

Distribution of clinical findings and evaluation of infrequent subcutaneous emphysema in naturally infected Simmental and Holstein cattle with bovine ephemeral fever

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Abstract

Bovine ephemeral fever (BEF), also known as three-day sickness, is a vector-borne viral disease of cattle. It causes significant economic losses due to high fever and a sudden decline in milk yield and is characterized by low mortality but high morbidity (80–100%). The aim of this study was to determine the distribution of acute clinical findings in naturally infected Holstein and Simmental cattle with BEF, to evaluate breed-related differences, and to identify subcutaneous emphysema. A total of 32 PCR-confirmed cattle were evaluated. Vital parameters and clinical findings were assessed and recorded within the first 12 hours after the onset of clinical signs. The distribution of clinical findings was as follows (n=32): Appetite was affected in all animals, with complete anorexia observed in 75% of cases; jugular vein distension, recumbency, tremor and salivation (34.4%); lameness (31.2%); mastitis and complete loss of milk production (12.5%); constipation (6.25%); and subcutaneous emphysema (3.1%). No statistically significant difference was found in the distribution of clinical findings between Holstein and Simmental breeds. Subcutaneous emphysema had the lowest incidence among the observed clinical findings. Additionally, jugular vein distension, a clinical finding not commonly described in the literature, was recorded in both breeds during the acute phase of BEF. The combined evaluation of the clinical findings identified in this study may facilitate the diagnosis of BEF, particularly under field conditions, increase clinical suspicion, and support the early initiation of treatment protocols. Additionally, complications such as jugular vein distension, which has not been widely emphasized in previous studies, and subcutaneous emphysema, which is less common, have been observed and should be considered in clinical evaluation.

Keywords: Bovine ephemeral fever, cattle, clinical findings, jugular vein distension, subcutaneous emphysema

INTRODUCTION

Bovine ephemeral fever (three-day sickness or BEF) is a vector-borne viral disease caused by an RNA virus belonging to the genus *Ephemerovirus* and the family *Rhabdoviridae* (Nadeem et al., 2024). BEF primarily affects cattle and buffalo herds and causes economic losses due to a sudden drop in milk yield (Akakpo, 2015; Ayvazoğlu and Demir, 2022; Çamkerten et al., 2019; Paksoy et al., 2024). BEF is prevalent in temperate, tropical, subtropical, and other regions of Australia, Asia, Africa, and the Middle East. The morbidity rate of the disease is quite high, reaching 80–100% in outbreaks, while the mortality rate is generally below 1%. However, in some severe outbreaks, this rate can reach 10–20% (Walker and Klement, 2015).

Approximately 24 hours after infection, BEF virus induces leukocytic alterations, including neutrophilia. The resulting endothelial cell damage contributes to the development of clinical signs and lesions. Hypocalcemia observed in BEF is thought to be associated with this systemic inflammatory response, leading to a marked decrease in calcium levels and subsequent neuromuscular dysfunction (Ashraf et al., 2023). High levels of interferon production by infected cells lead to toxemia and inflammation in blood vessels, mucous membranes, and joints. Plasma fibrinogen is observed in the peritoneum, joints, heart, and pleural cavities. Characteristic clinical

signs of BEF include sudden onset of biphasic fever, a sudden and severe decrease in milk production, anorexia, depression, eye and nasal discharge, drooling, muscle rigidity, lameness, ruminal stasis, lying in a sternal position, infertility in male bulls, and abortion and other inflammatory responses in dams (Walker and Klement, 2015; Walker et al., 2012). BEF is one of the rare viral diseases that can be treated with rational medical interventions (St George, 2004). Nonsteroidal anti-inflammatory drugs (NSAIDs) are highly effective in preventing the onset of clinical symptoms when administered daily during the incubation period of the disease, and they support faster recovery when administered after clinical symptoms have appeared (Uren et al., 1989). Clinical signs of hypocalcemia such as ruminal stasis, paresis, decreased and/or absent reflexes disappear with subcutaneous or intravenous administration of calcium borogluconate (St George, 2004). Although numerous experimental and commercial vaccines have been developed, utilization rates are generally low due to the irregular nature of epizootics and the need for multiple repeated doses, and there is scarce published data on the evaluation of vaccines in the field (Walker and Klement, 2015).

