Malignant Edema in Some Sheep Flocks of Iran

Abstract

Malignant edema is a severe and swiftly fatal disease affecting domestic and wild livestock. The disease manifests following the introduction of *Clostridium* spp. into wounds or skin damage. *Clostridium septicum* is commonly linked with malignant edema. This disease, characterized by edema, doughy swelling, and skin necrosis, is underreported in Iran, leading to a lack of awareness among clinicians. Given its underreporting, addressing this issue is essential, prompting current research efforts to understand better its prognosis, bacteriological and molecular diagnosis, clinical signs, and treatment. Upon detecting suspicious signs of malignant edema in three separate flocks with imported breeds, investigations ensued, including regular clinical exams and sample collection from subcutaneous tissue. The impacted livestock consists of five Île-de-France sheep and two Romane rams, with one Île-de-France ram succumbing to the disease. The bacteriological procedure, including Gram staining and isolation of the causative agent, was meticulously carried out using the standard method. The PCR assay was conducted to validate the existence of *C. septicum* and reject the presence of *Clostridium chauvoei* by employing specific primers. The diagnosis of malignant edema in the affected sheep was confirmed through clinical, macroscopic, and bacteriological examinations, all of which corroborated the presence of *C. septicum*. The PCR assay demonstrated the presence of the *C. septicum*, verifying the bacteriological procedure. Initial signs of the infection included depression, weakness, high fever, and colic, followed by regional pain, crepitation, swelling characterized by a doughy consistency, edema, pain, and necrosis. The study highlights the potential for preventing malignant edema-related fatalities through early diagnosis and antibiotic intervention (Penicillin and Streptomycin). However, it notes a persistent challenge: the inability to repair necrotic tissue at the lesion site. Malignant edema, not being a prominently warned disease and with vaccinations available against its causative agent, has received comparatively less focus from clinicians and researchers in Iran.

Keywords: Malignant edema, *Clostridium septicum*, Sheep, Crepitation, Gas gangrene.

1. Introduction

Malignant edema (gas gangrene) is an acute and often fatal disease affecting both domestic and wild animals, resulting from contamination of wounds by one or more members of the "gas gangrene" group of clostridial organisms. One potential explanation for its occurrence is that the causative bacteria are soil-borne organisms, gaining entry into livestock primarily through grazing on low-lying, wet pastures. This disease is common in cattle and sheep but infrequently in goats, with young rams most commonly affected. It is hypothesized that the nature of the fleece and the relatively sparse presence of skin folds and body pleats in goats result in fewer shearing wounds, thereby leading to less secondary invasion of clostridial spores (1-5).

Clostridium chauvoei, *Clostridium septicum*, *Clostridium sordelli*, and *Clostridium novyi* are rod-shaped, Gram-positive, toxin-producing, spore-forming, ubiquitous, and anaerobic bacteria. They are commonly found in soil and can persist for extended periods due to sporulation. Additionally, these bacteria may inhabit healthy livestock's intestinal tract and liver without causing disease. "False" blackleg, more accurately known as malignant edema, is primarily caused by *C. septicum*, although *C. novyi*, *C. sordelli*, *C. chauvoei*, and even *Clostridium perfringens* have been isolated from lesions characteristic of malignant edema (1, 6, 7).

Malignant edema can develop when spores enter tissues through penetrating wounds, which commonly arise during routine management practices like vaccinations, blood sampling, parturition, shearing, docking, castration, disbudding, and particularly shearing activities (1, 2, 5, 8). Fighting and head-butting among bucks can also facilitate the introduction of spores, leading to a specific type of malignant edema known as "big head." The tissue damage associated with wounds creates anaerobic conditions that promote bacterial proliferation. As anaerobic conditions develop in body tissues, the organisms multiply and release several exotoxins that have local and systemic effects. These toxins induce severe localized inflammation and generalized, fatal toxemia. Local inflammation typically manifests as tissue necrosis accompanied by the accumulation of edema and gas. Subcutaneous and connective tissue are often more prominently affected than muscle tissue, although muscle involvement can occur (1, 2).

