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Gangrenous *Clostridium perfringens* Infection and Subsequent Wound Management in a Rhesus Macaque (*Macaca mulatta)*

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A 10-y-old female rhesus macaque presented acutely with 3 large (diameter, greater than 4 cm), malodorous, ulcerogangrenous skin wounds on the left caudal thigh and calf. Limb radiographs revealed free gas infiltrating deep tissues, and histologic examination confirmed myonecrosis. *Clostridium perfringens*, *Staphylococcus aureus*, and *Prevotella intermedia* were isolated from the wounds. Antimicrobials, analgesics, and aggressive debridement of necrotic skin and muscle resulted in immediate clinical improvement of the primate. At 1 wk prior to presentation, the animal had received several intramuscular injections in close proximity to the site of infection. Repeated intramuscular injections through excrement-contaminated skin possibly contributed to the pathogenesis of infection. Continued therapy consisted of biweekly wound debridement and nonadherent bandage changes for 7 wk. The macaque regained full use of the affected leg and remains in good physical condition at our facility. Our management of this case led to improvements in training regarding intramuscular injection practices in our macaque colony. This case study is the first report of *Clostridium perfringens* myonecrosis in a laboratory nonhuman primate. We discuss various methodologies for the diagnosis and treatment of necrotizing clostridial infections.

Abbreviations: HBO, hyperbaric oxygen; IM, intramuscular

Clostridial wound infections are among the most widely recognized anaerobic infections in both humans and animals. With few exceptions, *Clostridium* spp. are ubiquitous spore-forming anaerobic gram-positive rods found in dust, soil, or vegetation. The most common sources of the organism are human and animal excrement.[1,5,9,11,15] Wounds infected with this organism can be classified into 3 categories based on clinical features.[5,12] Gas gangrene (clostridial myonecrosis) is the most severe outcome, resulting in shock and organ failure in 50% of affected human patients.[15] Pain, marked swelling, and systemic toxemia are hallmarks of 'classic' gas gangrene.[11] Aggressive treatment, including radical debridement and amputation, often is required for successful therapy; however, fulminant gas gangrene carries a poor prognosis.[1,6,20] The second category of clostridial wound infection is anaerobic cellulitis. This condition occurs when the degree of devitalized wound tissue is able to support bacterial growth. Gas production remains localized, and bacterial invasion of healthy tissues does not occur.[12,14] Pain and systemic signs of toxicity are minimal, and patient prognosis is good with aggressive early treatment. The third and least serious category of clostridial infection is superficial wound contamination. The specific purpose of this report is to describe a case of gas gangrene and myonecrosis caused by *Clostridium perfringens* in a rhesus macaque. To our knowledge, clostridial wound infections in nonhuman primates maintained in research colonies have not been documented previously.

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Aspects of this clinical case were presented at both the Association for Primate Veterinarians (APV) conference in Park City, UT, and the American Association for Laboratory Animal Science (AALAS) conference in Salt Lake City, UT, in October 2006.

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Case Report

A 10-y-old female rhesus macaque (*Macaca mulatta*), 10.6 kg in weight, was reported to the veterinary staff for reddened lesions on the left leg. This animal was individually housed in a stainless-steel nonhuman primate cage and was fed a commercial primate diet (Lab Diet 5038, PMI Nutrition International, Brentwood, MO) and automated water ad libitum.

This rhesus macaque was used for respiratory assay in a dose-response study of opioid analgesics; the study was approved by the institutional animal care and use committee. The experimental history included a single intramuscular (IM) injection of morphine sulfate (3.2 mg/kg; National Institute on Drug Abuse, Bethesda, MD) given daily for 3 consecutive days. On the fourth day, a dose-response study was performed. At this time, the animal was placed in a restraint chair while conscious and received 4 IM injections of the kappa opioid receptor agonist bremazocine HCl (Research Biochemicals, Natick, MA) 30 min apart. All injections were administered in the left thigh musculature. Single-use sterile needles and syringes were used for all injections.

