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Mast cell tumor in an eastern kingsnake (*Lampropeltis getulus getulus*)

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A 16-year-old captive-bred male eastern kingsnake (*Lampropeltis getulus getulus*) was presented to the Veterinary Medical Teaching Hospital, University of Florida for evaluation of a bleeding mass which was first noted 2 months prior to presentation. The grayish mass was 2 × 2 × 5 cm, firm, ovoid, raised above the ventral scales, and located ventral to the liver. The snake was anesthetized with isoflurane in 100% oxygen, and the mass was found firmly attached to the fascia of the skin and the underlying skeletal musculature. Because of the invasiveness of the mass into the surrounding tissue, complete excision was not possible. Tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (HE), and sections were examined by light microscopy.

Microscopically, the mass was composed of sheets and interwoven bundles of contiguous neoplastic cells ranging from round to polygonal to fusiform. Neoplastic cells had scant to abundant, eosinophilic, foamy to fibrillar cytoplasm and variably distinct borders. Nuclei were moderately anisokaryotic, round to elongate, and euchromatic to vesicular and frequently contained single or multiple nucleoli of various sizes. Mitotic figures were rare, and vascular invasion by tumor cells was not detected. Neoplastic tissue invaded adjoining skeletal muscle and extended to the surgical margins (Fig. 1). No bacterial or fungal organisms were detected by Goodpasture, Fites, or Gomori methenamine silver stains. An anatomic diagnosis of undifferentiated sarcoma was made. Given the invasive nature and the incomplete excision, recurrence was considered likely.

After 6 months, the mass had regrown at the original site and was approximately the same size as the previous mass. To stage this neoplastic disease, a diagnostic evaluation was performed. Whole body radiographs were within normal limits. An ultrasonogram revealed a heteroechoic mass extending from the skin into the coelomic cavity, displacing the lung. The liver was uniform in echogenicity and texture. Hematologic parameters revealed a leukocytosis (11,300 cells/μl), lymphocytosis (5,760 cells/μl), and anemia (packed cell volume [PCV] = 17%). Plasma biochemical parameters were within normal limits. When the mass was reexcised, it was found to be firmly attached to the surrounding skeletal musculature and invading the coelomic cavity.

Portions of the mass were processed as described above for histopathologic evaluation. Findings were similar to those of the initial biopsy except for the following. Most neoplastic cells contained a few periodic acid–schiff (PAS)-positive and weakly metachromatic granules (Giemsa stain) (Fig. 2). Mitotic figures were present at 1–4/400× field and were occasionally abnormal. Neoplastic cells invaded skeletal muscle and extended into the superficial dermis, which contained scattered heterophils. Neoplastic emboli were present within lymphatics. The high mitotic index and the lymphatic invasion was suggestive of increased anaplasia or tumor progression when compared with the original biopsy results. Cells were weakly positive for chloroacetate esterase.

Formalin-fixed tissues were postfixed in 4% glutaraldehyde for 2 hours, embedded in epon-araldite, and sectioned at 80 nm for transmission electron microscopy. Thin sections were stained with uranyl acetate and lead citrate. Most neoplastic cells were ovoid and had frequent, usually small pseudopodia (Fig. 3). Neoplastic cells usually contained 1–6 irregular, electron-dense cytoplasmic granules consisting of membrane-bound structures containing multiple linear, curved, electron-dense structures in scroll-like arrangements. These findings were characteristic of mast cell granules (Fig. 3).[3] The cytoplasm also contained frequent mitochondria and small profiles of rough endoplasmic reticulum.

Histologic examination of liver tissue samples obtained with ultrasound guidance 2 months after the second excision revealed multifocal hemosiderosis. On ultrasound examination, the liver was uniform in echogenicity. No evidence of metastases was present. Hematology at that time showed persistent anemia (PCV = 16%) with leukopenia (6,500 cells/μl) and lymphopenia (1,170 cells/μl). A decision to apply local radiation therapy was made because there was no evidence of systemic disease.

A planar interstitial implant using ^{192}Iridium seeds was planned to deliver a total dose of 5,000 cGy to 0.5 cm over 5 days. Standard afterloading technique was used while the patient was under general anesthesia.[19] A computerized dose distribution chart of the implant was generated using orthogonal radiographic views with magnification correction. The implant included the previous site of the mass and the surgical incision. The snake recovered from anesthesia and was held in radiation isolation until the implant was removed. No complications were noted during the isolation period. Six weeks later, the scales were mildly curled and opaque at the implant site, indicating radiation damage.

