PEER REVIEWED

Blackleg (*Clostridium chauvoei* Infection) in Beef Calves: A Review and Presentation of Two Cases with Uncommon Pathologic Presentations

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Abstract

Blackleg (*Clostridium chauvoei* infection) has long been recognized as a cause of death in calves grazing summer pastures. Clinical signs are not often observed in affected calves due to the peracute nature of the disease, but may include fever, lameness, and swelling and crepitation over large muscle groups, followed by collapse and death. Typical gross and histopathologic lesions in these muscle groups are well-described and include gelatinous, gassy subcutaneous changes, along with well-defined muscle necrosis. Areas of necrosis can also be present in myocardium, diaphragm, and tongue, among other sites. Primary gross lesions consisted of pleuritis and pericarditis in animals examined in this report. More typical blackleg lesions were found in some but not all calves, including necrosis in thigh and abdominal muscles, dark discoloration of diaphragm muscle, and histologic evidence of necrotic myocarditis. Practitioners finding these lesions in animals found dead on pasture should consider *C. chauvoei* infection as the diagnosis regardless of whether muscle necrosis lesions are present.

Keywords: bovine, calves, blackleg, *Clostridium chauvoei* infection

Résumé

Le charbon symptomatique (infection causée par *Clostridium chauvoei*) est reconnu depuis longtemps comme une cause de mortalité chez les veaux au pâturage durant l'été. Les signes cliniques ne sont pas toujours observés chez les veaux atteints en raison de la nature sous-aiguë de la maladie mais peuvent inclure la fièvre, la boiterie de même que l'enflure et le crépitement dans certaines masses musculaires suivis d'un collapsus entraînant la mort. Les lésions macroscopiques et histopathologiques typiques dans ces masses musculaires sont bien connues et incluent des changements sous cutanés gélatineux et gazeux de même que des nécroses musculaires bien démarquées. Des zones de nécrose peuvent aussi être présentes dans le myocarde, le diaphragme et la langue par exemple. Les lésions macroscopiques primaires chez les animaux examinés étaient associées à des pleurésies et à des péricardites. Des lésions de charbon symptomatique plus typiques se retrouvaient chez certains mais pas chez tous les veaux atteints et incluaient une nécrose dans les muscles de la cuisse et de l'abdomen, une décoloration foncée du muscle du diaphragme et des indices histologiques de myocardite nécrotique. Les praticiens qui trouvent ces lésions chez des animaux morts au pâturage devraient considérer l'infection par *C. chauvoei* dans leur diagnostic peu importe si des lésions musculaires nécrotiques sont présentes ou non.

Introduction

It is often difficult to confirm the cause of death when calves die on summer pasture. Remote, expansive pasture locations, high environmental temperatures during the summer months, and predators often contribute to conditions that preclude accurate and timely diagnoses of these cases. Common differential diagnoses of calf death loss on pastures in the northern plains and elsewhere include blackleg (*Clostridium chauvoei* infection), lead poisoning, lightning strike, anthrax, respiratory disease and perforated abomasal ulcers, to name several. In many cases, postmortem decomposition complicates an accurate diagnosis.

Blackleg is considered one of the oldest and most recognized causes of death in calves.[7] A review of the common veterinary references and current literature regarding this disease is presented, along with a description of two cases of blackleg which had pathologic presentations not readily mentioned in most common reference texts.

Review of *C. chauvoei* Infection (Blackleg)

Occurrence of blackleg

Blackleg is primarily a disease of pastured cattle, although sheep may also be affected. It preferentially affects animals under two years of age, with most cases occurring in cattle from four to 24 months of age.[8] Blackleg tends to affect animals in good condition that exhibit rapid growth rates.[8] A seasonal pattern exists, with most cases occurring during summer months and periods of hot weather. It is often associated with moist conditions and periods of rapid pasture forage growth.[8,14]

Occurrence of the disease is worldwide, although it tends to be localized, even to certain farms or to certain pastures. Because of this localization, it is assumed that *C. chauvoei* is soil borne, but likely does not grow in soil. The bacteria grow readily in the intestinal tract of cattle, and may be recycled through fecal contamination of the soil. Once exposed to the environment, *C. chauvoei* readily forms spores, which may survive for long periods (many years) in the soil.[14]

The pathogenesis of blackleg is still not completely understood, but certain aspects of disease progression have been confirmed. Cattle are exposed and become infected with *C. chauvoei* through ingestion of spores from pasture.[9] Spores, either those directly ingested or those formed after germinating in the gut, are then carried across the intestinal mucosa. There they are taken up by macrophages that distribute the spores throughout the body. *C. chauvoei* spores can be found in many tissues throughout the body of normal animals, including muscle, where they are stored for long periods of time in phagocytic cells.[3,14]

