR633345.1.

### 2.4 | Phylogenetic Analysis of Partial G Gene

A FASTA file consisting of 25 G gene sequences was generated from the GenBank database, including the OR633345 sequence generated in this study, representing the spatiotemporal diversity of BEFV but with a focus on the ME group. Sequence alignment and phylogenetic analysis were performed using MEGA11 software (version 11.0.11) (Tamura et al. 2021). The phylogenetic tree was constructed using the minimum evolution method for evolutionary history and the maximum composite likelihood



**FIGURE 1** | Google Map showing the study area with disease onset/offset data collection points, as well as daily average temperature and monthly precipitation data, marked by drop pins. Blue or green drop pins indicate blood sample collection sites, whereas green pins specifically denote outbreak data collection locations. Coloured polygons represent irrigated areas by the Atatürk Dam: red for Suruç Plain, purple for Şanlıurfa and Harran Plains and brown for Ceylanpınar and Mardin Plains. The yellow line delineates international borders.

method for evolutionary distances, and bootstrapping was performed on 1000 replicates. Pairwise deletion was applied to handle ambiguous positions. The per cent identity between the OR633345.1 sequence and other sequences was also calculated using the same software.

## 2.5 | Timing of Cases and Descriptive Epidemiology

In December 2020, private veterinary practitioners treating BEF cases in districts marked with drop pins on the map were contacted, and it was confirmed whether the affected animals met the criteria for the probable case definition (rapid onset, biphasic fever, lethargy, anorexia, cessation of lactation, unsteady gait, respiratory distress, muscle tremors and rapid recovery, as diagnosed by a veterinarian) (Dicker et al. 2006). The start and end dates of cases meeting this definition were determined.

Additionally, morbidity, mortality and case fatality rates for the outbreak period were collected from two pure-breed dairy herds: one high-yielding Holstein-Friesian (HF) herd (average milk yield 42–44 kg/day) and one Simmental herd (average milk yield 28–30 kg/day), both managed by the state in the Ceylanpınar district. Data were categorized on the basis of breed, age and sex. In these herds, the probable case definition was consistently applied and pathological examinations were carried out for some, though not all, of the deaths to confirm diagnoses.

## 2.6 | Collection of Precipitation, Temperature, Wind and Volume of Irrigation Data

Monthly precipitation data for Southeastern Anatolia for 2020, along with long-term annual data (1981–2010), were obtained from the Turkish State Meteorological Service (Anonymous 2021a). Daily average temperature and monthly precipitation data for each drop pin location in 2020 were also provided by the Turkish State Meteorological Service. Wind speed and direction data were specifically collected for Cizre in March 2020

to assess potential windborne vector transmission (Anonymous 2021b). Additionally, the monthly irrigation volumes supplied from the Atatürk Dam to the Şanlıurfa plains were obtained from the General Directorate of State Hydraulic Works (Anonymous 2021c).

## 2.7 | Statistical Analysis

The significance of differences in the frequency of BEF cases and BEF-related mortality in Ceylanpınar farms by breed, sex, age group or production status was assessed using chi-square ( $\chi^2$ ) tests (The Jamovi Project 2022). A significance level of  $p < 0.001$  was applied. This analysis ensures robust evaluation of the impact of these factors on BEF incidence and mortality.

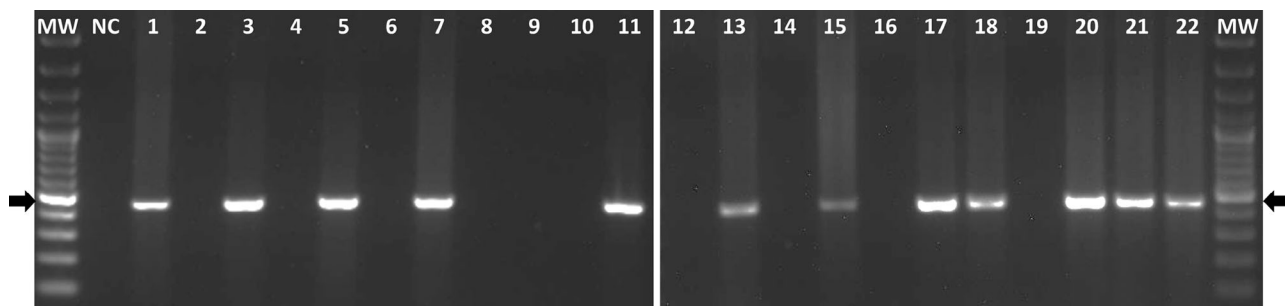
Logistic regression analysis was conducted to assess the association between environmental factors (temperature, precipitation and irrigation volume) and the occurrence of BEF outbreaks across 12 locations in Southeastern Anatolia (Table S1). The dependent variable was defined as the presence or absence of BEF cases, and the independent variables included daily average temperature, monthly precipitation and monthly irrigation volume. Odds ratios ( $\text{Exp}(B)$ ) and 95% confidence intervals were calculated to quantify the relationship between each environmental variable and BEF incidence. Data were analysed using SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY), and a significance level of  $p < 0.05$  was applied to determine statistical significance.