The animal was received from the Tulane Primate Facility in December 1997; she had an excellent health history at our facility for the 8 y prior to the described incident and an unremarkable semiannual physical examination 2 wk prior to presentation. Initial examination revealed diffuse, nonpitting edema of the left pelvic limb, a 4 × 6-cm skin ulceration in the caudal thigh with subjacent *biceps femoris* necrosis characterized by black discoloration, subcutaneous pockets extending along fascial plana, and dark skin discoloration of the lateral thigh and calf (Figure 1). Shaving revealed the skin discoloration to be 2 malodorous, irregularly shaped, circumscribed, well-demarcated necrotic areas of the lateral thigh (lesion size, 7 × 5 cm) and lateral calf (6 × 5 cm, Figure 2). All lesions were malodorous, and the lateral left thigh and calf lesions were well demarcated from

Figure 1. Day 0. Lateral view of the left leg after removal of hair on initial examination. Note the ulceration on the caudal thigh. The dark color seen in this wound is the muscle belly of the *biceps femoris*.

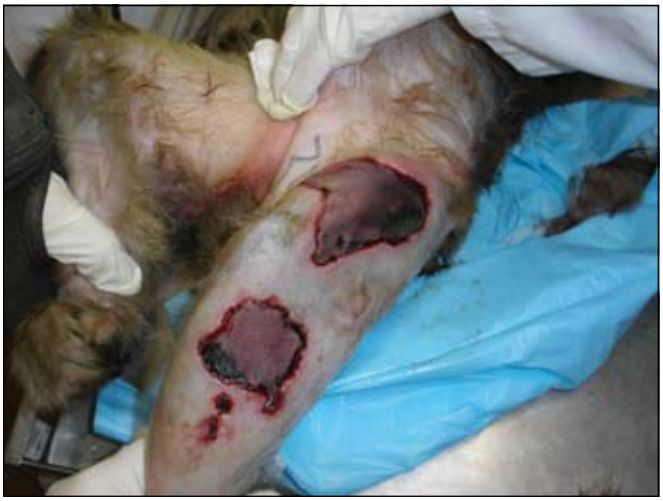

Figure 2. Day 0. Anterior view of the affected leg. The leg is diffusely edematous. The well-demarcated lesions shown (lateral thigh and calf) were not appreciable on cageside examination and required clipping the hair from the leg for visualization.

the surrounding healthy skin and circumscribed by a 0.5- to 1.0-cm skin defect. The animal's left popliteal lymph node was enlarged markedly. The left stifle circumference was 26.5 cm, compared with the unaffected right stifle circumference of 22 cm. The remainder of the exam was unremarkable: the animal was normothermic; palpation of the affected limb revealed no appreciable crepitus; and distal limb and toe temperature were normal to the touch. Blood was obtained from the right femoral vein for complete blood count and serum biochemical analysis. A wedge biopsy was taken from the margin of the lateral thigh wound for histologic examination. Exudate samples from a subcutaneous pocket in the caudal thigh lesion were submitted to the Animal Diagnostic Laboratory (aerobic) and University of Michigan Hospital Microbiology Laboratory (anaerobic) for culture and sensitivity.

All lesions were scrubbed and lavaged with 2% chlorhexidine (Nolvasan, Fort Dodge Animal Health, Fort Dodge, IA). Initial treatments included IM administration of enrofloxacin (5 mg/kg; Baytril, Bayer Healthcare, Shawnee Mission, KS), cefazolin sodium (30 mg/kg; Ancef, SmithKline Beecham; Research Triangle Park, NC), and ketoprofen (2 mg/kg; Ketofen, Fort Dodge Animal Health) for alleviation of discomfort. For the remainder of this report, the day of presentation will be referred to as day 0.

Complete blood cell counts were within reference ranges. Serum chemistry revealed a creatine phosophokinase level of 3838 (normal, 0 to 436) U/l, indicating muscular injury. Preliminary results from anaerobic culture revealed gram-positive rods. Because we suspected a clostridial wound infection, twice-daily IM injection of procaine penicillin G (30,000 U/kg; Pro-Pen-G, Bimeda, Irwindale, CA) was added to the antibiotic regimen on day 2, before final anaerobic culture and sensitivity results were available. Aerobic culture revealed β-hemolytic *Staphylococcus aureus* that was susceptible to all of our antibiotic choices. Our hypothesis of an anaerobic infection was confirmed on day 4, when *C. perfringens* and *Prevotella intermedia* were identified by culture. In light of the culture and sensitivity results, enrofloxacin was discontinued and replaced with cleocin phosphate (15 mg/kg; Clindamycin, Pharmacia and Upjohn, Kalamazoo, MI) IM twice daily for improved anaerobic coverage. The antibiotic protocol of twice-daily IM cefazolin, procaine penicillin G, and clindamycin continued until resolution of clinical disease. Daily IM ketoprofen injections continued for 1 mo duration. These injections were administered in separate regions of the right (unaffected) thigh.

