PEER REVIEWED

Myositis due to *Mannheimia haemolytica* Infection in a Beef Heifer

Catherine G. Lamm[1], *DVM, DACVP;* **D. L. Step**[2], *DVM, DACVIM;* **Clint R. Krehbiel**[3], *PhD;*
Brenda C. Love[1], *DVM, PhD, DACVM*
[1]*Oklahoma Animal Disease Diagnostic Laboratory*
[2]*Department of Veterinary Clinical Sciences*
[3]*Department of Animal Science, Oklahoma State University, Stillwater, OK 74078*
Corresponding author: Catherine G. Lamm, Oklahoma State University, Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, OK 74078

Abstract

A 10-month-old, 447 lb (203 kg), mixed-breed beef heifer was presented with an elevated rectal temperature, weight loss, and clinical signs suggestive of respiratory disease. This heifer was from a group of 360 mixed-breed, high-risk heifers purchased and transported to the research feedlot at the Oklahoma State University Willard Sparks Beef Research Center. Despite extensive consecutive treatment with antimicrobials, the heifer continued to have an elevated rectal temperature and weight loss. The heifer died 22 days after arrival at the feedlot. Necropsy examination revealed an unusual localized necrotizing myositis that concentrated along the musculature of the ventral cervical area and into the adjacent right axilla. Severe bronchopneumonia, confirmed grossly and histologically, was the presumed cause of death. A pure culture of *Mannheimia haemolytica* was cultured from the skeletal muscle and the lung. The strain of *M. haemolytica* isolated from the lung and muscle was resistant to oxytetracycline and tilmicosin, and susceptible to ceftiofur and enrofloxacin by antimicrobial sensitivity testing. This infection was likely due to septicemia following severe fibrinous bronchopneumonia and pleuritis caused by *M. haemolytica*.

Keywords: bovine, antibiotics, cattle, *Mannheimia haemolytica*, myositis, pneumonia

Résumé

Une taure de boucherie de 10 mois de race mélangée et pesant 447 lb (203 kg) a été présentée avec une température rectale élevée, une perte de poids et des signes cliniques compatibles avec une maladie respiratoire. Cette taure faisait partie d'un groupe de 360 taures de race mélangée à haut risque achetées et transportées au parc d'engraissement du Willard Sparks Research Center de l'Oklahoma State University. En dépit de traitements répétés avec des antimicrobiens, la taure avait toujours une température rectale élevée et perdait du poids. La taure est morte 22 jours suivant son arrivée au parc d'engraissement. La nécropsie a révélé une myosite nécrosante localisée inhabituelle qui se concentrait tout au long des muscles de l'aire cervicale ventrale et dans l'aisselle droite adjacente. Une broncho-pneumonie sévère, confirmée par examen macroscopique et histologique, était la cause présumée de la mort. Une culture pure de *Mannheimia haemolytica* a été isolée des muscles squelettiques et du poumon. La souche de *M. haemolytica* isolée du poumon et du muscle était résistante à l'oxytétracycline, à la tilmicosine, et susceptible au ceftiofur et à l'enrofloxacine suite à des tests de résistance aux antimicrobiens. Cette infection était probablement causée par la septicémie suivie d'une broncho-pneumonie sévère et d'une pleurite causée par *M. haemolytica.*

Introduction

Bovine respiratory disease (BRD) is one of the leading causes of morbidity and mortality in feedlot cattle, resulting in severe economic losses.[7] Bacterial bronchopneumonia is a significant contributing factor in BRD.[7] Common bacterial causes of bronchopneumonia include *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*. Bacterial infection is often predisposed by stress and/or viral infection, resulting in severe fibrinous bronchopneumonia with pleuritis.[2,5,8,10] Infection with *M. haemolytica* has been rarely reported in systems other than the respiratory tract.[4,7,9,14,18] This case report documents infection with *M. haemolytica* within the muscle of a beef heifer diagnosed with *M. haemolytica* bronchopneumonia and pleuritis.

History

A group of 360 mixed-breed heifers was purchased over a four-day period and transported to the Oklahoma

State University Willard Sparks Beef Research Center (WSBRC) on two different shipment dates. The heifers were from multiple auction-market sources in the southeastern United States, categorized as high-risk for BRD, and had unknown medical histories. Upon arrival at the WSBRC, average weight of the heifers was 515 lb (234 kg).

