DOI: 10.7589/2016-09-221

*Journal of Wildlife Diseases*, 53(3), 2017, pp. 695–698


# Myocardial Necrosis Associated with *Clostridium novyi* Infection in a Bighorn Sheep (*Ovis canadensis*)

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ABSTRACT: We describe a case of myocardial emphysema and necrosis in a bighorn sheep (*Ovis canadensis*), associated with infection by *Clostridium novyi*, diagnosed through necropsy, histopathology, and fluorescent antibody testing. We documented rapid onset of disease in an apparently healthy wild sheep and discuss our findings in the context of reported clostridial infections in humans, domestic animals, and wildlife.

Domestic animals are susceptible to disease caused by several species of the bacterial genus *Clostridium*. When anaerobic conditions trigger germination of clostridial spores that have previously been ingested, circulated, and deposited in tissues, bacterial proliferation results in tissue damage through toxin production. Although *Clostridium novyi* infection is common in domestic animals, including sheep, cattle, dogs, and pigs (Garcia et al. 2009; Cullen and Stalker 2016), there is only one reported case in wildlife: a single forest reindeer (*Rangifer tarandus fennicus*; Voigt et al. 2009). The only report of clostridial-linked mortality in bighorn sheep (*Ovis canadensis*) was in a group exposed to a water source contaminated with *Clostridium botulinum* toxin (Swift et al. 2000). Here we present a case of myocardial necrosis associated with *C. novyi* infection in a wild, free-ranging bighorn sheep.

In July 2015, an adult female bighorn sheep collared 5 mo earlier for research on psoroptic mange near Penticton, British Columbia, Canada was found dead in sternal recumbency (49°25′48″N, 119°36′48″W). The motion-sensitive global positioning system radio collar (VECTRONIC Aerospace GmbH, Berlin, Germany) indicated normal travel between location fixes prior to collar stasis as of 1936 hours, 18 July 2015. The intact carcass was recovered at 0315 hours, 19 July 2015, and a field necropsy was performed at 1430 hours. Ambient temperature dropped from 27.5 C at time of stasis to 15.2 C overnight, rising to 35.2 C at time of necropsy; daily maxima in July ranged from 26.7 to 36.9 C (Environment Canada 2015). The ewe weighed 73 kg with thick renal fat deposits and no evidence of trauma. There was crusting in the ear canals typical of psoroptic mange, the heart was soft, and the kidneys were blistered and discolored (interpreted as autolytic changes). Heart, lungs, liver, kidneys, and spleen were frozen within 2 hr of necropsy and stored at −20 C before shipping to the Animal Health Centre in Abbotsford, British Columbia, Canada for diagnostic workup.

Tissues were thawed at room temperature at the Animal Health Centre and examined grossly. On cut section, the left ventricular wall had a locally extensive, well-demarcated area of green discoloration with gas pockets and crepitation, which floated in formalin (Fig. 1A). There were no other gross abnormalities.

Organs were fixed in 10% buffered formalin, routinely processed and stained with H&E for histopathology. All organs but skeletal muscle had changes compatible with autolysis. Histopathological lesions were confined to the heart, where foci of cardiomyocyte degeneration, necrosis, and emphysema infiltrated by Gram-positive rod-shaped bacteria were observed (Fig. 1B).

Heart lesions were screened for *C. novyi*, *Clostridium septicum*, *Clostridium sordellii*, and *Clostridium chauvoei* by direct fluorescent antibody (FA). With the use of aseptic technique, heart sections were pressed onto sterilized glass slides to make impression smears. Slides were allowed to air dry, then