The disease typically occurs sporadically but can also manifest as outbreaks. For instance, an outbreak of this disease in Brazil was likely triggered by using a shared needle to vaccinate a flock of 1000 sheep. Clinical signs observed before death in this outbreak included severe

depression, swelling around the vaccination site, subcutaneous edema, lameness, and crepitation (2, 9). Morris et al. reported a case of malignant edema in a 1-year-old Friesian sheep following blood sampling from the jugular vein. Clinical signs in this Friesian sheep were similar to those observed in the previously mentioned outbreak. Additionally, during necropsy, the animal was in very good physical condition. The skin in the swollen area exhibited blue discoloration, and crepitation was evident in the subcutaneous tissue. *C. septicum* and *C. sordellii* were isolated from the lesions and were further confirmed by a direct fluorescent antibody test (8).

Sheep breeding stands as the cornerstone of Iran's livestock production sector, particularly for meat consumption, reflecting its popularity among the populace (10). However, the industry faces significant threats from diseases such as malignant edema, which poses a pervasive risk owing to its ubiquitous agents. Despite its potential danger, there remains a notable dearth of comprehensive studies on this disease in sheep, particularly within Iran and for local livestock. The limited literature available underscores the pressing need for increased research and understanding of malignant edema's epidemiology and management. Since vaccination against its causative agent is typically performed, and the disease is not considered a significant threat, clinicians and researchers tend to overlook it. Consequently, despite its lethality, fewer cases of this disease are documented. Recognizing the importance of addressing this gap, the current study aims to comprehensively explore the clinical manifestations, post-mortem observations, laboratory diagnosis techniques involving both bacteriological and molecular approaches, and treatment modalities related to malignant edema across three imported sheep industrial farms.

2. Materia and Methods

2.1.Animals and Sampling

Following the observation of concerning signs of malignant edema in three separate flocks in 2021—comprising two Île-de-France flocks and one Roman flock—a research initiative was initiated to pinpoint the causative agent and establish a definitive diagnosis for the ailment. The disease impacted two ewes within one Île-de-France flock, along with two ewes and one ram in another flock of the same breed, and two rams within a Roman breed flock. It's noteworthy that these sheep breeds were imported into Iran. Seven months prior to the occurrence of the disease, these animals had been administered the Enterotoxemia Quadrivalent Bacterin Vaccine from the

Razi Vaccine & Serum Research Institute of Iran. This vaccine includes *C. perfringens* types D, C, B, and C. *septicum* Bacterins.

Regular clinical examinations were conducted whenever signs were observed. Subsequently, subcutaneous swellings were aseptically aspirated using sterile syringes from the affected areas. The samples were promptly transported to the bacteriology laboratory within two hours under cold chain conditions (+ 2/+ 8 °C in an icebox). Antibiotic treatment (Penicillin G 8,000 IU/kg + Streptomycin 10 mg/kg, administered once daily intramuscularly for seven days) was administered to these animals following sample collection (5). Unfortunately, one of the Île-de-France rams succumbed to the disease, and the animal underwent necropsy within 2 hours of death.

2.2.Bacteriological procedure

Samples from the affected areas were processed into smears using standard conditions and then subjected to Gram staining. Subsequently, a bacteriological procedure was conducted to isolate the disease-causing agent.

Samples were inoculated onto 5% sheep blood agar and Cooked Meat Medium (Oxoid, UK) and incubated aerobically and anaerobically at 37°C for 48 hours. Anaerobic media were supplied with anaerobic gas kits (Anaerocult A, Merck). The identification process involved selecting suspicious *Clostridium* colonies for further analysis, relying on their distinctive features such as morphology, including shape, consistency, size, and color. The selected colonies were placed on fresh agar plates, including 'Stiff' blood agar (3% agar) and normal blood agar, to obtain pure cultures of *Clostridium* bacteria. The isolates were identified using conventional biochemical techniques (11).

The biochemical tests encompassed in the analysis comprised Indole Production, Motility (via stab inoculation into semi-solid media), Gas production, Starch hydrolysis, Gelatin hydrolysis, inoculation in Egg Yolk Agar for examination Lecithinase and Lipase Production, Casein digestion, Nitrate reduction, and sugar fermentation, which involved Glucose, Lactose, Sucrose, and Maltose (11, 12).

2.3. Molecular assay (PCR)

To validate the molecular identification of *C. septicum* after bacteriological techniques, a PCR assay was conducted following the protocol used by Khiav and Paradise (13). In the current study, the presence of the Alpha toxin gene, a critical virulence factor involved in the pathogenesis of gas gangrene caused by *C. septicum*, was investigated using PCR. Considering that *C. chauvoei* shares similar biochemical characteristics and is often isolated alongside *C. septicum*, the growth colonies were also screened for the presence of *C. chauvoei* using a species-specific PCR assay. The primers and PCR conditions employed in the present study are detailed in **Table 1**.

