Clostridial Myonecrosis in the Equine patient

Clinicopathologic conference

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Introduction

Clostridial myonecrosis is an acute, rapidly progressive, non-pyogenic, invasive clostridial infection of the muscles. It is characterized by massive death of tissue and a variable degree of gas production.[1] The majority of equine cases occur iatrogenically as a result of intramuscular administration of non-antibiotic injectables; however, muscle trauma or direct inoculation of wounds may also result in clostridial myositis.[2] Horses with clostridial myonecrosis demonstrate swift toxemia that may progress to circulatory collapse and multi-organ failure over just a few hours.[3] The peracute nature of the disease and high mortality rates necessitates rapid diagnosis and treatment.[4]

History and Presentation

A history involving blunt trauma, laceration, or intramuscular injection is common in cases with clostridial myonecrosis. Perivascular leakage of irritating intravenous substances has also been reported.[5] Onset of clinical signs typically occurs within 6-72 hours of the inciting wound or injection.[2,3,4] A wide array of pharmacologic and biologic preparations have been incriminated as inciting causes of clostridial myonecrosis through IM injection, including nonsteroidal anti-inflammatory drugs, vitamins, and prostaglandins.[3] The most commonly affected sites are those where IM injections are given most frequently: the cervical musculature, the gluteal muscles, and in the semimembranosus and semitendinosus muscles.[4,6] The most frequently reported pharmacologic agent associated with the development of clostridial myonecrosis is flunixin meglumine, with the most cases occurring in the cervical region.[3] The most common presentation in these cases is post IM NSAID administration for colic pain. Clostridial myonecrosis is also associated with wounds particularly those associated with a significant amount of soft tissue trauma. This is most often arises from stallions fighting with

one another resulting in severe bite wounds. There are suspicions that colic may be linked to clostridial myositis, such as through enteric colonic absorption of the bacteria, as this is one of the ways that blackleg can occur in cattle.[7] However, this method of introduction to the muscle has not been proven.

Affected animals may be found recumbent or dead, as the disease can progress extremely quickly.[8] In less severe cases or those caught early enough, mild to severe lameness, colic, or obtunded mentation due to overwhelming septicemia may be the presenting complaint.[5] Systemic signs often include hyperthermia, dehydration, tachycardia, tachypnea, poor capillary refill time, and congested mucous membranes.[8,9] Localized signs include a painful muscular swelling, as well as flocculence or gas crepitation.[2] The overlying skin may initially feel hot then progressively become cool, tough, firm, and insensitive, indicating a loss of circulation as the local vessels become necrotic.[7] The animal is usually very stiff and reluctant to move.[8] Disseminated intravascular coagulation is the cause of acute death in many horses with clostridial myonecrosis.[3]

On bloodwork, azotemia is common, which can be attributed to dehydration due to depression or an inability to lower their neck to drink.[10] Elevation of creatine phosphokinase is common; however, the magnitude of elevation rarely correlates with the degree of muscle damage observed clinically and histopathologically.[2] This discrepancy may be the result of vascular compromise of affected muscles with reduced systemic absorption of muscle cell enzymes. There is also the potential for pigment nephropathy due to hemolysis.[2] Alterations in the total white blood cell count are commonly present due to neutrophilia or neutropenia, with or without a left shift.[2,3] Hemoconcentration is typically noted due to fluid sequestration into necrotic muscles as a result of increase in vascular permeability.[2] There may be

hyperproteinemia with a long-standing walled-off abscess, but more typically hypoproteinemia occurs because of exudative losses into the necrotic tissue.[10]

Immune mediated hemolytic anemia has also been seen as a result of clostridial infections in horses.[5] It is not certain whether hemolytic events are a direct effect of the clostridial infection. Some clostridial species, predominantly *C. septicum*, can produce exotoxins that may damage the RBC membrane, exposing new or altered antigens that induce an immune response.[11] Alternatively, hemolysis may potentially be caused by beta-lactam antibiotic-induced red cell hemolysis, or other drug therapy.[3,12] Hemolytic anemia occurs inconsistently, perhaps because of variability in toxin production by different clostridium species or variability in absorption of toxins into the blood. The presence of type III echinocytes and spheroechinocytes may be useful for identifying hemolytic anemias associated with clostridial infections.[11]

