Acute Lymphoblastic Leukemia in a Diamondback Terrapin, *Malaclemys terrapin*

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**Abstract:** Lymphoblastic leukemia was diagnosed in a diamondback terrapin, *Malaclemys terrapin*, based on hematology, bone marrow biopsy, and immunocytochemistry. Diagnostic imaging procedures included radiographs, magnetic resonance imaging, and computed tomography. The turtle was treated with chlorambucil, cytosine arabinoside, and prednisone. A treatment effect was achieved 24 d after the chemotherapy was initiated, but the patient died 46 d following the start of chemotherapy. This article chronicles the clinicopathologic features of leukemia in this patient, elaborates on the diagnostic procedures performed as related to a chelonian, outlines the patient’s response to the treatment protocol, and includes the postmortem findings, morphologic and staining features of the disease.

**Key words:** diamondback terrapin, *Malaclemys terrapin*, B-cell, chemotherapy, cytochemistry, immunocytochemistry, leukemia.

**Case Report**

An 850 g, female, diamondback terrapin, *Malaclemys terrapin*, at least four years of age, presented with a one week history of anorexia, mucoid ocular discharge, and mild right forelimb tremors. Physical examination was performed at this time and at two, seven, eight and nine weeks after initial presentation.

The physical examination findings at initial presentation included mild right forelimb trembling and bilateral mucoid ocular discharge. A survey radiograph was obtained, and there were no radiographic abnormalities observed. Differential diagnoses included central nervous system disease, nutritional imbalance, metabolic neuropathy, endoparasitism, and bacterial infection. Empirical therapy with enrofloxacin (Baytril, Bayer Inc., Shawnee, KS; 10 mg/kg, SQ q 48 hr for 10 d) was instituted.

At the second presentation (one week after the first presentation), a fecal parasite examination and a complete blood count and serum chemistry were performed. The results of the CBC and serum chemistry profile are tabulated in Table 1. At the third presentation (seven weeks after initial presentation), a bone marrow biopsy was performed. Anesthesia was induced with tiletamine HCl-zolazepam HC1 (Telazol, Fort Dodge, Fort Dodge, IA; 8 mg/kg SQ q 48 hr for 10 d) was instituted.

At the second presentation (one week after the first presentation), a fecal parasite examination and a complete blood count and serum chemistry were performed. The results of the CBC and serum chemistry profile are tabulated in Table 1. The physical examination findings included amelioration of the forelimb tremors and ocular discharge, yet the patient remained lethargic and anorexic. Microscopic examination of blood films revealed a preponderance of atypical mononuclear cells. These cells had scant deeply basophilic cytoplasm, mild anisokaryosis, round, cleaved, or lobulated nuclei containing one or multiple prominent nucleoli, and rare bi-nucleation. Small well-differentiated lymphocytes, heterophils, basophils, and thrombocytes were also present in much fewer numbers. A marked lymphocytic leukocytosis was interpreted, making leukemia to be one of the suspected differential diagnoses. In addition to mature erythrocytes, occasional polychromatophilic were also detected. Heterophils occasionally were immature (Figures 1a and 1b). Hemoparasites were not seen. The serum chemical profile (Table 1) was within ISIS physiological reference ranges (International Species Information System, Apple Valley, MN).

*Coccidian oocysts* and Cryptosporidia sp., were identified in a direct fecal smear. Metronidazole suspension (Flagyl, Searle, Chicago, IL; 125 mg/kg, PO q 72 hr for three treatments) and sulfadimethoxine suspension (Albon, Roche, Eaton, PA; 90 mg/kg, PO initially, 45 mg/kg, PO q 72 hr for three treatments) were administered via an oral-gastric tube.