Activation of latent spores resulting in infection and disease is an area of conjecture, but evidence suggests that conditions resulting in reduced local oxygen content or muscle damage may enable the spores to germinate. These conditions could include muscle bruising, such as with transport, handling, intramuscular injections, or strenuous exercise.[14] Stress, with resultant increases in cortisol levels and subsequent release of catecholamines, was considered to induce sufficient physiological change in heart muscle to cause myonecrosis in lambs.[3] After germination and proliferation, *C. chauvoei* releases several toxins and enzymes that result in severe local muscle damage and systemic organ dysfunction,[10] ultimately resulting in very rapid progression of clinical signs and death.[12]

Clinical presentation

Clinical signs of blackleg have been well-documented, but due to the sudden onset and often peracute nature of the disease, are not often observed in field cases.[2,6,11] The course of clinical disease is rapid, and most animals die within 12 to 36 hours of the onset of clinical signs.[2,8] Many cases are found dead with no prior clinical signs.[9] When a limb is involved, as is commonly the case, acute lameness and pronounced muscle swelling in the upper limb may be noted. However, if lesions are deep within the muscle group, swelling may not be evident externally.[2,6,11] In some cases, the swelling may be present in the back, brisket, neck, or elsewhere.[2,6] Regardless of the site, the swollen area is initially hot and painful, but becomes cold, insensitive, and crepitant as the condition progresses.[2,6,8,11] Edema and emphysema are commonly present in the tissue, and the overlying skin may become dry and cracked.[2,11] Regional lymphadenopathy may be observed.[11]

Other signs observed in affected animals include depression, complete anorexia, rumen stasis, tachycardia, and tachypnea.[2,4,6,8,10,11] Fever (104-106°F or 40-41.1°C) is present initially, but body temperature quickly drops to normal or subnormal levels as other signs progress.[6] Terminally, signs of ataxia, tremors, and dyspnea quickly progress to recumbency, coma, and death.[8]

Pathology

Lesions resulting from *C. chauvoei* infection are typically within the larger muscle groups of the limbs. The affected area is dark red, within which small areas of necrosis may be observed.[2,6,8,10,11] The muscle is typically dry and spongy, with small gas bubbles separating muscle bundles, with little edema centrally.[6,8,11] Around the periphery of this central lesion, the muscle is also dark red, but much moister, filled with serosanguineous fluid.[8,10,11] The subcutaneous tissues in the area are frequently engorged with serosanguineous fluid and gas bubbles as well.[2,8] When incised, affected muscle may have a characteristic odor, variably described as "sweetish,"[6] "rancid,"[2,11] or similar to that of rancid butter.[8] Lymph nodes in the area will be enlarged.[11]

Less commonly, the lesion(s) associated with blackleg will not be present in the large limb muscles, but found in areas such as the base of the tongue,[2,5,8,11] myocardium,[2,3,6] diaphragm,[2,5,6,8] udder,[2,11] the musculature around the vulva,[8] masseter muscles, intracostal muscles, and ventral neck.[5] In locations such as the tongue, myocardium, and diaphragm, the lesion may be small and escape detection during postmortem examination, but still producing enough bacterial toxins to be lethal to the animal.[2]

Myocarditis, with or without concurrent lesions in skeletal muscle, has been reported in calves[13] and lambs.[3] Such lesions consist of dark red areas of necrotic tissue, sometimes extending through the full thickness of the atrial and ventricular walls.[13] Heart muscle may be friable, degenerated, and feature endocardial hemorrhages.[8] Fibrinous epicarditis has been reported in affected lambs.[3] Pulmonary edema, congestion, and

hemorrhage may also be found in the lungs.[8] In addition, there has been one report of meningitis caused by *C. chauvoei* in a calf.[5]

Although blood clots rapidly in animals that have died due to blackleg,[2,11] blood-stained froth or fluid is observed to discharge from body orifices, such as the nose and anus.[2,8] Carcasses are often found bloated, and degeneration of internal organs progresses rapidly, usually more quickly than normal postmortem autolysis due to the action of clostridial exotoxins on the body.[8] References disagree on the usual pathology of the spleen and liver in blackleg cases. Both may be of normal size but may be distended with gas,[11] or the spleen may be enlarged and friable, with the liver pale and friable as well.[8]

