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Research article

Serological evidence of bovine ephemeral fever in Jordan

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Abstract

Bovine ephemeral fever (BEF) is an economically significant vector-borne viral disease of cattle and buffaloes caused by an *Ephemerovirus* in the family *Rhabdoviridae*. The disease causes production losses and trade restrictions. Although BEF has been reported in several neighboring countries, it had not previously been documented in Jordan. This study describes two suspected BEF outbreaks in Jordan in 2018 and 2021, focusing on clinical and laboratory findings. In 2018, 14 cattle farms in the Jordan Valley were investigated, and 315 animals were examined. Affected cattle exhibited sudden fever, reduced feed intake, and spontaneous recovery within 2–4 days. The morbidity rate was 8.6%, and no deaths were recorded. A total of 122 paired blood and serum samples were collected from clinical, non-clinical, and neighboring animals. Qualitative BEF antibody ELISA testing detected antibodies in three clinical cases, whereas all non-clinical animals were negative. In contrast, during the 2021 investigation, four farms were examined, and 90 cattle showed similar clinical signs. However, BEF antibody testing of 20 serum samples collected from the investigated cattle was negative, and no morbidity or mortality was recorded. Viral RNA was not detected by RT-PCR in both years. These findings confirm the clinical and serological occurrence of BEF in Jordan in 2018, whereas the 2021 event showed no serological or molecular evidence of infection. Continued surveillance and laboratory monitoring are essential for detecting and controlling BEF in the region.

Keywords: Bovine ephemeral fever, Cattle, ELISA, Jordan, PCR

Introduction

Bovine ephemeral fever (BEF) is an acute, febrile viral disease of cattle and buffaloes caused by an *Ephemerovirus* in the family *Rhabdoviridae* [1]. The disease is transmitted primarily by biting insects, including mosquitoes and midges, which serve as biological vectors. BEF is widely distributed in tropical and subtropical regions, with outbreaks frequently reported in Asia, Australia, and Africa [2]. Its occurrence is strongly influenced by climatic conditions, vector abundance, and animal movement, all of which favor the emergence and spread of infection.

The disease has a significant economic impact. Outbreaks often cause substantial losses due to sharp reductions in milk yield, decreased weight gain, impaired fertility, and, in some cases, mortality among affected animals [3]. Moreover, BEF can disrupt national and international trade in live animals and animal products, further amplifying its economic burden. Clinically, BEF is marked by a sudden onset of high fever, anorexia, muscle stiffness, salivation, ocular and nasal discharge, lameness, and ruminal stasis. Affected animals may become recumbent. Although the disease is generally self-limiting, with recovery within two to four days, the associated production losses are significant [4].

Globally, BEF is recognized as an emerging and re-emerging transboundary animal disease. Its occurrence is sporadic but often epidemic, with outbreaks influenced by seasonal rainfall, wind patterns, and changes in vector populations [5]. In recent decades, climate change, intensification of livestock production, and increased animal trade have facilitated the spread of the BEF virus (BEFV) to new areas and contributed to recurrent outbreaks in previously endemic regions [6]. As a result, BEF has become an increasing concern for veterinary authorities, both for its direct production losses and for its role in limiting international livestock movements.

Although BEF has been reported in several Middle Eastern countries, including those bordering Jordan, the disease has not been clinically confirmed in Jordan to date. Serological evidence from 2012 suggested prior exposure to BEFV in the Jordan Valley [7], but no documented outbreaks were recorded. In 2018 and again in 2021, cattle farmers in the Jordan Valley reported a sudden disease syndrome marked by a sharp decline in milk production, reduced feed intake, pyrexia, muscular stiffness, and temporary recumbency, followed by spontaneous recovery within a few days. These descriptions were consistent with BEF, raising concern that the virus may be circulating in the region.

