An outbreak of malignant edema in cattle

Surto de gangrena gasosa em bovinos

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Summary: This report describes an outbreak of malignant edema in cattle in Itacarambi, Minas Gerais, Brazil, resulting in high mortality. In a herd of 500 Nelore calves of ages varying between 14 and 18 months, 58 animals (11.6%) died 30 days after having been vaccinated against clostridial diseases, rabies and foot and mouth disease. Clostridium septicum was isolated in pure culture and was detected by fluorescent antibody testing in the muscle smears from the vaccination area and in the liver of four necropsied animals.

Resumo: Este relato descreve um surto de gangrena gasosa em bovinos em Itacarambi, Minas Gerais, Brasil, resultando em alta mortalidade. Em um rebanho de 500 bovinos da raça Nelore com idade variando entre 14 a 18 meses, morreram 58 animais (11,6%), 30 dias após terem sido vacinados contra doenças clostridiais, raiva e febre aftosa. Clostridium septicum foi isolado em cultura pura e detectado pela técnica de imunofluorescência direta em impressões de músculo da área de inoculação e fígado dos quatro animais necropsiados.

In several parts of the world, C. septicum is considered to be one of the most common microorganisms responsible for malignant edema in cattle. In Brazil, both C. septicum and C. chauvoei, acting alone or in combination, are the most frequently reported agents of malignant edema (Baldassi et al., 1985). In Minas Gerais, Brazil, 12,291 cases of malignant edema and black leg were recorded between 1990 and 2001 (Solange Olinda, personal communication). However, in spite of the large number of cases of myonecrosis in Brazil, the etiological information about malignant edema is scant (Correa et al., 1980; Baldassi et al., 1985). Most of the diagnosis are based only on clinical and necropsy findings.

We report here an outbreak of malignant edema in cattle in Itacarambi, Minas Gerais, Brazil, related to a previous vaccination against clostridial and other diseases.

In a herd of 500 Nelore calves bred under extensive grazing conditions, 58 animals (11.6%), aged between 14 and 18 months, died with clinical signs suggestive of clostridial myonecrosis. The animals had received one subcutaneous dose of a clostridial vaccine containing C. septicum at 4-6 months of age. Thirty days before the outbreak they received a booster of a vaccine containing C. septicum and they were also vaccinated against rabies and foot and mouth disease. A cypermethrin pour-on was also used on the animals the same day as the vaccination. The vaccines were applied in the cervical area and a new, sterile needle was used after every 40 animals. Prior death the animals showed walking difficulties, depression and recumbency. Most of the animals died 24 hours after the onset of clinical signs, but a few survived up to 3 days.

Four animals were necropsied approximately five
after inoculation, consisting of depression and swelling around the point of inoculation. This area was hot and it showed blue discoloration and a moderate degree of crepitation. At necropsy, severe subcutaneous and muscular edema with gas was observed in the underlying tissues. Large pleomorphic Gram-positive rods sporulated or not were seen in the muscle and liver smears of these animals. Rich, pure cultures of *C. septicum* were obtained on blood-agar inoculated with samples of muscle from the guinea pigs.

No microorganisms were cultured from the vaccines, and they produced no side-effects when they were inoculated into guinea pigs.

The pathological and microbiological findings confirmed a diagnosis of malignant edema. FAT on direct smears is a specific technique, as demonstrated by the results obtained with control strains in this study, and results such as ours are considered sufficient for a definitive diagnosis of malignant edema (Sterne e Batty, 1975).

According to the history of the case, the only wounds that occurred before the outbreak were those of the vaccinations performed 30 days before the outbreak. This is surprising, since usually the incubation period of malignant edema is 1 to 3 days (Smith, 1984). However, we can not rule out other injuries that were not reported in the history of the case and that were not detected during the post-mortem examination.

It is also possible that the oil adjuvant of the foot and mouth disease vaccine provided protection to the clostridial spores. It has been shown that spores of several clostridial species have an increased resistance to the environmental conditions when suspended in oil (Ababouch e Busta, 1987). If this was the case, germination may have occurred when for reasons unknown to us, anaerobic conditions were reached, 30 days after the vaccination. Most vaccine oil adjuvants are tissue irritants (Harwood, 1984) and they generate chronic progressive granulomas at the injection sites. Harwood (1984) reported four separate incidents of apparent iatrogenic clostridial myositis in cattle, following the intramuscular injection of various non antibiotic drugs and reported that the irritant nature of many commonly used preparations will also produce marked local tissue damage.

If the spores were admitted into the tissues by the needle at the time of vaccination, since the needles and syringes were originally sterile, it is likely that the contaminant microorganisms were present in the environment and/or the skin of the animal and that they were admitted into the subcutaneous and muscular tissues during the vaccination procedure. A case of malignant edema in sheep was reported by Morris *et al.* (2002), in which the predisposing factor was routine bleeding and the causing microorganisms were suspected to be in the wool or the environment.

The animals in this study were vaccinated against *C. septicum*; the presence of this microorganism in

Figure 1 – Muscle of the vaccination area, presenting large and irregular dark red areas.

hours after death by a field veterinarian. Samples of muscular tissue from the area of vaccination, liver, heart and lung from the affected animals were aseptically collected and submitted refrigerated to the laboratory of Anaerobe of the Veterinary School (UFMG), Belo Horizonte, Minas Gerais, Brazil. The samples were inoculated onto 5% sheep blood-agar plates and thioglycollate broth (Barcelona, Dignolab, Spain) and incubated under anaerobic conditions at 37 °C for 48 hours. Impression smears of the tissues were obtained and stained by Gram and a fluorescent antibody technique (FAT) as previously described (Assis *at al.*, 2001), using conjugates for *C. septicum*, *C. chauvoei*, *Clostridium sordellii*, *Clostridium novyi* and *Clostridium perfringens*. International reference strains of these clostridia were used as controls for each fluorescent preparation. Additional samples of muscle from the area of vaccination were macerated under sterile conditions and inoculated intramuscularly into guinea pigs as described by Shapton e Board (1971). The animals were observed for 2 days and they were killed and necropsied as soon as they showed clinical symptoms. Samples of muscle and liver of the guinea pigs were obtained and processed as described for the calves. Samples of the vaccines were also submitted to the Laboratory of Animal Support of the Ministry of the Agriculture and Provisioning (LARA), Pedro Leopoldo, Minas Gerais, Brazil for routine sterility and inocuity testing.

At necropsy, all the calves showed large, irregular dark red areas in the muscle of the vaccination area (Fig 1). Subepicardial hemorrhages, congested lungs and severely congested livers were also observed. In smears from the muscle and liver, large pleomorphic, sporulated or not Gram-positive rods, identified as *C. septicum* by the FAT were seen. Each fluorescent antibody preparation stained its corresponding control and none of the others. Similar results were obtained when smears prepared from blood-agar plates inoculated with samples of muscular tissue and liver were processed by Gram staining and FAT. The guinea pigs presented clinical symptoms between 14 and 18 hours
the infection could have been related to a sub-optimal vaccination procedure; an individual lack of immune response; an unusually high challenging dose; and/or the poor quality of the vaccine used. In Brazil only *C. chauvoei* and *Clostridium botulinum* vaccines are systematically controlled for potency by the Ministry of the Agriculture and Provisioning; in Porto Alegre, Rio Grande do Sul and Pedro Leopoldo, Minas Gerais, respectively.

This report stresses the need for maintaining strict hygienic measures during procedures that generate wounds, for careful management of the animals to avoid traumas, and for proper vaccination in order to prevent malignant edema.

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**References**