## 3 | Results

### 3.1 | Screening of Field Samples by RT-PCR and Phylogenetic Analysis

The BEFV genome was identified in field blood samples by RT-PCR. Using previously defined primers (Niwa et al. 2015), the cDNA yielded a 448 bp band. Of the 22 samples collected, 12





**FIGURE 2** | RT-PCR detection of the bovine ephemeral fever virus genome. Partial G gene (448 bp) fragments were amplified from genomic RNA extracted from blood samples. Lanes 1–22 correspond to samples collected from locations marked by blue or green drop pins in Figure 1. NC represents the non-template control. Arrows indicate 500 bp.

tested positive: 2 from Suruç, 3 from Haliliye-Kavaklı and 7 from Ceylanpınar (Figure 2).

Phylogenetic analysis of the BEFV G gene revealed four main clades: ME, East Asia (EA), Australia and Africa. The ME clade consists of taxa belonging to the 1985–2020 period. However, from 2018 to 2020, a subclade diverged from the main ME clade, and the sequence produced in this study (OR633345.1) was included in this recently diverged group. This clade also included sequences from India, Iran and Türkiye (Figure 3a). When sequence homology was analysed, the newly identified strain exhibited >99% identity with other sequences in the subclade, except for MN781183/IND/2018, which showed slight divergence (Figure 3b).

### 3.2 | Descriptive Epidemiology

To assess the impact of the BEF outbreak in the region, data were collected from a state-owned dairy farm in Ceylanpınar. Morbidity, case fatality rates and mortality rates recorded between 28 August and 7 November are summarized in Table 1. A total of 8085 cattle from two separate herds—comprising Simmental and high-yielding HF breeds—of different ages and sexes were examined. Clinical BEF was observed in 36.7% of the animals.

High-yielding HFs exhibited a significantly higher prevalence (58.4%) than Simmentals (28.1%). This disparity was especially notable among dairy cows (95.4% vs. 32.9%) and heifers (74.8% vs. 44.7%). Across both breeds, females (44.5%) and dairy cattle (50.7%) showed higher disease incidence compared to males (10.4%) and nondairy cattle (27.1%) (data not shown).

Interestingly, calves under 6 months old and young bulls aged 15–24 months were unaffected. Mortality followed similar trends, with 8.7% of affected animals succumbing to the disease. High-yielding HF dairy cows had the highest case fatality rate (15.5%), nearly double that of Simmental cattle (7.6%).

### 3.3 | Meteorological Data and Disease Occurrence

To elucidate the outbreak dynamics, data on daily average temperatures, monthly rainfall and irrigation practices were analysed alongside BEF case data (Figure 4). Additionally, long-

term rainfall averages (1981–2010) and monthly rainfall data for the entire southeastern Anatolian region, as well as monthly irrigation data, were included in the evaluation (Figure 5).

BEF cases began in August and lasted for an average of 83 days (range: 36–168 days). This period coincided with the peak irrigation demand and a decrease of high temperatures (below 35°C), which aligns with optimal vector activity. However, in Suruç, Silopi, Cizre, Nusaybin and Kızıltepe, cases manifested earlier and persisted even during peak temperatures. Logistic regression (Table S1) revealed a significant association between BEF outbreaks and daily temperatures ranging from 15°C to 35°C ( $p < 0.05$ ). Temperatures exceeding 35°C were associated with reduced outbreak odds, likely due to suppressed vector activity.

