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Source: Journal of Zoo and Wildlife Medicine, 41(4):745-748. 2010.
Published By: American Association of Zoo Veterinarians
DOI: 10.1638/2010-0096.1
URL: http://www.bioone.org/doi/full/10.1638/2010-0096.1

Journal of Zoo and Wildlife Medicine 41(4): 745–748, 2010

SQUAMOUS CELL CARCINOMA WITH VASCULAR INVASION IN A DIAMONDBACK RATTLESNAKE (*CROTALUS ADAMANTEUS*)

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Abstract: Squamous cell carcinoma (SCC) is a common neoplasm diagnosed in domestic and wild animals, including several species of reptiles. However, reports of SCC invading vasculature or metastasizing in snakes are lacking. This report documents a case of SCC in an adult male eastern diamondback rattlesnake (*Crotalus adamanteus*) with a unique presentation and invasion into several small- to medium-sized vessels, suggestive of a metastatic process. What was initially suspected to be an abscessed tail was ultimately determined to be SCC originating at the base of the rattle.

Key words: Diamondback rattlesnake, *Crotalus*, neoplasia, squamous cell carcinoma.

BRIEF COMMUNICATION

As captive care of nondomestic animals improves, increasing life spans are accompanied by more observations of neoplasia. In reptiles, neoplasia has been identified rarely in all major groups, with snakes having the highest prevalence.[4,5,8,15] Squamous cell carcinoma (SCC) has been identified in lizards, turtles, and snakes.[4,5,7,12,15] The oral cavity, cloaca, hemipene, scent glands, and skin are the primary sites affected.[4,5,7,12,15] In snakes, SCC usually comprises well-differentiated neoplastic epithelial cells arranged in cords and nests that invade and disrupt the surrounding tissues. Reports of SCC metastasis in snakes are lacking. Here, we present a case of SCC in an eastern diamondback rattlesnake (*Crotalus adamanteus*), with a unique presentation, localization, and vascular invasion suggestive of a metastatic process.

An adult male diamondback rattlesnake was wild caught in August 2004 in eastern North Carolina for a public aquarium display. It was housed in a 1,600-L glass-fronted fiberglass display tank in a covered shelter on an outdoor boardwalk exhibit. Substrate consisted of natural sand, sticks, and leaf litter. The snake was fed frozen thawed rats (*Rattus norvegicus*) weekly to biweekly. No medical problems were noted until September 2005, when the lower left lip appeared to be drooping. The snake was treated periodically between December 2005 and June 2006 for recurrent left fang abscessation. Treatment included enrofloxacin (15 mg/kg s.c. once weekly for 8 wk, diluted 1:1 in saline; Baytril®, Bayer AG, D-51368 Leverkusen, Germany), manual debridement, topical dilute chlorhexidine, and topical 1% silver sulfadiazine cream (SSD; Thermazene, Kendall Co., Mansfield, Massachusetts 02048, USA). A culture of the lesion showed multiple gram-negative bacteria. The fang abscess was ultimately treated successfully with surgical debridement and extirpation of the fang. Histopathology of the excised tissue showed mild heterophilic gingivitis and submucosal edema. A culture at the time of surgical extirpation yielded no anaerobic or aerobic organisms. Postoperatively, the snake was treated with clindamycin (Hospira Inc., Lake Forest, Illinois 60045, USA) at 5-mg/kg i.m. once daily for 10 days and ceftazidime (Fortaz®, GlaxoSmithKline, Research Triangle Park, North Carolina 27709, USA) at 20 mg/kg s.c. every 72 h for 10 doses, and the snake made a full recovery.

No further medical problems were noted until 23 April 2009, when keepers observed a dry, crusting lesion near the tail tip after normal ecdysis. Topical therapy with 1% SSD was initiated. The snake was in good body condition, weighing 3.86 kg. The tail lesion consisted of necrotic tissue extending deep into the underlying musculature and involving approximately 40% of the tail circumference. Ceftazidime at 20 mg/kg

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i.m. every 72 hr for eight doses was initiated because of initial suspicion of an infection. The lesion was treated with sterile saline irrigation and topical SSD on days the animal was handled for injections. Hematology showed a hematocrit of 32% and a white blood cell count of 4,000 cells/μl with 21% heterophils, 52% lymphocytes, and 27% azurophils. The hematocrit fell within acceptable ranges for most snakes, whereas the white blood cell count was mildly decreased.[3,10] The increased percentage of heterophils and azurophils supports a chronic inflammatory response despite the low absolute numbers, whereas absolute numbers of lymphocytes were normal.[2,10] Culture of an aspiration from the lesion yielded an *Enterococcus* sp. and nonreproductive hyphae that could not be identified. No anaerobic bacteria were cultured. The *Enterococcus* sp. was susceptible to commonly used antibiotics, including a third-generation cephalosporin.

