Original Article

# Malignant Edema in Some Sheep Flocks of Iran

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## ABSTRACT

Malignant edema is a severe and rapidly fatal disease that affects both domestic and wild livestock. The disease manifests following the introduction of Clostridium spp. into wounds or skin damage, with Clostridium septicum being commonly linked with malignant edema. This disease, characterised by oedema, doughy swelling and skin necrosis, is underreported in Iran, leading to a lack of awareness among clinicians. Addressing this issue is imperative, as evidenced by current research efforts aimed at enhancing our understanding of the disease's prognosis, bacteriological and molecular diagnosis, clinical signs, and treatment.The present study was initiated after the detection of suspicious signs of malignant edema in three separate flocks with imported breeds. Investigations included regular clinical exams and sample collection from subcutaneous tissue. The affected 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, thereby verifying the bacteriological procedure.Initial signs of the infection included depression, weakness, high fever, and colic, followed by regional pain, crepitation, swelling characterised by a doughy consistency, edema, pain, and necrosis. The study emphasises the significance of early diagnosis and antibiotic intervention (Penicillin and Streptomycin) in preventing fatalities due to malignant edema. Nevertheless, it is important to note the persistent challenge of 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 that affects both domestic and wild animals. It is caused by 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. The disease manifests most commonly in young rams and is prevalent in cattle and sheep, though it is uncommon in goats. The hypothesis 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, is supported by evidence. The bacterium responsible for this condition is a rod-shaped, Gram-positive, toxin-producing, spore-forming, ubiquitous, and anaerobic bacterium. These bacteria are commonly found in soil and can persist for extended periods due to sporulation. Additionally, these bacteria may inhabit the intestinal tract and liver of healthy livestock 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). The development of malignant edema can occur as a result of the entry of spores into tissues through penetrating wounds. Such wounds commonly arise during a range of routine management practices, including vaccinations, blood sampling, parturition, shearing, docking, castration, and disbudding. Notably, shearing activities have been identified as a significant risk factor for the development of malignant edema (1, 2, 5, 8). Additionally, fighting and head-butting among bucks has been observed to 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, with the organisms multiplying and releasing exotoxins that have local and systemic effects. These toxins induce severe localized inflammation and generalized, fatal toxemia, with local inflammation typically manifesting 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 manifests sporadically, but can also be transmitted as an outbreak. For instance, an outbreak of the disease in Brazil was likely triggered by the use of a shared needle to vaccinate a flock of 1000 sheep. The 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, with clinical signs similar to those observed in the aforementioned outbreak. Additionally, during necropsy, the animal was found to be in very good physical condition, with blue discoloration evident in the skin of the swollen area and crepitation 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 is 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 paucity of research in this area is underscored by the limited extant literature, which highlights the pressing need for increased research and understanding of the epidemiology and management of malignant edema. The disease is not considered a significant threat, as it is typically prevented through vaccination of its causative agent. Consequently, the lethality of the disease is under-documented. It is therefore vital to recognize the importance of addressing this gap in knowledge, and 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. Materials and Methods

### 2.1. Animals and Sampling

In 2021, three distinct flocks exhibited signs of malignant edema, including two Île-de-France flocks and one Roman flock. This prompted the initiation of a research initiative to identify the causative agent and establish a definitive diagnosis for the condition. The disease manifested in 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 is noteworthy that these sheep breeds were imported into Iran. Seven months prior to the onset of the disease, these animals had been administered the Enterotoxaemia Quadrivalent Bacterin Vaccine from the Razi Vaccine & Serum Research Institute of Iran. This vaccine contains 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 (+8/+18 °C in an icebox). Following sample collection, antibiotic treatment was administered to the animals (Penicillin G 8,000 IU/kg + Streptomycin 10 mg/kg, administered once daily intramuscularly for seven days). Unfortunately, one of the Île-de-France rams succumbed to the disease and underwent necropsy within two 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 then 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 the selection of suspicious Clostridium colonies for further analysis, based on their distinctive features, including morphology, 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)**

In order to validate the molecular identification of C. septicum following bacteriological techniques, a PCR assay was conducted in accordance with the protocol established by Khiav and Paradise (13). The present study investigates the presence of the Alpha toxin gene, a critical virulence factor involved in the pathogenesis of gas gangrene caused by C. septicum, using PCR. Given the biochemical similarity between C. chauvoei and C. septicum, and the concomitant isolation of both species, 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 subjected to a process of centrifugation and dilution in a solution of Tris-EDTA (TE) that had been treated with lysozyme at a concentration of 1 mg/mL. Following the addition of 10% SDS, the mixture was then subjected to incubation at a temperature of 37°C for a minimum duration of 30 minutes. Thereafter, proteinase K at a concentration of 50 mg/mL was introduced and the mixture was incubated at a temperature of 56°C for a period of one hour. The extraction process was then initiated by the use of phenol and chloroform solutions in equivalent amounts. Sodium acetate (1:10 v/v) and isopropanol (1 v/v) were then 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. The quality and quantity of the DNA were measured using a NanoDrop instrument (Nanodrop Technologies, USA).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.