For 1 wk after initial presentation, the animal remained nonweightbearing on the affected limb. In addition, periodic recumbency (atypical for this animal) was noted. The limb edema did not improve, and the necrotic skin became escharotic (Figure 3). On day 7, the presence of subcutaneous gas, a sequela to the clostridial infection in the deep tissues of the left limb, was documented on lateral and ventrodorsal radiographs (Figure 4).

The primate was prepared for surgical exploration of the wounds and debridement of necrotic tissues. Additional blood was taken to repeat the complete blood count and serum chemistry and to assess titers for measles, simian retrovirus, simian immunodeficiency virus, *Cercopithecine herpesvirus* 1, and simian T-lymphotrophic virus. All viral samples were negative.

The animal was sedated with tiletamine–zolazepam (8 mg/kg IM; Telazol, Fort Dodge Animal Health), intubated, and maintained on isoflurane (Isoflo, Abbott Laboratories, Abbott Park, IL) with 100% oxygen for the duration of the procedure. The caudal thigh wound was expanded parallel to the leg by use of a scalpel and explored with a probe. Necrotic tissue was excised with a combination of blunt and sharp dissection by using Metzenbaum scissors and scalpel. Exploration of the caudal thigh wound revealed a 3 × 3-cm abscess near the left popliteal lymph node that provided common communication among all lesions (Figure 5). One of the more severely affected muscles, the *biceps femoris*, was discolored yellow, waxy, and nonviable. The left caudal thigh wound was closed around a Penrose drain using absorbable suture (PDS II, Ethicon, Somerville, NJ). The lateral calf and thigh wounds were debrided in a similar fashion and the margins trimmed. Tissue viability was determined by color, texture, and vascularity. Tissue of questionable viability was scraped with a scalpel until bleeding occurred. The lateral thigh and calf wounds were not closed but allowed to heal by second intention. Buprenorphine HCl (0.5 mg/kg IM; Buprenex, Reckitt Benckiser Pharmaceuticals, Richmond, VA**)** was given twice daily for 7 d to provide additional analgesia.

Immediately after debridement, 1% silver sulfadiazine cream (Silvadene, Monarch Pharmaceuticals, Bristol, TN) was applied to open wounds, and a wet-to-dry 3-layer modified Robert-Jones bandage was placed. The primary layer consisted of sterile

Figure 3. Day 7. Prior to surgical debridement, the skin is escharotic. The wound has not increased in size, but the leg remains edematous.

saline-soaked 4 × 4-in. gauze. This material was covered with roll cotton and a layer of roll gauze for support. The final layer consisted of flexible tape (Elastikon, Johnson and Johnson, New Brunswick, NJ) and elastic bandage material (Vetrap, 3M Animal Care Products, St Paul, MN). Future references to bandaging of this leg refer to the same 3-layer bandage (with noted modifications). Bandages were changed every 3 to 4 d.

Samples of the laboratory's opiate compounds, bremazocine and morphine, were submitted for aerobic and anaerobic culture. All submitted samples were negative for growth. Histopathologic examination of the muscle biopsy revealed marked polymorphonuclear cell infiltration, suppurative myositis, and severe, widespread coagulation necrosis.

The day after surgery (day 8), the recumbent behavior ceased. On day 9, the animal was sedated with tiletamine–zolazepam for bandage-changing and wound inspection. The diffuse edema was decreased by approximately 2 cm (left stifle circumference: current, 24.5 cm; initial, 26.3 cm). On day 12, the circumference of the left stifle had returned to that of the unaffected right stifle (22 cm). Approximately 50% of the wound beds contained fleshy, pink granulation tissue; the remainder contained necrotic tissue, biofilm, and adherent fibrin and were surgically debrided during bandage changes (Figure 6).