The high morbidity rate, increased mortality in severe cases, and associated productivity losses, together with limitations in treatment and prevention strategies, underscore the importance of early diagnosis. Thus, this study aimed to characterize the clinical spectrum of the acute

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phase of PCR-confirmed BEF in cattle, to compare the distribution of clinical findings between Holstein and Simmental breeds, and to identify clinical manifestations that may increase the suspicion index of the disease under field conditions. By providing these data, the study aims to facilitate earlier diagnosis, promote timely treatment, and enhance the effectiveness of supportive medical interventions.

MATERIALS AND METHODS

Animal material

The animal material consisted of 46 cows clinically suspected of BEF that were presented to the Internal Medicine Clinic of the Faculty of Veterinary Medicine, Harran University, for diagnosis and treatment purposes between September 25, 2024, and November 30, 2024.

Clinical examinations

Vital parameters, including respiratory rate (breaths/min) and heart rate (beats/min), as well as clinical findings such as fever, lameness, recumbency, appetite status, mastitis, tremor, salivation, jugular vein distension, milk yield changes, defecation status, and subcutaneous emphysema, were recorded for all animals included in the study.

Ultrasonographic examination

In cases where subcutaneous emphysema was suspected during detailed physical examination, ultrasonographic evaluation was performed using a 5 MHz convex probe (Mindray Z60, Bio-Medical Electronics Co., Ltd., Shenzhen, China).

Blood sampling and storage

To confirm the presence of BEF, approximately 5 mL of blood was collected from the jugular vein via venipuncture. The samples were placed in anticoagulant-free tubes, centrifuged at 3,000 rpm for 10 minutes, and the serum was stored at -80°C until analysis (approximately 2 months). Polymerase chain reaction (PCR) was subsequently performed, and only PCR-positive animals were included in the study.

RNA extraction and RT-PCR

BEFV RNAs were purified using the QIA amp Viral RNA kit (Qiagen, Germany) according to the manufacturer's instructions and stored at -80°C . cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher, USA). A size of 800 bp for G gene of BEFV was amplified using primers 420F and 420R. In short, PCR conditions: 94°C 5 min; 35 cycles (94°C 40 sec, 46°C 60 sec, 72°C 40 sec); final 72°C 10 min (Zheng and Qiu, 2012). Then, PCR products were visualized by a transilluminator (Bio Rad, USA) following 1% agarose gel electrophoresis (Figure 1).

Inclusion/exclusion criteria

Animals were eligible for inclusion if they exhibited typical clinical signs of BEF and had a medical history indicating disease onset within the previous 12 hours. During differential diagnosis, traumatic reticulopericarditis (TRP), which may cause jugular vein distension, was excluded using ferroscopy and pain tests. Pseudo pericarditis associated with hemoparasitic infections was ruled out based on peripheral blood smear examination (using light microscope, x100 magnification, Olympus, Japan) and tick inspection. Chronic cardiac diseases were excluded based on cardiac auscultation findings, medical history, and the acute onset of high fever.

Statistical methods

The distribution characteristics of continuous variables (body temperature, heart rate, and respiratory rate) were evaluated using the Shapiro–Wilk normality test. Since the analysis results showed that the variables did not meet the assumption of normal distribution, non-parametric methods were preferred for comparisons between two independent groups. The Wilcoxon rank-sum test (Mann–Whitney U test) was used for comparisons of continuous variables between the two breeds, and W was reported as the test statistic. For comparisons of categorical variables such as clinical findings, Fisher's Exact Test was applied due to the limited sample size. Descriptive statistics are presented as median, first quartile (Q1), third quartile (Q3), minimum, maximum, and interquartile range (IQR) for continuous variables; and as

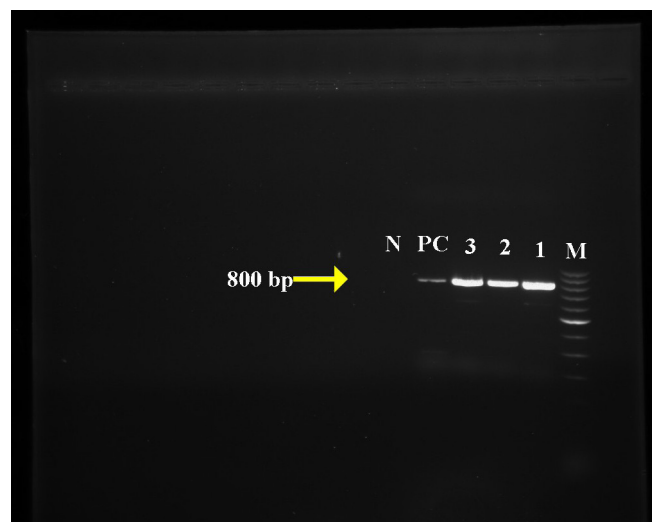


Figure 1. PCR amplification products using BEFV primers. Lane M: 100 bp DNA ladder marker (Thermo Fisher Scientific, USA), Lane 1, 2 and 3: Positive amplification PCR products, NC: Negative Control (distilled water), PC: Positive Control (800 bp).

