Case report

History

In a sheep breeding farm around the town of Yenisehir in Bursa, Turkey, animals of one flock showed lameness, anorexia, recumbency, subcutaneous oedema including vulva, limbs, abdomen, and discoloration of the skin. The flock consisted of 45 sheep, which were 2–4 years of age and had a body weight of 45–58 kg. In detailed history, the owner mentioned that a combined vaccine against enterotoxaemia, blackleg, malignant oedema, black disease and tetanus had been administered to each sheep by a veterinary practitioner 2 days before the first appearance of clinical signs. There is always a local lesion at the site of infection consisting of a soft swelling with erythema accompanied by pain on palpation. Emphysema can be felt. At later stage, skin necrosis and gangrene occur. High fever is present and toxæmia develops. Death occurs within 1 to 2 days (2).

Clinical examination

On our clinical examination, 20 sheep were found to be suffering from different stages of malignant oedema. They had high fever (41.4–42.2 °C), depression, tachycardia, muscle tremor, stiffness, and hind limbs. Subcutaneous oedema and emphysema was commonly detectable. Local lesions at the site of infection consisted of a soft, hot, doughy swelling with local erythema accompanied by pain on palpation. The sheep had dark reddish brown skin lesions, and some showed dermal necrosis. In pregnant ewes swelling of the vulva accompanied by the discharge of a reddish-brown fluid could be seen (Fig. 1).

Diagnostics

Routine haematological values including total white blood cell and differential leukocyte count, haematoцит, haemoglobin, erythrocyte and platelet counts were evaluated by a haemocell counter (Cell-Dyn 3500®, Abbott Inc., USA). The results were within normal limits except for total white blood cell count and differential leukocyte count. Fifteen sheep had leukocytosis (13800–23700/μL, reference value: 4000–12000/μL) along with neutrophilia (11250–20500/μL, reference value: 700–6000/μL) (9).

As biochemical parameters, aspartate aminotransferase (AST) and creatin kinase (CK) were assessed by Reflotron® (Boehringer Mannheim Inc., Germany). Serum AST (452–717 U/L) and CK (745–885 U/L) activities exceeded the reference range (AST: 48–128 U/L, CK: <350 U/L) (9).

Smears for bacterial culture and confirmation of diagnosis were prepared from the centre of the damaged tissue. Smears were air dried and fixed in anhydrous acetone during 30 minutes at −20 °C.

Outbreak of malignant oedema in sheep following vaccination with a multivalent clostridial bacterin-toxoid

H. Cihan; E. Yalcin; Z. Mecitoglu; S. Senturk
Department of Internal Medicine, Faculty of Veterinary Medicine, University of Uludag, Bursa, Turkey

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Introduction

Malignant oedema is a fatal disease of several animal species, produced by one or more members of the Clostridium genus including C. oedematiens type A, C. chauvoei, C. perfringens, C. sordelli, and C. septicum. Animals of all ages and species are susceptible, especially sheep. The disease generally occurs sporadically. However, outbreaks may occur in sheep after intramuscular injection of vaccines and some drugs including antimicrobials and nutritional supplements (1). Clinical signs appear rapidly after infection. There is always a local lesion at the site of infection consisting of a soft swelling with erythema accompanied by pain on palpation. Emphysema can be felt. At later stage, skin necrosis and gangrene occur. High fever is present and toxæmia develops. Death occurs within 1 to 2 days (2).

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and processed for a fluorescence antibody test (FAT) based on the methodological guide (3–5). Neither *Cl. septicum* nor *Cl. chauvoei* were isolated from the lesions.

In 20 sheep that had died necropsy was performed. The carcasses were generally markedly oedematous. Affected muscles had a dark red colour and showed inclusion of gas bubbles. Gangrene was seen around the site of injection. The oedema fluids were gelatinous, blood-stained and contained gas bubbles. Subserous haemorrhages in all tissue and accumulation of haemorrhagic, serosanguineous fluid in body cavities were detected. As a characteristic, pathological findings in 15 sheep were dominated by acute multifocal locally extensive necrotising and haemorrhagic myocarditis (Fig. 2).