On day 14, hematology and serum chemistry tests were repeated and revealed that the hematocrit had increased from 31% (day 0) to 37%. In addition the creatine phosophokinase level had returned to 149 U/l (within normal limits). Buprenorphine was discontinued on day 15 in light of concerns about a diminished appetite that had been observed since administration of narcotic analgesia. Appetite returned to normal within 2 d.

On day 22, the primary bandage layer was changed to 4 × 4-in. biofilm hydrogel dressing (Transorbent, Swiss-American Products, Dallas, TX). The hydrogel dressing provided adherence to the wound and surrounding skin with absorption of exudate but protected newly forming granulation tissue. This type of dressing remained the primary layer in the bandage until case resolution. Complete closure of the left lateral thigh wound was accomplished on day 26 with the use of a tension-relieving suture pattern and placement of a Penrose drain.

The size and position of the calf wound would not permit edge-to-edge apposition of skin margins, so a 'walking suture' technique was used to reduce the wound size.[17] After surgical debridement, the skin margin was undermined 1 to 2 cm along the natural plane between the dermis and subcutis to relieve undue tension. The skin edge then was stretched to the center by use of gentle traction with nonabsorbable suture (Prolene, Ethicon) and sutured to the underlying granulation tissue (Figure 7). The walking-suture method allows for rapid

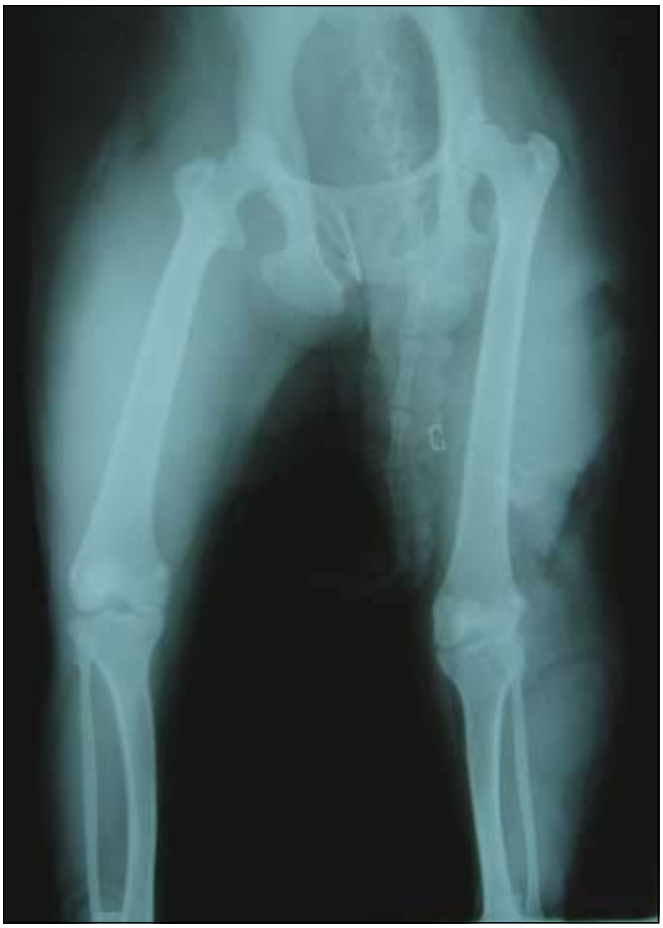

Figure 4. Day 7. Ventrodorsal radiograph of the pelvic limbs. Note the accumulation of gas between muscle fascial planes in the right calf region of this animal. Additional evidence of gas-producing bacteria is present on the right lateral thigh, near the knee. The surgical staple in this radiograph was used to close a biopsy site.