Clinical Findings and Therapeutic Management

No clinical signs consistent with BRD were observed in any of the heifers during unloading. Several heifers, however, had blepharospasm and lacrimal discharge. All heifers were unloaded from the trucks, calmly moved into a dry pen, and allowed to rest for two to four hours before each animal was individually weighed and identified with a numbered ear tag. Upon closer examination during the weighing process, this particular heifer, as well as several others, had corneal edema and conjunctivitis consistent with infectious bovine keratoconjunctivitis (IBK).[12] Heifers diagnosed with IBK were administered long-acting oxytetracycline[a] subcutaneously at 9 mg/lb (20 mg/kg) body weight. The clinical signs of IBK in all affected heifers resolved following one dose of this treatment.

After heifers were individually weighed and ear tagged, they were allowed to rest and acclimate to new surroundings before being administered their initial health processing protocol. Initial processing occurred within 36 hours of arrival. During the time period before processing, heifers were fed good quality long-stem grass hay and water *ad libitum* from an automatic watering device. At initial processing (day 0 after arrival), calves were vaccinated with a combination modified-live viral (MLV) vaccine[b] containing bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV) types 1 and 2, parainfluenza type 3 virus (PI3V) and bovine respiratory syncytial virus (BRSV). In addition, a 7-way clostridial bacterin/toxoid,[c] pour-on formulation of moxidectin,[d] and a trenbolone acetate-estrogen combination growth-promoting implant[e] were administered. After processing, heifers were housed in a 40 x 100 feet (12.2 x 30.5 m) dry-lot open-air pen with automatic watering devices shared between two adjacent pens. Some 24 to 30 heifers were randomly assigned to each pen. The feed ration consisted of 45% corn, 15% distiller's grain, 35% hay, and 5% supplement, and met 1996 National Research Council requirements.[11]

Each animal was subjectively evaluated for abnormal clinical signs consistent with undifferentiated BRD. Heifers exhibiting abnormal signs were assigned a severity score of 1 to 4 (1 = mild; 2 = moderate; 3 = severe; 4 = moribund). The day after initial processing (day 1), the heifer in this report was mildly depressed (clinical severity score of 1), and was removed from her home pen for further objective evaluation. At that time, the heifer had a rectal temperature of 106.8°F (41.5°C). Because of the mild clinical signs of BRD and fever, the heifer was treated with tilmicosin[f] at 4.54 mg/lb (10 mg/kg) of body weight subcutaneously with a five-day post-treatment interval prior to retreatment.

Although the heifer appeared to respond initially, on day 6 after arrival she again showed mild depression (clinical severity score of 1) and had a rectal temperature of 107.3°F (41.8°C). Enrofloxacin[g] was administered at 4.5 mg/lb (9.9 mg/kg) body weight subcutaneously, utilizing a two-day post-treatment interval.

The heifer appeared to respond clinically for three days following retreatment. On day 9 following arrival, the heifer was moderately depressed (clinical severity score of 2) and had a rectal temperature of 105.8°F (41.0°C). Ceftiofur HCl[h] was administered subcutaneously (1.0 mg/lb; 2.2 mg/kg body weight), and repeated in approximately 48 hours. The fever (106.0°F [41.1°C]) persisted and the heifer was found dead in the pen on day 22.

Laboratory Findings

A complete postmortem examination was performed. Reflection of the right forelimb revealed a locally extensive area in which the skeletal muscles and connective tissue of the ventral cervical area and right axilla (including areas of the pectoral, latissimus dorsi, and brachiocephalicus muscles) were markedly expanded by edema and fibrin. The muscle within this area was mottled tan, red, and white. There was no extension of the affected area into the adjacent joints or thorax. Changes in the muscle were consistent with severe, acute, locally extensive myositis. Regional lymph nodes were diffusely enlarged and edematous. In addition to the myositis, over 90% of the lung lobes were dark red, firm, and the pleural surface was covered with a thick layer of fibrin. Numerous abscesses ranging in size from 0.9 to 2.7 inches (2 to 6 cm) were scattered throughout the affected areas of the lung. Changes within the lung were consistent with severe, subacute fibrinous bronchopneumonia, and the lesions were considered the cause of death. All other organ systems examined, including the joints, were grossly within normal limits.

