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Case Study

Use of Fluconazole-impregnated Beads to Treat Osteomyelitis Caused by *Coccidioides* in a Pigtailed Macaque (*Macaca nemestrina*)

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A 3-y-old male pigtailed macaque (*Macaca nemestrina*) presented for swelling of the left distal forearm and decreased use of the arm. The monkey had been raised at an indoor-outdoor facility in Arizona and transferred to an indoor facility in Washington 2 mo prior to presentation. A preliminary diagnosis of fungal osteomyelitis of the radius was made based on radiographs and *Coccidioides* titers. In addition to systemic antifungal treatment, surgery was performed to debride the bony lesion and implant polymethylmethacrylate beads impregnanted with the anti-fungal fluconazole. Histologic examination of the debrided material confirmed the diagnosis of fungal osteomyelitis. The surgical procedure resulted in clinical improvement, as evidenced by weight gain and decreased *Coccidioides* titers. The beads were removed in a second surgery, and the bony lesion completely resolved. With continued systemic fluconazole treatment, the monkey remained healthy with no further evidence of osteomyelitis. *Coccidioides* is an emerging pathogen that causes significant morbidity and mortality in both humans and animals. Bone infections can be resistant to systemic treatment, and the implantation of fluconazole-impregnated beads may offer a successful treatment strategy for fungal osteomyelitis.

Abbreviations: PMMA, polymethylmethacrylate; VF, Valley Fever

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Introduction

Coccidioidomycosis, commonly known as Valley Fever (VF), is a fungal disease caused by *Coccidioides immitis* or *Coccidioides posadasii*.[41] VF has been reported in a wide variety of domestic species including dogs, cats, and horses,[3,9,12,20,21,39,40] and in wildlife species such as bats, wallabies and kangaroos, tapirs, and dolphins.[37,40] Naturally-occurring VF infections have also been found in multiple species of nonhuman primates[29] (NHP), including rhesus,[7,31] cynomolgus,[35] and Japanese macaques[17] in Texas, and rhesus and bonnet macaques in California.[4] NHP have also been used as experimental models to study various aspects of VF,[5] including evaluation of potential VF vaccines.[25] Recrudescence of VF was recently reported after treatment of a pigtailed macaque.[18] When a macaque's history includes housing in a VF-endemic area, coccidioidomycosis must be considered in the differential diagnosis of lesions consistent with this diagnosis.

Osteomyelitis is a difficult condition to treat because of the poor penetration of systemic antibiotics into bone.[26] One option for the treatment of chronic osteomyelitis is the implantation of antibiotic-impregnated polymethyl methacrylate (PMMA) spheres.[6,26] While initial recommendations included the removal of the PMMA implants at 6 to 8 wk in humans,[26] some patients have shown clinical improvement when the PMMA spheres remain in place.[6] Moreover, the use of antibiotic-impregnated PMMA has been used successfully to treat osteomyelitis in a rhesus macaque (*Macaca mulatta*).[27] Nevertheless, because of concerns regarding bone healing, the use of biodegradable materials to replace the PMMA has also been studied in both human and veterinary medicine.[16,26]

Antibiotic-impregnated calcium sulfate beads are frequently used in the management of bacterial infections associated with prosthetic implants in humans.[1,2] The beads are more successful if the implant is removed than if it remains in place.[1] Fungal infection is rare around these implants and is usually treated with systemic antifungal agents[2,10,24,28] either with or without local antifungals.[24] Long-term antifungal therapy both before and after surgical debridement is necessary for successful treatment.[43] Voriconazole-impregnated calcium sulfate beads[32] and amphotericin B-impregnated PMMA[44] have been used to successfully treat fungal infections associated with total knee arthroplasty. We are not aware of any reports of antifungal-impregnated implants for the treatment of osteomyelitis in veterinary medicine.