Pericarditis was presented as a distinct feature of a set of 29 blackleg cases examined in the 1980s.[5] Of those cases, 21% had gross lesions that consisted only of pericarditis. The lesions consisted of a 1-5 mm thick layer of fibrinous exudates external to the pericardial capsule. An additional 28% of cases had pericarditis in conjunction with skeletal muscle lesions. The investigators concluded that a significant percentage of cases could be overlooked if clostridial myositis is considered to be the feature lesion.[5] Despite these reports, only one[8] out of four popular food animal clinical reference texts[2,6,8,11] mentioned fibrinohemorrhagic pleuritis, but not pericarditis, as a common gross lesion in blackleg cases. Excess fluid in the chest and abdominal cavity with varying amounts of fibrin was listed by one reference.[2] Peritonitis was not mentioned in the lists of possible lesions caused by blackleg in any of the references reviewed.

Diagnosis

Initial suspicion of blackleg as the cause of death in an animal is often the result of the history and postmortem lesions, along with clinical signs if observed.[1] A history of rapid onset of signs or death in young, well-nourished cattle indicates that blackleg should be included on the differential diagnosis list.[6] Often, characteristic lesions described above are present and highly suggestive of blackleg.

C. chauvoei is a facultative, anaerobic gram-positive coccus. Anaerobic culture of sections of affected muscle or fluid from the lesion may be employed to identify the causative organism. The bacterium, however, is quite fastidious in its growth requirements. Also, affected tissue is often rapidly overgrown with other clostridial contaminants from the gastrointestinal tract, further complicating culture of the causative organism.[2] *C. septicum* may be isolated from the lesion along with *C. chauvoei*.[6] Physically and biochemically, *C. chauvoei* is very similar to *C. septicum*, further confusing diagnostic efforts.[4]

Fluorescent antibody (FA) testing is a rapid, reliable method of detecting *C. chauvoei* in affected tissue; it is recognized as the diagnostic method of choice.[1,4,6,8,8,11] Organisms do not have to be viable to be identified when using this method. The specimen of choice is affected muscle tissue, which should be chilled when shipped to the diagnostic laboratory. Polymerase chain reaction (PCR) techniques have also been employed to identify *C. chauvoei*.[4]

Case Reports

Case 1

A southeastern South Dakota cattle herd consisting of approximately 60 cow-calf pairs was dispersed into four pastures early in the summer of 2008. No vaccinations were given to the calves prior to turnout. Several weeks after turnout, calf deaths occurred on one of the pastures, which contained 30 cow-calf pairs. A total of six calves, three to four months of age, and one two-week-old calf, had died by August 01. Because of time constraints and warm ambient temperatures, the producer and veterinarian were unable to confirm a diagnosis, but enterotoxemia was suspected in one of the cases. Accordingly, calves in this pasture were gathered and given a 7-way clostridial bacterin-toxoid on August 01.

Three days later, another calf was found moribund on the same pasture and submitted to the South Dakota State University (SDSU) Animal Disease Research and Diagnostic Laboratory (ADRDL) for necropsy and diagnostic workup. Gross examination of this calf revealed severe fibrinous pleuritis, pericarditis, and epicarditis. Other than pericarditis and epicarditis, no other gross lesions in the heart muscle were noted. Pleural adhesions were extensive, and adhesions from the diaphragm to the reticulum were present. These lesions were suggestive of traumatic reticulitis, but no foreign objects were retrieved. No lesions were noted elsewhere, including diaphragm and skeletal muscles.

Histopathologic examination of tissues from this calf revealed severe suppurative and necrotizing pneumonia and suppurative and fibrinous pleuritis. Heart muscle showed severe suppurative and necrotizing myocarditis with extensive necrosis. In addition, multifocal areas of suppurative inflammation were noted in the thymus, and mild colitis was observed. There were areas of centrilobular necrosis in the liver. Fecal floatation procedures showed evidence of coccidia, strongyle, and *Nematodirus* spp infection. Fluorescent antibody procedures were negative for bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis virus (IBRV), and bovine respiratory syncytial virus (BRSV); virus isolation procedures did not recover viruses from lung, spleen, or kidney. Aerobic cultures did not yield

pathogenic bacteria from lung, heart, liver, intestine, or brain.