Pathophysiology

Clostridial myonecrosis is a form of moist gangrene caused by the invasion of gas producing bacteria of the genus clostridium.[13] These organisms have the ability to induce necrosis and then live a saprophytes in the dead tissue, thus it is a progressive condition. In order for anaerobic clostridia to grow and cause disease, an altered local environment and the presence of clostridial spores is required.[5] An area of lowered oxidation reduction potential, caused by circulatory failure in a local area of by extensive soft tissue damage and necrotic muscle tissue, is created.[14] The absence of a tissue inflammatory response is a hallmark feature of these infections. This is in striking contrast to soft-tissue infections caused by organisms such as *S. aureus*, in which significant influx of PMNs localizes the infection without adjacent tissue or vascular destruction.[14] The term clostridial myonecrosis is often used interchangeably with malignant edema, but there is a difference in pathology between the two. Malignant edema is

caused by the same organisms, but is confined to the subcutaneous tissue as cellulitis rather than muscle involvement.[15] Malignant edema of the connective tissues has the capacity to progress to an anaerobic infection of the muscles if the blood supply to them is damaged, however, it does not always develop into a myositis.[15]

Clostridial myonecrosis is caused by anaerobic, spore-forming, gram positive encapsulated bacilli of the genus *Clostridium*.[7,14] There are over 100 different species of Clostridium, but the ones most commonly isolated from equine clostridial myonecrosis are *C. perfringens, C. septicum, C. novyi,* and *C. chauvoi.*[15] Clostridium species are widespread in nature because of their ability to form endospores that persist in the environment. They are commonly found in soil and marine sediments, as well as in human and animal intestinal tracts.[14] Although species such as *C. perfringens* can frequently be cultured from the environment and soil wherever livestock are found, the means by which spores or vegetative organisms gain access to areas of affected soft tissue is not fully understood. Vegetative growth of these clostridial species is accompanied by production of dermonecrotizing and vasoactive toxins that lead to gas production, extensive tissue damage, and necrosis, as well as rapidly developing, life-threatening systemic toxemia.[3,7] There has been preliminary evidence presented that it may be possible for dormant clostridial spores to reside within the equine skeletal muscle, thus presenting another potential route of infection.[16]

Of the species of bacteria known to cause clostridial myositis, the most information is known about C. perfringens.[14] *Clostridium perfringens* is a heterogenous group of bacteria that is classified into five major types, identified as types A through E.[7] All five types are capable of causing clostridial myositis in horses; however, the most commonly involved is type A, which produces alpha-toxin.[2] This toxin is a calcium-dependent phospholipase that hydrolyzes

phospholipids within cell membranes.[7] It causes capillary endothelial damage, resulting in increased vascular permeability.[7] In experimental animals, intramuscular α toxin injection caused a rapid, irreversible decline in muscle blood flow and concomitant ischemic necrosis of tissue because of the formation of occlusive intravascular aggregates of destroyed platelets, leukocytes, and fibrin.[1,2,14]

Clostridium septicum is less commonly isolated, and is thought to be more pathogenic than *Clostridium perfringens.*[2] The survival rate of infection with *C. septicum* is less than 50%.[4] *C. septicum* also possesses an alpha toxin, which is a pore-forming hemolysin that induces rapid necrosis of cultured cells by causing efflux of intracellular potassium and ATP depletion.[4] In humans, *C. septicum* is documented to be associated with spontaneous gas gangrene, which occurs in the absence of an obvious portal of bacterial entry.[7] Translocation of the more aerotolerant *C. septicum* from the gut to the bloodstream via a colonic lesion, such as neoplasia, with subsequent hematogenous metastatic infection, is thought to occur.[7]