Three weeks after initial presentation, the lesion had progressed to encompass the caudal 2 cm of the tail. During routine cleaning of the lesion, the tissue sloughed, causing the loss of the rattle and entire tip of the tail. The lesion was manually debrided, revealing apparently healthy granulation tissue. Ceftazidime therapy was initially continued but was switched 27 days later to amikacin (Hospira Inc.) at 5 mg/kg i.m. initial dose, then 2.5 mg/kg i.m. every 72 hr for seven treatments because of lack of improvement with ceftazidime. Subcutaneous fluids (0.9% saline at 10 ml/kg) were also administered every second amikacin treatment to maintain hydration.

The tail lesion remained ulcerated and bled easily when manipulated. Cytologic examination of purulent material aspirated from the lesion showed moderate numbers of bacteria, inflammatory cells, and what appeared to be reactive epithelial cells. Radiographs revealed marked bony lysis of the distal three to four caudal vertebrae (Fig. 1). Amputation of the distal portion of the tail was elected because of the severity of the bone lesion and lack of response to medical treatments. The animal was manually restrained in a snake tube and induced with 10 mg/kg i.m. ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA), combined with 0.05 mg/kg dexmedetomidine (Dexdomitor®, Pfizer Animal Health, Exton, Pennsylvania 19341, USA) and 0.5 mg/kg midazolam (Baxter Healthcare Corp., Deerfield, Illinois 60015, USA). Additionally, 6 mg/kg danofloxacin i.m (Pfizer Animal Health) was administered, and 2.7 mg/kg (0.5 ml) 2% lidocaine (Hospira Inc.) diluted in sterile saline was used to perform a ring block around the base of the tail. Minimal sedation was obtained after 15–20 min, so 5.4 mg/kg propofol i.v. (Hospira Inc.) was administered. A surgical plane of anesthesia was obtained within 5 min. An elliptical incision was made circumferentially around the tail, approximately 2 cm proximal to the affected tissue, to remove the distal 7 cm of the tail. The soft tissues were sharply dissected, and the vertebrae were removed with rongeurs. The site was thoroughly flushed with sterile saline, and the soft tissues were closed over the exposed vertebrae with the use of 3-0 polydioxanone (PDS II®, Ethicon, Somerville, New Jersey 08876, USA) in a combination of horizontal mattress and simple interrupted patterns. The skin was closed with 3-0 polyglyconate (Maxon®, United States Surgical, Norwalk, Connecticut 06850, USA) in a horizontal mattress pattern, and SSD cream was applied. Vitamin B complex (12 mg/kg as thiamine) in 30 ml s.c. of reptile Ringer's (1:1:1; 0.9% NaCl, lactated Ringer's solution, 5% dextrose) and 1 mg/kg ketoprofen i.m. were administered, and the dexmedetomidine was reversed with 0.5 mg/kg atipamazole i.m. (Antisedan®, Pfizer Animal Health). The snake remained minimally responsive to stimuli the following morning. An additional 35 ml of reptile Ringer's solution s.c. and a half dose of atipamazole (0.25 mg/kg i.m.) were administered to aid recovery. The snake was alert and acting normally the second day after surgery and remained stable thereafter.

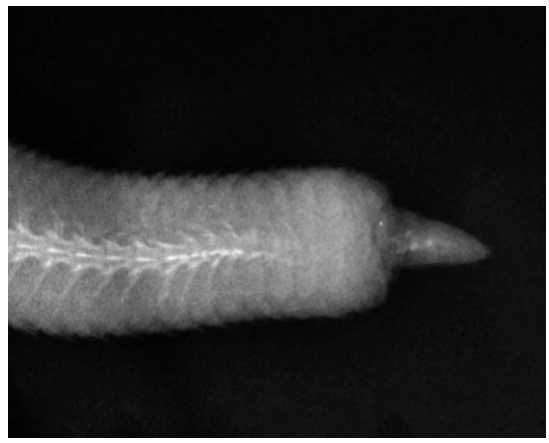

Figure 1. Dorsoventral radiograph showing the bony lysis associated with a necrotic tail lesion from squamous cell carcinoma in a diamondback rattlesnake.

Approximately 1 mo after surgery, a necrotic plug in the center of the surgery site was removed through gentle debridement, revealing a large defect and one and a half exposed vertebrae. The exposed vertebrae were removed under local anesthesia, and the wound was allowed to heal by second intention. Periodic cleaning with dilute betadine followed by topical SSD was continued, and the wound healed completely without further complications.