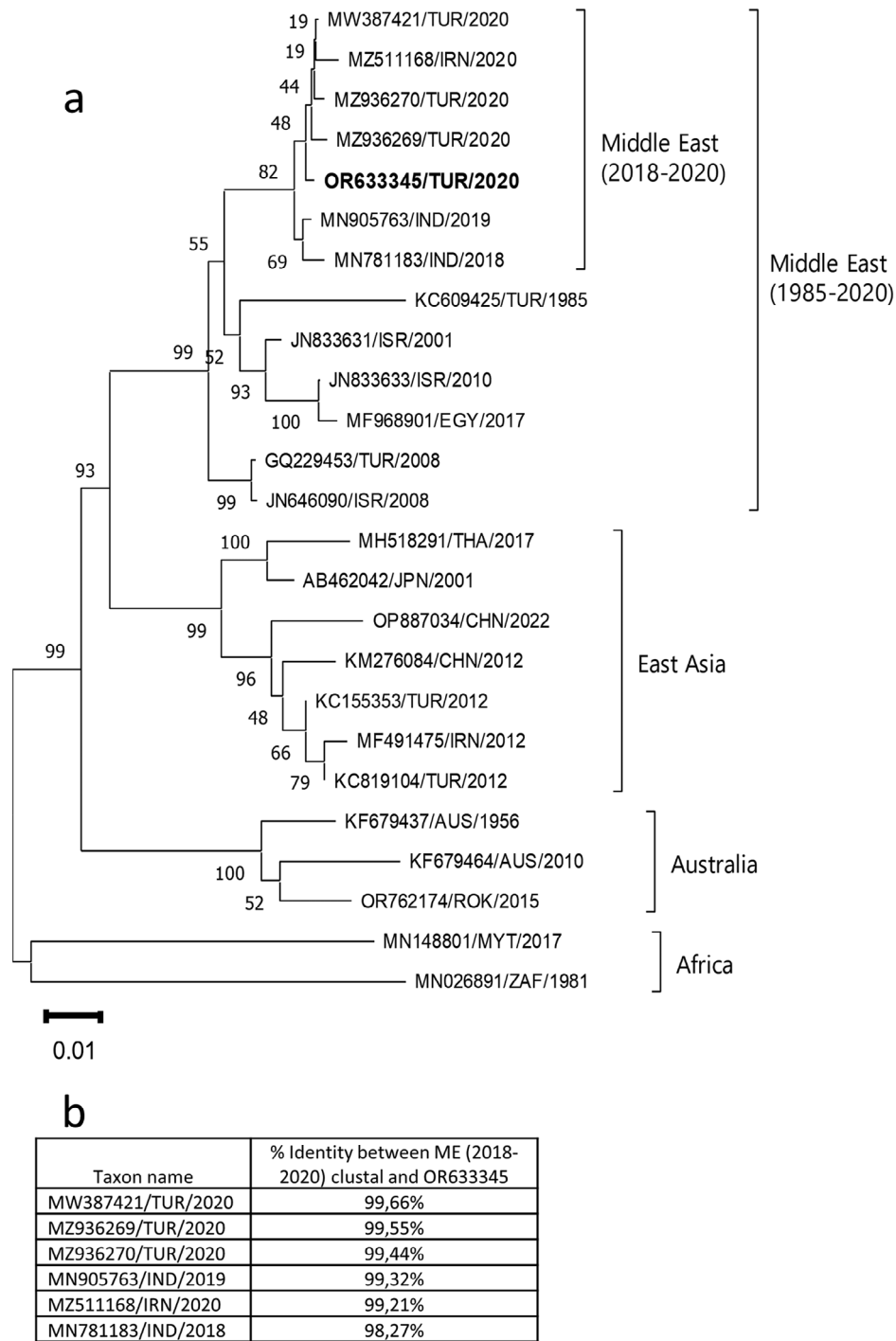
Increased irrigation volumes during August–September correlated with higher BEF occurrence, suggesting that human-managed irrigation practices create optimal breeding conditions for vectors. Conversely, when temperatures fell below 15°C in November, BEF cases ceased across all locations ( $p < 0.05$ ).

Precipitation levels varied widely, and although March rainfall in most plains where the Atatürk Dam is used for irrigation was close to or below the long-term average, Cizre and Silopi received three and two times more rainfall than normal, respectively. In Cizre, this excessive rainfall in March coincided with the onset of the first cases in the study area. However, the logistic regression analysis indicated that precipitation was not a significant predictor of BEF outbreaks.

Wind data collected specifically for Cizre in March 2020 showed a prevailing southeast-to-northwest wind pattern, with wind speeds averaging 4–5.6 m/s (Figure 6). Notably, 14–15 March experienced the strongest winds in this direction, coinciding with the earliest BEF cases in Cizre. These conditions were compatible with potential cross-border movement of infected vectors from neighbouring regions such as Iraq or Iran, consistent with the early manifestation of BEF in Cizre.

## 4 | Discussion

This study aimed to identify the environmental factors and breed-specific vulnerabilities contributing to the emergence of BEF outbreaks in Şanlıurfa, a region prone to vector-borne diseases.



**FIGURE 3** | Phylogenetic relationship of the BEFV G nucleotide sequence from this study (bold) with 24 global BEFV G sequences. (a) The phylogenetic tree, constructed using the neighbour-joining method with 2000 bootstrap replications, is scaled by evolutionary distances calculated via the *p*-distance method. Subtrees, indicated by brackets, are labelled on the basis of previously identified taxa. GenBank accession number, geographical origin and collection date are shown for each sequence. Analysis performed using MEGA11. (b) Percentage identity between OR633345 and members of the ME (2018–2020) subgroup. The table shows base homology percentages between OR633345 and other sequences in the same sub-branch, using pairwise deletion to remove ambiguous positions. BEFV, bovine ephemeral fever virus; ME, Middle East.

By integrating climatic and management variables—such as daily average temperature, precipitation and irrigation data—with clinical case timing, a robust dataset was generated. Logistic regression analysis revealed significant associations between BEF occurrence and temperatures of 15°C–35°C as well as increased irrigation volumes. Wind data from 14 to 15 March in Cizre

indicated a strong southeast-to-northwest flow, suggesting cross-border vector movement from Iraq or Iran. These findings highlight the importance of enhanced environmental monitoring and cross-border surveillance to support early warning systems for BEF. The study also confirmed that HF cattle, characterized by high milk yield, experienced significantly higher morbidity, case

**TABLE 1** | Number of animals affected and died in Ceylanpinar State Farm.

Groups by age	n		Morbidity (%)		Case fatality rate (%)		Mortality (%)	
	Simmental	Holstein-Friesian	Simmental	Holstein-Friesian	Simmental	Holstein-Friesian	Simmental	Holstein-Friesian
Cows >24 months	2350	937	32.9	<b>95.4</b>	7.6	<b>15.5</b>	2.5	<b>14.8</b>
Young bulls (15–24 months)	165	142	0	0	0	0	0.0	0.0
Heifers (15–24 months)	1525	461	44.7	<b>74.8</b>	2.5	<b>8.1</b>	1.1	<b>6.1</b>
Weaned female Calves (6–15 months)	n/a	219	n/a	37.9	n/a	6	n/a	2.3
Weaned male Calves (6–15 months)	558	240	<b>31.2</b>	7.5	5.2	5.6	1.6	0.4
Calves female (0–6 months)	588	161	0	0	0	0	0.0	0.0
Calves male (0–6 months)	606	133	0	0	0	0	0.0	0.0
Total	5792	2293	28.1	<b>58.4</b>	5.2	<b>12.9</b>	1.5	<b>7.5</b>
Total (sum of two herds)	8085		36.7		8.7		3.19	