Another calf died on the same pasture the day the first calf was taken to the ADRDL. This calf exhibited rapid onset of dyspnea and died shortly thereafter. The referring veterinarian performed a necropsy on the calf, and reported the same gross lesions as in the calf submitted to the ADRDL: suppurative and fibrinous pleuritis, pericarditis, and epicarditis. Tissues, including lung, liver, kidney, heart, and spleen, were submitted to the ADRDL. Histopathologic examination of these tissues revealed fibrinopurulent pleuritis and atelectasis in the lung, and fibrinopurulent epicarditis and patchy areas of myocarditis with necrosis in the heart. Pathogenic bacteria were not recovered from aerobic culture of lung, heart, intestine, cecum or lymph nodes. Fluorescent antibody tests were negative for BVDV, IBRV, and BRSV, and virus isolation procedures did not yield virus from lung, kidney, or spleen. Liver chemistry analysis was unremarkable, and mycoplasma culture was also negative. Based on the findings of pleuritis and pericarditis in the two calves, the remaining calves in this pasture were vaccinated with an intranasal IBR – PI3 vaccine,[a] and treated with tulathromycin[b] (1.14 mg/lb or 2.5 mg/kg subcutaneously, once). No further death losses occurred on this pasture.

Three weeks later, a calf housed on another pasture was found dead, and was necropsied by the attending veterinarian. In this calf, there were no pleuritis or pericarditis lesions, although hemorrhage was present on the epicardial surface. The veterinarian also found one small, golf-ball-sized area of muscle necrosis and hemorrhage in the right caudal thigh muscle. Tissues from this calf were submitted to the ADRDL for diagnostic workup.

Laboratory examination of the muscle tissue revealed microscopic lesions of focally extensive necrotizing myositis. No pathogens were isolated from muscle and heart samples with anaerobic culture; the FA test for *C. chauvoei* was negative as well.

Despite the negative culture and FA test, the muscle lesions were strongly suggestive of blackleg, and the previous case that featured pleuritis and pericarditis lesions was reopened. Heart muscle was recultured anaerobically, and a pool of lung and heart tissues were tested for *C. chauvoei* utilizing FA. Both the culture and FA test were positive, confirming the presence of *C. chauvoei*.

On the day following the most recent submission, a calf was found dead in a third pasture where there had been no prior losses. Gross necropsy findings included severe necrosis of the caudal thigh and caudal abdominal muscles with gas formation. Lungs and pleural cavity were grossly normal. The heart had extensive superficial hemorrhage, but no indication of pericarditis. Tissues were submitted to the ADRDL and *C. chauvoei* was cultured anaerobically from muscle, and FA tests were positive for *C. chauvoei*. Histopathologic examination of skeletal muscle specimens revealed extensive necrotizing myositis with hemorrhage, edema, and emphysema. Mild sub-epicardial hemorrhage was present in the heart, but no other lesions were present in the submitted specimens.

In total, the owner reported that approximately 30 calves from the 60 cow-calf pairs that went to summer pasture had died. Except for the cases previously described, none of the remaining dead calves were submitted for necropsy examination. The cattle owner indicated that cattle using those pastures had not been vaccinated for blackleg for at least the past 20 years. He recalled that his grandfather had experienced losses due to blackleg prior to that, but blackleg vaccination was discontinued after perceived adverse vaccine reactions.

Case 2

A cattle producer in eastern South Dakota reported that four calves from a group of 30 cow-calf pairs on summer pasture had died. A diagnosis was not made on the first three calves, but the fourth calf, found dead on pasture on October 17, 2008, was submitted to the SDSU ADRDL for diagnostic evaluation.

Gross necropsy examination revealed that the calf, a four-month-old crossbred, was in good condition. Fibrinous pleuritis was present throughout the thoracic cavity (Figure 1). No abnormalities in skeletal muscles were observed; however, the ventral portion of the diaphragm was dark in appearance. Peritonitis was present

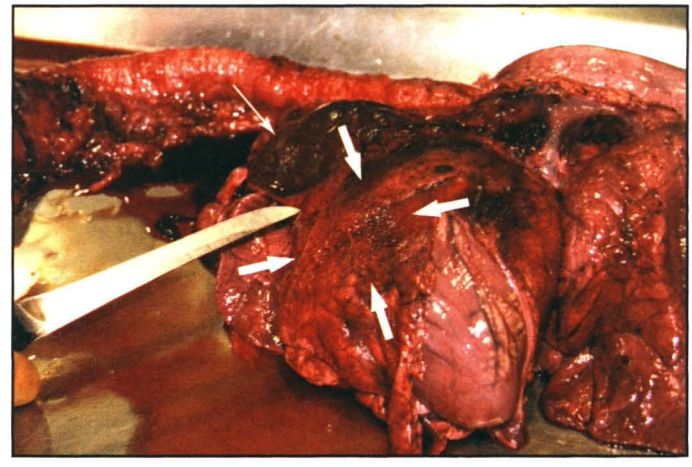

Figure 1. Heart and lung from a case of blackleg in a four-month-old crossbred heifer calf. Pleuritis is evident on the pleural surface of the pericardial mediastinum (larger arrows). The thymus is congested (thin arrow).

in areas of the abdomen adjacent to this area (Figure 2). There was congestion in the thymus, and the spleen was moderately enlarged.