Representative samples were fixed by immersion in 10% buffered formalin, routinely processed for paraffin embedding, and stained with hematoxylin and eosin. Histologically, the architecture of the affected portion of the muscle was diffusely replaced by coalescing areas of liquifactive necrosis, inflammatory cells, and fibrin with rare remnant myofibers. The areas of necrosis were characterized by lakes of eosinophilic amorphous debris surrounded by a rim of nuclear debris admixed with degenerate neutrophils. These areas were surrounded by

loosely associated plump reactive fibroblasts, aggregates of fibrin, neutrophils, lymphocytes, and plasma cells. Histologic changes were consistent with severe, acute, locally extensive necrosuppurative myositis, confirming the gross findings.

Fresh samples of affected skeletal muscle were obtained before opening of the thoracic or abdominal cavities. A sample of affected lung was also collected, and both muscle and lung were cultured for aerobic bacteria. Muscle and lung tissue were inoculated onto separate blood agar plates[i] containing 5% citrated sheep blood[j] and on Tergitol-7 Agar plates.[k] The plates were incubated overnight at 98.6°F (37°C) in 5% CO_2. Small, hemolytic colonies grew from both tissues and were identified as *Mannheimia haemolytica* based on Gram's stain reaction (small, gram-negative coccobacilli), and biochemical reactions (catalase positive; oxidase positive; produces acid on triple sugar iron [TSI]; indole negative; citrate negative; urease negative; methyl red negative; Voges-Proskauer negative; lysine decarboxylase negative and nitrate positive). The isolate was serotyped as *M. haemolytica* serotype 1. Antimicrobial sensitivity testing was performed on the BOPO6F (Trek Diagnostics, Bovine, Porcine, 6F) panel using the Sensititre™ semi-automated system.[l] The isolate was susceptible to ampicillin, ceftiofur, chlortetracycline, enrofloxacin, and trimethoprim/sulfonamide. It was resistant to clindamycin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, spectinomycin, sulfadimethoxine, and tilmicosin.

Fluorescent antibody testing for *Clostridium chauvoei*, *C. novyi*, *C. sordelli*, and *C. septicum* in sections of affected muscle was negative. *Mycoplasma* spp enrichment cultures of the lung were negative. Fresh sections of lung were negative for BVDV, BRSV, BHV-1 and PI3V with fluorescent antibody testing.

Discussion

M. haemolytica (biotype A) is commonly isolated in cases of BRD and is associated with the "shipping fever" complex. *M. haemolytica* infection is often predisposed by stress and/or viral infection and results in severe fibrinous bronchopneumonia with pleuritis.[2,5,8,10] *M. haemolytica* infection has also been sporadically reported in cases of otitis, mastitis, sepsis, and abortion.[4,7,9,14,18]

M. haemolytica can be transmitted via aerosol with resulting colonization of the upper airways. Under certain conditions, such as periods of stress or immune suppression, the bacteria can replicate within the airways and spread into the alveoli, inciting severe fibrinosuppurative bronchopneumonia.[6,9,19] The pneumonic lesions are attributed in part to localized release of bacterial virulence factors, including leukotoxin and lipopolysaccharide.[2,19] Typically, *M. haemolytica* infection remains restricted to the respiratory system.

In this case, the *M. haemolytica* infection appears to have spread to the muscle, inciting a necrosuppurative myositis. However, the route of the muscle infection could not be determined. Given the location of the lesion within the ventral cervical area and axilla, it is possible that the bacteria could have spread directly from the thoracic cavity into the surrounding tissues. Extensive dissection of the muscle did not reveal a grossly evident tract between the thorax or the scapulohumeral joint and the affected regions of muscle. Another possible route of infection could be through direct inoculation via injection. However, the anatomic distribution of the lesions was not within an area typically used for injections at this facility. It is also possible that the myositis was secondary to septicemia. Given the chronicity and severity of the pneumonia compared to the acute nature of the myositis, it is probable that the pulmonary lesions developed first. The pulmonary infection could have progressed into a peracute septicemia with development of infection at distant sites (i.e., the muscle). Infection in visceral organs or joints was not grossly evident. Unfortunately, blood cultures were not performed, thus bacteremia could not be diagnosed if present.