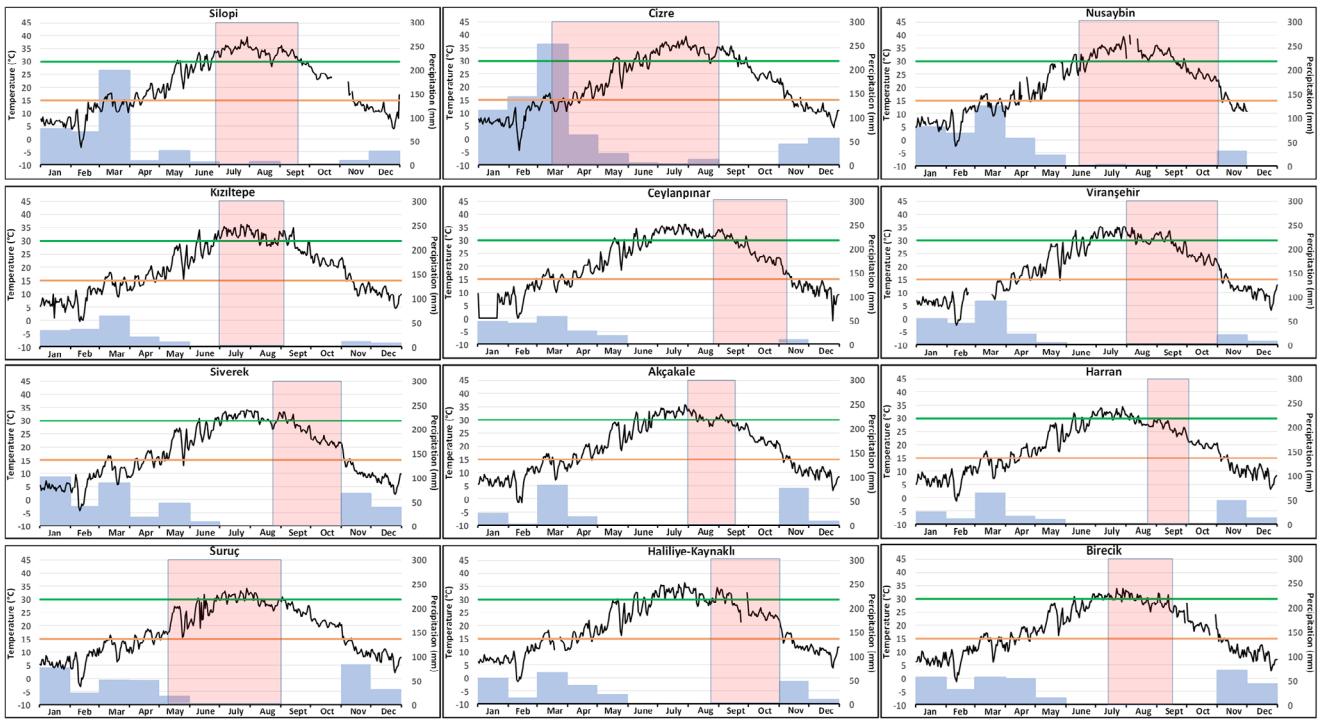
Note: Significant values are in bold ( $p < 0.001$ ).

fatality rates and mortality compared to Simmentals, likely due to increased metabolic stress. Tailored management practices are recommended to mitigate these breed-specific risks and reduce the impact of future outbreaks.