positive case number and percentage (%) for categorical variables. Statistical analyses were performed using the R programming language (version 4.3.2). All statistical analyses were performed using two-tailed methods, and the significance level was set at $\alpha = .05$.

RESULTS

Animals

Following clinical, laboratory, and PCR evaluations of 46 cattle suspected of BEF, 32 animals met the inclusion criteria and were enrolled in the study. Of these, 14 were Simmental (43.8%) and 18 were Holstein (56.2%). All animals were between 3 and 6 years of age.

Clinical examination results

Fisher's Exact Test results, used to compare clinical findings, also revealed no statistically significant relationship between the breeds in any of the variables of lameness, lying down, mastitis, muscle tremor, salivation, jugular vein distension, and complete loss of appetite (all p values > 0.05). These results show that the frequency of the evaluated clinical findings is similar in both breeds (Table 1). No statistically significant difference was observed between Holstein and Simmental breeds in

terms of body temperature ($W = 144.0$, $p = 0.505$), heart rate ($W = 150.0$, $p = 0.371$), and respiratory rate ($W = 137.5$, $p = 0.676$). These findings indicate that Holstein and Simmental breeds have similar distributions in terms of these physiological parameters (Table 2). When descriptive statistics were examined, it was observed that respiratory rate showed a wider distribution in the Holstein breed (IQR = 24.8), while body temperature showed a narrower distribution in the Simmental breed (IQR = 1.20) (Table 3). However, these differences were not found to be statistically significant in the statistical analyses.

Findings detected during the acute phase were, in order: complete loss of appetite (75%), recumbent position (34.4%), muscle tremor (34.4%), hypersalivation (34.4%), jugular vein distension (34.4%), lameness (31.2%), mastitis (12.5%), constipation (6.25%), and subcutaneous emphysema (3.1%) (Figure 2).

During the general examination, palpation of the skin revealed the presence of generalised subcutaneous emphysema (Figure 3). Furthermore, an E-line indicating emphysema was identified on ultrasound in the area where crepitus was detected in the same case (Figure 4).

Table 1. Fisher's Exact test results for clinical findings by breed

Clinical Findings	Holstein (n = 18)	Simmental (n = 14)	p
Lameness	5 (27.8%)	5 (35.7%)	0.712
Recumbency	7 (38.9%)	4 (28.6%)	0.712
Mastitis	2 (11.1%)	2 (14.3%)	1.000
Muscle Tremor	7 (38.9%)	4 (28.6%)	0.712
Hypersalivation	5 (27.8%)	6 (42.9%)	0.465
Jugular Vein Distension	7 (38.9%)	4 (28.6%)	0.712
Complete Loss of Appetite	13 (72.2%)	11 (78.6%)	0.733

Data are presented as percentages of the number of cases. All comparisons were made using Fisher's Exact Test.

Table 2. Mann-Whitney U test results for continuous variables by breed

Variable	W	p
Body temperature (°C) *	144.0	0.505
Heart rate (bpm)	150.0	0.371
Respiratory rate (breath/min)	137.5	0.676

Bpm: Beats per minute, all analyses were performed using the two-tailed Mann-Whitney U test. The significance level was accepted as $\alpha = 0.05$.

*Rectal.

Table 3. Descriptive statistics of continuous variables by breed

Variable	Breed	n	Median	Q1	Q3	Min	Max	IQR
Body temperature (°C)*	Holstein	18	40.00	38.70	40.20	38.00	42.10	1.55
	Simmental	14	39.90	39.30	40.50	38.60	41.20	1.20
Heart rate (bpm)	Holstein	18	70.50	58.20	74.00	52.00	79.00	15.80
	Simmental	14	70.50	62.20	78.20	49.00	82.00	16.00
Respiratory rate (breath/min)	Holstein	18	65.50	53.80	78.50	45.00	94.00	24.80
	Simmental	14	73.50	59.00	79.00	49.00	85.00	20.00

Bpm: Beats per minute, Q1 = first quartile, Q3 = third quartile, IQR = interquartile range. Continuous variables are presented with median and interquartile range (IQR). *Rectal.