The most common cause of bacterial myositis in cattle is related to *Clostridium* spp infection.[3,20] However, localized abscesses due to *Arcanobacterium pyogenes* and sporadic infections, such as coliform and *Histophilus somni* myositis, have also been reported.[13,15] Typically, muscular infection with *Clostridium* spp results in blackleg or malignant edema, depending on the organism isolated and the gross appearance.[13] Manifestations of these infections typically follow a traumatic event, such as bruising, in which the embedded organisms within the muscle are exposed to an anaerobic environment. Grossly, the affected muscle is dark red to black, dry, and foul smelling. Gas bubble formation within the affected areas of muscle can also be seen with some species of *Clostridium*. In the present case and in contrast to typical clostridial cases, gross lesions were limited to slight muscle discoloration and expansion of the fascial planes by edema and fibrin. Histologically, clostridial myositis is characterized by a severe necrosuppurative myositis, as was seen in the present case; however, clostridial infection was ruled out based on the gross appearance and negative fluorescent antibody testing for *Clostridium* spp. *M. haemolytica* was the only significant organism cultured from the muscle with aerobic cultures, providing support that it was the causative agent of the myositis.

Though *M. haemolytica* can be isolated from both treated and non-treated cattle with bronchopneumonia, there has been an overall increase in antibiotic-resistant strains of *M. haemolytica* isolated from cases of BRD *in vitro*.[1,7,16] A previous study at the Oklahoma Animal Disease Diagnostic Laboratory reported that *M. haemolytica*

isolates had decreased sensitivities to erythromycin, florfenicol, spectinomycin, and tilmicosin.[17] A national study in German cattle also found increased antibiotic resistance in *M. haemolytica* isolates, especially for ampicillin, cephalothin, nitrofurantoin, streptomycin, and tilmicosin.[14] The isolate of *M. haemolytica* in the present case was susceptible to ceftiofur and enrofloxacin by antimicrobial sensitivity testing. Interestingly, the heifer was treated with these medications for BRD and appeared to respond immediately post-treatment. However, despite initial response to each antimicrobial used, the heifer continued to relapse and eventually died. Furthermore, lung and muscle lesions in the present case were quite extensive, suggesting progression of the lesions despite treatment.

Isolation of an organism from muscle that was susceptible to two antibiotics used for treatment of BRD is interesting. It is possible there was inadequate penetration of the antimicrobial medications to the site of infection, resulting in persistence of the organism within the tissue and eventual septicemia and death. Inactivation of the antimicrobial at the site of infection could also have played a role. Finally, the disease may have progressed despite administration of two antimicrobial medications that the organism was susceptible to because the drugs were administered late in the course of disease.

The present case highlights the fact that *M. haemolytica* has the potential to spread beyond the respiratory tract. The use of appropriate diagnostics in dead feedlot cattle, including a complete postmortem examination with bacterial cultures and antibiotic sensitivity testing, were critical in the identification of *M. haemolytica* within this unusual location.

Conclusion

Mannheimia haemolytica is a rarely diagnosed cause of myositis in beef cattle. The myositis in this case may be a reflection of terminal septicemia secondary to bronchopneumonia.

Endnotes

[a]Bio-Mycin® 200, Boehringer Ingelheim Vetmedica Inc., St Joseph, MO
[b]Pyramid® 5 with MetaStim®, Fort Dodge Animal Health, Fort Dodge, IA
[c]20/20 Vision® 7 with Spur®, Intervet Inc., Millsboro, DE
[d]Cydectin® Pour On, Fort Dodge Animal Health, Fort Dodge, IA
[e]Component® E-H with Tylan®, VetLife, West Des Moines, IA
[f]Micotil® 300, Elanco Animal Health, Indianapolis, IN
[g]Baytril® 100, Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, KS
[h]Excenel® RTU, Pfizer Animal Health, New York, NY.
[i]Blood Agar Base, BBL, Sparks, MD
[j]Citrated sheep blood, Colorado Serum Company, Denver, CO
[k]Tergitol-7 Agar plates, BBL, Sparks, MD
[l]Sensititre™, Trek Diagnostics, Cleveland, OH