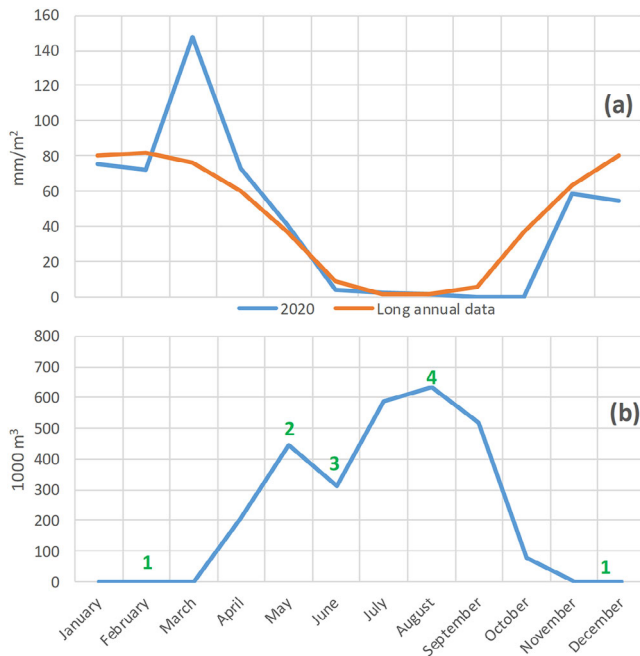
Phylogenetic analysis of the BEFV G gene revealed four main clades: ME, EA, Australia and Africa. The phylogenetic branches exhibit continuity in tropical and subtropical regions where the disease is endemic (Trinidad et al. 2014), whereas regions like Türkiye, which experience intermittent epidemics, exhibit distinct independent branches. Excluding the outbreak caused by an exotic strain from the EA group in 2012, it has been confirmed that the BEF outbreaks observed in the ME have consistently been associated with strains from the ME clade, with distinct subgroups sampled since 1985 (P. J. Walker 2005). In 2018, a new subgroup within the ME clade (BEFV-ME-2018-2020) was reported in India and Iran, subsequently causing outbreaks in the southeastern Anatolia region of Türkiye in 2020 (Karayel-Hacioglu et al. 2021; Pyasi et al. 2020; Rezatofghi et al. 2022). The G gene sequences from this study and others associated with the 2020 outbreak in Türkiye showed >99% similarity with the sequences from India and Iran, confirming their inclusion in this subgroup. This genetic homogeneity, despite broad geographic distribution, suggests a consistent reservoir within the endemic niche.

The BEFV-ME clade is known to cause irregular seasonal epidemics in Israel, Palestine, Iran, Iraq, Kuwait, Jordan, Lebanon, Saudi Arabia and Türkiye. However, its primary endemic region has not been precisely identified (P. J. Walker and Klement 2015). Findings from this study, combined with historical outbreak data, suggest the Tigris River region may serve as a key transmission corridor. In March 2020, simultaneous BEF cases were reported in Cizre, Türkiye (37.318199° N, 42.180457° E), and Khuzestan, Iran (31.428687° N, 49.047526° E), pointing to cross-border spread (Rezatofghi et al. 2022). Wind data from 14 to 15 March in Cizre—indicating strong southeast-to-northwest winds—align with this hypothesis. The Babylon region of Iraq, downstream along the Tigris, reported a 2019 outbreak, reinforcing the corridor's significance (Al-Khafaji et al. 2019). These findings are similar to observations for African horse sickness in 1960, another vector-borne disease first detected in the Cizre and Silopi districts (Hakioğlu 1963), underscoring the strategic importance of Cizre as an early warning surveillance point for vector-borne diseases in Türkiye.

In general, morbidity rates in BEF outbreaks can reach 100%, whereas mortality rates are reported to be less than 1% (P. J. Walker and Klement 2015). However, various determinants such as strain, breed, maintenance, nutrition, stocking density, climate and comorbid diseases can influence these rates. For instance, data from two studies on the BEFV-EA 2012 outbreak in Türkiye showed morbidity and mortality rates ranging between 35%–90% and 15%–26%, respectively (Alkan et al. 2017; Tonbak et al. 2013). Data from a recent BEF-ME-2018-2020 strain outbreak in a herd of 750 cattle reported a morbidity rate of 40%, a mortality rate of 12.7% and a case fatality rate of 31.6% (Arserim et al. 2022). In this study, with treatment and care properly managed, an analysis of 8085 cattle (ungrouped by age or breed) showed a morbidity rate of 36.7%, a mortality rate of 3.19% and a case fatality rate of 8.7%.



**FIGURE 4** | Time series of meteorological data and BEF case occurrence. Daily average temperature (black curve) and monthly total precipitation (blue bars) are shown on the right and left y-axes, in °C and mm/m<sup>2</sup>, respectively. Red transparent rectangles indicate periods when BEF cases were observed. Brown and green lines represent the upper and lower thresholds for *Culicoides* activity. The locations of the 12 places listed in table headings are shown on the map in Figure 1. BEF, bovine ephemeral fever.