During radiation therapy, the snake became anorectic and less active. Three months after the start of radiation therapy, the snake died spontaneously. Gross necropsy following spontaneous death 394 days after initial presentation revealed tumor invasion into the internal surface of the ventral body wall. There was a tan, 4- × 4- × 3-cm mass associated with the lateral body wall and the adventitia of the esophagus. There were multiple nodules of various sizes in the

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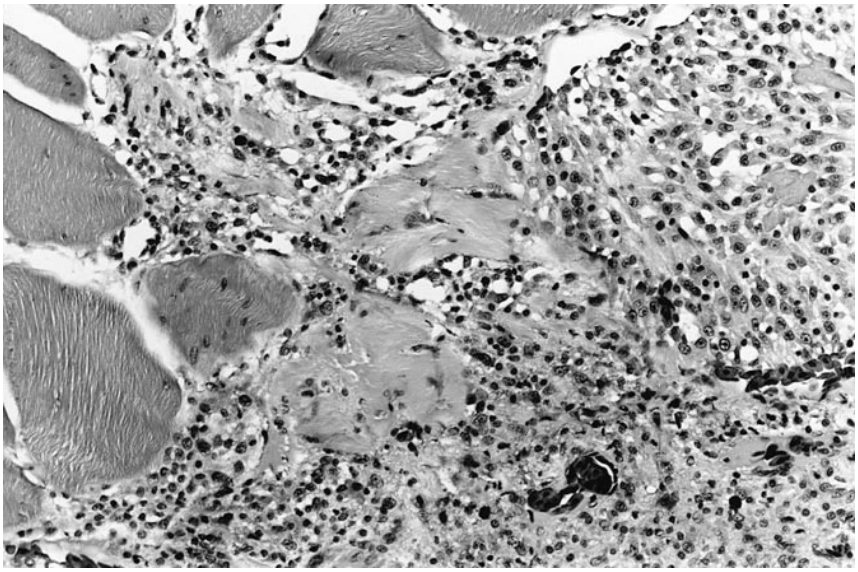

Figure 1. Neoplastic cells form dense cellular sheets and are invading skeletal muscle of an eastern kingsnake at the deep tumor margin. HE. 200×.

liver, lung, spleen, endocardium, kidney, and fat body. Metastatic nodules ranged in size from 0.1 to 2.0 cm and from tan to pale green. A 3- × 2- × 2-cm pedunculated mass was located at the cranial pole of the liver. Histologically, sections of the liver revealed nonencapsulated foci of neoplasia of various sizes, which were well demarcated from the adjoining parenchyma. Perisinusoidal spaces were often markedly widened and comprised of cords of closely packed ovoid to fusiform neoplastic cells, with staining features described above. Within sections of the heart, multiple irregularly shaped nodules of neoplastic tissue of various sizes were found. Sections of the kidney contained nonencapsulated neoplastic nodules similar to those described above. Several large renal vessels were completely occluded by neoplastic tissue. Small tumor foci were observed within the tunica intima of large renal vessels covered by endothelial lining. In the lung, partially obstructed airways and tumor emboli in vascular lumina were observed.

Neoplasms of all major organ systems have been described in reptiles. Most neoplasms have been reported in the order Squamata because of the popularity of these animals, whereas only a few have been seen in the orders Crocodylia and Chelonia. Most neoplasms have been detected upon postmortem histopathologic evaluation of tissues, and information about the etiology, diagnosis, and treatment of various tumors is scant.[2,4,7,12] In some instances, abscesses containing predominantly gram-negative organisms and granulomas are incorrectly diagnosed as neoplasms.[15]

