

Systemic *Mycobacterium terrae* Infection in an Eastern Box Turtle, *Terrapene carolina carolina*

Hillary Noyes¹, DVM, Ellen Bronson², DVM, Sharon L. Deem³, DVM, PhD, DACZM, Carlos Sanchez³, DVM, Suzan Murray³, DVM, DACZM

1. VCA San Diego Emergency Animal Hospital and Referral Center
San Diego, CA 92129, USA

2. Maryland Zoo in Baltimore, Druid Hill Park, Baltimore, MD 2121, USA

3. Department of Animal Health, Smithsonian National Zoological Park,
3001 Connecticut Ave NW, Washington, DC 20008, USA

ABSTRACT: An adult female eastern box turtle, *Terrapene carolina carolina*, presented with a proliferative soft tissue mass adjacent to the tail. Initial blood work revealed a moderate leukocytosis. Over the next five months, the lesion progressed despite treatment with a combination of surgical debridement and antibiotic therapy. Ultimately, *Mycobacterium terrae* was isolated from the locally extensive cutaneous lesion. Euthanasia was elected, and necropsy revealed disseminated mycobacteriosis.

KEY WORDS: box turtle, *Terrapene carolina*, *Mycobacterium terrae*, mycobacteriosis, granulomatous disease, zoonoses.

INTRODUCTION

Mycobacteriosis affects animals across many taxa. Although mycobacteriosis is considered relatively uncommon in reptiles, several cases of infection with atypical *Mycobacterium* spp. have been reported in the literature (Rhodin and Anver, 1977, Friend and Russell, 1979, Quesenberry, *et al*, 1986, Olsen, *et al*, 1987, Ariel, *et al*, 1997, Morales and Dunker, 2001, Hernandez-Divers and Shearer, 2002, Greer, *et al*, 2003, Orós, *et al*, 2003). This report describes the first published case of *M. terrae* infection in an eastern box turtle, *Terrapene carolina carolina*.

CASE REPORT

A 420 g, adult, female, captive eastern box turtle, *Terrapene carolina*, presented with a 1.5 cm diameter soft tissue mass dorsal and to the right of the tail on the day it was noticed by caretakers. The turtle had been housed at the National Zoological Park Reptile House for two years with two conspecifics in an exhibit containing mulch, dirt and a pool. The diet was considered adequate for the species. The turtle had had no previous medical problems. On initial examination, the turtle was alert, responsive, and in excellent body condition. A bright pink-red mass surrounded by a soft white substance was firmly attached to the skin at the tail base. Blood was collected from the subcarapacial sinus for complete blood count and serum biochemistry. The mass was aspirated with a 25 ga needle for cytologic evaluation. Two incisional biopsies, one of the white material and one of the mass, were collected at this time, the results of which were reported on Day 58. Pending results, the turtle was given lactated Ringers solution (1 ml/kg intracoelomic [IC]; Hospira, Inc. Lake Forest, IL) and ceftazidime (20 mg/kg IM q 48 hr x for 3 doses initially; GlaxoSmithKline, Research Triangle Park, NC) and admitted to the National Zoological Park vet-

erinary hospital. Initial differential diagnoses included granulation tissue from a healing traumatic injury, prolapsed cloacal tissue, neoplasia, infection or other inflammatory disease.

Blood work revealed mild leukocytosis ($13.4 \times 10^3/\mu\text{L}$ $15.1 \times 10^3 \times 2.3/\mu\text{L}$). Diff-Quik (VWR Scientific, Chicago, IL.) and Gram stained cytologic preparations of the fine needle aspirate showed rare gram-negative pleomorphic bacteria. The turtle remained hospitalized and continued to be bright. Due to a lack of visible improvement in the mass, it was surgically removed using local anesthesia (Xylocaine 2% jelly, Astra USA, Inc. Westborough, MA) on Day 8. The turtle was monitored off-exhibit for two weeks.

By Day 19, a 5 – 10 mm deep, irregular ulcer filled with a thick yellow exudate had formed at the surgical site. Exudate was submitted for aerobic and anaerobic bacterial culture and sensitivity. Empirical treatment with ceftazidime was initiated (20 mg/kg IM q 72 hr x 7 doses) and switched to amikacin sulfate (2.5 mg/kg IM q 72 hr x 7 doses; Amiglyde-V, Fort Dodge Animal Health, Fort Dodge, IA) based on culture and sensitivity results, which yielded *Citrobacter brackii*, *Enterobacter cloacae* and non-group D *Streptococcus* sp.