Case Report

A 3-y-old male pigtailed macaque (*Macaca nemestrina*) presented for swelling of the left distal forearm and decreased use of the arm. The monkey had been raised at an indoor-outdoor facility in Arizona as part of a specific pathogen free breeding colony on a University of Washington IACUC-approved protocol. The macaque was confirmed negative for tuberculosis, simian immunodeficiency virus, simian retrovirus, simian lymphotropic virus, and *Macacine alphaherpesvirus-1* and then

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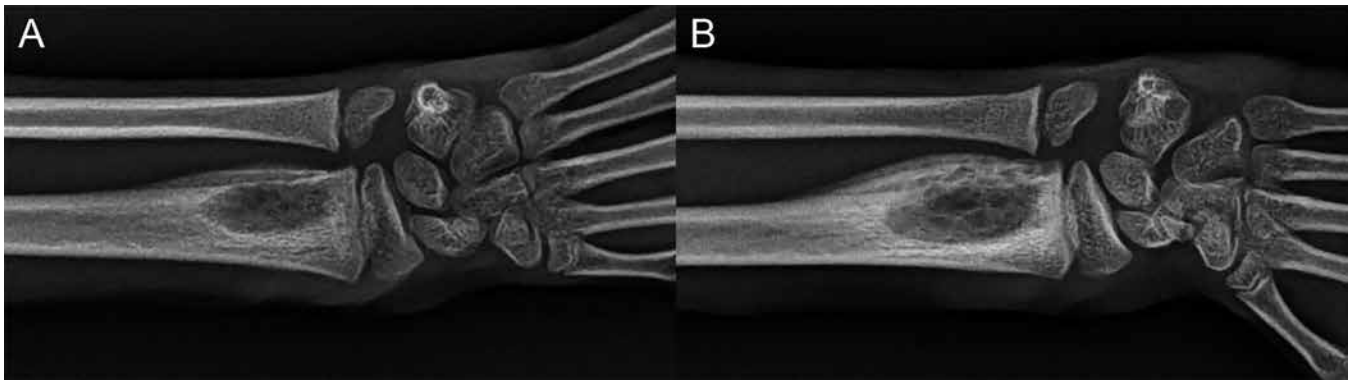

Figure 1. Fungal osteomyelitis in the distal radius of a *M. nemestrina*. A) Initial presentation. A single osteolytic lesion is seen within the left radius, with cortical thinning and periosteal reaction on the lateral surface. B) After 4 wk of systemic fluconazole treatment, there was increased periosteal proliferation with loss of cortical definition on the lateral surface, irregular density within the lesion, and decreased definition at the margins of the lesion.

transferred to an indoor facility in Washington 2 mo before clinical presentation. At both AAALAC-accredited facilities, the monkey was maintained under ABSL-2 conditions, fed a commercial diet (LabDiet 5045 or 5049, St. Louis, MO) supplemented with fresh produce twice a day, and given water ad libitum. Environmental enrichment consisted of social contact (full or protected contact depending on compatibility with partner), manipulanda, and foraging opportunities. Five months before presentation, the macaque experienced an episode of respiratory illness characterized by rales on auscultation. At that time, radiographs revealed a mild bronchial pattern in the dorsocaudal lung lobes; serologic titers for *Coccidioides* were negative at that time. Treatment included albuterol (0.66 mg/kg PO BID) and nutritional support, and clinical signs resolved within 2 wk.

At the time of presentation for the forearm swelling, physical exam findings included 10% weight loss (from 3.0 to 2.7 kg) and intermittent expiratory stridor and stertor. A complete blood count revealed a mild monocytosis (1.8 thou/mL) and serum chemistry displayed a moderate hyperglobulinemia (6.7 g/dL) and hypoalbuminemia (2.1 g/dL). Radiographs of the left arm revealed a lytic lesion in the distal radius (Figure 1A). Analgesics (buprenorphine-SR at 0.2 mg/kg SC and meloxicam at 0.2 mg/kg PO loading dose followed by 0.1 mg/kg SID) and antibiotics (amoxicillin/clavulanate at 12 mg/kg PO BID) were administered initially. Serum samples were submitted to a commercial laboratory (ProtaTek Reference Laboratory, Mesa, AZ). Titers for *Coccidioides* were positive at 1:64 (IgG) and 1:16 (IgM). Based on these results, a preliminary diagnosis of fungal osteomyelitis was made, and therapy with fluconazole (8 mg/kg PO BID) was initiated.

Although the use of the arm improved with treatment, the swelling did not resolve. Weight loss stopped, but body weight did not increase as expected for a young, growing macaque. Whole body radiographs were then obtained to determine if any other bony lesions were present. All other bones appeared to be unaffected; however, the lesion in the left radius had progressed (Figure 1B). Therefore, the decision was made to surgically debride the lesion and implant PMMA beads containing fluconazole. This technique had been used in humans for treatment of *Candida* infection after hip replacement.[8] Appropriate materials were identified, ordered, and received, and surgery was performed approximately 6 wk later.