At the third presentation (seven weeks after initial presentation), a bone marrow biopsy was performed. Anesthesia was induced with tiletamine HCl-zolazepam HCl (Telazol, Fort Dodge, Fort Dodge, IA; 8 mg/kg SQ). Core biopsies were collected using a 14 ga hypodermic needle. The needle was used to bore a hole at the bony bridge of the plastron and gular projection, where accessible marrow cellular density was likely to be most representative (Garner, et al, 1996). The bone marrow was preserved in 10% neutral buffered formalin. The biopsy sites were sealed using cyanoacryilc surgical...
Table 1. Sequential complete blood count and serum chemistry data from a diamondback terrapin, *Malaclemys terrapin*, with lymphoid leukemia before and after chemotherapy. (1) International Species Information System, Apple Valley, Minnesota 55124, USA. a. 2 monocytes and 58 undifferentiated, unidentified cells.

<table>
<thead>
<tr>
<th>Reference Interval (1)</th>
<th>First Presentation lymphatic dilution (results may be spurious)</th>
<th>Sixth Presentation</th>
<th>Seventh Presentation lipemia 2+</th>
<th>Eight Presentation</th>
<th>Ninth Presentation</th>
<th>Tenth Presentation</th>
<th>Eleventh Presentation</th>
<th>Twelfth Presentation</th>
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<tbody>
<tr>
<td></td>
<td>T = 0</td>
<td>T = 7 d</td>
<td>T = 14 d</td>
<td>T = 24 d</td>
<td>T = 34 d</td>
<td>T = 46 d</td>
<td></td>
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<tr>
<td>Hematocrit %</td>
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<td>12/13/2000 a</td>
<td>2/17/01</td>
<td>2/24/01</td>
<td>3/3/01</td>
<td>3/13/01</td>
<td>3/23/01</td>
<td>4/4/01</td>
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<tr>
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<td>1.5</td>
<td>200</td>
<td>65</td>
<td>33</td>
<td>10</td>
<td>16</td>
<td>28</td>
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<tr>
<td>Absolute Polys</td>
<td>10780</td>
<td>1020</td>
<td>8000</td>
<td>7800</td>
<td>6930</td>
<td>4800</td>
<td>6880</td>
<td>4480</td>
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<tr>
<td>Absolute Lymphs</td>
<td>140140</td>
<td>180</td>
<td>192000</td>
<td>57200</td>
<td>5940 (a)</td>
<td>2700</td>
<td>4800</td>
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<tr>
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<td>300</td>
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<td>0</td>
<td>20130</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>2.2-6.6 mmol/L or 40-120 mg/dL</td>
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<td>279</td>
<td>31</td>
<td>47</td>
<td>163</td>
<td>131</td>
<td>177</td>
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<td>31.8</td>
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<td>64.6</td>
<td>37.8</td>
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<td>33</td>
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<td>2.3</td>
<td>2.4</td>
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<td>1.7</td>
<td>1.4</td>
<td>0.9</td>
<td>0.7</td>
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<td>131</td>
<td>118</td>
<td>133</td>
<td>134</td>
<td>142</td>
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<td>2.7</td>
<td>5.7</td>
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<td>4.1</td>
<td>2.6</td>
<td>2.1</td>
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<td>91</td>
<td>84</td>
<td>100</td>
<td>96</td>
<td>111</td>
<td>94</td>
<td>102</td>
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<td>81</td>
<td>1396</td>
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<td>1332</td>
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<td>850</td>
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<td>89.2</td>
<td>714</td>
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</table>

Volume 17, No. 3, 2007

Journal of Herpetological Medicine and Surgery
Figure 1. Blood films of a diamondback terrapin, *Malaclemys terrapin*. a) Note large numbers of intermediate to large sized lymphocytes in the Giemsa-stained film. Bar = 40 μm. b) Note Giemsa-stained atypical lymphocytes have scant deeply basophilic cytoplasm, and frequent cleaved or lobed nuclei with prominent nucleoli (arrow). Bar = 20 μm c) Atypical lymphoid cells have positive cytoplasmic staining for BLA36, a mammalian B cell marker. Bar = 20 μm d) Normal circulating lymphocytes have positive cytoplasmic staining for CD3, a mammalian T cell marker. Bar = 20 μm e) Atypical lymphocytes stain focally and diffusely positive for acid phosphatase. Bar = 20 μm.