The turtle was readmitted to the hospital on Day 29 for intensive wound care and treated with fluids (lactated Ringer's solution, 10 ml/kg IC q 72hr x 7 doses) and daily flushing of the lesion and topical triple-antibiotic ointment. The turtle continued to eat well and behave normally. Cytologic examination of the wound exudate with Diff-Quik, Gram and acid-fast stains showed predominantly gram-positive bacilli with rare acid-fast bacteria and a large amount of cellular debris. The exudate was also submitted for mycobacterial culture, the results of which were not available until Day 85. Cephalexin monohydrate (20 mg/kg PO SID x 7 d; Lupin Limited, Mumbai, India) was added empirically to the treatment regimen.

On Day 34 a 2-cm fracture of the carapace was noted.

Complete blood count and serum biochemistry results were not remarkably different than on presentation. No abnormalities were noted on dorsoventral and caudorostral radiographs. Due to lack of improvement on current therapy, antibiotic treatment was switched to ceftazidime (20 mg/kg IM q 72 hr x 5 doses)

On Day 36, the eleventh right marginal scute became loose and was easily removed with digital manipulation. Fluids (0.9% NaCl Injection, USP, B. Braun Medical Inc., Irvine, CA, 15 ml/kg SQ) and ketoprofen (2 mg/kg IM; Ketofen, Fort Dodge Animal Health, Fort Dodge, IA) were administered. Cytologic examination of the underlying exudate with gram and acid-fast staining showed Gram-positive bacteria and numerous acid-fast bacilli. Due to the repeated presence of acid-fast bacteria, mycobacteriosis was suspected.

On Day 39, the affected tissue was surgically excised. Anesthesia was induced with ketamine (7.5 mg/kg IM; Phoenix Scientific, Inc., St. Joseph, MO) and midazolam (1 mg/kg IM; Hospira, Inc., Lake Forest, IL) and maintained on isoflurane via endotracheal tube and facemask. Supplemental ketamine was administered during the procedure (10 mg/kg intralymphatic at 32 min, 25 mg/kg IM at 46 min and 35 mg/kg IM at 63 min). Four papillomatous masses were excised at the right caudal carapace. The turtle received fluids (lactated Ringer's solution 10 ml/kg SC SID), ketoprofen (2 mg/kg IM once), and enrofloxacin (5 mg/kg IM BID; Baytril, Bayer Healthcare LLC, Animal Health Division, Shawnee Mission, KS). After seven days, cephalexin (20 mg/kg PO SID x 7 doses) was added to the antimicrobial regimen and injectable enrofloxacin was replaced with oral (2.03 mg PO SID) to reduce muscle irritation. The surgical site was cleaned daily and packed with silver sulfadiazene cream (Silvadene 1% Cream, Hoechst Marion Roussel, Kansas City, MO). The turtle continued to exhibit normal behavior, eating and defecation habits.

Unfortunately, cutaneous infection was progressive. On Day 58, biopsy results from tissue collected on Day 1 became available. Histologic changes included necroulcerative dermatitis with edema and intralesional and intrahistiocytic acid-fast bacilli. On Day 64, amikacin (5 mg/kg IM once, then 2.5 mg/kg IM q 72 hr x 13 doses) was added back to the treatment regimen based on a report of a similar case (Greer *et al*; 2003). During the next month, local improvement was noted with development of new carapacial tissue. No renal compromise was evident on periodic blood work, so enrofloxacin and amikacin treatments were continued with concurrent fluid administration.

On Day 85, culture results revealed growth of *Mycobacterium terrae* complex. Due to the apparent clinical improvement over the previous month, treatment was continued.

The lesion continued to improve clinically over the next two months. On Day 152, however, necrotic tissue was noticed at the edges of the carapacial lesion and further surgical debridement elected. Anesthesia was induced with ketamine (10 mg/kg IM), medetomidine (0.01 mg/kg IM, Orion Corporation, Espoo, Finland) and propofol (6.68 mg/kg IV, Abbott Laboratories, N. Chicago, IL) and maintained on isoflurane (5 – 1%; Vedco, Inc., St. Joseph, MO). Supplemental ketamine was administered throughout the procedure (55 mg/kg IM at 41 min and 27 mg/kg IM at 79 min). Approximately, two-thirds of the lesion was necrotic and surgically resected.