Materials and Methods

On the day of surgery, anesthesia was induced with ketamine (15 mg/kg IM) and maintained with inhalational isoflurane. A 3 cm skin incision was made over the dorsal aspect of the left radius at the level of the distal metaphysis centered approximately on the firm swelling on the radius. The subcutaneous tissue was dissected between the second and third extensor tendon compartments, and through the periosteum to the bone. During subcutaneous dissection, a cystic soft tissue was seen protruding from a cortical defect in the dorsal surface of the radius (Figure 2A, B). A fine needle aspirate of the lesion was obtained and submitted to the UW Research Testing Services for routine aerobic bacterial culture. Prior to submission, a fresh smear was made of the aspirate and stained with Wrights-Giemsa for cytology. The soft tissue structure was excised, fixed in formalin, and submitted for routine H and E histopathology. The bone was burred away at the site of the cortical defect to expose the medullary cavity (Figure 2C). The cancellous bone was curetted, and the medullary cavity was debrided of all visible abnormal tissue (Figure 2D).

Fluconazole (3 g powder) was mixed aseptically with one package (40 g) of bone cement powder (Palacos LV, Heraeus Medical, Yardley, PA). Aliquots of this mixture were catalyzed by the addition of methylmethacrylate/toluidine per the manufacturer's instructions and formed into PMMA beads (approximately 0.2 g/bead) by hand. Two beads were inserted into the excavated medullary cavity (Figure 2E) and all the subcutaneous tissues were closed in a single layer with absorbable suture. The skin incision was closed with simple continuous absorbable suture and infiltrated with bupivacaine. Radiographs were obtained immediately before and after surgery (Figure 3A, B). Analgesics (one injection of buprenorphine-SR at 0.2 mg/kg SC and meloxicam at a loading dose of 0.2 mg/kg PO, followed by 0.1 mg/kg SID) and antibiotics (cephalexin at 25 mg/kg PO BID) were administered after surgery. The arm was bandaged for a total of 5 d after surgery, with a bandage change at day 3.

Fifteen weeks after the initial surgery, a second surgery was performed to remove the nonresorbable fluconazole-cement beads. Surgical preparation and approach were essentially the same as in the initial surgery. Additional blunt dissection was required due to the presence of scar tissue. The beads were partially exposed on the dorsal surface of the radius (Figure 4A) , as indicated in preoperative radiographs (Figure 5A). A surgical drill was used to remove bead material, taking care not to damage healthy bone. Once each bead was loosened, the remaining bead fragments were removed using a periosteal elevator (Figure 4B). The resulting cavity was flushed copiously with sterile saline, and the subcutaneous tissue and skin were closed routinely and were infiltrated with bupivacaine. Analgesics (one