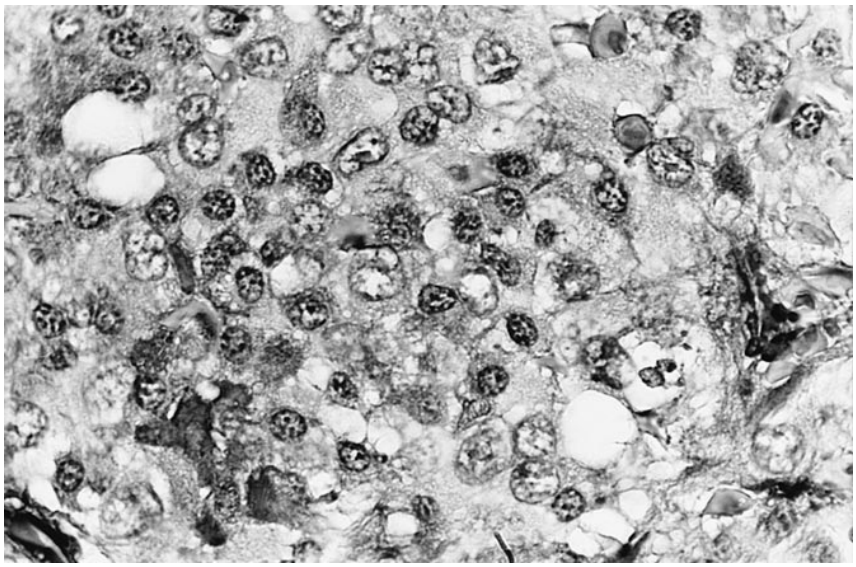

Figure 2. Eastern kingsnake. Neoplastic cells are ovoid and often have large vesicular nuclei. Cytoplasmic granules can be seen within a few cells. PAS. 1,000×.

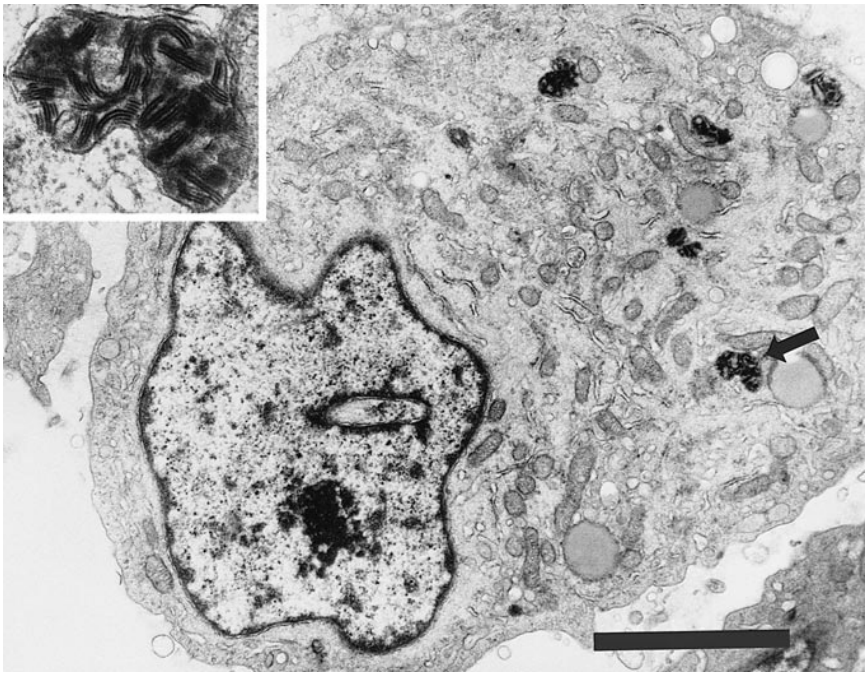

Figure 3. Eastern kingsnake. Neoplastic mast cell containing small granules (arrow). *Inset:* Granules are membrane bound and have characteristic scroll-like arrangement. Bar = 2 μm.

In dogs, mast cell tumors originate most often from the dermis and subcutaneous tissues, as seen in this snake.[1] Although previous chronic inflammation and possibly a viral etiology have been associated with the development of mast cell tumors,[13] no infectious agent could be identified by light microscopy in this snake. When first submitted for histopathologic evaluation, the mass was diagnosed as an undifferentiated sarcoma. Recurrence was considered likely, and repeated surgical excisions of the mass were performed. A definitive diagnosis of mast cell tumor was made based on suggestive staining characteristics and ultrastructural findings. This tumor was highly anaplastic based upon the low number of cytoplasmic granules, the weak metachromasia, the fusiform shape of many neoplastic cells, the high mitotic rate, and the presence of large numbers of metastases. This anaplasia led to the initial misdiagnosis of an undifferentiated sarcoma, such as a fibrosarcoma. Weak metachromasia is not unexpected in poorly differentiated mast cell tumors because neoplastic cells have low content of heparin and possibly of other glycosaminoglycans.[16] Mast cells of snakes have granules that stain with acid stains such as PAS, as do some mammalian mast cells.[17,18] There are no previous reports of mast cell tumors; however, given the occurrence of sarcomas in reptiles, staining of these tumors for the presence of mast cell granules may be indicated and may result in correction of a previous misdiagnosis.