160 days after initial presentation, due to persistent and worsening local disease (Figure 1) and zoonotic risk associated with handling, the turtle was sedated with 0.1 ml/kg propofol (intralymphatic) and euthanized with 1 ml sodium pentobarbital (Euthasol[®], Virbac AH, Inc., Forth Worth, TX) injected into the intervertebral sinus.

Post-mortem examination revealed a 12 x 9 x 9 mm cavity in the carapace with proliferation of the underlying tissue associated with the primary cutaneous lesion in addition to multiple granulomas in the liver, spleen, lungs and kidneys.

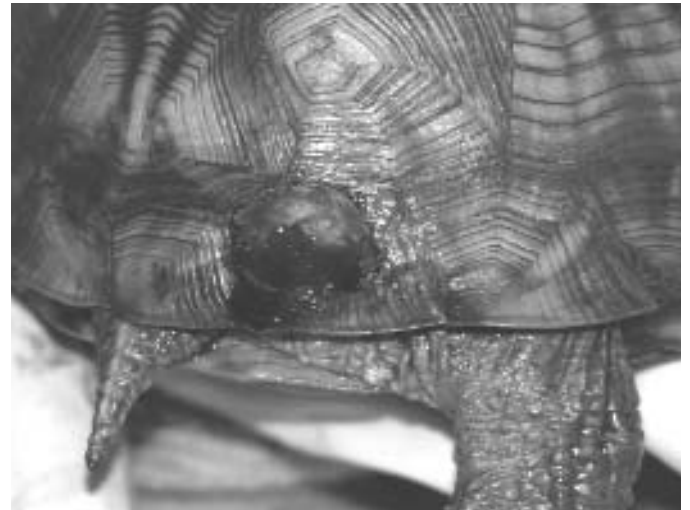


Figure 1. Carapacial lesion at Day 160.

DISCUSSION

Mycobacterium spp., are aerobic, slender, acid-fast bacilli. Many species are saprophytes residing in soil or water and cause sporadic, opportunistic infections. They can cause clinical disease in many animals, including mammalian, avian, and reptilian species. Diagnosis and appropriate treatment of mycobacteriosis is complicated by the slow-growth of many *Mycobacterium* spp., in culture. Treatment in humans is typically long-term and has included isoniazid, rifampin, ethambutol, aminoglycosides, and their various combinations (Smith, *et al*, 2000). To minimize adverse effects of long-term antibiotic treatment and reduce the potential for development of drug-resistant mycobacterial species, combination antibiotic regimens have been employed

No previous reports of infection with *Mycobacterium terrae* in reptiles or amphibians were found, but occasional reports of infection by other atypical *Mycobacterium* spp., were identified. Treatment in these cases was typically empirical due to the prolonged culture time characteristic of *Mycobacterium* sp. Despite a wide variety of reported antibiotic treatment protocols in several different affected reptile and amphibian species, medical treatment of mycobacterial infection was typically found to be unrewarding (Quesenberry, *et al*, 1986, Olsen, *et al*, 1987, Greer, *et al*, 2003).

Mycobacterium terrae is considered an opportunistic pathogen with only rare reports of infection in humans or animals including a cat (Henderson, *et al*, 2002), elk (Rohonczy, *et al*, 1996), cattle (Dvorska, *et al*, 2004), raw milk (Kazwala, *et al*, 1998) and oysters (Hosty and McDermont, 1975). *Mycobacterium terrae* infects primarily immunocompromised

humans, but it has also been reported in immunocompetent individuals (Krisher, *et al*, 1988, Peters and Morice, 1991, Fodero, *et al*, 1999, Bartralot, *et al*, 2000, Smith, *et al*, 2000). A pseudo-outbreak was recently reported in 20 humans from two hospitals in a six-day period (Bettiker, *et al*, 2006). In the reported feline case, apparent resolution of a solitary subcutaneous *Mycobacterium terrae*-associated granuloma was achieved after several months of combination antibiotic therapy including enrofloxacin, rifampicin, and clarithromycin (Henderson, *et al*, 2002).

Although the cutaneous lesion described in this report displayed partial response to treatment, eventual progression and the impracticality of reintroducing the turtle to its exhibit led to the decision to euthanize. Mycobacteriosis should be included among the differential diagnoses for chronic infections in reptiles, especially infections displaying little or no response to aggressive antibiotic treatment.

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