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Clostridium sordellii in a Brown Bear (*Ursus arctos*) from Spain

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ABSTRACT: *Clostridium sordellii* is found in the environment and occasionally in animal (including human) intestines and may cause myonecrosis and large outbreaks of enterotoxemia. A few cases of fatal clostridial infection in bears (*Ursus* spp.) have been described worldwide but none attributed to *C. sordellii*. We describe a fatal case of septicemia caused by *C. sordellii* in an illegally trapped brown bear (*Ursus arctos*). At necropsy, acute gangrenous myositis was the primary lesion. Serohemorrhagic edema was observed in the abdominal cavity, thorax, pericardium, and skeletal muscle, mostly affecting femoral, humeral, and scapular muscles. Hemorrhage was observed in the heart, skeletal muscles, stomach, and intestine. Liver, spleen, and kidney appeared with loss of consistency, hemorrhages, and edema. Microscopically, primary lesions were in skeletal muscle, stomach, and small intestine, with gram-positive, clostridial-like bacilli. Biochemical and molecular tests identified *C. sordellii* in cultures from liver, muscle, and intestine. Sequences showed a homology of >99% with the 16S rRNA gene sequence of *C. sordellii*. The severity of effects of the *C. sordellii* infection reveal the importance of this pathogen as a wildlife health risk with conservation concerns, as well as the need to consider possible infection with this pathogen in management actions involving immobilization, stress, or severe muscular activity of wild brown bears.

Key words: Brown bear, *Clostridium sordellii*, Spain, *Ursus arctos*.

Clostridium sordellii, *Clostridium septicum*, *Clostridium perfringens*, *Clostridium chauovei*, and *Clostridium novyi* are gram-positive (G+), spore-forming anaerobic rods that alone, or in combination, are responsible for malignant edema and myonecrosis. These pathogens remain an important cause of animal morbidity and mortality worldwide (Bryant and Stevens, 2010).

Clostridium sordellii is found in the environment and occasionally in animal (including human) intestines. It causes myonecrosis, uterine infections, and sepsis in humans (Abdulla and Yee, 2000), and myonecrosis, large outbreaks of enterotoxemia, sporadic cases of necrotic and hemorrhagic enteritis, and sudden death syndrome in domestic animals (Coleman et al., 1975; Richards, 1982; Al-Mashet, 1983; Lewis and Naylor, 1998; Clark, 2003). This bacterium produces several exotoxins, which contribute to extensive tissue destruction, increase in vascular permeability, edema, and shock (Geny et al., 2007; Carter et al., 2011), progressing so rapidly that death often precedes diagnosis.

There are few described cases of fatal clostridial infection in bears (*Ursus* spp.) worldwide, and none of them attributed to *C. sordellii*. Clostridial myonecrosis was first described in 1979 in a black bear (*Ursus americanus*) in the United States (Barnes and Rogers, 1980). Later, a brown bear (*Ursus arctos*) was found dead in India (Rao et al., 1988). *Clostridium septicum*, *C. chauovei*, and *C. novyi* were isolated from the black bear and *C. perfringens* from the brown bear. In 1998, myopathy and clostridial bacteriemia due to *C. chauvoei* were diagnosed as the cause of death in a brown bear in Somiedo (43°5′34″ N, 6°15′16″W), Asturias, Spain, after the animal was fitted with a radio-tracking collar (Naves et al., 1999). The stress of capture could have triggered the clostridial infection.