References

1. Autio T, Pohjanvirta T, Holopainen R, Rikula U, Pentikäinen J, Houvilainen A, Rusanen H, Soveri T, Sihvonen L, Pelkonen S: Etiology of respiratory disease in non-vaccinated, non-medicated calves in rearing herds. *Vet Microbiol* 119:256-265, 2007.
2. Confer AW: Immunogens of *Pasteurella*. *Vet Microbiol* 37:353-368, 1993.
3. Cule AP: Fatal clostridial myositis in a cow. *Vet Rec* 123:427, 1988.
4. Duarte ER, Hamdan JS: Otitis in cattle, an aetiological review. *J Vet Med B Infect Dis Vet Public Health* 51:1-7, 2004.
5. Duff GC, Galyean ML: Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. *J Anim Sci* 85:823-840, 2007.
6. Ewers C, Lübke-Becker A, Wieler LH: *Mannheimia haemolytica* and the pathogenesis of enzootic bronchopneumonia. *Berl Munch Tierarztl Wochenschr* 117:97-115, 2004.
7. Gagea MI, Bateman KG, van Dreumel T, McEwen BJ, Carmen S, Archambault M, Shanahan RA, Caswell JL: Diseases and pathogens associated with mortality in Ontario beef feedlots. *J Vet Diagn Invest* 18:18-28, 2006.
8. Gonzalez CT, Maheswaran SK: The role of induced virulence factors produced by *Pasteurella haemolytica* in the pathogenesis of bovine pneumonic pasteurellosis: review and hypotheses. *Br Vet J* 149:183-193, 1993.
9. Jericho KW, Kozub GC: Experimental infectious respiratory disease in groups of calves: lobar distribution, variance, and sample-size requirements for vaccine evaluation. *Can J Vet Res* 68:118-127, 2004.
10. Kaspar H: Results of the antimicrobial agent susceptibility study raised in a representative, cross-sectional monitoring study on a national basis. *J Med Microbiol* 296:69-79, 2006.
11. *Nutrient Requirements of Beef Cattle*, ed 7. Washington DC, National Academy Press, 1996.
12. Step DL, Smith RA: Nonrespiratory diseases of stocker cattle. *Vet Clin North Am Food Anim Pract* 22:413-434, 2006.
13. Van Vleet JF, Valentine BA: Muscle and tendon, in Maxie GM (ed): *Pathology of Domestic Animals*, ed 5. Edinburgh, Saunders Elsevier, 2007, pp 559-565.
14. Visser IJ, Zalsman C, Wouda W: *Pasteurella haemolytica* serotypes in cattle. *Tijdschr Diergeneeskd* 117:35-37, 1992.
15. Wallace LL, Fagin B, Schmidt D, Fales WH: Coliform myositis in a calf. *J Am Vet Med Assoc* 197:1487-1488, 1990.
16. Wallmann J: Monitoring of antimicrobial resistance in pathogenic bacteria from livestock animals. *Int J Med Microbiol* 296:81-86, 2006.
17. Welsh RD, Dye LB, Payton ME, Confer AW: Isolation and antimicrobial susceptibilities of bacterial pathogens from bovine pneumonia: 1994–2002. *J Vet Diagn Invest* 16:426-431, 2004.
18. Wenz JR, Barrington GM, Garry FB, McSweeney KD, Dinsmore RP, Goodell G, Callan RJ: Bacteremia associated with naturally occuring acute coliform mastitis in dairy cows. *J Am Vet Med Assoc* 219:976-981, 2001.
19. Whiteley LO, Maheswaran SK, Weiss DJ, Ames TR, Kannan MS: *Pasteurella haemolytica* A1 and bovine respiratory disease: pathogenesis. *J Vet Intern Med* 6:11-22, 1992.
20. Williams BM: Clostridial myositis in cattle: bacteriology and gross pathology. *Vet Rec* 100:90-91, 1977.