**3. Results**

**3.1. Microbiological Findings**

The microscopic examination of the smears, in conjunction with the bacteriological procedures involving the isolation of the causative agent, revealed the presence of C. septicum, a finding that was subsequently corroborated through a 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 manifested as either sporulated or non-sporulated (spores oval and subterminal) and exhibited 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, characterised 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. However, acid production from sucrose was not observed. These biochemical findings have verified the existence of C. septicum based on referenced sources (11). Collectively, these findings provide substantial evidence to 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. 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). This 270 bp amplicon aligns with the

**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<br>R- AGTCTTTATGCTTCCGCTAG | 270 bp | 94 °C 1 min<br>55 °C 1 min<br>72 °C 1 min<br>(30 cycles) | (13) |
| *C. chauvoei* flagellin gene (*fli*C) | FlaF-AGAATAAACAGAAGCTGGAGATG<br>FlachR-TACTAGCAGCATCAAATGTACC | 535 bp | 94 °C 1 min<br>55 °C 1 min<br>72 °C 90 s<br>(30 cycles) | (3, 27) |

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

expected size for the hemolysin gene, thus providing further validation of the molecular diagnosis of C. septicum infection in the affected sheep. Conversely, the absence of a 535 bp amplicon corresponding to the C. chauvoei flagellin gene (fliC) was observed, thereby confirming the exclusion of C. chauvoei presence in the lesions.

### 3.2. Clinical observations

The clinical and macroscopic findings, in conjunction with the bacteriological observations, provided unequivocal confirmation of the diagnosis of malignant edema, along with the presence of C. septicum in the lesions observed in the infected sheep. With the exception of the Île-de-France ram, which was found to be deceased, the remaining animals were treated, resulting in the resolution of the signs of gas gangrene within a period of three weeks following the administration of antibiotic therapy. However, the skin in the affected area exhibited signs of necrosis and failed to heal, as depicted in 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 (Figures 2 and 3). Typically, skin gangrene accompanied by subcutaneous and intermuscular connective tissue edema surrounding the infection site became apparent. The study revealed signs of prostration in three animals, with subcutaneous crepitation – a crackling sensation caused by gas within tissues – being clearly detectable in four cases. However, evidence of subcutaneous gas production was less pronounced than in blackleg disease cases. The clinical features observed included cellulitis at the injury site, characterized by minimal gangrene and gas formation (crepitation). Further observations included tissue swelling due to edema, discoloration of the overlying skin, and a sensation of coldness. 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.

## 4. Discussion

The diagnosis of malignant edema in ovine subjects is dependent on a combination of clinical, macroscopic and microbiological findings. The clinical examinations conducted in this study 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 ovine subjects (2, 4, 5, 14). Crepitation, observed as a macroscopic feature at the site of the lesion, was evident in the present study, corroborating observations documented in other studies involving similar cases. The present investigation diagnosed malignant edema and administered treatment to six sheep, resulting in their recovery. However, the unfortunate demise of one animal serves to underscore the grave consequences associated with this malady when it is not diagnosed and treated in a timely manner (1, 2, 8, 14, 15). The present study is noteworthy for being the sole investigation to document the occurrence of malignant edema disease in Île-de-France and Romane sheep breeds. It is noteworthy that the majority of malignant edema cases in breeding farms involve imported sheep breeds from Iran, while there is an absence of this disease in local breeds. In the present study, the following symptoms were observed in sheep with malignant edema: subcutaneous edema, doughy swelling, taut skin, and crepitation. These symptoms are consistent with those reported in other cases of malignant edema (3, 8, 14, 16).The production of Alpha toxin has been shown to result 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 exhibits structural and functional similarity to the Epsilon toxin of C. perfringens type B and D and the Aerolysin of Aeromonas hydrophila. However, in contrast to C. perfringens, the Alpha toxin of C. septicum leads to the infiltration of immune system cells into the infection site. Furthermore, the study observed that in infections caused by C. septicum, the presence of Alpha toxin leads to microvascular destruction, which in turn results in