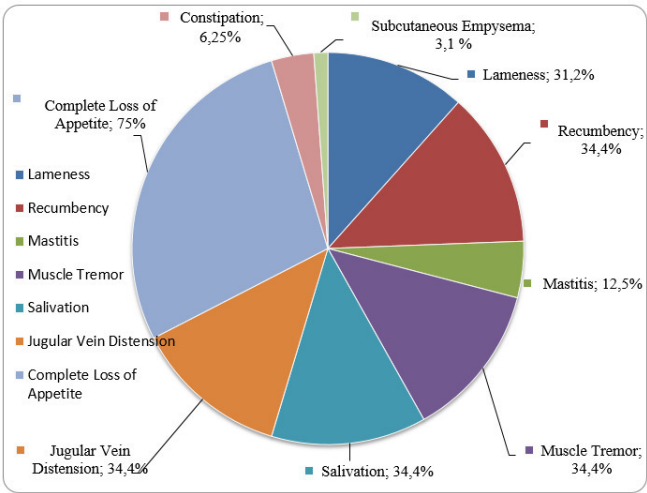


Figure 2. Distribution of clinical findings in all cattle included in the study (n = 32).



Figure 3. Clinical appearance of subcutaneous emphysema in the dorsal region of a BEF-positive cow. Note the accumulation of subcutaneous air, evidenced by crepitus on palpation.

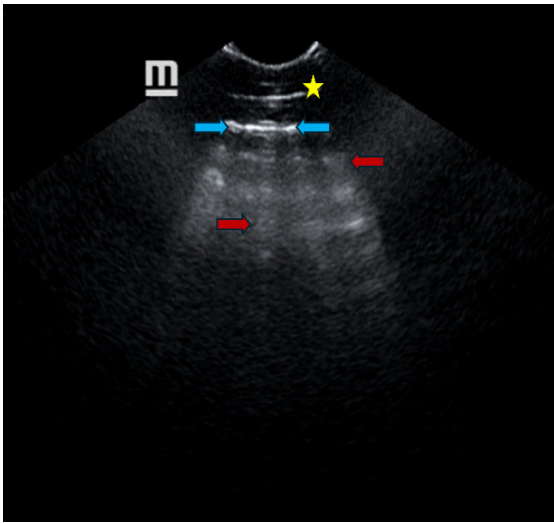


Figure 4. Ultrasound image of subcutaneous emphysema showing the emphysematous area (blue arrow) and E-lines (red arrow). The asterisk indicates the peritoneal line.

DISCUSSION

BEF outbreaks have both immediate and delayed economic impacts, including the culling of valuable cows and dramatic reductions in milk yield, leading to substantial farm income losses for several months following clinical diagnosis (Lavon et al., 2023). When treatment and other productivity losses are considered together, they cause significant economic losses averaging \$85 per dairy cow and \$148 per beef animal (Ayvazoglu and Demir, 2022). Therefore, accurate assessment of clinical signs for early diagnosis of the disease is crucial in terms of treatment costs. Although BEF is generally associated with high morbidity and low mortality, mortality rates of 10–20% have been reported in some herds (Walker and Klement, 2015). However, they have indicated that various factors such as strain, breed, breeding, nutrition, animal density, climate, and accompanying diseases can affect these rates (Özyörük et al., 2025). Thus, rapid recognition of clinical findings that suggest the presence of BEF is crucial in preventing the spread of the disease through early diagnosis and reducing economic losses (Ayvazoglu and Demir, 2022; Mosa, 2025). Estimating the probability of a disease is, by its nature, a multivariate process. Every clinician considers patient characteristics (signals), clinical symptoms, and test results together when assessing the probability of a disease. Although rare, the probability of a disease can sometimes be determined based on a single predictor. A multivariate diagnostic prediction model is a mathematical equation that relates the probability of a particular clinical outcome occurring in a given individual, based on multiple predictors (Collins et al., 2015; Moons et al., 2015). Clinical findings are objective terms used to describe abnormalities detected during clinical observation or examination of an animal (Fentener Van Vlissingen et al., 2015; Percie du sert et al., 2020). The introduction of scoring systems for clinical findings has been reported to improve the assessment of clinical well-being, particularly in studies employing novel approaches (Percie du sert et al., 2020). Thus, in the accurate clinical evaluation of diseases, recognizing the distribution of symptoms, taking into account the frequency of each clinical finding and their co-occurrence, is of great importance. Early diagnosis, timely treatment, and recognition of relevant clinical signs are critical for improving outcomes and shortening recovery in BEF (Mosa, 2025; Walker and Klement, 2015). In this study, during the first 12 hours following symptom onset, the observed clinical signs in the animals ($n = 32$) were as follows: loss of appetite (75%), recumbency (34.4%), tremor (34.4%), salivation (34.4%), lameness (31.2%), mastitis with complete loss of milk production (12.5%), constipation (6.25%), and subcutaneous emphysema (3.1%) (Figure 2). In addition, all animals experienced a decrease in milk yield and a reduced or complete loss of appetite ($n:32$, %100). The incidence of recumbency and lameness during the acute phase of the disease was 34.4% and 31.2%, respectively. These findings suggest that the virus induces an acute inflammatory response in muscle and joint tissues, resulting in the release of prostaglandins and cytokines, which in turn lead to fever and muscle pain (Abdel-Hamied et al., 2026; Mosa, 2025; Walker and Klement, 2015). The manifestation of clinical findings such as muscle tremor, recumbency, and lameness can also be considered a response to the acute inflammatory process occur-