In summary, bacterial cells were centrifuged and diluted in TE solution containing lysozyme (1 mg/mL). After adding 10% SDS, the mixture was then incubated at 37°C for a minimum of 30 minutes. Proteinase K (50 mg/mL) was introduced and incubated at 56°C for an hour. Extraction was performed using phenol and chloroform solutions in equivalent amounts. Sodium acetate (1:10 v/v) and isopropanol (1 v/v) were added to the mixture, which was left to incubate overnight at -20 °C. The DNA was pelleted by centrifugation at 12500 rpm for 10 minutes at 4°C. The resulting sediment was washed with 70% ethanol, dried, and finally dissolved in TE buffer. A NanoDrop instrument (Nanodrop Technologies, USA) was used to measure DNA quality and quantity.

The final reaction mixture volume was 30 µL, comprising 1.8 µL of 25 mM MgCl2, 3 µL of 10X PCR buffer (SinaClon, Iran), 0.6 µL of 10 mmol dNTPs, 0.5 µL of Taq DNA polymerase (5 units/mL) (CinnaGen, Iran), 2.5 µL of DNA template (100 ng/µL), 1 µL of each primer (10 pmol/mL) **(Table 1)** (CinnaGen, Iran), and 20 µL of distilled water. Distilled water and type D *C. perfringens* served as negative controls, while the *C. septicum* vaccine strain acted as a positive control. Subsequently, the PCR product was combined with 2 µL of 6x gel loading dye, electrophoresed, stained with ethidium bromide (0.5 µg/mL), and visualized using a UV transilluminator.

Table 1. The primers and PCR conditions utilized in this investigation

Target gene	Sequences (5′-3′)	Amplicon sizes	PCR conditions	References
C. septicum Alpha toxin gene*	F-ATCGGAAACATGAGTGCTGC R- AGTCTTTATGCTTCCGCTAG	270 bp	94 °C 1 min 55 °C 1 min 72 °C 1 min (30 cycles)	(13)
C. chauvoei flagellin gene (*fli*C)	FlaF-AGAATAAACAGAAGCTGGAGATG FlachR-TACTAGCAGCATCAAATGTACC	535 bp	94 °C 1 min 55 °C 1 min 72 °C 90 s (30 cycles)	(3, 27)

* The hemolysin gene sequence is exclusive to *C. septicum* and does not exhibit similarity to other clostridial toxins (13).

3. Results

3.1. Microbiological findings

The microscopic examination of the smears, coupled with the bacteriological procedures involving isolation of the causative agent, revealed the presence of *C. septicum*, a finding subsequently corroborated through PCR assay.

Microscopic examination following Gram staining revealed the presence of large Gram-positive bacilli in the Gram-stained smears obtained from muscle and subcutaneous tissues. These bacilli appeared either sporulated or non-sporulated (Spores oval and subterminal) and displayed pleomorphic characteristics, suggesting variability in their cellular forms. In anaerobic conditions, turbidity was observed at the bottom of the Cooked Meat Medium tube, and *C. septicum* was successfully isolated upon reculture on blood agar. Conversely, no growth was observed during the incubation of samples under aerobic conditions. The colonial appearance of the isolates exhibited traits consistent with those of *C. septicum*, characterized by swarming and spreading, along with hemolytic growth on normal agar. On 'stiff' agar, the colonies appeared irregular, featuring a rhizoid edge.

The biochemical test outcomes indicated negative Indole production, positive motility, positive gas production, positive starch hydrolysis, positive Gelatinase production, negative Lecithinase and Lipase activity, positive Nitrate reduction, and fermentative ability and acid production with sugars Glucose, Lactose, and Maltose. Acid production from sucrose was not observed. Biochemical findings have verified the existence of *C. septicum* based on referenced

sources (11). Overall, these findings collectively support the diagnosis of malignant edema caused by *C. septicum* in the affected sheep.

The amplification of the hemolysin gene (Alpha-toxin gene) of *C. septicum* bacteria using specific primers yielded an amplicon with a size of 270 base pairs (bp). The 270 bp amplicon aligns with the expected size for the hemolysin gene, further validating the molecular diagnosis of *C. septicum* infection in the affected sheep. No 535 bp amplicon corresponding to the *C. chauvoei* flagellin gene (*fli*C) was detected, leading to the exclusion of *C. chauvoei* presence in the lesions.