Clostridium novyi and *chauvoei* have also been isolated from horses affected by clostridial myonecrosis, but with less frequency than cattle. Neither of these clostridial species has been found to act as the sole pathogen in cases of clostridial myonecrosis in horses and are generally found in conjunction with other clostridial species.[4] The a-toxin produced by *Clostridium novyi* is a necrotizing lethal toxin and increases capillary permeability, resulting in tissue edema.[2] *Clostridium chauvoei* is the most common cause of blackleg in cattle, which is also a gangrenous myositis. Blackleg is characterized by activation of latent spores in the muscles, rather than traumatic introduction, as is most often the case in clostridial myonecrosis in horses.[15]

Differential Diagnoses

Not all myositis/cellulites cases are due to clostridial organisms. *Streptococcus equi* can produce a suppurative myositis with similar clinical findings, although antibiotic therapies may be vastly different.[15] Anaerobes such as the gram negative Fusobacterium or Bacteroides rods, gram-positive Peptococcus and Peptostreptococcus cocci, or non–spore-forming Propionibacterium and Eubacterium gram positive rods may also be found in aspirates.[10] Gram stains are often helpful stall side, but culture and sensitivity should always be performed. Other differentials include any disease that causes significant systemic signs of illness.[3] One should also consider a penetrating wound into the thoracic cavity or trachea as the cause of crepitus if there is history of trauma.

Diagnostic Approach

A presumptive diagnosis of clostridial myositis is usually straightforward and can be reached from clinical signs. Ultrasonographic evaluation of the affected tissues reveals fluid accumulation within the subcutaneous tissues and between fascial planes, with or without gas echoes.[2] The fluid may be hypoechoic with flocculent echodensities.[10] This may be difficult to differentiate from cellulitis, which usually appears as an echogenic, homogeneous thickening of the subcutaneous tissues, also dissecting along tissue planes. Sometimes hypoechoic pockets are seen in an area of cellulitis, but lack the gas shadows that are frequently observed in anaerobic infections.[10] Aspirate will most likely reveal a malodorous, nonclotting fluid, with or without gas.[17] Impression smears of muscle or tissue exudate allow for cytological evaluation for bacteria via Gram's stain. The finding of large gram-positive rods, and potentially spores, provides support for a presumptive diagnosis.[2] Definitive diagnosis is depends on anaerobic culture of the affected muscle or exudate. Clostridial organisms are relatively easy to culture; however,

inoculation of culture media within 1 hour of collection is recommended to minimize exposure to oxygen.[2] Definitive diagnosis of *C. chauvoei* and *C. septicum* can also be achieved by performing fluorescent antibody staining of smears.[4]

Treatment:

A combination of aggressive medical and surgical treatment and good nursing care is required to provide a better outcome in clostridial myonecrosis cases. Antibiotic therapy needs to be instituted as soon as possible after presentation.[8] Typically high doses of potassium penicillin at 44,000 IU/ kg q6-8h (minimum, 22,000 IU/kg q6h) are used.[3] Caution should be exercised, as the treatment of horses with penicillin has resulted in immune-mediated hemolytic anemia.[17] Oral metronidazole (15-25 mg/kg q6h) should be administered if the use of oral medication is possible, although poor tissue levels achieved may limit its use (Munroe). Oxytetracycline is an alternative IV antimicrobial agent (6.6 mg/kg IV q12–24h).[9] In-vitro susceptibility testing alongside data from a rodent model of clostridial myositis suggests that tetracycline, chloramphenicol and metronidazole may have greater efficacy than penicillin G in the treatment of experimental *Clostridium perfringens* myositis.[18] However, the majority of horses that have survived the disease were administered penicillin, and it remains the antibiotic of choice.[17]

Prompt surgical inspection and debridement of devitalized tissue are mandatory to remove necrotic tissue, disrupt the anaerobic environment, and slow the spread of the bacteria (Reed). Numerous vertical incisions, approximately 2.5cm apart, should be made extending down through the muscles in the affected area.[5] Multiple surgical debridements over the course of several days may be required.[14] Veterinarians should warn owners of the significant soft tissue and skin sloughing that will likely ensue over coming days to weeks.[3] Long-term wound care will often be needed, with many cases taking weeks to months before granulation and second-

intention skin healing are complete. It is recommended that the wounds be lavaged daily, at minimal with hydrotherapy from a garden hose, and with antibiotic solutions if funds allow.[5,9] Cosmetically, some horses may heal with pigmentation changes and significant cicatrix formation.[3]