We present a description of a fatal case of septicemia in a brown bear caused by

C. sordellii in Cangas del Narcea (43°10′37″N, 6°33′7″W), Asturias, Spain. An adult male brown bear was illegally trapped by the left forelimb for several hours. The animal showed depression, and 20 min after being sedated in order to manage its release from the trap and provide veterinarian assistance, the animal died. Necropsy was performed, and samples for histopathology were taken from lungs, kidney, liver, spleen, heart, gastrointestinal tract, central nervous system, and skeletal muscles (longissimus dorsi, femoral, humoral, scapular, neck, and pectoral). Samples were fixed in 10% neutral-buffered formalin and processed routinely. Semiserial sections (4 μm) were cut and stained with HE, Gram, or Masson's trichrome staining. Samples of liver, small intestine, and skeletal muscle were tested by bacteriologic techniques. After homogenizing 1 g of tissue in 9 mL of saline, 10 μL of suspension was inoculated on to 5% sheep blood agar and McConkey agar (bioMérieux, Madrid, Spain) and incubated at 37 C (±1) for 24–48 hr in aerobic and anaerobic conditions. Colonies resembling *Clostridium* spp. were selected and identified biochemically using the ANC ID card in the VITEK 2 System (bio-Mérieux) and by sequencing the 16S rRNA gene (Lane et al., 1985). The PCR product (480 base pairs) was purified, sequenced, and compared with sequences available in GenBank databases.

External examination during necropsy showed fracture of one distal phalanx of the left forelimb and loss of hair in the metacarpus. Slight subcutaneous edema in the skin down through the trap was observed. Acute gangrenous myositis was the main lesion present. Serohemorrhagic edema was observed in the abdominal cavity, thorax, pericardium, and skeletal muscle, mostly affecting femoral, humeral, and scapular muscles. Hemorrhages were observed in the heart, skeletal muscles, stomach, and intestine. Liver, spleen, and kidney appeared with loss of consistency and with hemorrhages and edema. No lesions were observed in the central nervous system.

Microscopically, the main lesions observed were in skeletal muscle, stomach, and small intestine. Hyperacute myodegeneration consisted of myonecrosis, edema, gas, extravasation of fibrin into the interstitial spaces, and lacunar dissolution of myofibers in skeletal muscles (Fig. 1A, B). Vascular damage, confirmed by trichrome staining, was characterized by loss of endothelial cells and hyaline degeneration, mainly in the arteries. In the heart, subendocardic hemorrhages in both ventricles and diffuse and hyperacute myocarditis were observed. The stomach showed hemorrhagic gastritis with degeneration and lysis of Paneth cells. Hemorrhagic enteritis was observed mainly affecting the small intestine. Gram stain showed G+ clostridial-like bacilli, most numerous in skeletal muscle, heart, stomach, and intestine (Fig. 1C, D).

Growth was observed on plates incubated in anaerobic conditions. Biochemical and molecular tests identified *C. sordellii* in cultures from liver, muscle, and intestine. Sequences obtained showed a homology of >99% with the 16S rRNA gene sequence of *C. sordellii* (GenBank accession No. DQ978216.1).

Clostridium subterminale was also isolated from the intestine and liver and *C. perfringens* from the intestine. Isolation of multiple species of *Clostridium* is not unusual (Barnes and Rogers, 1980), but the presence of *C. sordellii* in muscle and the severity of lesions, not produced by *C. perfringens* or *C. subterminale*, confirm the septicemia and cause of death in this bear.

The importance of *C. sordellii* as a pathogen has increased in recent years (Bryant and Stevens, 2010). It has been described in Spain in foals (*Equus ferus caballus*) and captive lions (*Panthera leo*; de la Fe et al., 2006; Ortega et al., 2007); however, no cases have been reported in free-ranging wildlife species. This bacterium is mainly found in the digestive tract