Although mast cell tumors in dogs have been associated with gastrointestinal ulceration, no lesions of the gastrointestinal tract could be identified in this snake.[6] The observed delayed wound healing at the surgical site may have been caused by the release by the tumor of proteolytic enzymes and vasoactive amines.[10] The hemosiderosis may have reflected chronic changes in red blood cell destruction due to neoplasia and chronic disease.

Recommended treatment for mast cell tumors in dogs includes surgical excision, cryosurgery, chemotherapy, radiation therapy, or a combination of these.[13] Because ultrasonography, radiography, and liver biopsies failed to detect metastatic mast cell tumors, a decision for local radiation therapy was made. Brachytherapy uses sealed radioactive sources for surface, interstitial, or intracavitary applications.[14] Interstitial implant brachytherapy may be used to treat neoplasms of the body wall when vital organs are contiguous with the treatment field. In this snake, the surgical site was implanted after resecting the recurrent tumor. One major advantage of brachytherapy is the high dose differential between tumor and normal tissues. ^{192}Iridium, with a half-life of 74.2 days, was used for nonpermanent brachytherapy because it can be implanted using an afterload technique. Neither the use of interstitial implants for tumor treatment nor the normal tissue response to radiation have been reported for reptiles.

Despite local therapy in this case, metastases developed in the lung, liver, kidney, heart, and spleen. In the dog lung, metastases occur in only 15–34% of cases of mast cell tumor. Regional lymph nodes (76–96% of cases), spleen, and liver are the most common locations for metastases.[5] There is little information about the therapy of neoplasms in reptiles. Most commonly, surgical removal of the neoplasm is elected. Success of surgical excision depends on early identification of the tumor, its localization, and clear demarcation from surrounding tissues. In addition to surgical removal of a neoplasm, radiation therapy may be beneficial. A malignant chromatophoroma in a yellow rat snake (*Ela-*

phe obsoleta quadrivittata) was treated with radiation therapy, which delayed local recurrence.[11] Cobalt therapy was unsuccessful in the treatment of a lymphosarcoma in an Indian python (*Python molurus*) and an angiosarcoma in a spitting cobra (*Naja nigrocollis*).[7,11] Chemotherapy has been used rarely in reptiles.[8] Cytosine arabinoside has been unsuccessfully used in the treatment of a lymphosarcoma in a rhinoceros viper.[9] In this kingsnake, chemotherapy was not selected because the tumor appeared localized and without metastases.

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Typhlocolitis caused by *Clostridium difficile* in suckling piglets

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Clostridium difficile, a gram-positive to gram-variable, spore-forming, anaerobic bacterium, is a common inhabitant of soil, water, and the intestinal tract of various mammals, birds, and reptiles.[16] It has been identified as a cause of pseudomembranous colitis in humans,[2,4,21,24,27] enterocolitis in foals,[16] nosocomial diarrhea and typhlocolitis in adult horses,[20,22] typhlitis in adult hamsters,[3,7] and cytopathic toxin production in guinea pigs treated with penicillin.[2] Spores are heat resistant, can survive for months or years, and are carried readily on hands and equipment.[21] Recovery of *C. difficile*, in association with *Balantidium coli* and *Salmonella typhimurium*, from 8-week-old pigs with a naturally occurring enterocolitis has been reported.[15] Here, we describe a herd problem of mesocolonic edema and typhlocolitis in suckling piglets from which *C. difficile* was identified as the putative etiology.

An unusual outbreak of disease began in a newly established herd of 600 gilts 3 weeks after the first litters were farrowed. Initially, piglets were affected at about 2 weeks of age. Soon, newborn piglets also became ill. An average of 25 litters were farrowed weekly, with the number of live births per litter ranging from 9.9 to 10.3 piglets. Weekly preweaning mortality prior to the outbreak and subsequent to its resolution ranged from 7% to 9%. During the several months in which clinical signs of disease were noted, weekly losses from all causes ranged from 7% to 58% ($\bar{x}$ = 25%). The practitioner estimated that the disease described here accounted for at least 90% of the losses, but necropsies were

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