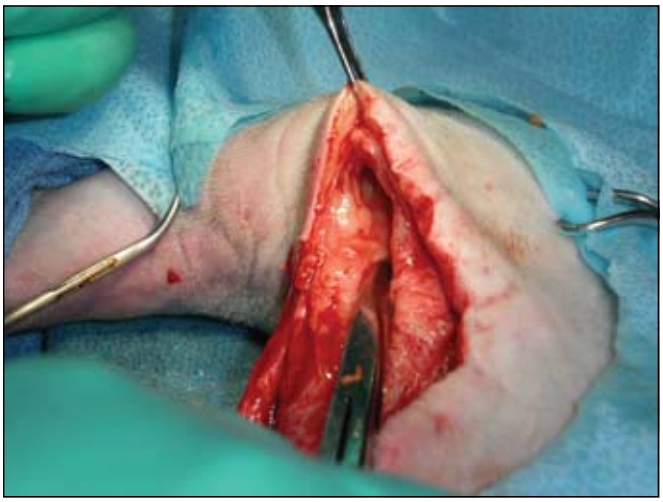

Figure 5. Day 7. An abscess was discovered adjacent to the left popliteal lymph node. Purulent material is shown draining from the deep cavity toward the tip of the instrument. This abscess communicated with the lesions on the lateral calf and thigh. The caudal thigh wound was the logical location for the site of the original injury.