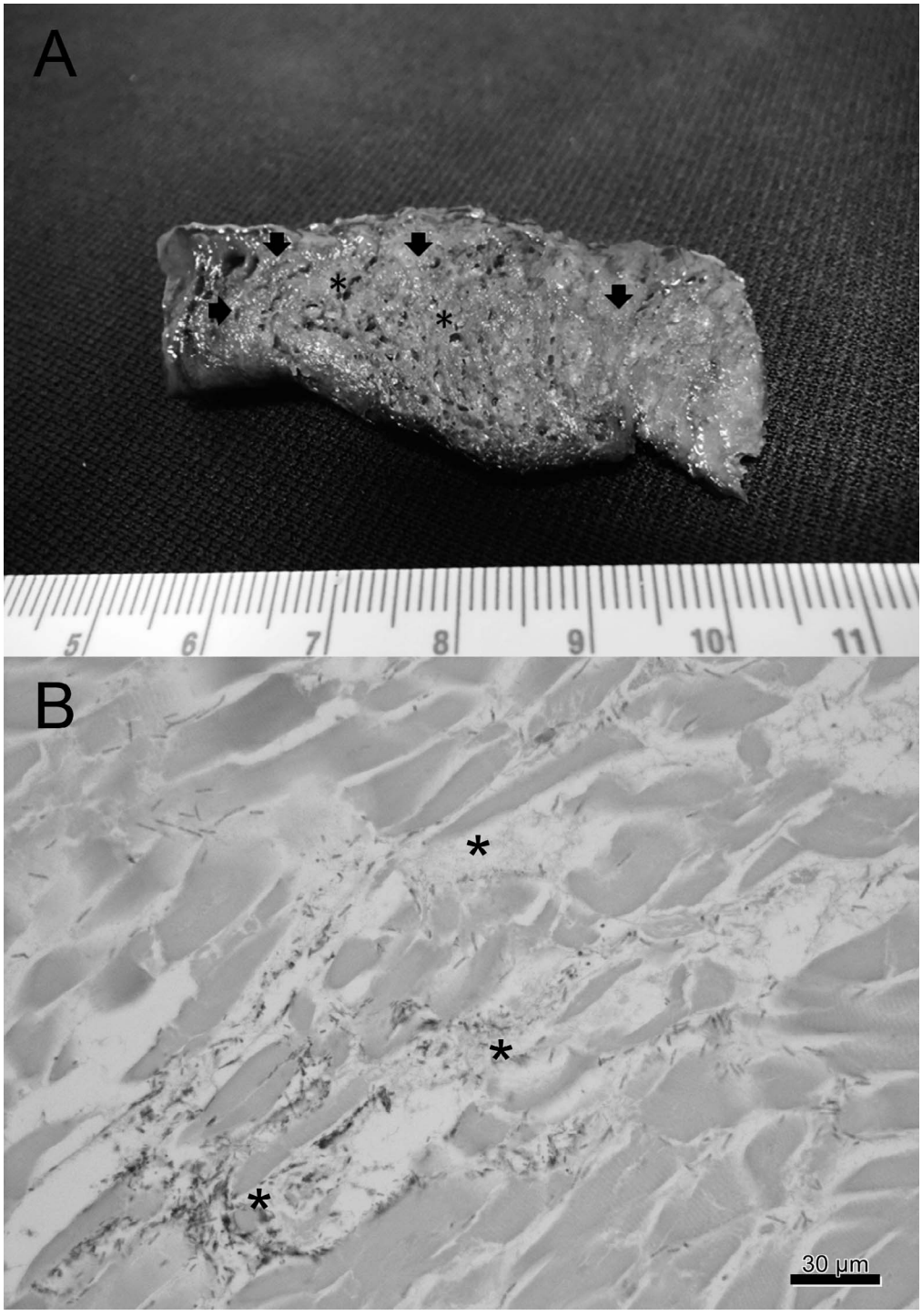


FIGURE 1. Myocardial necrosis associated with *Clostridium novyi* infection in an adult female bighorn sheep, *Ovis canadensis* that died suddenly near Penticton, British Columbia, Canada. (A) Gross examination. Note the sharp demarcation of the lesion (arrows) and areas of gas pocket formation (asterisks). (B) Histopathology. Areas of myocyte degeneration and necrosis (asterisks), gas pocket formations, and rod-shaped bacteria. H&E stain. 400× magnification.

fixed in acetone at room temperature for 20 min and left to dry completely. Direct FA conjugate for each bacterium (VMRD, Pullman, Washington, USA) was applied to the impression smears and to a positive control *Clostridium* spp. four-way FA substrate slide (VMRD) and incubated in a humidity chamber at 37 C for 30 min. Slides were gently rinsed with FA rinse buffer to remove excess conjugate, and soaked in FA rinse buffer for 10 min. Slides were drained, dried, coverslipped, and scanned for brightly fluorescing rods at 100–250× magnification on a fluorescence microscope. Confirmation of a positive smear with moderate numbers of *C. novyi* bacilli was made under 400× magnification; *C. chauvoei*, *C. septicum*, and *C. sordellii* were not detected. *Clostridium perfringens* was isolated from anaerobic culture of heart tissue, and aerobic bacterial culture of blood-filtering organs yielded low numbers of *Streptococcus* sp. and *Enterobacter* sp., consistent with postmortem overgrowth. Myocardial necrosis associated with *C. novyi* infection was diagnosed in the ewe based on gross findings, histopathology, and FA testing.

*Clostridium novyi* lesions in ruminants typically occur in the liver (black disease, caused by *C. novyi* type B), triggered by necrosis and/or inflammation, often the result of trematode migration, which creates an anaerobic environment permissive for clostridial proliferation (Robles et al. 2000; Cullen and Stalker 2016; Uzal et al. 2016). Liver histopathology revealed no changes compatible with clostridial proliferation in this case. Black disease in sheep is associated with rapid death preceded by reluctance to move (Stalker and Hayes 2007; Uzal et al. 2016). Collar transmissions indicated movement of the bighorn sheep ewe until just prior to death, which, in the absence of trauma, is indicative of the rapid onset of disease. This is compatible with the myocardial necrosis we observed and with *C. novyi* detection.

Myocardial necrosis associated with *C. novyi* is uncommon. Of 17 reports of clostridial myocardial infections in humans, all but one involved species other than *C. novyi* (Ma et al. 2007). Cattle occasionally have myocardial lesions with *C. chauvoei* infection (Cooper and Valentine 2016). A cause for spore germination in the current case is unknown. Myocardial infarction can create an anaerobic environment favorable for clostridial proliferation. However, no evidence of acute myocardial infarction or pre-existent disease that might have triggered infarction, such as vegetative endocarditis, thrombosis, or vasculitis, was observed. Acute infarction was considered less likely to cause the lesion because of the lack of thrombosis, although it cannot be completely ruled out. The cardiomyocyte necrosis was acute and interpreted to be related to clostridial toxin production.

The time that elapsed between collar stasis and necropsy suggests carcass autolysis progressed for up to 18 h following death. Autolysis is mediated by proteolytic enzymes released from organs; tissues with high enzyme levels, such as liver, pancreas, and kidney, autolyze quicker than those with low levels, such as muscle and heart. Clostridial disease is associated with advanced autolysis of organs because clostridial toxemia can mimic natural autolysis (Cooper and Valentine 2016). Because heart and muscle autolyze slower than other tissues, autolysis in the heart in this case was likely the result of *C. novyi* proliferation (Stalker and Hayes 2007). This case emphasizes the need to consider domestic animal diseases when evaluating free-ranging animals.

We recognize technical assistance by Jaime Osei-Appiah and Giselle Hughes, and financial support provided by the British Columbia Habitat Conservation Trust Foundation.

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*Submitted for publication 27 September 2016.*
*Accepted 23 February 2017.*