**Diagnosis**

Diagnosis of malignant oedema was based on the clinical and pathological findings and history.

**Treatment and outcome**

Initially, crystalline penicillin and streptomycin (Vetimisin, VETAS, Istanbul, Turkey) were administered intramuscularly (60000 IU/kg BW) to each sheep at 8-hour-intervals for one day. Then, treatment was continued with penicillin G procaine and penicillin G potassium (Iecilline®, I.E. Ulugay, Turkey) (40000 IU/kg BW i. m., q12 h, 10 days) and flunixin meglumine (Flumed®, Alke, Istanbul, Turkey) (2.2 mg/kg, i. m., q24 h, 3 days). In spite of therapy, there was no clinical improvement and the animals developed severe toxemia. By day 5, a total of 20 sheep had died.

**Discussion**

A detailed history, clinical sings including high fever (41.4 to 42.2 °C), subcutaneous oedema with emphysema around the perineal region, abdomen and hind limbs as well as skin necrosis, and swelling of the vulva accompanied by discharge of a reddish-brown fluid give evidence to suggest malignant oedema. A definitive diagnosis of this disease needs identification of the causative microorganisms (2). The FAT is frequently used for identification of these microorganisms on tissue or culture smears (5). The FAT is considered the gold standard and a positive result of this test is one of the criteria for diagnosis of blackleg and malignant oedema. However, these procedure is not always successful due to difficulties in obtaining, submitting and processing the samples in the laboratory. Also, some of the Clostridia causing blackleg and malignant oedema are extremely sensitive to oxygen, and they tend to be easily overgrown by other microorganisms present in the samples (3). In the present report, diagnosis was made based on the clinical and post-mortem findings. *Cl. septicum* and *Cl. chauvoei* could not be isolated from affected animals. This may be associated with the previous use of antibiotics or with one of the aforementioned reasons. There was leukocytosis and neutrophilia in 15 sheep which could be associated with bacterial infection. AST and CK activities were high in the same sheep, probably due to myonecrosis.

In all affected sheep, post-mortem findings were similar. In addition, it was detected that extensive myocardial necrosis occurred in 15 affected sheep, as found in lambs (7), cattle (8) and calves (10).

The first clinical signs appeared on the third day following the application of a multivalent clostridial vaccine to the sheep. Similarly, Costa et al. (3) reported an outbreak of malignant oedema in sheep caused by *Clostridium sordellii*, following routine vaccination. A flock of 1000 Santa Ines sheep aged 12–18 months was vaccinated subcutaneously with a commercial vaccine. A veterinarian visited the farm 3 days post-vaccination and found 15 animals with extensive subcutaneous oedema and crepitation surrounding the area of vaccination, lameness and severe depression. All these animals died within 3 hours after the onset of clinical signs (3). Morris (6) reported a case of malignant oedema in a 1-year-old Friesian sheep after a blood sample had been collec-
ted from the jugular vein. *Clostridium septicum* and *Clostridium sordellii* were isolated from the lesions and also demonstrated by a fluorescent antibody test.

In this report, it was considered that the malignant oedema might be associated with the vaccination. It could not be determined whether it resulted from the multivalent clostridial vaccine or inappropriate hygienic measures. However, the most likely predisposing factor was the wound caused by the needle, which presumably allowed access of microorganisms or its spores to the subcutaneous tissue. The vaccine had been administered intramuscularly. In general, vaccines should be applied subcutaneously according to hygienic rules to avoid possible contaminations. Furthermore the injection site should be a clean part of the sheep such as the lateral thorax or the area behind the ear.

This case report emphasizes the need for maintaining strict hygienic measures together with immunity acquired by proper vaccination in order to prevent clostridial infections.

### References