The present study was therefore conducted to investigate these suspected outbreaks through serological testing using indirect ELISA. Our objectives were to confirm the presence of the BEF virus in Jordan, to provide baseline epidemiological data on its occurrence, and to explore potential transmission routes. Establishing evidence of BEFV circulation in Jordan is critical for guiding disease prevention and control strategies,

safeguarding livestock productivity, and minimizing potential trade restrictions.

Material and methods

Clinical history of the herd

In 2018 and 2021, the official veterinary services in Jordan received two reports suspecting BEFV outbreaks in the Jordan Valley. However, these reports were not confirmed by laboratory testing. To investigate these outbreaks, clinical and epidemiological data were collected and analyzed.

Study area

Samples were collected from cattle raised in eight locations within the Jordan Valley region of Jordan: Kraimah, Al-Masharee, Al-Masharqa, Northern Al-Adassiyah, Abu Obaida, Al-Balawneh, Al-Zor, and Deir Alla (Figure 1). These areas are administratively located within the Jordan Valley districts of Balqa Governorate and Irbid Governorate, mainly within the lower Jordan Valley. The Jordan Valley is an important agricultural and livestock-producing area, including dairy cattle farming, due to its favorable climate and abundant irrigated agricultural land. The region is bordered by Israel to the west, across the Jordan River, and by Syria to the north (via the Yarmouk River). The selected locations were included because clinical signs consistent with the disease under study had previously been reported in cattle in 2018 and 2021.

