Outbreak of malignant oedema in sheep caused by *Clostridium sordellii*, predisposed by routine vaccination

J. L. N. COSTA, M. M. D. OLIVEIRA, F. C. F. LOBATO, M. F. SOUZA JÚNIOR, N. E. MARTINS, A. V. A. CARVALHO, E. J. FACURY FILHO, V. L. V. ABREU, R. A. ASSIS, F. A. UZAL

MALIGNANT oedema is considered to be an exogenous infection caused by one or more of the following microorganisms: *Clostridium chauvoei, Clostridium septicum, Clostridium sordellii, Clostridium novyi* type A and *Clostridium perfringens* type A (Sterne and Batty 1975). These microorganisms enter the body through skin or mucous membrane wounds such as those caused by neutering, docking, shearing, bleeding, parturition and vaccination (Smith 1984, Assis and others 2002, Morris and others 2002), and proliferate locally under appropriate conditions (anaerobiosis), producing several exotoxins that act both locally and systemically. This short communication describes an outbreak of malignant oedema in sheep in Pintadas, Bahia, Brazil, caused by *C sordellii* and predisposed by clostridial vaccination.

A flock of 1000 12- to 18-month-old Santa Inês sheep bred under grazing conditions was vaccinated subcutaneously on the neck with a commercial vaccine containing *C sordellii, C chauvoei, C novyi, C septicum, C perfringens* types B, C and D and *Clostridium botulinum* types C and D antigens adsorbed in aluminium hydroxide. The same needle (40 mm x 18 G) that was originally sterile was used to vaccinate all the flock without sterilisation or disinfection between animals. The skin was not disinfected before vaccination. The animals had not been vaccinated against clostridial diseases previously. According to the owner of the animals, 63 sheep died with clinical signs consisting of severe depression, swelling around the vaccination area and lameness, between 24 hours and three days after vaccination.

A veterinarian visited the farm on the third day after 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 three hours after the onset of clinical signs. A sample of subcutaneous exudate from one animal was aseptically collected by needle aspiration. A field postmortem examination was then performed on another animal, and samples of the affected subcutaneous tissue and liver were aseptically collected and submitted, refrigerated, to the Laboratório de Anaeróbios da Escola de Veterinária da UFMG, Belo Horizonte, Minas Gerais, Brazil.

At the laboratory, the samples were inoculated into thioglycollate broth (Dignolab) and on to 5 per cent sheep blood agar plates, and incubated under aerobic and anaerobic conditions at 37°C for 48 hours. Smears prepared from the subcutaneous exudate of both animals and the liver from the animal examined postmortem were stained with Gram stain and also examined by the fluorescent antibody test (FAT) for *C chauvoei, C septicum, C sordellii, C novyi* and *C perfringens,* as described by Assis and others (2001), using conjugates from Pragma. International reference strains of the clostridial species were used as controls for each fluorescent preparation. Additionally, samples of the vaccine used were evaluated for sterility and safety according to previous descriptions (Anon 1993).

The animal that underwent postmortem examination had extensive oedema, emphysema and haemorrhage of the subcutaneous tissue in the area of vaccination and an excess of clear pleural fluid. In the smears of subcutaneous exudate and liver, sporulated (central or subterminal) or non-sporulated, Gram-positive rods, identified as *C sordellii* by the FAT, were observed. The FAT gave negative results when conjugates against other clostridia were used. A pure culture of Gram-positive rods with the same morphology described above was obtained from all the specimens incubated on sheep blood agar plates in anaerobiosis. No growth was observed on the plates incubated aerobically. The colonies were approximately 3 to 7 mm in diameter, with ridged contours, and crenated and tentacular margins. Profuse growth was observed in the thioglycollate broth inoculated with the samples. When subcultured anaerobically on to sheep blood agar, a pure culture of colonies identical to those isolated directly on sheep blood agar in anaerobiosis was obtained. These colonies were identified as *C sordellii* by conventional biochemical reactions (Quinn and others 1994) including lecithinase, lipase, indole and urease production, gelatin and casein hydrolysis and fermentation of glucose, lactose, maltose and sucrose. No bacteria were isolated from the vaccine (sterility control) and the product did not produce clinical or pathological alterations when inoculated into guinea pigs used as controls. Thus, the pathological and microbiological findings confirmed a diagnosis of malignant oedema caused by *C sordellii*.

Disease caused by *C sordellii* in sheep has been associated with enteritis (Al-Mashat and Taylor 1983) and the poorly defined, so-called 'sudden death syndrome' (Lewis and Naylor 1998). *C sordellii* has also been isolated, together with *C novyi*, from a case of malignant oedema associated with blood sampling in a sheep (Morris and others 2002). However, to the authors' knowledge, only one case of malignant oedema produced by *C sordellii* alone has been reported in sheep (Vannelli and others 1996). The condition has been reported in cattle (Williams 1977). In the present case, the most likely predisposing factor for malignant oedema was the wound caused by the needle, which presumably allowed access of *C sordellii*, or its spores, to the subcutaneous tissue. Because the needle used to vaccinate the entire flock was originally sterile, and no microorganisms were isolated from the vaccine used, it is thought that the contaminant microorganism was present in the environment or on the skin of the animals.

In the same region of the country, an outbreak of clostridial myonecrosis caused by *C chauvoei* after caseous lymphadenitis vaccination in sheep has recently been reported (Assis and others 2004), so it is possible that the soil in this area is heavily contaminated with spores of *Clostridia* species. Malignant oedema occurs frequently in areas with soils rich in organic matter and high humidity. These conditions, characteristic of the area where the outbreak occurred (data not shown), appear to favour the survival of spores for many years, creating one of the predisposing factors needed for clinical cases of malignant oedema. In the present case, the lack of an aseptic vaccination technique, coupled with the appropriate environmental conditions for spore survival, created the ideal conditions for the disease outbreak.

This case emphasises the need for maintaining strict hygienic measures during procedures that generate wounds and proper vaccination in order to prevent clostridial infections.

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J. L. N. Costa, DVM, MSc, PhD,
M. M. D. Oliveira, DVM, Universidade Federal da Bahia, Salvador, Brazil
F. C. F. Lobato, DVM, MSc, PhD,
M. F. Souza Júnior, DVM,
N. E. Martins, DVM, MSc,
E. J. Facury Filho, DVM, MSc, PhD,
V. L. V. Abreu, DVM, MSc, PhD,
R. A. Assis, DVM, MSc, PhD,
Laboratório de Anaeróbios da Escola de Veterinária da UFMG, Belo Horizonte, Minas Gerais, Brazil
A.V.A. Carvalho, DVM, MSc,
Ministério da Agricultura, Pecuária e Abastecimento, LARA-MG, Pedro Leopoldo, Minas Gerais, Brazil
F. A. Uzal, DVM, MSc, PhD, DipACVP,
California Animal Health and Food Safety Laboratory System, San Bernardino, 105 West Central Avenue, CA 92408, USA

Correspondence to Dr Uzal

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