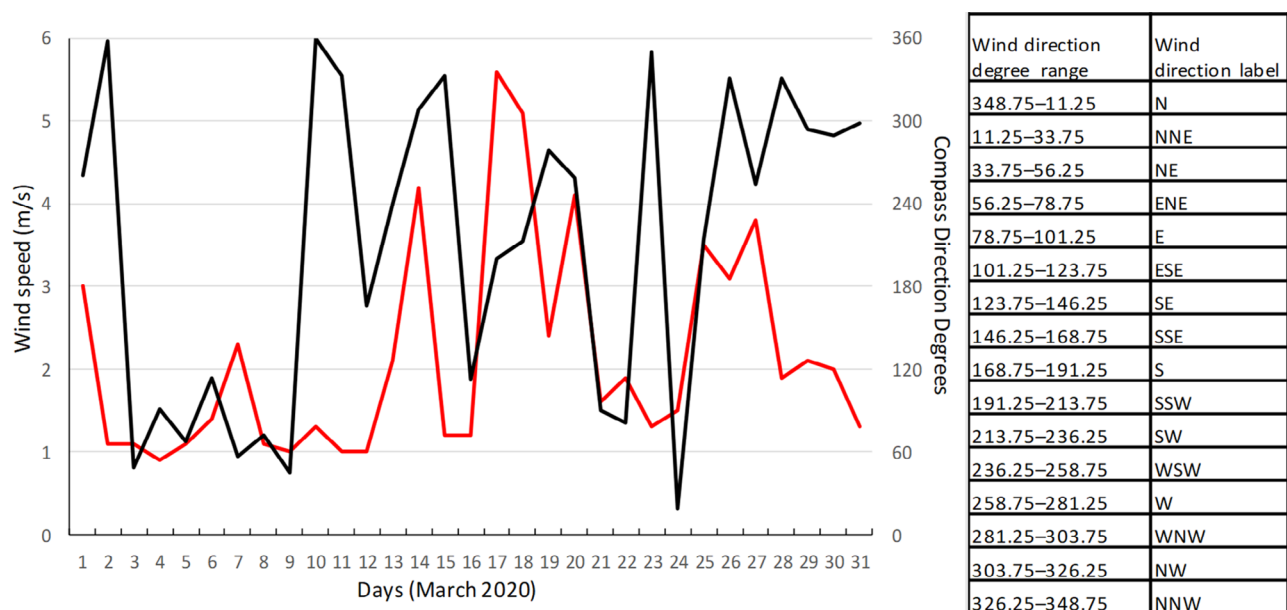


**FIGURE 5** | Precipitation and agricultural irrigation data for 2020. (a) Monthly precipitation data as a percentage difference from the 1981 to 2010 annual average for Southeastern Anatolia. (b) Monthly volume of water provided by the Atatürk Dam for irrigation in the Şanlıurfa Plain. Green numbers represent crop stages based on water demand: 1—no crop; 2—wheat and cotton; 3—wheat harvest; 4—cotton and maize.

The susceptibility of HF cattle to BEF was confirmed in this study, consistent with previous findings (Sayed et al. 2001; Yeruham et al. 2003). Despite controlling variables such as maintenance, nutrition, climate and treatment, HF cattle exhibited significantly higher morbidity (58.4%), case fatality (12.9%) and mortality (7.5%) rates compared to Simmentals, particularly among cows over 24 months and heifers aged 15–24 months. In contrast, Simmentals displayed lower morbidity (28.1%), case fatality (5.2%) and mortality (1.5%) rates, suggesting greater resilience or recovery potentials. Elevated haptoglobin, serum amyloid A and ceruloplasmin levels in BEF-infected cattle (Paksoy et al. 2024) reflect a systemic inflammatory response, which may disproportionately impact metabolically stressed breeds like HFs. Heightened metabolic and oxidative stress, driven by higher milk production demands, likely compromises disease resilience in HFs (De Matteis et al. 2021). Simmentals, with their more stable metabolic profiles, appear less vulnerable to severe outcomes.

The 2020 BEF outbreak in Şanlıurfa began in March, approximately 300 km east of Şanlıurfa, with significantly higher rainfall near the Tigris River compared to long-term averages. Similar rainfall patterns during BEF outbreaks have been documented (Finlaison et al. 2010; Davies et al. 1990). Although rainfall alone was not a significant predictor of BEF occurrence ( $p > 0.05$ ), logistic regression showed that irrigation volume and temperatures between 15°C and 35°C were strongly associated with outbreaks ( $p < 0.05$ ).

As the outbreak progressed, cases spread to irrigated areas in the study's central region, coinciding with an extremely dry period



**FIGURE 6** | Wind speed and direction trends in Cizre. Daily wind speed (black line, in m/s on the left y-axis) and corresponding compass direction (red line, in degrees on the right y-axis) are displayed for March 2020. Compass direction degrees correspond to specific labels, as indicated in the table on the right (e.g., 348.75°–11.25° = North (N), 11.25°–33.75° = North-Northeast (NNE), etc.), covering all 16 cardinal and intercardinal directions.

that began in the summer. Comparable increases in BEF during hot, dry months after rainy seasons, linked to stagnant water and marshland environments, have been reported in Saudi Arabia (Abu Elzein et al. 1999). In this study, peak irrigation volumes between July and September likely supported higher vector activity, particularly in areas with flood irrigation of cotton and maize crops. These practices increased local humidity, creating ideal conditions for vector proliferation.

The extrinsic incubation period of BEFV within vectors is shortened at temperatures near 30°C but extends significantly at 15°C, highlighting the critical role of temperature in outbreak dynamics. Logistic regression results confirmed a significantly higher likelihood of BEF cases during months with temperatures between 15°C and 35°C ( $p < 0.05$ ), with the highest risk observed as temperatures approached 30°C. This pattern aligns with other arboviruses, where irrigation practices are closely linked to vector proliferation, as seen in Japanese encephalitis and increased cases of western equine and St. Louis encephalitis in the United States (St.George and Standfast 1988).

A primary challenge in identifying the endemic niche of the BEFV-ME-2018-2020 strain remains the lack of international data-sharing. BEF is not listed by the World Organisation for Animal Health, limiting access to genetic sequence data from neighbouring regions like Iraq and Syria. Although the inclusion of wind data for Cizre in March 2020 addressed some gaps, broader wind datasets are needed for a comprehensive assessment of vector movements. Similarly, the absence of real-time vector surveillance (e.g., *Culicoides* density) precludes direct correlations between vector dynamics and outbreak patterns. Future studies should prioritize systematic vector monitoring, expanded meteorological data, and robust international collaboration to clarify BEF's transmission dynamics and endemic zones.