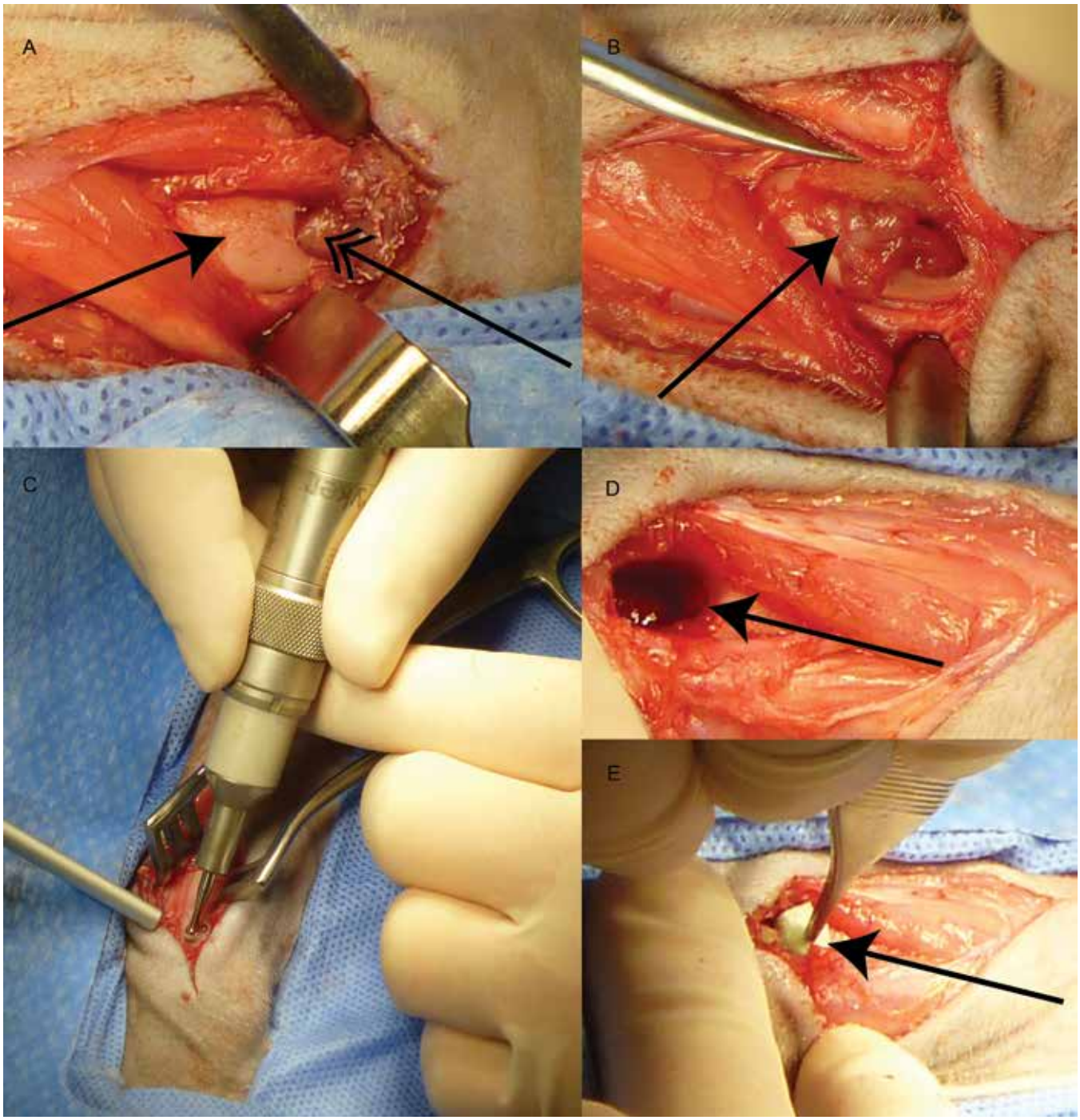

Figure 2. Bead implantation surgery. A) Incision over dorsal surface of distal radius showing both bony and soft tissue swelling. Solid arrow = radius bone, double arrow = soft tissue in bony defect. B) Pyogranulomatous tissue with reactive fibroplasia (arrow) partially removed from the medullary cavity of the distal radius, C) Expansion of the cortical bone defect using a surgical drill, D) Medullary cavity (arrow) after debridement, E) Fluconazole-impregnated PMMA beads (arrow) within the medullary cavity.

injection of buprenorphine-SR at 0.2 mg/kg SC and meloxicam at 0.2 mg/kg PO loading dose followed by 0.1 mg/kg SID) and antibiotics (cephalexin at 25 mg/kg PO BID) were administered after surgery. Recovery from surgery was uneventful.

Results

The monkey did well after the first surgery with no evidence of discomfort and was using the arm normally within 2 wk afterwards. Bacterial culture of the soft tissue cyst was negative. Histopathology revealed multifocal granulomas and pyogranulomas with reactive fibroplasia. Fragments of spherules were seen on histopathology, and intact, variably sized, double-walled, nonbudding spherules were seen on cytology, consistent with *Coccidioides* organisms. Fungal culture was not attempted, because germination of arthroconidia could pose a laboratory hazard.[36] While BSL-2 practices are appropriate for handling animal tissues that may contain *Coccidioides*, BSL-3 practices are recommended for manipulating cultures.[36] The cytologic appearance, in combination with the serologic titers and history of residence in an endemic area, combined for