Strong supportive care will need to be given to affected horses. Pain and discomfort in the affected horse should be controlled using IV flunixin meglumine or opioids. Fluid therapy and a nasogastric tube are also warranted if the horse is unwilling or unable to drink. [2,5,8] If the cervical muscles are affected, severe dependent facial edema and edema of the larynx and pharynx, may necessitate the placement of a tracheostomy tube until the swelling abates.[10] The head may also need to be mechanically elevated for set periods of time to prevent further edema from forming.[10]

Alternate therapy options include hyperbaric oxygen (HBO) therapy and clostridial anti-toxin. In humans, hyperbaric oxygen therapy has been used with some success to halt the disease process of clostridial myonecrosis locally.[14] While HBO has been used for other maladies in the equine patient, experimental studies in animals have failed to demonstrate therapeutic efficacy of HBO alone or in conjunction with antibiotic therapy.[2] Similarly, while clostridial anti-toxins have been used in people, they are cost prohibitive in horses and have not been proven to be safe or efficacious.[7,17]

Prognosis

Prognosis for recovery from clostridial myonecrosis is guarded to poor, depending on whether the patient has *C. perfringens, C. septicum*, or a combination of other clostridial bacteria, as well as the location of the origin of infection.[6] Those with *C. septicum* or multiple types of clostridial bacteria have a poorer prognosis.[4] In the largest study performed, which

retrospectively gathered information on clostridial myonecrosis cases, 73% of the horses survived following treatment for clostridial myonecrosis, with a 78% survival rate for *C. perfringens* and a 50% survival rate for *C. septicum*.[4] The treatment methods used also has a large impact on the outcome of cases. Only 2 of the horses that survived in the previously mentioned study did not undergo myotomy/fenestration. Other factors that may affect prognosis include the size of the inoculum, time until treatment is initiated, and competency of the host response.[2]

Prevention

A retrospective study performed by Brown et al found there is no difference in the occurrence of infection when an injection site is aseptically cleaned before intramuscular injection or not, although they concluded that disinfection of the site with alcohol may still have some benefit and should not be ignored.[19] While proper disinfection of the IM injection site is the provides the best chance of preventing infection, even the latest skin disinfection methods are incapable of completely sterilizing the skin from bacteria.[6] It has been shown that clipping the hair before antiseptic site preparation does reduce bacterial numbers, but actually causes more skin cores to be transferred into the site.[6] Therefore, the benefits of removing the hair are balanced out by the higher chance of infection from skin cores and hair follicles transferred into the site. The site of injection, however, does have the capacity to make a difference. The larger, more vascularized gluteal muscles have shown less chance of being infected, but if they do become infected, they are far harder to drain.[6]

Specific immunization of horses against clostridial myonecrosis is not practiced in North America, due to lack of availability of an appropriate vaccine and the sporadic nature of the disease.[7]There are vaccines against *C. perfringens* type A in use elsewhere in the world.

However, as this is only one of the many possible causative agents of clostridial myonecrosis, its usefulness is questionable.[7]

Conclusion

Clostridial myositis is a rapidly progressing and often fatal disease in equine patients. Introduction of anaerobic clostridial bacteria into the muscle by intramuscular injection is the most common cause of the disease, although trauma has been known to cause it as well. Multiple species of clostridium are ubiquitous in the environment and the gastrointestinal tract, making it difficult to prevent introduction of the bacteria into the muscle through trauma. Even with rapid treatment, case mortality rate is between 30 and 50%. Horses surviving clostridial infections commonly suffer from permanent disfigurement or scarring due to extensive muscle necrosis. The key to preventing death due to clostridial myonecrosis is swift treatment intervention. Clients should be encouraged to report all injection-site reactions to their veterinarian as soon as they are noticed and to monitor closely for changes in condition suggestive of clostridial infection.

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