## 5 | Conclusion

This study identified the environmental and management factors contributing to the BEFV-ME 2018-2020 outbreak in Şanlıurfa, Türkiye, highlighting the roles of temperature, irrigation practices, and geographic location. Logistic regression analysis confirmed that temperatures between 15°C and 35°C and high irrigation volumes were significantly associated with BEF outbreaks. HF cattle exhibited markedly higher morbidity, case fatality and mortality rates than Simmental cattle, reflecting their metabolic vulnerability.

The first cases detected in Cizre, near the Tigris River, underscore the region's strategic importance for early warning surveillance. Wind data suggesting possible cross-border vector movement and outdated flood irrigation systems exacerbate vector proliferation, intensifying disease transmission.

To mitigate future outbreaks, integrated management strategies addressing climatic and agricultural factors are essential. Targeted interventions, particularly for HF cattle, should be prioritized. Enhancing international cooperation, data-sharing protocols, and expanded meteorological datasets—including wind patterns and vector surveillance—will be critical for controlling future outbreaks and safeguarding the dairy industry in this agriculturally vital region.

## Author Contributions

Fuat Özyörük contributed to the conceptualization, data curation, investigation, validation, formal analysis and writing of the original draft. İrfan Özgünlük, Sena İnel Turgut, Yılmaz Şengül, A. Akif Yiğman and Veli Gülyaz were involved in the methodology and investigation. İrfan Özgünlük, Sena İnel Turgut, Yılmaz Şengül and A. Akif Yiğman also



contributed to visualization. Veli Gülyaz provided supervision and project administration. All authors participated in writing, reviewing and editing the manuscript.

## Ethics Statement

This study was conducted in accordance with the local ethics committee decision HRÜ-HADYEK-01-06.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Peer Review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/vms3.70257>.