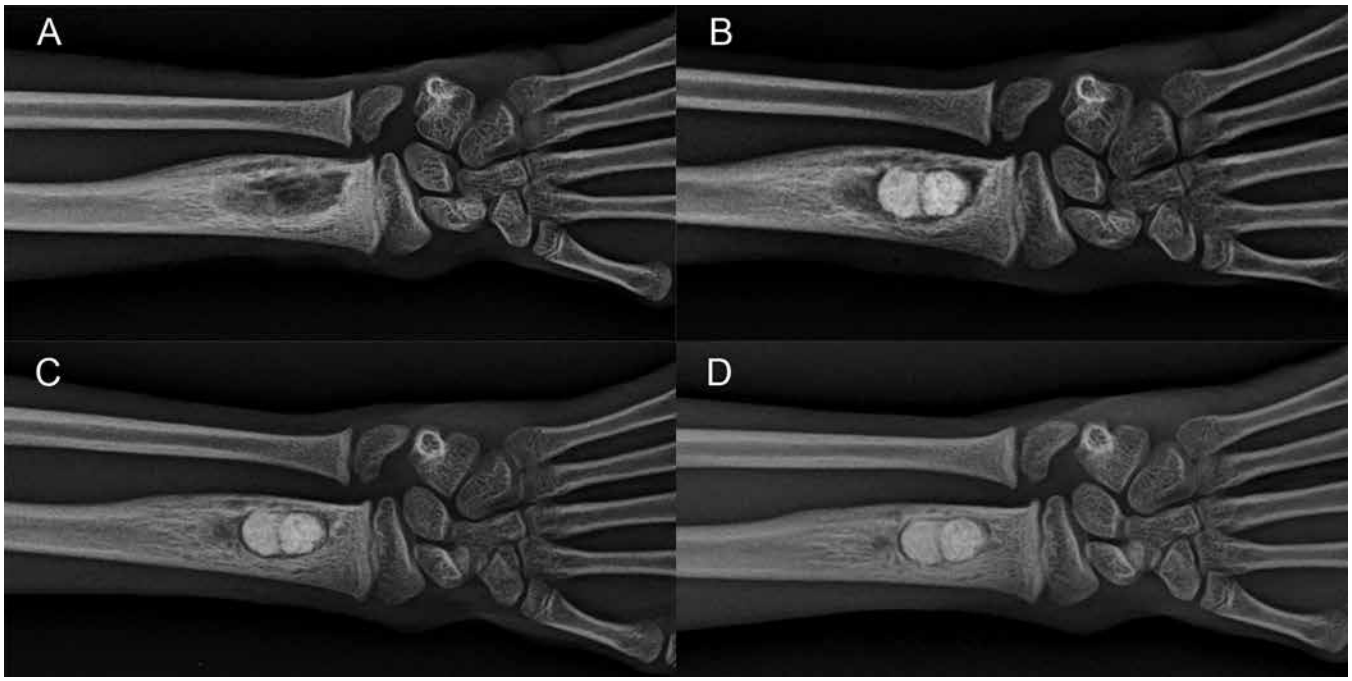

Figure 3. Radiographs associated with bead insertion. A) Preoperative, approximately 10 wk after initial presentation. After additional systemic fluconazole treatment while preparing for surgery, the periosteal reaction appears to be resolving. However, the osteolytic lesion in the radius is unchanged in size. B) Immediately after surgery. Placement of 2 fluconazole-impregnated beads within the radius was confirmed. An area of radiolucency is present proximal to the beads. C) 4 wk after implantation. Radiodensity has increased around the beads. D) 8 wk after implantation. Radiodensity has further increased around the beads and has the appearance of trabecular bone.

a presumptive diagnosis of coccidioidomycosis. Follow up examination one month after surgery revealed healing of the surgical incision and a decrease in external swelling of the arm. Serum globulin had returned to normal (3 g/dL), and albumin was higher (2.5 g/dL), although still below the reference range for this species. *Coccidioides* titers were measured periodically and fell over time, although some regression occurred, and a cure, as indicated by negative titers, was not achieved within one year (Table 1). Radiographs (Figure 3C, D) showed a consistent increase in bone density around the beads over the 8 wk period after surgery.

After the surgery to remove the beads, the monkey showed no evidence of discomfort, was using the arm normally within one week, and remained in good health, as evidenced by steady growth after surgery (Figure 6). The animal was euthanized more than a year later (510 d after bead removal) as part of an unrelated research project. At necropsy, the bone appeared radiographically and histologically normal, although a positive VF titer at that time indicated a reservoir of *Coccidioides* organisms within the animal.