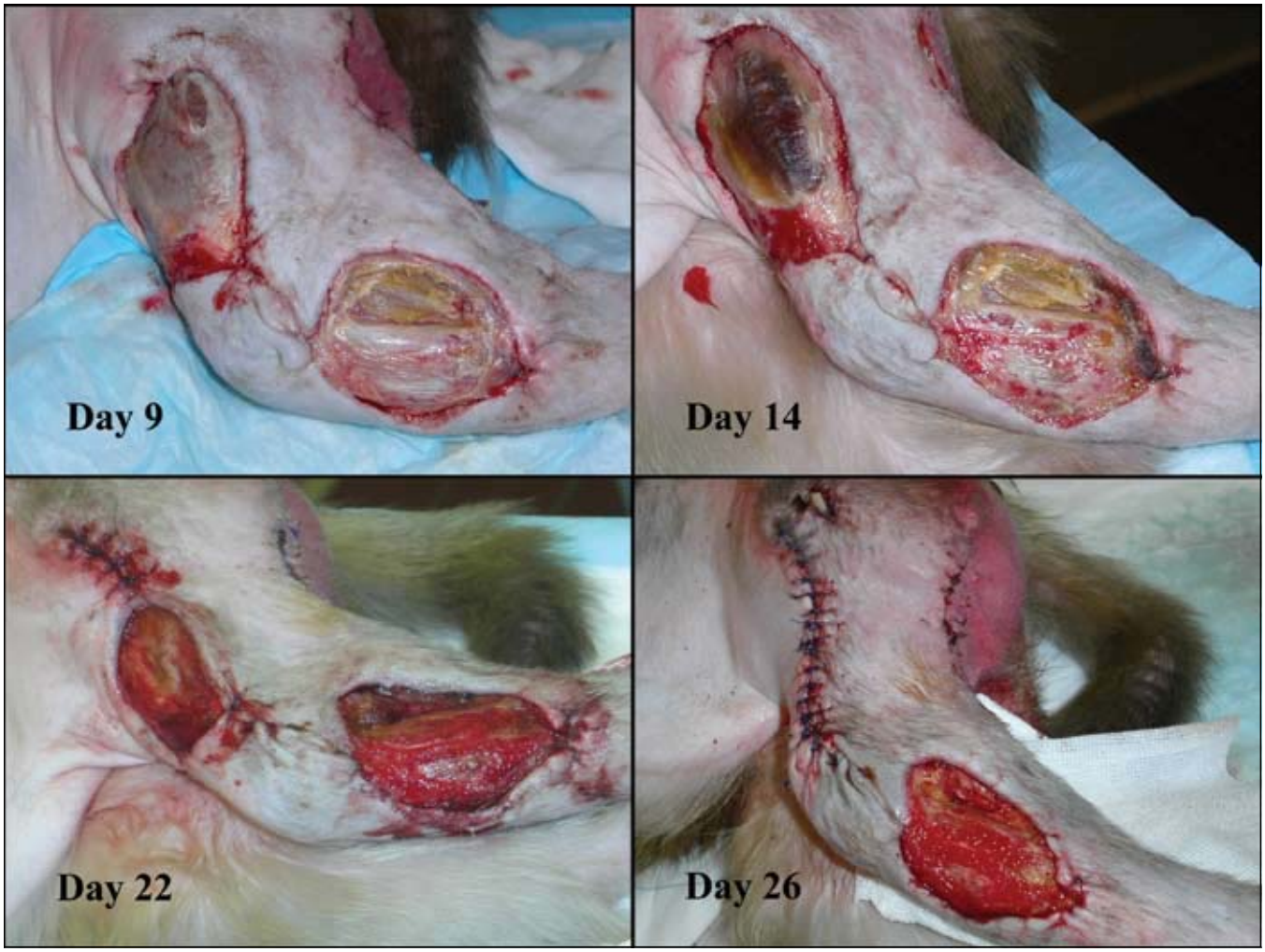

Figure 6. Day 9: appearance of the thigh wound 2 d after extensive surgical debridement and removal of initial wet-to-dry bandages. The regions of necrotic muscle and fibrin are evident by their yellow appearance in the wound bed. Stifle circumference is improved (24.5 cm) but remains abnormal. Day 14: 7 d after debridement. Stifle diameter is normal (22 cm). Granulation tissue is present in the lower halves of both wound beds. Conversely, black, dry leathery tissue is present on proximal portions of these wounds, suggestive of further necrosis and possibly continued infection. Day 22: margins of the lateral thigh wound were trimmed and closure started at the proximal and distal aspects of this wound. Note the granulation tissue in both wounds has increased substantially from day 14. Day 26: lateral thigh wound is closed with the aid of a Penrose drain. The shape and orientation of this wound permitted closure in 2 surgical procedures. The calf wound bed now is covered completely in granulation tissue.

approximation of wound margins to facilitate closure. This technique was repeated on days 29, 35, and 42, at which time bandaging was discontinued, walking sutures were removed, and the wound, now 1 cm wide, was allowed to heal by second intention. The animal's use of the affected leg improved during the weeks after removal of the bandage. At 8 mo post-treatment, the only noticeable change is a smaller left pelvic limb due to the surgically debrided muscle tissue (Figure 8). Range of motion in the stifle is normal. At this time, experimental use of this animal has resumed, and it remains clinically normal and otherwise healthy in our animal colony.

Discussion

This case study is the first report of a *Clostridium* necrotizing soft tissue infection in a nonhuman primate maintained in a research colony. The diagnosis of *C. perfringens* gas gangrene was confirmed by culturing the causative agent, by radiographic evidence of modest gas production, and by histologic confirmation of myonecrosis. Pathogenesis of gas gangrene requires altered environmental conditions (that is, compromised tissue perfusion) that permit clostridial spores to induce disease.[9] Tissue damage combined with impaired blood flow results in decreased oxygen tension, facilitating anaerobic growth.[9] In humans, coinfection with *Streptococcus* spp. or *Staphylococcus* spp. is common.[6,22] Similar to human polymicrobial infections, we isolated *S. aureus* and the anaerobe *P. intermedia* from these wounds. Although both bacterial species have been associated with opportunistic infections, they also are part of normal skin and oral flora.[4] Our antibiotic selection targeted these organisms while the focus was maintained on the presumed primary pathogen *C. perfringens.* Although we were unable to identify the specific source of the bacteria, we hypothesized that the animal's own gastrointestinal tract was the source of the clostridial spores necessary for infection. Further, the series of IM injections in the affected leg 1 wk prior to presentation provided opportunity for both tissue injury and spore inoculation. The initial series of 3 daily morphine injections was administered in the left thigh because this primate was trained to present this leg to technicians. In addition, the animal received 4 injections in the left thigh during the dose–response study 8 d prior to presentation.