## References

- Abu Elzein, E. M. E., A. A. Gameel, A. I. Al-Afaleq, et al. 1999. "Observations on the Recent Epizootic of Bovine Ephemeral Fever in Saudi Arabia." *OIE Revue Scientifique Et Technique* 18, no. 3: 672–680.
- Alkan, F., H. Albayrak, M. O. Timurkan, E. Ozan, and N. Coskun. 2017. "Assessment of the Molecular Epidemiology of Bovine Ephemeral Fever in Turkey." *Veterinarski Arhiv* 87, no. 6: 665–675.
- Al-Khafaji, M. A., M. U. Hashim, and H. H. O. Al-Sultany. 2019. "Estimation the Hematological Changes and Physical Examination in Cattle Infected With Bovine Ephemeral Fever in Iraq." In *9th International Conference for Sustainable Agricultural Development*, 432–439. Fayoum University.
- Arserim, N. B., M. Gürçay, A. Sait, and M. Türkdogan. 2022. "Detection of a New Variant in Outbreak of Bovine Ephemeral Fever in Turkey, 2020." *Indian Journal of Animal Research* 1–6. <https://doi.org/10.18805/IJAR.BF-1438>.
- Davies, F. G., P. Ochieng, and A. R. Walker. 1990. "The Occurrence of Ephemeral Fever in Kenya, 1968–1988." *Veterinary Microbiology Elsevier Science Publishers B.V* 22: 129–136.
- De Matteis, G., M. C. Scatà, G. Catillo, et al. 2021. "Comparison of Metabolic, Oxidative and Inflammatory Status of Simmental × Holstein Crossbred With Parental Breeds During the Peripartur and Early Lactation Periods." *Journal of Dairy Research* 88, no. 3: 253–260. <https://www.cambridge.org/core/product/77D302335BD9529B0A5A4E4FE10F8F45>.
- Dicker, R. C., F. Coronado, D. Koo, and R. G. Parrish. 2006. *Principles of Epidemiology in Public Health Practice: An Introduction to Applied Epidemiology and Biostatistics*. 3rd ed. Centers for Disease Control and Prevention (CDC). <https://stacks.cdc.gov/view/cdc/6914>.
- Erganiş, O., S. Yavru, Z. Sayin, et al. 2010. "The Development of Vaccine for Bovine Ephemeral Fever Infection." In *Proceedings of the 9th National Congress of Veterinary Microbiology*, 60–61. Turkish Veterinary Microbiology Association.
- Ergunay, K., F. Gunay, O. E. Kasap, et al. 2014. "Serological, Molecular and Entomological Surveillance Demonstrates Widespread Circulation of West Nile Virus in Turkey." *PLoS Neglected Tropical Diseases* 8, no. 7: e3028.
- Finlaison, D. S., A. J. Read, and P. D. Kirkland. 2010. "An Epizootic of Bovine Ephemeral Fever in New South Wales in 2008 Associated With Long-Distance Dispersal of Vectors." *Australian Veterinary Journal* 88, no. 8: 301–306.
- General Directorate of Meteorology. "Precipitation Evaluation." 2021a. <https://www.mgm.gov.tr/index.aspx>.
- General Directorate of Meteorology. "Meteorological Data Information System (MEVBIS)." 2021b. <https://www.mgm.gov.tr>.
- General Directorate of State Hydraulic Works, GAP 15th Regional Directorate. 2021c. Monthly water consumption report: Harran Main Canal, Şanlıurfa Main Canal, and Mardin Main Canal. Internal report.
- Girgin, H., A. D. Yonguç, A. Akçora, and E. Aksak. 1985. "Preliminary Report of an Outbreak of Bovine Ephemeral Fever in Turkey (Türkiye'de İlk Bovine Ephemeral Fever Salgını)." *Etlik Veteriner Mikrobiyoloji Enstitüsü Dergisi* 5, no. 10–12: 5–14.
- Hakioğlu, F. 1963. "Türkiye'de At Vebası: Yayılışı, Klinik Belirtileri, Patolojik Anatomisi, Mücadele ve Koruyucu Tedbirleri." *Türkiye Veteriner Hekimler Derneği Dergisi* 33, no. 7–8: 297–303.
- Karayel-Hacıoğlu, I., S. Duran Yelken, Y. Vezir, N. Unal, and F. Alkan. 2021. "Isolation and Genetic Characterization of Bovine Ephemeral Fever Virus From Epidemic-2020 in Turkey." *Tropical Animal Health and Production* 53, no. 2: 1–8.
- Niwa, T., H. Shirafuji, K. Ikemiyagi, et al. 2015. "Occurrence of Bovine Ephemeral Fever in Okinawa Prefecture, Japan, in 2012 and Development of a Reverse-Transcription Polymerase Chain Reaction Assay to Detect Bovine Ephemeral Fever Virus Gene." *Journal of Veterinary Medical Science* 77, no. 4: 455–460.
- Oğuzoğlu, T., A. Ertürk, S. G. Çizmeci, B. T. Koç, and Y. Akça. 2015. "A Report on Bovine Ephemeral Fever Virus in Turkey: Antigenic Variations of Different Strains of EFV in the 1985 and 2012 Outbreaks Using Partial Glycoprotein Gene Sequences." *Transboundary and Emerging Diseases* 62, no. 5: e66–e70.
- Paksoy, N., C. Balıkcı, O. Merhan, E. Dinçer, A. Şahan, and K. Bozukluhan. 2024. "Evaluation of Acute Phase Response in Cattle With Naturally Infected Bovine Ephemeral Fever Virus." *Acta Veterinaria Brno* 93, no. 2: 129–134.
- Pyas, S., B. P. Sahu, P. Sahoo, et al. 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, no. 5: 2226–2232.
- Rezatofighi, S. E., K. Mirzadeh, and F. Mahmoodi. 2022. "Molecular Characterization and Phylogenetic Analysis of Bovine Ephemeral Fever Viruses in Khuzestan Province of Iran in 2018 and 2020." *BMC Veterinary Research* 18, no. 1: 1–12. <https://doi.org/10.1186/s12917-021-03119-x>.
- Sayed, A. S., A. H. Sadiek, and A. A. Ali. 2001. "Bovine Ephemeral Fever in Assiut Governorate: Clinical, Laboratory and Therapeutic Studies." *Assiut Veterinary Medical Journal* 44, no. 88: 157–175.
- St George, T. D., and H. A. Standfast. 1988. "Bovine Ephemeral Fever." In *The Arboviruses Epidemiology and Ecology*, edited by T. P. Monath II, 71–86. CRC Press.
- Tamura, K., G. Stecher, and S. Kumar. 2021. "MEGA11: "Molecular Evolutionary Genetics Analysis Version 11".*" Molecular Biology and Evolution* 38, no. 7: 3022–3027. <https://doi.org/10.1093/molbev/msab120>.
- The Jamovi Project. "Jamovi. (Version 2.3) [Computer software]." Published 2022. <https://www.jamovi.org>.
- Tonbak, S., E. Berber, M. D. Yoruk, A. K. Azkur, Z. Pestil, and H. Bulut. 2013. "A Large-Scale Outbreak of Bovine Ephemeral Fever in Turkey, 2012." *Journal of Veterinary Medical Science* 75, no. 11: 1511–1514.
- Trinidad, L., K. R. Blasdel, D. A. Joubert, et al. 2014. "Evolution of Bovine Ephemeral Fever Virus in the Australian Epizootic." *Journal of Virology* 88, no. 3: 1525–1535.
- Walker, P. J. 2005. "Bovine Ephemeral Fever in Australia and the World." *Current Topics in Microbiology and Immunology* 292: 57–80.
- Walker, P. J., and E. Klement. 2015. "Epidemiology and Control of Bovine Ephemeral Fever." *Veterinary Research* 46, no. 1: 1–19.
- Yenmez, N. 2005. "A New Example of Increasing Soil Salinities of Plains: Harran Plain." *Balıkesir Üniversitesi Sosyal Bilimler Enstitüsü Dergisi* 8,

no. 14: 199–235. <https://dergipark.org.tr/tr/pub/baunsobed/issue/50338/651838>.

Yeruham, I., M. V. A. N. Ham, D. Bar, H. Yadin, and D. Tiomkin. 2003. "Economic Aspects of the 1999 Outbreak of Bovine Ephemeral Fever in Dairy S Cattle Herds in the Jordan Valley in Israel." *Veterinary Record* 153: 180–182.

### **Supporting Information**

Additional supporting information can be found online in the Supporting Information section.