Discussion

We used surgical implantation of fluconazole-impregnated PMMA beads to treat fungal osteomyelitis in a pigtailed macaque, which resulted in resolution of the bone lesion. In humans, most deaths due to VF occur in California and Arizona.[23]

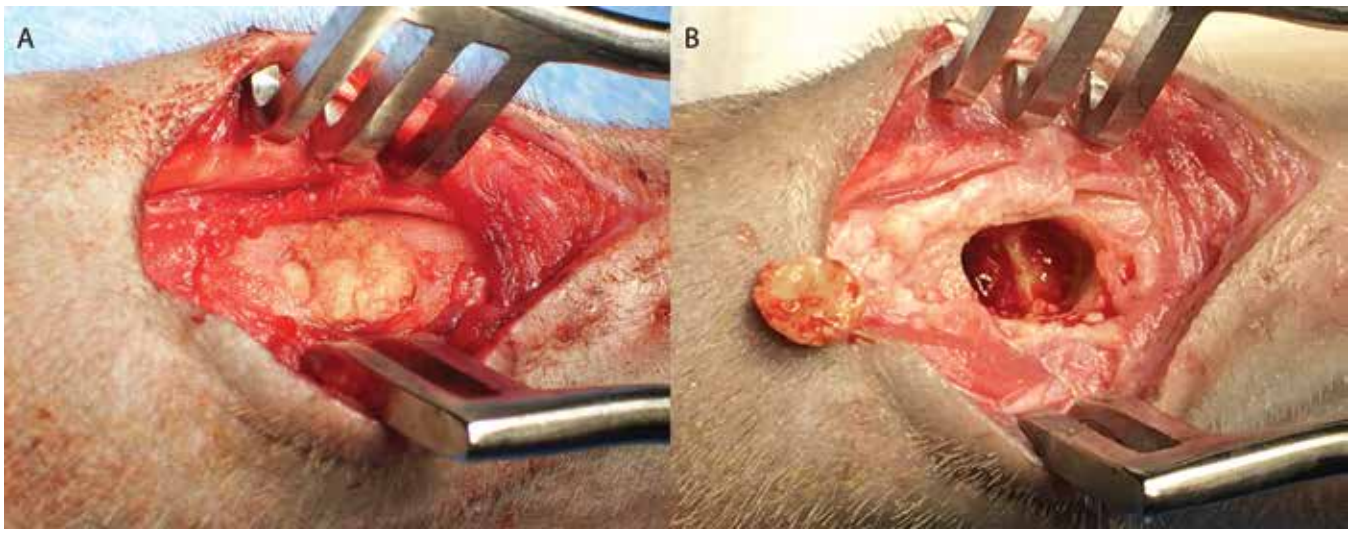

Figure 4. Bead removal surgery. A) Beads visible in situ within radius, B) Partial bead that had been removed shown next to cavity after bead removal. Note ridge in center of cavity where bone had remodeled around beads.

Table 1. Changes in *Coccidioides* titers over time.

Day	IgG Titer Result	IgG Titer Value	IgM Titer Result	IgM Titer Value
Prior to presentation	–	< 1:1	–	< 1:1
Presentation	+	1:64	+	1:16
Implant + 30	+	1:16	+	1:2
Implant + 63	+	1:4	+	1:1
Implant removal (Implant + 106)	+	1:2	–	< 1:1
Removal + 33	+	1:2	–	< 1:1
Removal + 128	+	1:2	–	< 1:1
Removal + 177	+	1:4	–	< 1:1
Removal + 239	+	1:1	–	< 1:1
Removal + 338	–	< 1:1	–	< 1:1
Removal + 350	–	< 1:1	–	< 1:1
Removal + 510	+	1:2	–	< 1:1

The first sample shown was collected 5 mo prior to presentation. Later sample time points are indicated by the number of days after specific events.

thus indicating a risk of naturally occurring VF infections in a captive population of pigtailed macaques in Arizona. Because the primary route of exposure is inhalation, the organ most commonly affected by VF in all species is the lung, although dissemination to multiple organs may occur. Bone is frequently reported as a secondary site of infection; in dogs, the appendicular skeleton is more often affected, while in humans and NHPs, the axial skeleton appears to be more commonly affected.[29]

Treatment for VF most commonly consists of antifungal medication and supportive care. Fluconazole is the most frequently prescribed antifungal in both humans and animals, based on its effectiveness, cost, and the feasibility of once-a-day dosing.[40,42] However, fluconazole is less effective in treating disseminated VF as compared with pulmonary VF, and surgical intervention is often necessary to treat skeletal VF.[22,38,41] Surgical procedures used in humans with VF include incision and drainage, surgical debridement, synovectomy, and bone resection.[22,38]