**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.

decreased blood flow in the infection site. This phenomenon, known as ischemia, has been shown to support the survival of C. septicum in the absence of external trauma (3, 14, 15, 17, 20). In general, C. septicum has been observed to demonstrate a positive response to various medications, including Penicillin G, Ampicillin, Chloramphenicol, Clindamycin, Cephaloridine, Oleandomycin, Erythromycin, Lincomycin, and Tetracyclines (5, 17, 21). In the present investigation, the prompt identification of clinical signs in affected animals, indicating the onset of the disease's early stages, and the administration of appropriate treatment, contributed to the prevention of animal fatalities, despite the untreated presence of necrotic skin lesions. This outcome underscores the potential benefits of antibiotic therapy, particularly in mitigating fatalities attributed to C. septicum infection when the disease is diagnosed early. The findings of this study demonstrate that early intervention with antibiotics not only aids in halting the progression of the infection but also contributes to minimising 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). The paucity of research in this field is evidenced by the limited number of studies comparing malignant edema in sheep cases caused by C. septicum. This 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. In 2018, Gazioglu et al. conducted a study that identified nine goats with malignant edema, exhibiting clinical signs and necropsy findings similar to the present research. Moreover, the method employed for detecting and isolating C. septicum aligned with the present study. Among the affected goats, four 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, the researchers conducted a PCR assay similar to the method described in the present study, observing 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, and the clinical manifestations observed in this outbreak closely resembled those documented in the present study. Subsequent 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), which lends further support to the hypothesis that the clinical manifestations associated with malignant edema are consistent 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 aforementioned 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). The role of microbiological analysis in confirming the diagnosis was crucial. 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, a microbe 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. The rapid dissemination of the pathogen 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. The process of microbiological and biochemical identification can be a time-consuming process, further complicated by the challenge of distinguishing between Clostridia, notably C. chauvoei and C. septicum, due to their similarities (13). The present study attempted to introduce a standardized protocol for the isolation of C. septicum, which could serve as a valuable resource for future research endeavors seeking to isolate and identify the causative agent of malignant edema. The molecular findings confirmed 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. The differentiation 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, although 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 could not 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. It can be posited that additional interventions or supportive care might be required to manage the necrotic tissue and facilitate healing in these affected areas (2, 14). It is imperative to implement measures aimed at averting malignant edema in sheep, with a view to mitigating its deleterious effects on livestock health and economic interests. To this end, several strategies can be employed to reduce the risk of disease occurrence. Primarily, adherence to stringent hygiene and sanitation practices in sheep housing and handling areas is crucial in minimizing exposure to Clostridium spores in the environment. Moreover, regular cleaning and disinfection of equipment utilized for routine management practices, such as vaccinations and shearing, can further contribute to the reduction of wound contamination. In addition, vaccination against Clostridium species, including C. septicum, should be considered a preventative measure (14, 15, 25).The industrial flocks investigated in the present study were vaccinated against C. septicum, and cases of malignant edema have sporadically occurred within these flocks, thereby underscoring the significance of vaccination as a preventative measure against the disease. The sporadic occurrence of malignant edema within vaccinated flocks underscores a pivotal element of disease prevention strategies. While vaccination is a highly effective approach in mitigating the overall incidence of the disease, its efficacy may not be infallible in preventing every instance of infection. Variations in vaccine efficacy, environmental conditions, and individual animal susceptibility can contribute to sporadic breakthrough infections despite vaccination efforts, thereby maintaining a high disease potential even within vaccinated populations. However, the sporadic nature of these cases suggests that vaccination plays a significant role in disease prevention by reducing outbreaks' overall frequency and severity. This 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. The importance of prompt and thorough wound cleaning and disinfection, along with timely veterinary intervention, cannot be overstated in this context. Such measures can play a pivotal role in minimizing the risk of infection and subsequent development of malignant edema. It is imperative to educate sheep farmers and livestock handlers about the signs, risk factors, and preventive measures for malignant edema. 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, a meticulous approach is imperative for the diagnosis of malignant edema in sheep, encompassing clinical assessment, microbiological scrutiny, and histopathological examination. Preventive measures, such as maintaining proper hygiene, administering vaccinations, and effectively managing wounds, are crucial to mitigate disease risk and safeguard the well-being and productivity of sheep herds. The increasing incidence of malignant edema in small ruminants across Iran underscores the need for a more thorough investigation of this disease. This study provides valuable insights into the prognosis of malignant edema, and given the absence of recent research on the subject in Iran, greater 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. The data produced and/or analyzed during the present study is available upon request from the corresponding author.

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