3.2. Clinical observations

All clinical and macroscopic findings and bacteriological observations confirmed the diagnosis of malignant edema, and the presence of *C. septicum* in lesions created in infected sheep was confirmed. Except for the Île-de-France ram, which was dead, the rest of the animals were treated, and the signs of gas gangrene disappeared within three weeks following antibiotic therapy. Still, the skin of the area where the lesions were created became necrotic and did not heal (**Figure 1**).

The initial signs observed during the detailed clinical examination included impaired general condition, high fever (41.4 – 42.2 °C), tachypnea, tachycardia, weakness, and colic. These were followed by localized or regional pain, doughy swelling, edematous and painful swelling, local erythema, wetness, and gelatinous secretions (**Figure 1**). As time progressed, the swelling intensified, and the skin appeared dark and taut. Eventually, the taut skin cracked and assumed a yellow hue, with edematous fluid seeping from the cracks, varying from a thin serum to a gelatinous deposit **(Figure 2 and Figure 3)**. Typically, skin gangrene accompanied by subcutaneous and intermuscular connective tissue edema surrounding the infection site became apparent.

Signs of prostration were noted in three animals during the study. Subcutaneous crepitation, a crackling sensation caused by gas within tissues, was clearly detectable in four cases. However, evidence of subcutaneous gas production was less pronounced than in blackleg disease cases. Clinical features observed included cellulitis at the injury site, characterized by minimal gangrene and gas formation (crepitation). Additionally, tissue swelling due to edema, along with a discoloration of the overlying skin and a sensation of coldness, was noted. During the necropsy of

the deceased Île-de-France ram, the animal was found to be in very good physical condition, but clear macroscopic findings of crepitation were observed.