ring in muscle and joint tissues during BEF infection. Indeed, Dinçer (2024) reported a significant increase in inflammatory cytokines in BEF cases, which supports the findings of the present study. Additionally, naturally infected animals exhibited a notable decrease in blood calcium levels (Constable et al., 2017; Dinçer et al., 2024; Şahin et al., 2004). In BEF-infected animals, some exhibit severe constipation, whereas others develop diarrhea (Constable et al., 2017). In this study, constipation was observed in 6.25% of cases without diarrhea. This is likely due to dehydration associated with high fever (Grünberg and Constable, 2009), which can impair digestive system function and contribute to hypocalcemia in affected animals (Constable et al., 2017; Dinçer et al., 2024; Şahin et al., 2004). Zahid (2022) reported that serum calcium levels in cattle infected with BEF dropped to as low as 7.84 mg/dl. Clinical recovery takes 1-3 days, but some animals have been reported to experience prolonged paralysis and ataxia following the acute phase of the disease (Walker and Klement, 2015). The clinical presentation of BEF follows a typical pattern, with acute symptoms such as muscle stiffness, lameness, and sudden fever usually emerging after a short incubation period of two to four days (Pyasi et al., 2025). BEF can progress to lameness in the hind legs due to involvement of the axial muscles and pelvic girdle. This condition can be unilateral or bilateral. Sometimes, transient joint swelling or muscle tremors are observed (Mosa, 2025). In the present study, lameness was observed in 31.2% of animals, with some exhibiting unilateral hindlimb lameness and others bilateral involvement. Additionally, 34.4% of the muscle tremors observed in this study may be attributable to hypocalcemia (Zahid et al., 2022). Mastitis, which can occur as a clinical complication of BEF, has been associated with significant economic losses (El daous et al., 2025). A major contributor to these losses is reduced milk production resulting from mammary gland tissue damage (Zhao and Lacasse, 2008). In BEF cases, milk yield often decreases sharply, and the development of mastitis in affected animals can further exacerbate production losses (Ayvazoglu and Demir, 2022; Constable et al., 2017). In this study, it was determined that 12.5% of cases involved mastitis and 12.5% involved complete loss of milk yield. BEF reduces resistance to mastitis in dairy herds. This leads to significant economic losses due to decreased or complete cessation of milk production (Down et al., 2013; Lunagariya et al., 2015). It has also been reported that recovering cows do not return to their pre-disease yield levels (Lavon et al., 2023). Consistent with these findings, although not reported in the study results, post-study follow-up indicated that while cows of both breeds fully recovered clinically, their milk production did not return to pre-disease levels. Contrary to previous reports, not all symptoms were observed in every case in the present study. For comparison, a study conducted in Egypt in the summer of 2004 reported complete cessation of milk production in 84.16% of cows, salivation in 80.86%, tremor in 78.59%, recumbency in 71.03%, lameness in 83.37%, and mastitis in only 1.58%. The same study noted that clinical mastitis occurred in a very small proportion of cases (1–2%) and no instances of subcutaneous emphysema were reported (Ammar et al., 2005). The lower incidence of clinical findings observed in the present study compared to previous reports (Amin, 2016) suggests that symptom