Histopathologic examination of the diaphragm revealed severe, diffuse necrotizing myositis. In addition, there was pleuritis and mild interstitial pneumonia, which was not grossly visible. The spleen had mild, multifocal necrosis in the parenchyma, and capsulitis was present. Mild multifocal hemorrhage and mild capsulitis were present in the thymus. No pathogenic bacteria were cultured (aerobic) from lung, pericardium, thymus, spleen, and diaphragm muscle specimens. *Clostridium chauvoei* was isolated from diaphragm muscle using anaerobic culture techniques; a FA test on the diaphragm was positive for *C. chauvoei*, confirming a diagnosis of blackleg.

Following diagnosis of blackleg, the producer vaccinated the calves with a 7-way clostridial bacterin-toxoid and moved the cattle to a different pasture. No further losses were reported.

Discussion

The pleuritis, pericarditis, and peritonitis noted in some of these cases were unusual in our experience, and not as widely reported as the classic muscular lesions. Only one of the calves in Case 2 had any gross evidence of muscular involvement (dark appearance in the diaphragm), and myocardial lesions were only detectable with histopathology. The myocarditis and subsequent necrosis are believed to give rise to the pericarditis lesions observed in these cases, due primarily to the inflammatory response to the local muscle damage.[5] Fibrinous pleuritis, often hemorrhagic in nature, may then become extensive on the parietal and pulmonary pleural surfaces. The lung tissue itself is not usually affected except for congestion and pulmonary edema.[14] Myositis in the diaphragm muscle may likewise contribute to the development of peritonitis in the adjacent area of the abdomen, as was grossly evident in one of the calves examined in Case 2.

None of the calves in these affected herds had been vaccinated prior to disease onset. Vaccination of calves with 7-way clostridial bacterin-toxoid has proven effective for prevention of clinical illness and death due to blackleg; in many reports there has been good control when only one dose has been administered.[8,11] These cases represented an unusually high incidence of blackleg relative to normal years in these areas of the state. Moist pasture conditions in these areas may have contributed to the increased incidence of blackleg on these two beef cattle operations.

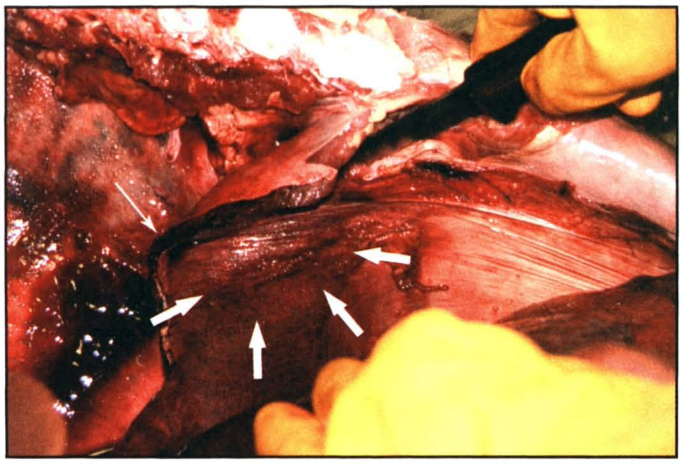

Figure 2. Abdominal surface of the diaphragm from a case of blackleg in a four-month-old crossbred heifer calf. The diaphragm appears darker than normal (thin arrow), and peritonitis is evident adjacent to the affected muscle (larger arrows).

Conclusions

These cases had blackleg lesions not commonly considered by practitioners or pathologists, and not consistently reported in common reference textbooks. Practitioners finding pleuritis and pericarditis lesions at necropsy in the absence of actual lung pathology, and in calves found dead on pasture, should consider blackleg as part of a differential diagnosis. Areas of peritonitis adjacent to the diaphragm suggest that blackleg lesions may be present in the diaphragm muscle. Vaccination against blackleg has proven effective for preventing these losses.

Endnotes

[a] TSV-2®, Pfizer Animal Health, New York, NY
[b] Draxxin, Pfizer Animal Health, New York, NY

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