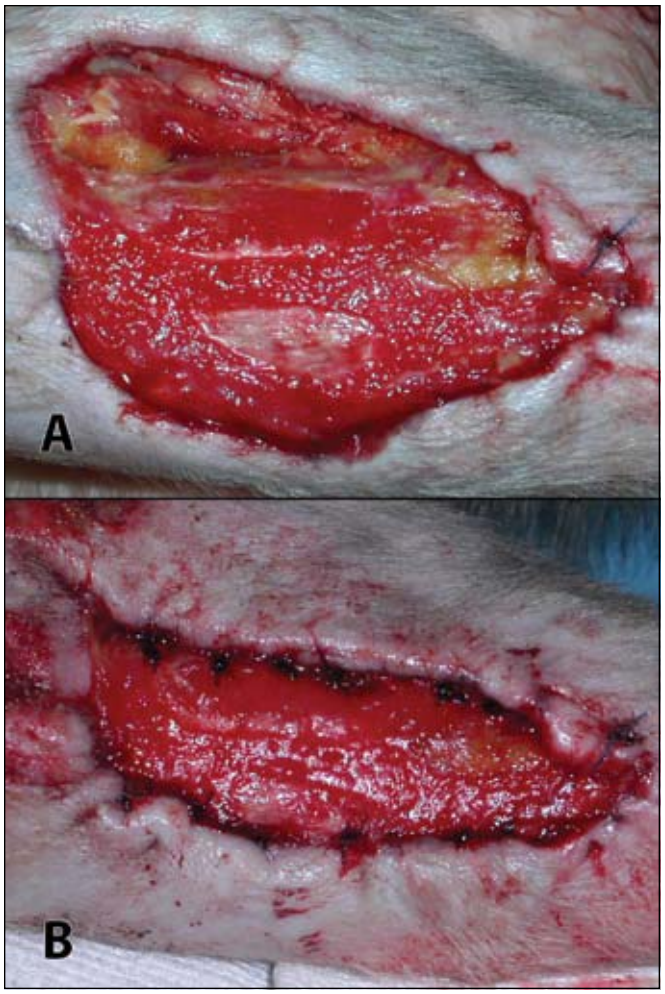

Figure 7. Day 29 (A) before and (B) after initial walking-suture procedure. The wound margins were undermined bluntly, and the skin was stretched toward the center of the wound and sutured to the underlying granulation tissue.

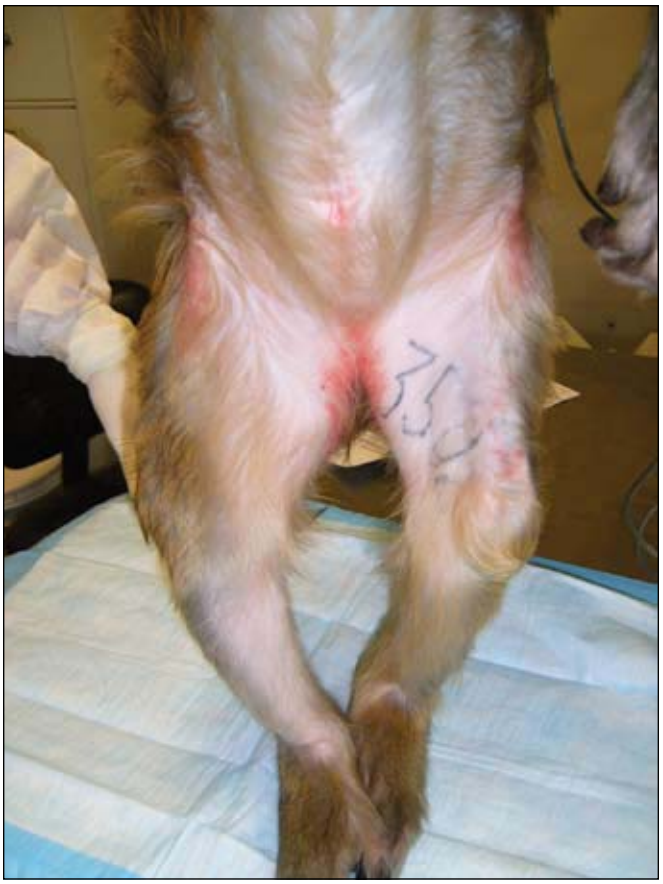

Figure 8. Eight months after injury. The primate has regained full use of the affected leg.

This experimental protocol has been used for several years in this macaque colony, including a similar study with the same animal 2 y prior. This case was the first time any complication potentially due to experimental protocol was encountered.