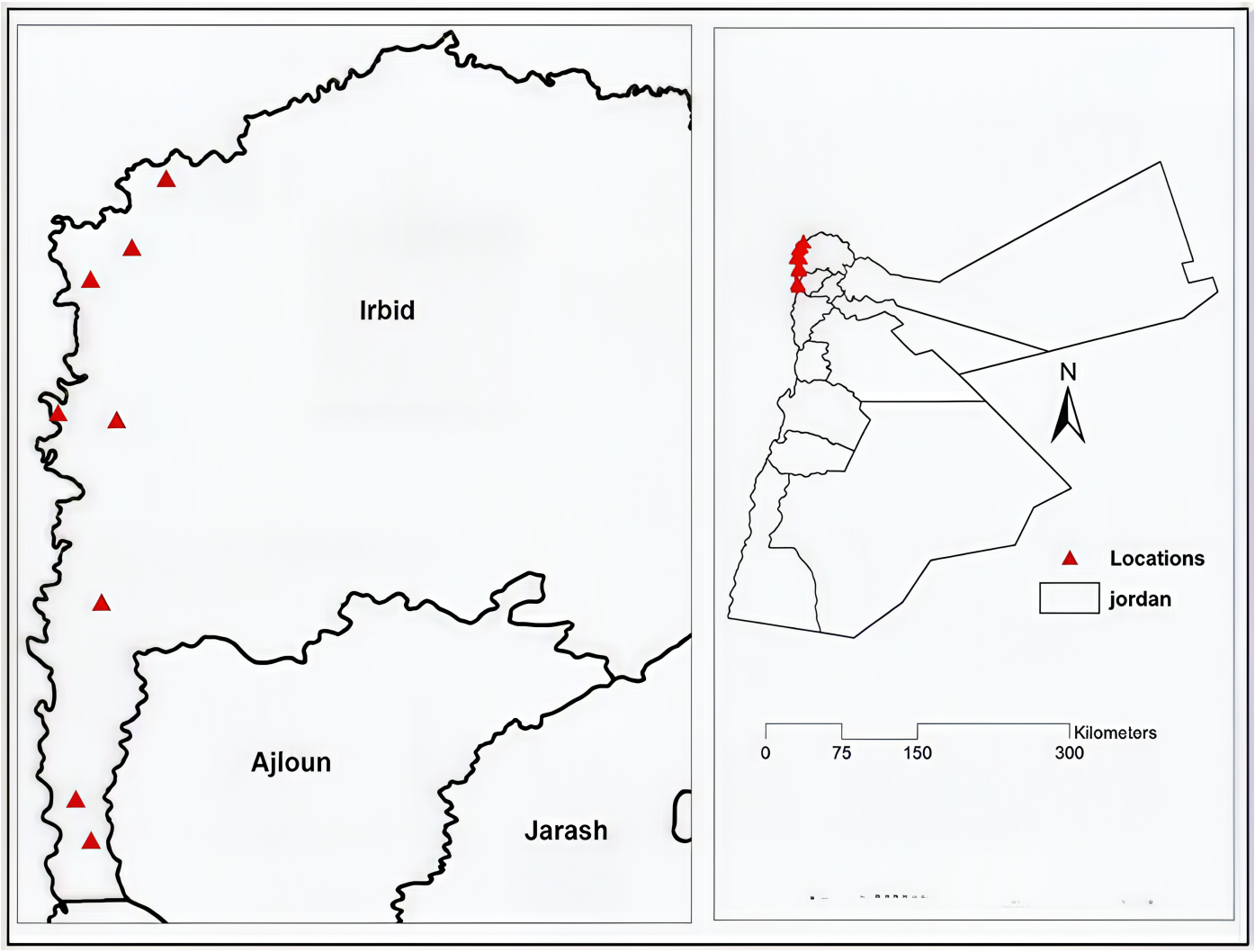

Figure 1: Bovine ephemeral fever study area in Jordan valley, maps prepared by GIS and RS Department/ NARC.

Data and samples Collection

A structured questionnaire was developed to collect clinical and epidemiological data on suspected cases of BEF infection. The questionnaire was administered through personal interviews with cattle owners. It included sections on herd information, farm location, animal demographics, clinical signs observed in infected cattle, treatment protocols, recovery rates, and animal mortality or loss.

Across the two suspected outbreaks (2018 and 2021), a total of 405 cattle from 18 farms were clinically examined, and 142 paired serum samples were collected (Table 1). In

2018, 122 paired serum samples were obtained from 62 cattle, including clinical cases and non-clinical animals from affected and neighboring farms. In 2021, 20 paired serum samples were collected from 10 clinically suspected cattle. The samples were collected at the onset of clinical signs and again two weeks later. The collected samples were stored at 2-4°C in a closed icebox and transported to the laboratory on the same day as the collection. All samples were kept at -20 °C until laboratory diagnosis.

Table 1: Summary of cattle examined and samples collected during suspected BEF investigations in the Jordan Valley (2018 and 2021).

Parameter	2018	2021	Total
Region/Locations	Jordan Valley (Kraimah, Al-Masharee, Al-Masharqa, Northern Al-Adassiyah)	Jordan Valley (Abu Obaida, Al-Balawneh, Al-Zor, Deir Alla)	Jordan Valley
Number of farms investigated	14	4	18
Total cattle clinically examined	315	90	405
Total number of sampled cattle			
Clinically affected/suspected animals	27	10	37
Non-clinical animals from affected farms	23	0	23
Non-clinical animals from neighboring farms	12	0	12
Number of paired serum samples			
From clinical animals	52	20	72
From non-clinical animals (affected farms)	46	0	46
From neighboring farms	24	0	24
Total paired serum samples collected	122	20	142

Determination of BEFV antibodies

Collected serum samples were tested using the BEFV-Ab ELISA kit according to the manufacturer's instructions (Sunlong Biotech Co., Ltd., Zhejiang, China). The kit's microplates were pre-coated with an antigen specific to BEFV antibody (BEFV-Ab). Positive and negative controls were provided by the manufacturer. Microplate readings were obtained by measuring optical density at 450 nm. The critical value (CUT OFF) was determined from the optical density (OD) values using the following formula:

Critical value (CUT OFF) = average value of negative controls + 0.15.

Samples with OD values greater than or equal to the CUT OFF were considered BEFV-Ab positive, while samples with OD values less than the CUT OFF were considered BEFV-Ab negative. The test had 100% sensitivity and 96.7% specificity [8].