Histologic examination of the amputated tail section revealed an unencapsulated, poorly circumscribed, infiltrative neoplasm consistent with well-differentiated SCC. The neoplasm was characterized by islands and nests of polygonal cells with abundant eosinophilic cytoplasm, distinct intercellular bridges, and round to oval nuclei with finely stippled chromatin and one to two prominent nucleoli. Neoplastic cells formed several circular keratinized foci consistent with keratin pearls and invaded, disrupted, and replaced adjacent skeletal muscle and vertebral bodies. Ventral to the vertebrae, nests of neoplastic cells were identified partially to completely occluding small- to medium-sized blood vessels (Fig. 2). Moderate inflammatory cell infiltrates were also present and consisted of heterophils, histiocytes, and fewer lymphocytes and plasma cells. Additional histologic sections distal to the original sections revealed approximately 95% of the normal tissue to have been replaced by the neoplasm. Proximal examined surgical margins were histologically free of neoplastic cells.

Additional diagnostics included thorough oral and cloacal exams, whole-body radiographs, and ultrasonography. No further evidence of metastasis or bone lysis was identified. Follow-up radiographs in October 2009 revealed no other bony lysis or abnormalities. At the time of writing, the animal continues to act normally and eat well with no evidence of disease progression.

Although SCC in reptiles is documented, in snakes it usually develops in the oral cavity, cloacal regions, or associated mucous membranes.[4,5,7,12,15] Reported cases indicate that SCC in reptiles can be invasive or destructive to surrounding tissues but have failed to demonstrate metastasis in snakes.[4,5,7,12,15] Metastasis by SCC to muscle, liver, lung, and kidneys has been reported in the loggerhead sea turtle (*Caretta caretta*).[11] In the present case, invasion and occlusion of several small vessels by neoplastic cells was evident on histologic examination of the tail sections, suggestive of metastatic behavior. The lack of neoplastic cells in the more proximal tail section suggests that the tumor might have started at the base of the rattle, progressed proximally, and invaded vasculature.

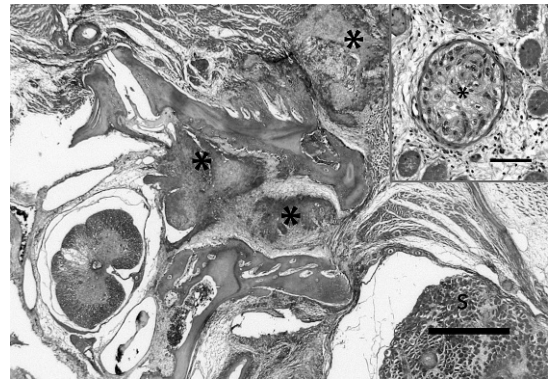

Figure 2. Transverse section through spinal cord of amputated tail segment. The vertebral body has been fractured by invasive squamous cell carcinoma (*). Ventral skeletal muscle bundle (S) has moderate atrophy of myofibers secondary to extensive vascular invasion by tumor. H&E, bar = 1.0 mm. Inset: Medium-sized blood vessel with lumen filled with squamous cell carcinoma (*). Surrounding interstitial tissues are edematous and have few scattered mononuclear cells. H&E, bar = 30 μm.

Several protocols have been used to address neoplastic disease in reptiles, including euthanasia, surgical debridement, cryotherapy, chemotherapeutics, photodynamic therapy, and radiation therapy.[1,6,9,13,14] In this case, treatments in addition to surgery were investigated but not pursued because of cost, difficulty of maintaining vascular access in snakes, and the added constraints on handling venomous snakes. No studies investigate the progression of SCC in reptiles, so the median survival time is unknown. No evidence of recurrence or spread of the SCC has been observed for at least 14 mo after tail amputation in this snake. Despite the loss of rattle and one fang, the snake remains an impressive exhibit animal.

What was initially thought to be an infected wound on the tail was later determined to be SCC. This case stresses the importance of considering neoplasia as a differential for any chronic, nonhealing skin lesion. This case also demonstrates that in addition to being locally destructive, SCC can invade vasculature in snakes. Although, clear distant metastasis was not observed, vascular invasion is an important preceding factor of the metastatic process.

At just under 15 months after tail amputation, the keeper noted an eruptive ventral mid-body swelling. Biopsy revealed extensive heterophilic and histiocytic inflammation with mixed bacterial and fungal infection superimposed over a dermal squamous cell carcinoma, confirming metastasis of SCC in this snake.

Acknowledgments: The authors thank the many residents and faculty at NCSU College of Veterinary Medicine, Raleigh, North Carolina, along with Dr. Roxanne Taylor and the staff at the Bridges Professional Park Animal Hospital, Morehead City, North Carolina, for their help with diagnostics and procedures performed during this case.

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Received for publication 9 June 2010