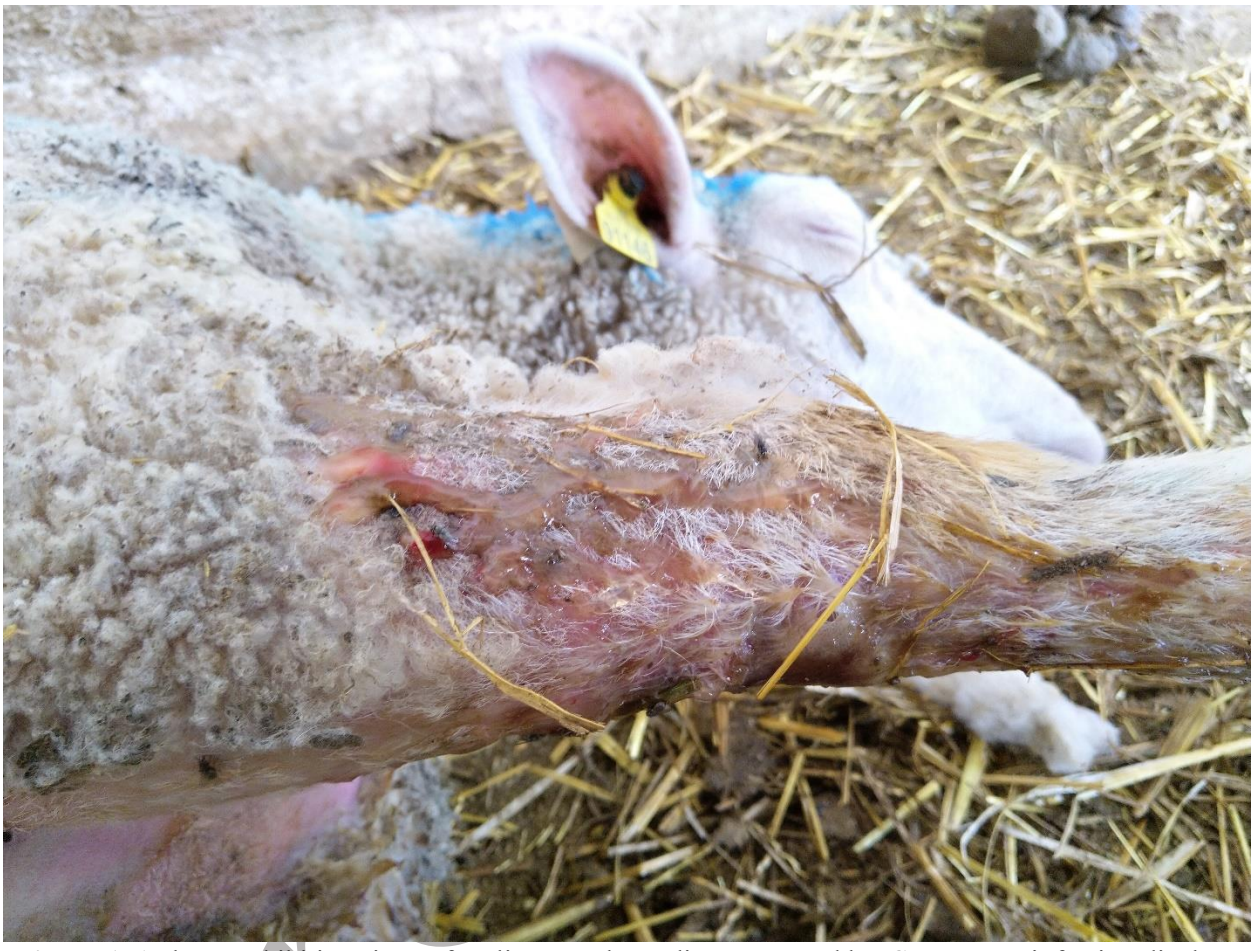

Figure 1. A sheep exhibiting signs of malignant edema disease caused by *C. septicum* infection displays signs of wetness, along with gelatinous secretions and edematous, doughy swelling.

Figure 2. Malignant edema in a sheep exhibits notable discoloration and gangrene of the subcutaneous tissue, alongside the presence of gelatinous secretions.

Figure 3. Tensile swelling, dark and taut skin with skin cracks, and eventually edematous fluid from cracks in a sheep affected by malignant edema. Tensile swelling refers to the abnormal swelling of tissues under tension. In the case of malignant edema, it's likely caused by gas and fluid buildup in the affected area. The tissues become stretched, leading to visible swelling.

4. Discussion

The diagnosis of malignant edema in sheep relies on a combination of clinical, macroscopic, and microbiological findings. This study's clinical examinations revealed a range of signs indicative of the disease, including impaired general condition, weakness, respiratory and cardiac abnormalities, localized pain, swelling, and high fever. These clinical manifestations are consistent with previous reports of malignant edema in sheep (2, 4, 5, 14). Crepitation, observed as a macroscopic feature at the lesion site of malignant edema, was evident in the current study. This finding corroborates observations documented in other studies involving similar cases. In the

current investigation, malignant edema was diagnosed, and treatment was administered to six sheep, resulting in their recovery. One animal succumbed to the illness, underscoring the fatal consequences of this disease when not promptly diagnosed and treated (1, 2, 8, 14, 15). The current research stands out as the sole investigation documenting the occurrence of malignant edema disease in Île-de-France and Romane sheep breeds. It's intriguing to observe that most malignant edema cases in breeding farms involve imported sheep breeds in Iran, while there are no occurrences of this disease in local breeds.

In the present study, subcutaneous edema, doughy swelling, taut skin, and crepitation were evident in sheep with malignant edema. This finding is in common with other reported cases of malignant edema (3, 8, 14, 16). Alpha toxin production has resulted in subcutaneous bloating, darkening of edematous skin areas, and interstitial hemorrhage in muscle tissue. Indeed, studies have shown that *C. septicum* needs to produce an Alpha toxin to manifest specific clinical signs (3, 6, 17, 18). In a 2005 study, Kennedy et al. reported a striking difference between alpha toxin-positive and negative strains in terms of virulence (19). The Alpha toxin of *C. septicum* is structurally and functionally similar to the Epsilon toxin of *C. perfringens* type B and D and the Aerolysin of *Aeromonas hydrophila*. On the other hand, the Alpha toxin of *C. septicum*, unlike C. perfringens, causes the infiltration of immune system cells into the infection site. In infections caused by *C. septicum*, hemorrhage due to Alpha toxin-induced microvascular destruction leads to decreased blood flow in the infection site. This ultimately leads to ischemia that will support the survival of *C. septicum* in the absence of external trauma (3, 14, 15, 17, 20).