Results of the bone marrow biopsy confirmed a preponderance of neoplastic lymphoid cells in the extrasinusoidal regions of the bone marrow (Figure 2). Increased osteoclastic activity was present in some areas of the trabecular bone. Extrasinusoidal myeloid cells and intrasinusoidal erythroid cells were scant. Fragmentation of the samples (attributed to collection and processing artifact) and small specimen size

...
impeded determination of any architectural effacement by the neoplastic population.

At the fourth presentation (eight weeks after the initial presentation), computed tomography was performed to investigate the extent of neoplastic involvement (Picker PQ2000, Marconi Medical Systems, Cleveland, OH). For the scan, the patient was sedated using tiletamine HCl-zolazepam HCl (Telazol, Fort Dodge, Fort Dodge, IA; 8 mg/kg SQ). A spiral scan was performed using a modified abdominal protocol. Axial images were obtained with a parameter of 3 mm slices. Computed tomography images of the stifles showed a mixed intensity signal pannus and osteophyte formation. Lesions were hazy white in comparison to the gray-white bone. Roentgen signs interpreted in the computed tomography findings of the soft tissue, brain, and lung windows obtained at week eight were consistent with lysis from gout induced degeneration of the stifles and tarsal regions.

Magnetic resonance imaging was performed at the fifth presentation (8 weeks after the initial presentation). Propofol (Diprivan, Astra Zeneca, Rochester, NY; 12 mg/kg, IV) was used to achieve immobilization anesthesia for the imaging procedure. Radiographs were also obtained. A fine needle aspirate was obtained from swelling around the left stifle. Interpretation of the MRI and radiographs indicated severe degenerative changes which corresponded to mineralization associated with tophaceous gout. Acid phosphatase (Figure 1c) demonstrated both diffuse and focal positive staining of the neoplastic cells.

A CBC and serum chemistry were performed, but the results were deemed to be spurious due to lymphatic dilution.

At the seventh presentation (ten weeks after the first presentation) a CBC and serum chemistry were performed. Elevated urea nitrogen, aspartate aminotransferase (AST), creatine phosphokinase (CPK), and uric acid were observed. The elevations observed in the serum chemistry were thought to be a result of the cancer and the gout. Concurrent treatments for the leukemia and articular gout were started on this day. Medications prescribed for the gout were colchicine (Colchicine, Abbott, Abbott Laboratories, Inc., Abbott Park, IL; 0.7 mg/kg PO q 48 hr), and allopurinol (Zyloprim, Glaxo Wellcome, Zebulon, NC; 6 mg/kg SQ q 48 hr). The anticaner therapy consisted of prednisone (Deltasone, Pharmacopia Upjohn, Kalamazoo, MI; 0.6 mg /kg PO q 48 hr), cytosine arabinoside (Cytosar-U, Upjohn Company, Kalamazoo, MI; 6 mg/kg SQ q 7 d for two treatments (seventh and eighth presentations)), and chlorambucil (Leukeran, Glaxo Wellcome, Zebulon, NC; 1 mg/kg PO q 7 d for two treatments (seventh and eighth presentations)).

The patient’s response to the treatment was monitored by a weekly CBC, serum chemistry profile (Table 1), and immunocytochemistry (nineth through twelfth presentations). At 24 d into the protocol, a marked improvement was measured in the patient’s white blood cell count. The keepers also
noted that the patient’s activity level and appetite were both returning to their pre-illness levels. The patient died 46 d after onset of treatment. At necropsy, extensive uremic mineralization was present in the joint surfaces of both tibia and tarsi as well as the soft tissues surrounding the vertebral column. Extensive tumor necrosis was seen histologically in numerous tissues in the turtle in this report. Microscopic examination of necropsy tissues revealed generalized lymphoma and lymphoid leukemia involving skeletal muscle, kidney, liver, spleen, heart, intestine, esophagus and bone marrow. Many of the malignant cells were necrotic, especially in the bone marrow (Figure 3), which suggests a treatment effect. Acute myocardial necrosis and focal chronic pericardial fibrosis, moderate renal tubular gout, and disseminated intravascular coagulation were also observed. Foci of acute fibrinocellular thrombosis was present throughout viscera. The authors feel that the distribution of cells and the cellular density in the marrow are consistent with a neoplastic infiltrate rather than normal cell populations, and that most of the neoplastic cells are necrotic. The authors feel that this degree of cellular necrosis, occurring in all neoplastic foci, would have caused significant toxemia, and likely precipitated the disseminated intravascular coagulation, which was the ultimate cause of death. Tissues were donated to the Registry of Tumors in Lower Animals, George Washington University Medical Center, Washington, District of Columbia, Accession number RTLA 7313, GO1-1380.