Although coccidioidomycosis is most prevalent in southwestern state of the U.S., its prevalence is increasing in Washington state.[13,33] The animal in this report was housed indoors in the western part of the state, where the causative organism is not known to be naturally present, making it unlikely that the initial infection was acquired in Washington and more likely that the initial infection was acquired in Arizona prior to transport. No clinical signs of VF were apparent on physical examination during the first month after arrival in Washington. This is consistent with a previous report that VF can remain latent for several months prior to development of clinical signs in macaques.[31] In humans, extrapulmonary dissemination of VF is more common in immunocompromised patients[30] and in monkeys, shipping stress can result in immune dysregulation,[15] suggesting that the shipment from Arizona to Seattle may have contributed to development of the bony VF lesion. Travel history must always be considered when developing differential diagnoses.

The standard treatment for VF is systemic antifungal medication. In our case, the initial treatment arrested weight loss but did not result in resumption of normal growth, and did not improve the osteomyelitic lesion. After failure of medical therapy, we determined that surgery would be necessary to address the bone infection. Options for surgery included debridement, debridement with implantation of an antifungal agent, or amputation. We elected to perform debridement with implantation of an antifungal agent to balance the probability of success with the least impact to the animal. Our approach was successful, as measured by improvement in bone integrity both

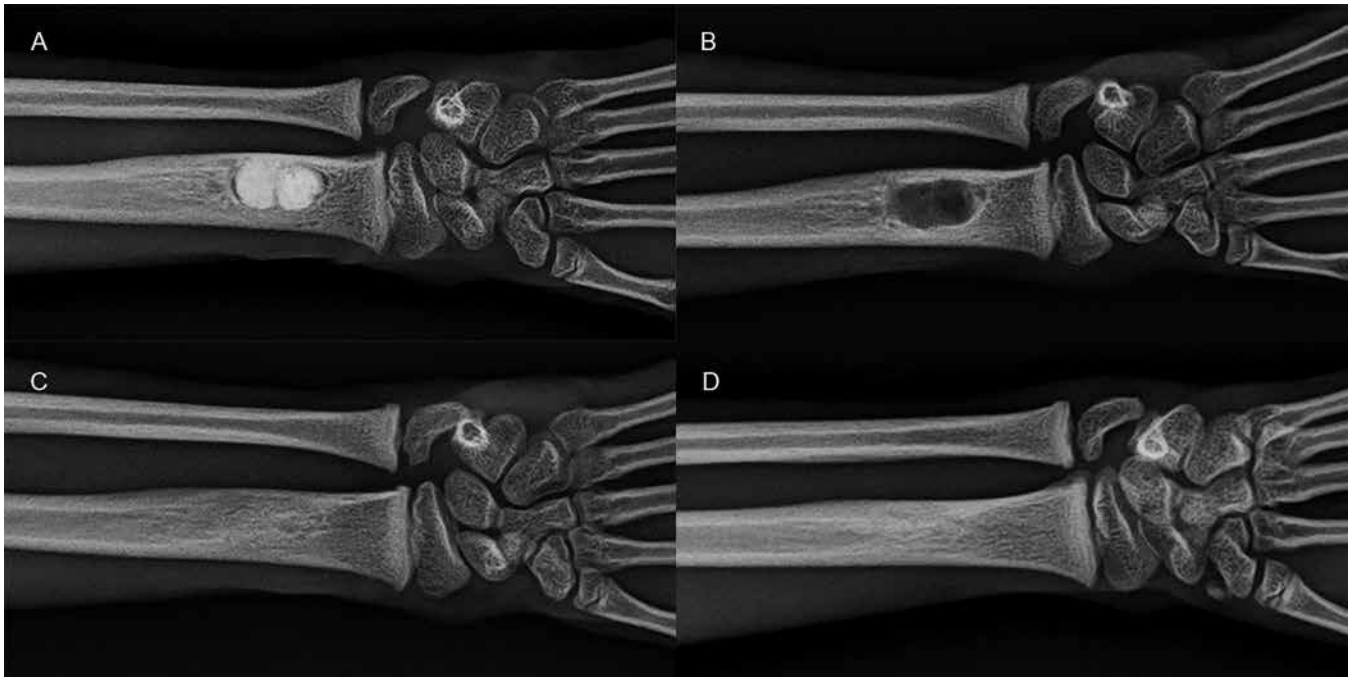

Figure 5. Radiographs associated with bead removal. A) Preoperative on the day of bead removal, 15 wk after the initial surgery. Beads appear unchanged and are surrounded by trabecular bone. B) 4 wk after bead removal. The void where the beads were persists, but the surrounding bone has normal trabecular architecture. C) 4 mo after bead removal. The defect where the beads had been has closed over. Some irregularity of the cortical/trabecular margins persists. D) 8 mo after bead removal. The bone shows normal trabecular architecture and smooth cortical contours.

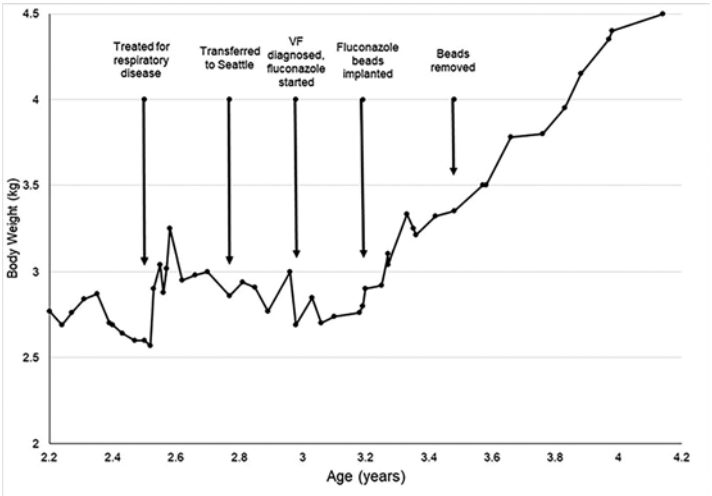

Figure 6. Body weight changes over time in relation to clinical events. VF = Valley Fever. A juvenile *M. nemestrina* is expected to steadily gain weight between 2 and 4 y of age. Weight gain stopped during clinical respiratory illness but rebounded after symptomatic treatment. Weight gain stopped again around the time of transfer to Seattle, and the diagnosis of VF was made after weight loss. Systemic treatment with fluconazole stabilized weight but weight gain still did not occur. After surgery to debride the fungal bony lesion and implant fluconazole-impregnated beads, normal weight gain resumed. Weight gain continued uninterrupted after surgical removal of the beads.