Total and viral RNA Extraction from Serum and Whole Blood

Viral RNA was extracted from 140–600 µl of serum, and total RNA was extracted from 1 mL of whole blood collected from clinically affected animals using the QIAamp Viral RNA Mini Kit (QIAGEN, Germany) and the QIAamp RNA Blood Mini Kit (QIAGEN, Germany), following the manufacturer's instructions.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Detection and differentiation of BEFV RNA was performed using the QIAGEN One-Step RT-PCR Kit according to the manufacturer's protocol. A specific primer pair targeting an 809 bp fragment of the glycoprotein (G) gene (GpF: TATTACCCTCCTGCCGGATGTTT; GpR: AGGTCTGTATTCGCACCAAGCTCT) was used as described by Aziz-Boaron et al. (2012) [9]. For direct detection of BEFV RNA, the VetPCR™ BEFV Detection Kit (Intron Biotechnology, Korea) was applied to RNA isolates obtained from serum and whole blood of clinically suspected animals, following the manufacturer's instructions.

Results

During the suspected BEF investigations conducted in the Jordan Valley, a total of 405 cattle from 18 farms were clinically examined during the 2018 and 2021 events (Table 1). In 2018, 315 cattle from 14 farms were investigated, whereas in 2021, 90 cattle from four farms were examined.

In the 2018 outbreak, 27 cattle showed clinical signs compatible with BEF, including sudden fever, reduced feed intake, muscular stiffness, recumbency, lameness, and a marked decrease in milk production. The affected animals recovered spontaneously within 2–4 days, and no mortality was recorded. The overall morbidity rate was 8.6%. Paired serum samples were collected from clinical and non-clinical animals. In the 2021 investigation, four farms were examined, and 90 cattle were evaluated due to similar clinical suspicion. Among these, 10 cattle with clinical signs were sampled, yielding 20 paired serum samples. No clinical progression, morbidity, or mortality was recorded during this investigation (Table 2).

Overall, across both investigations, 142 paired serum samples were collected, including 72 samples from clinically suspected animals and 70 samples from non-clinical animals (Table 1). BEFV antibody testing by ELISA detected antibodies only in the 2018 clinical cases; three samples were positive (two showed seroconversion between sampling periods, and one remained positive at both sampling times). All samples collected from non-clinical animals in 2018 and all samples collected during the 2021 investigation were negative for BEFV antibodies.

RT-PCR was performed on 44 whole blood and serum samples collected from cattle showing clinical signs suggestive of BEF during the 2018 and 2021 outbreaks; nonspecific reactions were observed. To increase assay sensitivity and specificity, nested PCR was carried out using the same two primer sets targeting the glycoprotein (G) gene in two successive reactions; however, nonspecific reactions were still observed in all tested samples. Additionally, 13 whole-blood samples from clinically suspected cattle were tested by conventional PCR, which also yielded only nonspecific reactions. These results indicate that BEFV RNA was not detected in any samples from either outbreak.

Table 2: Clinical and serological findings during suspected bovine ephemeral fever (BEF) outbreaks in the Jordan Valley in 2018 and 2021.

Parameter	2018 Outbreak	2021 Outbreak
Number of farms investigated	14	4
Locations	Kraimah, Al-Masharee, Al-Masharqa, Northern Al-Adassiyah	Abu Obaida, Al-Balawneh, Al-Zor, Deir Alla
Total cattle clinically examined	315	90
Clinically affected/suspected animals	27	10
Main clinical features	Acute fever, anorexia, muscular stiffness, lameness, recumbency; recovery within 2–4 days; no mortality	Similar clinical suspicion without progression, morbidity, or mortality
Morbidity rate	8.6%	0.0%
Mortality rate	0.0%	0.0%
ELISA testing	3/52 clinical samples were positive (2 seroconversion, 1 persistent positivity); All non-clinical samples were negative	All samples were negative
RT-PCR	All samples were negative	All samples were negative

Discussion

This study investigated suspected outbreaks of bovine ephemeral fever (BEF) in Jordan, focusing on clinical, serological, and molecular findings. The detection of seroconversion in clinically affected cattle provides evidence of BEFV infection in the 2018 outbreak investigation, supporting the occurrence of infection in the studied herds. A previous seroprevalence study in clinically healthy dairy cattle in northwestern Jordan reported a BEFV antibody prevalence of 45.37% [7], indicating that the virus may circulate in the region even in the absence of confirmed clinical outbreaks. The absence of a national vaccination program against BEF may further contribute to the continued circulation of the virus.