presentation can vary in BEF cases. Subcutaneous emphysema is a condition characterized by the accumulation of air or gas within the subcutaneous tissue. It may result from air entry through accidental or surgical wounds, extension of pulmonary emphysema, disruptions in the respiratory tract mucosa, gas migration following abdominal surgery, or infections such as gas gangrene (Görücü and Demirkan, 2021; Walker and Klement, 2015). In the present study, subcutaneous emphysema was regarded as a complication rather than a primary symptom. Ammar (2005), in a study of 397 BEF-positive cattle, reported no cases of subcutaneous emphysema, suggesting that its absence may be related to the low virulence of the virus and the immune status of the animals. In contrast to our study of naturally infected cattle, they noted that even when BEF was experimentally induced in 6-month-old animals and typical clinical signs developed, emphysema was not observed in either clinical or pathological examinations (Theodoridis and Coetzer, 1979). Although the exact reason for the absence of emphysema is not fully understood, factors such as animal age, viral virulence, and environmental conditions, including air temperature, may play a role, and further studies are warranted. Notably, some field studies have reported the occurrence of subcutaneous emphysema in severe outbreaks. For example, a 2017 study in Saudi Arabia found that subcutaneous emphysema was one of the key clinical signs of BEF, affecting 2% of cases (Zaghawa et al., 2019). In this study, subcutaneous emphysema was encountered in only 1 (n:32/ 3.1%) of the cases. The researchers noted that in some cases, pulmonary emphysema and fibrinous bronchiolitis were typical during necropsy of BEF cases, and that subcutaneous emphysema could also be seen in the dorsal regions (St George, 2004). It was previously reported that in the acute phase of BEF, significant respiratory signs such as tachycardia and tachypnea or dyspnea may occur, and pulmonary emphysema can develop in severe cases. Respiratory dysfunction resulting from pulmonary emphysema has been suggested as a primary cause of mortality in affected cattle (Çatik et al., 2015). In the present study, consistent with these findings, crepitations due to bilateral subcutaneous emphysema were detected around the costal, lumbar, and thoracic vertebrae on palpation of the dorsal region in cattle (Figure 3). In this study, jugular vein distension was observed in 34.4% of cases; this was considered a clinically significant finding in the acute phase of BEF. In cattle, jugular vein distension is generally considered a sign of increased right heart pressure and the resulting venous congestion (Buczinski et al., 2010). A previous study on chronic edema associated with cor pulmonale in cattle reported jugular vein distension as a prominent clinical finding. The study further indicated that pulmonary artery hypertension, resulting from lesions in the pulmonary vessels, caused right ventricular dilation and right heart failure, ultimately leading to jugular vein distension (Caymmi et al., 2025). Jugular vein distension may be a finding to consider when assessing the cardiovascular system in cases of BEF. Limitations of this study include the relatively small sample size, the absence of recorded lymph node and nasal discharge findings, the lack of hematochemical analyses, and the inability to measure serum calcium levels. The absence of cardiopulmonary investigations in animals exhibiting jugular

vein distension limits interpretations of the pathogenesis of the distension.

CONCLUSION

Jugular vein distension, a finding not adequately addressed in previous studies, was detected in both breed groups included in the study and can be considered a potentially clinically relevant finding in the acute phase of BEF. This distension observed in the acute phase can be considered an indicator that allows for the assessment of the systemic vascular effects of BEF and the developing secondary cardiopulmonary stress without the need for invasive intervention. However, further research is needed to better determine its pathogenesis and clinical significance. In contrast, subcutaneous emphysema, a rare complication rather than a primary feature of BEF, was also observed. The detection of crepitation on palpation and E-lines on ultrasonography can aid in its diagnosis. This study found no statistically significant differences in vital or clinical parameters between Holstein and Simmental cattle affected by BEF. However, small effect sizes may have been overlooked due to the limited sample size, and further studies with larger cohorts are warranted to confirm these findings. It was concluded that the clinical findings observed, particularly under field conditions, may help increase the BEF suspicion index and facilitate earlier treatment interventions, thereby preventing both animal and productivity losses.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

This study was conducted following the approval of the Local Animal Experiments Ethics Committee of Harran University (HRÜ-HADYEK/2025/009/06), Şanlıurfa, Türkiye.

Author Contributions

AS, CB, IG, EK, and EG designed the study. ED performed the PCR analyses for diagnostic confirmation. AS, CB, IG, EK, and EG contributed to the preparation and revision of the manuscript.

Availability of Data and Materials

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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