radiographically and as seen directly at surgery, as well as by the decrease in VF titers and resumption of normal weight gain.

We cannot determine how much of the clinical improvement (decreased VF titers, increased weight gain, improved the radiographic appearance of the radius) can be attributed to the implantation of fluconazole-impregnated beads as compared with manual debridement of infected bone. When a similar procedure was used in humans, the local concentration of fluconazole was measured in fluid collected from external drains.[8] However implanted drains are not feasible in a NHP due to the risk of interference by the patient. Therefore, we were not able to confirm that the beads provided an effective local concentration of fluconazole. However, we can state that we saw no adverse effects with this procedure.

PMMA bone cement was used as the vehicle to provide fluconazole locally. We chose this method based on published data[8] and our experience using it in other NHP implants. We removed the beads to allow regrowth of cancellous bone to maximize bone integrity at the site of the lesion, as the distal radius is the most common site for fractures in humans.[11] Removal of the implants required a second surgery, which is a source of stress that could affect the infection. Identification of a biocompatible material that could be used as a vehicle to maintain fluconazole locally, but would be resorbed slowly over time, would be a significant refinement of the described technique. Calcium sulfate has been used for antibiotic delivery,[14,19,34,45] and we tried this approach in a different macaque with a similar history. Unfortunately, that animal suffered a fracture after surgery, and we cannot determine whether that was related to excessive debridement in a smaller bone (the ulna) or to a factor related to the implant material. Given the effect of technique on bone integrity, we do not recommend the bead implantation technique for multifocal bone infections.

In conclusion, this report describes treatment of isolated osteomyelitis due to coccidioidomycosis in a pigtailed macaque by the use of implanted fluconazole-impregnated beads. While the lack of a negative titer suggests that the infection was not completely eradicated, the osteomyelitis lesion identified by radiology was successfully resolved. This technique is likely to be successful in other species as well and may provide an alternative to euthanasia or amputation.

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