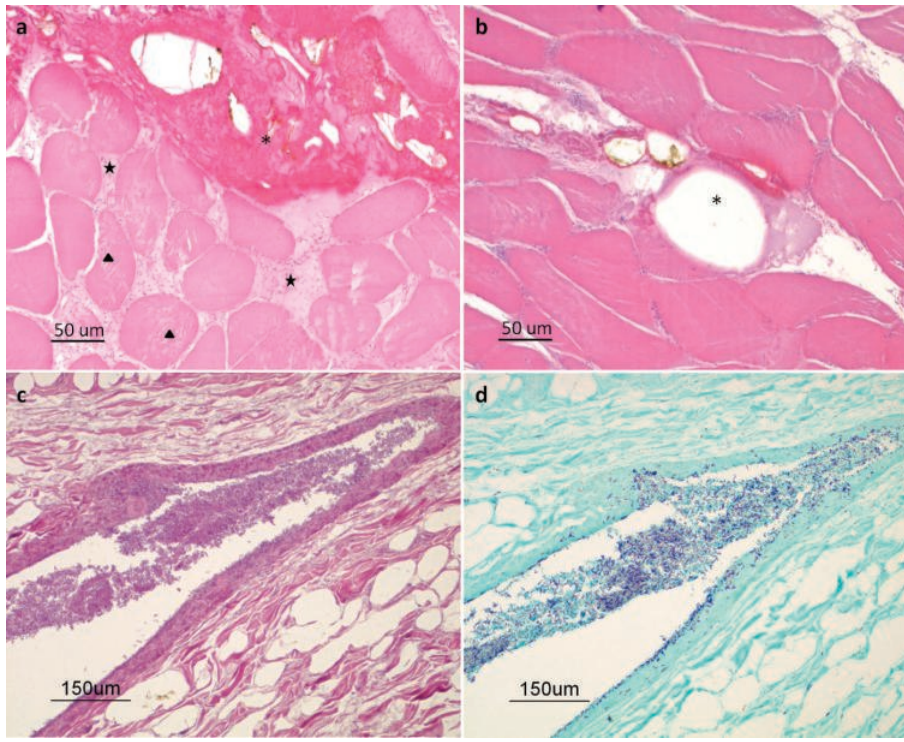

FIGURE 1. Histopathologic features in a brown bear (*Ursus arctos*) affected by *Clostridium sordellii* septicemia. (a) Skeletal muscle. Myodegeneration consisted of hemorrhages (asterisk), myonecrosis (arrowheads), and edema with presence of clostridia (stars). H&E staining. (b) Skeletal muscle. Presence of gas (asterisk) in the endomysium can be observed. H&E staining. (c) Stomach. Blood vessel in submucosa. Thrombus formed by clostridia can be observed in the lumen of the vessel. Endothelial cells are damaged, and presence of bacteria can be observed. H&E staining. (d) Stomach. Same blood vessel in submucosa as shown in (c). Gram staining demonstrates the presence of gram-positive clostridia-like bacilli.

and is eliminated in feces, persisting for long periods in the environment and contaminating soil, food, and water (Cunniffe et al., 1996).

The bear presented here died suddenly, suggesting that infection was acute or hyperacute. Although an exogenous infection reaching tissues by skin or mucosal lacerations (Barnes and Rogers, 1980) cannot be excluded, the presence of this bacterium in the gastrointestinal tract has been considered the most common origin in *C. sordellii* infections (Manteca et al., 2001). Spores can remain latent in the muscle of clinically healthy animals and germinate when predisposing factors such as trauma produce necrosis in an anaerobic environment. Presentation of lesions and the spread and number of bacteria in muscle, stomach, and intestine in this bear strongly suggest that the invasion of muscle would have been endogenously spreading from the gastrointestinal tract, which would have been facilitated by the stress and muscular activity experienced by the animal during capture in the trap. Once an active clostridial nidus is established, quick expansion may follow and progress within hours into profound prostration and systemic lethal toxemia (Carter et al., 2011).

Although a positive demographic trend has been observed in recent years, the Spanish brown bear population is still among the most endangered bear populations worldwide (Palomero et al., 2007). The lack of available data on the wild brown bear health status in Spain highlights the

need for further studies, including those on clostridial infections. The two fatal cases of clostridial infection described in Asturias, Spain, to date, suggest the need to explore if there may be a possible higher susceptibility of the Spanish brown bear population to these bacteria (perhaps related to a weaker immune system due to their lower genetic diversity [Valdiosera et al., 2008; Meli et al., 2010]). The severity of effects triggered by *C. sordellii* reported in the present work reveal the importance of this pathogenic agent as a wildlife health risk with conservation concerns and emphasize the need to consider *C. sordellii* in management actions involving immobilization, stress, or severe muscular activity in wild brown bears.

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