Figure 3. Histologic appearance of hematoxylin and eosin stained sections following chemotherapy (T = 46 d) in diamondback terrapin, *Malaclemys terrapin*. a) Note extensive necrosis of neoplastic lymphoid cells (arrow) and necrotic hepatocytes (arrowhead) in liver, bar = 115 μm. b) Note necrosis of lymphoid cells (arrows) in spleen, bar = 100 μm. c) In bone marrow, sinusoids (s) contain luminal foci of necrosis (arrowhead), and intrasinusoidal space (i) has focus of necrotic neoplastic cells (arrow), bar = 72 μm. d) Note necrosis of neoplastic cells in lumen of large pulmonary vein, bar = 100 μm.
DISCUSSION


Of the nine cases reported, four were leukemic (Cooper, et al, 1983, Frye and Carney, 1972, Harshbarger, 1974, Harshbarger, 1976, Machotka, 1984, Rosskopf, et al, 1981). Of these, three cases involved hematopoietic tumors: lymphosarcoma and lymphoreticular neoplasia (Harshbarger, 1974, Machotka, 1984). Treatment of these neoplasms was not described. The authors sought to fully investigate the extent of this patient's condition, as this appears to be the first reported case of neoplasia in a diamondback terrapin. Several of the diagnostic procedures performed required special considerations in the chelonian patient. The diagnostic procedures employed included bone marrow biopsy, CT, MRI, as well as cyto-chemical and immunocytochemical staining.

Because of the extensive bony trabecular network, conventional bone marrow aspirates in chelonians have low cell yield and are not useful for diagnostic purposes. Chelonian bone marrow architecture differs from that of mammalian and avian marrow in that most of the marrow is composed of bone. The marrow of chelonians is comprised of bony trabeculae, loose extrasinusoidal connective tissue, adipose tissue, and blood sinusoids. Small nests of extrasinusoidal myeloid cells, macrophages, lymphocytes, and plasma cells and intrasinusoidal erythroid cells constitute the marrow hematopoietic cell populations. The materials and methods for the collection of bone marrow in turtles have been described. Bone marrow may be harvested from the gular hypodermic needle, the sinusoidal architecture was fragmented in our sample. Bone marrow biopsy may be performed using a Jamshidi bone marrow biopsy needle or a Michelle trephine.

Immunocytochemistry allows determination of the cell of origin by detecting various membrane and cytoplasmic antigens. The specific set of antigens expressed by a cell population constitutes a unique phenotype. This technique has been applied to a variety of animal species, including reptiles (Monzon-Mayor, et al, 1998). The anti-CD3 reacts with the epsilon chain of the T-cell receptor. This antibody is directed against the intracytoplasmic domain, specifically amino acids 156-168. The CD3 antigen is expressed only in T-cells of many species. BLA36 is an antigen first detected in B-cells of Hodgkin’s lymphoma. The antibody reacts with a 36kD membrane glycoprotein. This antigen is associated with early, activated and malignant B-cells. Positive staining for BLA 36 was observed with this patient. The BLA36 antigen is known to stain a subset of B-cells in humans, and has been shown to stain mammalian cells which often correspond to CD79a-positive cells, a component of the B-cell receptor. In addition, one of the authors has shown that BLA 36 stains similar B-cell populations in several reptiles. The neoplastic cells were negative for CD3, a T-cell marker, thus supporting a B-cell origin for the neoplastic cells. A few well-differentiated small lymphocytes stained positive for CD3 and were likely T cells. Cytochemical staining for normal blood cells from this species has not been performed however several studies from other chelonian species are available (Alleman, et al, 1992, Garner, et al, 1996, Work, et al, 1998). In these reports, the normal blood and bone marrow lymphocytes are negative for many enzyme stains including acid phosphatase. Monocytes from these species are consistently positive for the enzyme. The presence of positive staining for acid phosphatase in this case would suggest that either these are monocytogenic or atypical lymphoid cells. A certain human B-cell leukemia termed hairy cell leukemia is frequently associated with diffusely acid phosphatase staining.