The clinical presentation observed in this study was characterized by a sudden onset of fever lasting two to five days, followed by spontaneous recovery. Affected cattle showed reduced feed intake, a marked decline in milk production, recumbency, and lameness, consistent with classical descriptions of bovine ephemeral fever reported in the literature [10]. Although BEF had not previously been confirmed in Jordan through integrated clinical and laboratory diagnosis, field veterinarians have long suspected its occurrence and have associated it with seasonal patterns [3].

In line with this, the present investigation evaluated suspected outbreaks that occurred in 2018 and 2021, while no comparable cases were officially reported in 2019 or 2020, according to the Jordanian Ministry of Agriculture. Laboratory confirmation of BEFV infection remains challenging due to the short duration of viremia, low viral loads in blood, RNA instability during transport, and potential pre-analytical limitations, all of which may reduce the sensitivity of RT-PCR-based detection [11, 12, 13]. Although virus isolation in cell culture is considered the reference standard, it was not performed in this study due to logistical and resource constraints [14].

Serological detection using ELISA is a practical and reliable approach for BEFV screening, with high reported sensitivity and specificity [15]. Previous studies have detected BEFV antibodies in multiple biological fluids, including synovial, pericardial, thoracic, and abdominal fluids from affected cattle [16]. Moreover, rising antibody titers in paired sera remain a robust indicator of recent infection [17, 18].

In the present study, paired sera collected during acute and convalescent phases demonstrated seroconversion in a subset of clinically affected cattle. Specifically, ELISA

testing detected BEFV antibodies only in the 2018 investigation, in which 3 of 52 clinical samples (5.8%) were positive, including two animals that seroconverted and one persistently positive case. All non-clinical cattle from affected and neighboring farms were negative. In contrast, all samples collected during the 2021 investigation were seronegative, and no BEFV RNA was detected by molecular assays. Therefore, the 2021 episode represents a clinically suspected but laboratory-unconfirmed event and cannot be classified as a confirmed outbreak. The absence of viral RNA detection in both investigations is consistent with the transient nature of BEFV viremia and the known diagnostic limitations of field sampling [19, 20].

Overall, the findings support the circulation of BEFV in Jordan, based solely on evidence from the 2018 investigation. These results highlight the need for strengthened national surveillance systems, improved laboratory diagnostic capacity, and enhanced awareness among veterinarians to ensure early detection and effective control of future outbreaks.

Conclusions

The present study provides the first laboratory-supported evidence of exposure to bovine ephemeral fever virus in cattle in Jordan, based on the 2018 investigation. Diagnosis was supported by compatible clinical signs and seroconversion in a subset of clinically affected animals. In contrast, the 2021 investigation represents a clinically suspected but laboratory-unconfirmed event, as no serological or molecular evidence of BEFV infection was detected. Therefore, it cannot be classified as a confirmed outbreak. The 2018 findings indicate probable BEFV circulation in the Jordan Valley, while the 2021 results emphasize the need for laboratory confirmation before declaring outbreaks. The inability to detect viral RNA in both investigations reflects the short viremic phase of BEFV and highlights the challenges associated with molecular diagnosis under field conditions. Strengthening surveillance systems, improving diagnostic infrastructure, and increasing veterinary awareness are essential for the early detection, confirmation, and control of future BEF outbreaks in Jordan.

Declarations and supplementary information

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