Clostridial Myositis: Evaluation of Normal Equine Skeletal Muscle for the Presence of Clostridial Spores

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Dormant clostridial spores are present in healthy skeletal muscle. Inflammatory events caused by a local and/or systemic insult may lead to development of an anaerobic environment suitable for germination of dormant spores in skeletal muscle and consequent clostridial myositis or clostridial myonecrosis. Author's address: University of Guelph, Ontario Veterinary College, Department of Clinical Studies, Guelph, Ontario, Canada N1G 2W1. © 2002 AAEP.

1. Introduction

Clostridial myositis or clostridial myonecrosis is a rapidly progressive condition with liquefactive necrosis of muscle, gas formation, and associated clinical signs of toxemia. It is an exotoxin-mediated inflammatory cascade caused by histotoxic or tissue-destroying clostridia. The most common causative organism in horses is Clostridium perfringens (type A); Clostridium septicum, Clostridium chauvoei, Clostridium novyi, and Clostridium fallax have also been reported. Left untreated, its rapid and aggressive progression is almost always fatal.1

The majority of equine cases are associated with intramuscular injection; however, intramuscular injection techniques seem to have no influence on the development of clostridial myositis/myonecrosis.2 Also, it is not clear whether clostridial organisms are inoculated or are pre-existing in the skeletal muscle. We evaluated the hypothesis that dormant clostridial spores are present in healthy equine skeletal muscle and are therefore capable of causing clostridial myositis/myonecrosis given proper environmental conditions.

2. Materials and Methods

Muscle samples were obtained from 35 horses that were euthanized for reasons unrelated to infectious disease, shock, or trauma. Aseptic surgical technique was used to obtain 1.5-cm³ muscle samples from the neck. Immediately after the muscle samples were obtained, they were plunged into a solution of 70% isopropyl alcohol for 1 minute, placed into a sterile container, and frozen at −80°C until analyzed further. For analysis, muscle samples were anaerobically cultured at 37°C in brain-heart-infusion broth for 4 d. After 4 d, 1 ml of broth/muscle homogenate was mixed in a sterile screw-cap tube with 1 ml of 95% ethanol. The specimen was
gently mixed at room temperature for 60 min. The ethanol-treated homogenate was inoculated onto blood agar and incubated anaerobically at 37°C for up to 5 d. Morphology and growth of colonies was assessed. Each colony was Gram stained and subcultured onto blood agar and incubated anaerobically. Identification was performed using the API rapid ID 32A biochemical identification test.

A skin swab was obtained from the site of incision after the skin was clipped and aseptically prepared for the procedure. Skin swabs were frozen and stored at −80°C. If the muscle sample from the respective horse showed any growth on blood agar, the skin swab from the same horse was processed as described above.

3. Results
A total of 11 isolates were cultured from 7/35 horses (20%). Two different strains of Clostridium sporogenes were isolated from horse 11 and a single isolate was isolated from horse 35. Clostridium histolyticum was isolated from horses 10 and 19. Clostridium glycolicum was isolated from horse 10. Two separate isolates of C. beijernickii/butyricum were isolated from horse 26 and a single isolate of this organism was obtained from horse 18. Clostridium clostridioforme was isolated from horse 18. Clostridium botulinum was isolated from horse 29.

Cultured skin swabs were all negative for the presence of anaerobic organisms.

4. Discussion
Our findings show that dormant clostridial spores are present in healthy equine skeletal muscle. C. histolyticum and C. sporogenes, isolated from healthy skeletal muscle in this study, have been encountered in human patients with myonecrosis; however, their pathogenic significance in horses is not as certain as that of C. perfringens. We are surprised that C. perfringens was not isolated in any muscle sample, based on the ubiquity of the organism in the environment and the fact that it is the most common isolate in clinical cases of clostridial myositis. Clostridial spores do not differ with respect to their mechanics, therefore, we do believe that C. perfringens is capable of gaining entry into the healthy skeletal muscle as well as isolates presented above and its isolation is most probably termed by the sample size.

C. botulinum, which was also isolated from a healthy skeletal muscle in this study, is capable of causing a life-threatening disease resulting from elaboration of botulinic toxin in vivo after growth of the organism in an infected tissue. However, it does not belong to a group of histotoxic or tissue-destroying clostridia and its determination in the healthy skeletal muscle only contributes to a speculation that the variety of clostridial organisms present in a healthy tissue correlate with their distribution in the environment.

Muscle samples were carefully selected from horses that were euthanized for reasons unrelated to infectious disease, shock, or trauma. All horses sampled resided in the Province of Ontario. There is no evidence that clostridial species in Ontario differ from those in other parts of North America; however, some dormant clostridial spores in a skeletal muscle tissue should follow their prevalence in certain environmental niches.

Cultured skin swabs were all negative for the presence of clostridial organism, which indicates that contamination of muscle samples was not likely to be due to contamination through the incision. The most probable source of clostridia is equine intestine. It is possible that clostridial organisms gain entry into systemic circulation, some of them being able to embed into the skeletal muscle. Spore formation may occur due to high oxygen tension in tissues and a local and/or systemic insult is needed to cause tissue inflammatory response consequently leading to a microenvironment suitable for germination of dormant clostridial spores.

There is only anecdotal evidence that specific drugs are more likely to cause clostridial myositis. Nevertheless, intramuscular injection is a traumatic event capable of causing an environment friendly for clostridial spores to germinate. Therefore, care should be taken that injections produce minimal trauma and irritation to skeletal muscle to prevent dormant clostridial spores in devitalized skeletal muscle and associated soft tissues from germinating.

References and Footnote

*BIOMERIEUX SA, Marcy-l’Etoile, France.