In general, *C. septicum* tends to respond well to medications like Penicillin G, Ampicillin, Chloramphenicol, Clindamycin, Cephaloridine, Oleandomycin, Erythromycin, Lincomycin, and Tetracyclines (5, 17, 21). In the present investigation, prompt identification of clinical signs in afflicted animals, indicating the onset of the disease's early stages and appropriate treatment, helped prevent animal fatalities despite leaving necrotic skin lesions untreated. This outcome underscores the potential benefits of antibiotic therapy, particularly in mitigating fatalities attributed to *C. septicum* infection when the disease is diagnosed early. Early intervention with antibiotics not only aids in halting the progression of the infection but also contributes to minimizing mortality associated with *C. septicum*. The effectiveness of antibiotic treatment in the early stages of the disease highlights the importance of timely diagnosis and intervention. Early

detection allows for the implementation of appropriate therapeutic measures, including antibiotic administration, which can arrest the spread of the infection and prevent the onset of severe complications (1, 14).

Comparisons of malignant edema in sheep cases caused by *C. septicum* are challenging due to the limited number of studies addressing this specific condition. The scarcity of comprehensive research on malignant edema in livestock, particularly those focusing on the involvement of *C. septicum*, hinders the ability to draw meaningful comparisons across different investigations. Gazioglu and colleagues conducted a study in 2018, identifying nine goats with malignant edema, showcasing clinical signs and necropsy findings akin to the present research—moreover, the method employed for detecting and isolating *C. septicum* aligned with the present study. Among the sufferers, four goats were treated following the technique outlined in the current research, and they exhibited successful responses to treatment. To validate the isolation of the disease agent, they conducted a PCR assay similar to the method described in the present study. Additionally, they observed an amplicon 270 bp, indicative of the alpha-toxin gene of *C. septicum*. (3). Lewis (2007) reported successful outcomes with early invasive antibiotic treatment in sheep afflicted with malignant edema (22).

In a documented incident in Brazil in 2006, a flock comprising 1200 sheep and goats experienced a significant loss of livestock, with 40 sheep and 20 goats succumbing to malignant edema within 24 hours to 5 days following non-standard vaccination procedures. These animals also had a vaccination history with *C. septicum* bacterin. The clinical manifestations observed in this outbreak closely resembled those documented in the present study. Analysis of samples collected from the lesion sites revealed the presence of *C. novi* and *C. septicum*, as confirmed by direct FAT testing (6).

In a case report by Cihan et al. (2010) in Turkey, 20 sheep were diagnosed with malignant edema, and the clinical signs closely resembled those observed in the present study. These animals also received *C. septicum* bacterin. (23). This finding underscores the consistency of clinical manifestations associated with malignant edema across different geographical regions and livestock populations. A case of malignant edema linked to umbilical infection was documented in a deceased Merino lamb within a flock comprising 50 ewes and 35 newborn lambs in Argentina. The sampling and diagnostic approach mirrored that of the present study, utilizing Gram staining

and direct FAT, yielding comparable results. During the post-mortem examination, despite the lamb being in good physical condition, macroscopic lesions consistent with those observed in the present study were identified (24). In the abovementioned study, the affected animals had a vaccination history with *C. septicum* antigen. However, akin to the present study, this disease still resulted in problems and damages. The presence of this microorganism in the infection may be attributed to a suboptimal vaccination method, a lack of individual immune response, or an unusual challenge dose (8).

Microbiological analysis played a crucial role in confirming the diagnosis. Gram staining of smears from affected tissues revealed the presence of large Gram-positive bacilli, characteristic of *Clostridium* spp. Further confirmation was achieved through the FAT, which specifically identified *C. septicum* in all cases. Culture and purification techniques supported these findings by isolating *C. septicum* from the affected tissues, particularly in anaerobic conditions. *C. septicum*, commonly found in soil, has also been detected in the feces of both humans and healthy animals. This pathogen, acting as a post-mortem invader, can swiftly disseminate throughout the body from the intestines of deceased or distressed animals, particularly ruminants. Its rapid spread raises the possibility of isolating *C. septicum*, potentially leading to misdiagnosis, even if a necropsy is performed immediately after the animals' demise (1, 13, 17). However, in this study*, C. septicum* was isolated not only from samples taken during necropsy but also from the skin lesions of live but diseased animals.