The use of CT and MRI for a tortoise has been described (Raiti and Haramati, 1997, Silverman, 2006, Wynen, 2006). CT scans provide excellent imaging of thoracic detail and bony structures, whereas MRI provides superb detail of soft tissue structures, including nervous tissue. Patient movement presented the biggest obstacle in obtaining scans of diagnostic quality. Chemical sedation was used to immobilize the patient for the duration of the imaging procedure. The patient was placed in a small cardboard box to minimize movement during the CT scan. Interpretation of the images can be augmented by scanning a normal turtle of similar size. In a case with a patient of small size, two turtles may be scanned simultaneously, reducing the cost to the operator. It was unexpected that the interpretation of the CT and MRI images did not include evidence of more diffuse neoplastic changes in the patient. The small size of the patient may have made it harder to appreciate the tumor effects radiologically.

The etiology of only a few reptilian tumors has been investigated. Many causative factors such as pollution, environmental, and geography have been investigated. Carcinogens such as trauma, plastics, toxins, as well as genetic predispositions have yet to be investigated in reptiles (Jacobson, 1981, Mouldin and Done, 2006). In the course of our investigation, we were unable to identify an etiology for our patient’s diagnosis. A complete blood count and serum chemistry was performed on a second diamondback terrapin that was housed with our patient. The second patient’s examination and lab work were within normal limits.

Treatment of chelonian neoplasia has only been reported in some tumors involving the integumentary and musculoskeletal systems (Mouldin and Done, 2006). Surgical excision was used to treat integumentary papillomas, fibromas, and fibropapillomas, while surgical excision and cryosurgery were used in the treatment of a neurilemmal tumor located under the plastron (Cooper, et al, 1983).

Cancer treatments have been reported in few other reptile species. Surgical excision of clinically apparent masses requires the same considerations as in mammalian tumors. Surgical debulking and radiation therapy were used to treat a malignant chromatophoroma in a yellow rat snake, Elaphe obsolete. Reduction of the tumor was achieved with only minor adverse effects (Leach, et al, 1991). Cobalt therapy was used for a lymphosarcoma in an Indian python, Python...
Photodynamic therapy using a chloroaluminum sulfonated phthalocyanine has been reported in three reptiles. The technique involves intravenous administration of a photosensitizing agent that selectively localizes in the tumor and is activated by exposure to an intense light source, usually a laser, producing a wavelength which both penetrates tissue and is specific for the absorption characteristics of the compound. The activated photosensitizer produces singlet oxygen or free radicals that are locally cytotoxic. As the presence of the photosensitizer is limited to the tumor tissue, selective tumor necrosis occurs. Use of photodynamic therapy was reported in a squamous cell carcinoma in a boa constrictor, *Boa constrictor*, a mixed carcinoma/sarcoma in a Burmese python, *Python molurus bivittatus*, and an adenocarcinoma in a European viper, *Viper berus berus*. Complete remission of the tumor was reported in all three cases; however, metastases were observed at necropsy in the European viper. Because this therapy requires both photosensitizer localization and light activation, it is more appropriate for the treatment of locally malignant disease rather than systemic neoplasia (Roberts, *et al.*, 1991).