Upon inoculation in the appropriate environment, *Clostridium* organisms multiply rapidly, producing a variety of toxins including hyaluronidase, collagenase, α toxin, and θ toxin. Hyaluronidase and collagenase promote local tissue necrosis, furthering the spread of infection; α toxin is locally cytotoxic and hemolytic, increases capillary permeability, and stimulates production of tumor necrosis factor and platelet-activating factor.[15] Systemic disease is attributed to direct and indirect effects of these toxins. In addition, α and θ toxin promote renal failure and cardiovascular shock by their synergistic contribution to hypotension, hypoxia, and reduced cardiac output.[15] The animal described in this case was not febrile, had a normal white blood cell count, and did not exhibit biochemical evidence of organ failure. An increased creatine phosophokinase and decreased hematocrit were noted during the first week of therapy; we attributed these changes to toxin production resulting in red blood cell lysis and muscle necrosis. The animal was neither immunosuppressed nor diabetic, both of which are conditions that increase the risk for anaerobic wound infections in humans.[2,6,9,18]

Early radical intervention was key to the successful management of this infection. After initial examination, clostridial infection was a primary differential diagnosis. Penicillin was added to the antibiotic regimen prior to final culture and sensitivity results, in light of the high index of suspicion for anaerobic infection. With confirmation of *Clostridium* from the reference microbiology laboratory, we investigated a more refined selection of antibiotic therapies. Penicillin has long been considered the drug of choice for *Clostridium* infection, but other appropriate antibiotic options (for example, clindamycin, rifampin, and metronidazole) have been documented.[14,16] We added clindamycin to the antibiotic regimen because it reduced in vitro expression of α toxin and demonstrated increased efficacy in mice experimentally infected with *C. perfringens*.[15,16] This difference may be attributable to the mechanism of action of clindamycin, which rapidly stops bacterial toxin production.[14] Interestingly, in a published series of experiments in mice, penicillin showed the lowest survival and poor attenuation of α toxin.[16] No controlled studies have compared antimicrobial efficacy in humans with clostridial infections.[14] Penicillin was continued in combination with clindamycin on the basis of reports in the human literature;[12,14,16] further, the regimen was well tolerated in this animal.

We initiated surgical debridement in response to the preliminary anaerobic culture report of *Clostridium* spp. (Day 4). Debridement is a cornerstone of successful management of necrotizing soft-tissue infections.[6,8,13,14,18,20] High levels of bacteria (>10^5/g tissue) inhibit healing and use necrotic tissue as a growth medium.[13] The removal of bacteria and necrotic tissues expedite the healing process by increasing the availability of growth factors promoting the formation of granulation tissue and subsequent epithelialization of the wound bed.[10,13,23] Be-

tween surgical debridements, we used mechanical debridement in the form of wet-to-dry bandages to remove residual exudate and necrotic tissues. As exudate and debris declined, the primary bandage was changed to a nonadherent hydrogel dressing to preserve the newly forming granulation tissue. The combination of surgical and mechanical debridement was essential to the successful management of this animal's wounds.

In humans, hyperbaric oxygen (HBO) therapy is used for a variety of conditions, including clostridial infections.[19,21] HBO is bactericidal to *C. perfringens* and, in conjunction with surgical and antimicrobial therapy, has proven beneficial in human patients.[7,19] HBO inhibits clostridial growth and inhibits α toxin production.[20] In an experimental study of *C. perfringens* myonecrosis in dogs, the survival rate was 50% in animals treated with antibiotics alone, 70% in animals treated with antibiotics and surgery, and 95% in animals treated with antibiotics, surgery, and HBO.[3] Although HBO is an appropriate and effective therapeutic adjunct, we had no access to this equipment in our nonhuman primate facility.

Nonhuman primates often undergo intramuscular injections for sedation and drug delivery. Experimental requirements, the personal safety of the animal handler, and noncompliance with oral formulations often make subcutaneous and IM routes of drug delivery a preference for these species. Although complications from parenteral injections are rare, the potential for pathogen introduction cannot be disregarded. Separate muscle groups, ideally separate limbs, should be used when multiple injections are administered. As we continued to monitor the healing process in this animal, the laboratory staff was trained in more appropriate methods of administering IM injections.

The cooperative behavior of this rhesus macaque was essential to the success of the intensive veterinary care required for wound treatment and healing. Throughout multiple bandage changes, debridement surgeries, and surgical drain placements, the macaque did not compromise any of the wound management materials.

This case study provides the first report of *C. perfringens* infection in a nonhuman primate. The infection most likely was procedurally induced through repeated IM injections near the same site. The rhesus macaque we described regained full use of the affected leg through a combination of aggressive medical and surgical intervention. Currently this primate is participating on an approved research protocol and remains in sound health and good physical condition.

Acknowledgments

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