Microbiological and biochemical identification can be a time-consuming process, compounded by the challenge of distinguishing between Clostridia, notably *C. chauvoei* and *C. septicum*, due to their similarities (13). The current study tried to introduce a standardized protocol for isolating *C. septicum*, which could serve as a valuable resource for future research endeavors seeking to isolate and identify the causative agent of malignant edema. Molecular findings validated the efficacy of the bacteriological method employed. In the current study, all *C. septicum* strains that tested positive in biochemical and microbiological assays exhibited the expected PCR product size (270 bp), confirming the presence of the bacterium in the samples. Differentiating between *C. septicum* and *C. chauvoei* alpha-toxin is only possible through PCR analysis, not immunological methods. The sequence of the hemolysin gene is unique to *C. septicum* and does not show homology with other clostridial toxins.

The current study noted that even though the signs of malignant edema diminished in the treated animals after antibiotic therapy, the skin in the region where the lesions had developed underwent necrosis and failed to heal. This implies that although the antibiotic treatment successfully managed the infection and alleviated systemic signs, it couldn't halt tissue damage and necrosis at the site of the initial lesions. As time elapsed, the damage inflicted by the disease remained unrepaired, indicating the severity and persistence of its effects. Additional interventions or supportive care might be needed to manage the necrotic tissue and facilitate healing in these affected areas (2, 14).

Preventing malignant edema in sheep is paramount to avoid its detrimental effects on livestock health and economic losses. Several measures can be implemented to mitigate the risk of disease occurrence. First and foremost, maintaining proper hygiene and sanitation in sheep housing and handling areas can help minimize exposure to *Clostridium* spores in the environment. Regular cleaning and disinfection of equipment used for routine management practices, such as vaccinations and shearing, can also reduce the risk of wound contamination. Furthermore, vaccination against *Clostridium* species, including *C. septicum*, should be considered a preventive measure (14, 15, 25). The industrial flocks investigated in the present study were vaccinated against *C. septicum*. Cases of malignant edema have sporadically occurred within these flocks. This underscores the significance of vaccination as a preventive measure against the disease.

Sporadic cases of malignant edema within the vaccinated flocks highlight an important aspect of disease prevention. While vaccination is an effective strategy for reducing the overall incidence of the disease, its effectiveness may not be absolute in preventing every single case. Factors such as variations in vaccine efficacy, environmental conditions, and individual animal susceptibility can contribute to occasional breakthrough infections despite vaccination efforts. Therefore, even in vaccinated populations, the "possibility" of disease occurrence remains high. However, the fact that these cases are sporadic suggests that vaccination still plays a significant role in disease prevention by reducing outbreaks' overall frequency and severity. It emphasizes the importance of increased prognosis and continued vaccination programs to maintain herd immunity and minimize the impact of the disease on livestock populations (6, 15).

Additionally, proper wound management practices are crucial for preventing the entry of *Clostridium* spores into tissues. Prompt and thorough cleaning and disinfection of wounds, along

with timely veterinary intervention, can help minimize the risk of infection and subsequent development of malignant edema. Educating sheep farmers and livestock handlers about the signs, risk factors, and preventive measures for malignant edema is essential for disease control and management. Awareness campaigns and training programs can empower farmers to implement appropriate biosecurity measures and vaccination protocols to safeguard their flocks against this potentially devastating disease (1, 13, 26).

In conclusion, diagnosing malignant edema in sheep necessitates a thorough approach encompassing clinical assessment, microbiological scrutiny, and histopathological examination. Prevention measures, such as maintaining proper hygiene, administering vaccinations, and effectively managing wounds, are imperative to mitigate disease risk and safeguard the well-being and productivity of sheep herds. The escalating instances of malignant edema in small ruminants across Iran underscore the urgency of giving this disease greater attention. This study furnishes valuable insights into the prognosis of malignant edema. Considering the absence of recent research on malignant edema in Iran, heightened investigation coupled with enhanced management practices and training holds promise for reducing disease incidence and averting economic losses.

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Authors' contribution

Study concept and design: H. E.
Acquisition of data: H. E. and S. M. J.
Analysis and interpretation of data: H. E. and S. M. J.
Drafting of the manuscript: S. M. J.
Administrative, technical, and material support: H. E.
Study supervision: H. E.

Ethics

The authors of this study affirm that all ethical standards were upheld in the preparation of the submitted article.

Conflict of interest

The authors declare that they have no conflict of interest.

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Data availability

The data produced and/or analyzed during the present study can be obtained from the corresponding author upon request.

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