Only a few reports describe the use of chemotherapy to treat neoplasia in a reptile. The lack of easily obtainable venous access creates a difficult situation for delivering intravenous chemotherapeutic agents, as well as the requisite monitoring of hematological indices (Mouldin and Done, 2006). Intralesional injections of cisplatin into fibrosarcomas have been reported (Ramsay and Fowler, 1992). A rhinoceros viper, *Bitis nasiicorna*, with lymphosarcoma was treated with two equal treatments of cytosine arabinoside (30 mg/kg), but died within 24 hr of the first treatment. It was not reported whether the viper died of complications involving the chemotherapeutic agent or other factors (Jackson, *et al.*, 1981). A corn snake, *Elaphe guttata*, with a sarcoma was treated with intravenous adriamycin via a vascular access port. The snake received a total of six doses over a 3 month period (Rosenthal, 1994). A king cobra, *Ophiophagus Hannah*, received a treatment regime of subcutaneous L-asparagine aminohydrolase, intravenous vincristine, and oral prednisone over a three week period. Initially a clinical response did occur, however, the tumors did metastasize. The protocol was later modified to one of oral prednisone and chlorambucil. The modified regime did achieve an apparent clinical effect, however, the authors reported the patient underwent a gradual decline in condition (Willette, *et al.*, 2001).

As there were no known published or anecdotal reports describing the use of antineoplastic drugs in a chelonian, established mammalian protocols for the treatment of leukemia served as the basis for the treatment plan for our patient. Based on the marked decreases in the patient’s white blood cell estimate, a positive response to the chemotherapy was observed. At 14 d post treatment, marked mononcytosis occurred that was likely related to tissue necrosis. An apparent effect of the tumor lysis was the severe increases in the serum chemistry indices, blood urea nitrogen (BUN), aspartate aminotransferase (AST), creatinine phosphokinase (CPK), and uric acid. Hepatocellular damage likely contributed to the elevated concentration of AST. At necropsy, extensive tumor necrosis was seen histologically in numerous tissues in the turtle in this report. This was interpreted as a treatment effect; however, it was considered possible that toxemia associated with tumor necrosis may have caused or contributed significantly to the turtle’s demise. It is possible that toxemia with subsequent myocardial necrosis and disseminated intravascular coagulopathy may have resulted from the extensive tumor necrosis. The extensive renal gout may have been a result of dehydration, drug therapy, toxemia, neoplastic infiltrate, tumor lysis, or a combination of these factors.

In this case, we feel the patient’s tumors did respond to the cancer chemotherapy. This case serves to add to the growing evidence that reptiles are viable candidates for cancer treatment using modified mammalian protocols.

ACKNOWLEDGEMENTS

Special thanks to Natalie Antinoff, DVM, Gulf Coast Veterinary Specialists, Houston, TX; David Brinker, DVM, Todds Lane Veterinary Hospital, Hampton, VA; John Hashbarger, PhD, Registry of Tumors in Lower Animals, Washington, DC; Michael Katz, MD, Children’s Hospital of the Kings Daughters, for diagnostic imaging services; Greg Lewbart, DVM and Nancy Love, DVM, North Carolina State University College of Veterinary Medicine for consultation and radiographic interpretation; Doug Mader, DVM, Marathon Animal Hospital for repeated consultations; Paul Raiti, DVM, Beverlie Animal Hospital for consultation; Charles Sedgwick, DVM, for consultation; Doug Thamm, DVM, University of Wisconsin School of Veterinary Medicine for consultation; J.L. VanSteenhouse, DVM, PhD, Antech Diagnostics for diagnostic services; Helmut Triessmann, MD, Beth Fairbanks, RT-R, & Bill Hart, RT-R, Orthopaedic Surgery and Sports Medicine Specialists, Newport News, VA, for diagnostic imaging services; Pam Wolf, Access Technologies, Skokie, IL, for technical support.

molurus, and an angiosarcoma in a spitting cobra, *Naja nigrocollis*; initial regression of the tumor was achieved in both patients, but metastases occurred later (Jackson, *et al.*, 1981). Radiation therapy was used to treat a sun gazer lizard, *Cordythus giganteus*. The patient survived 11 m following one whole body radiation treatment of 1 Gray (Martin, *et al.*, 2003).
REFERENCES


