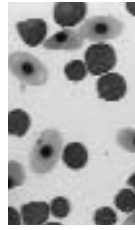


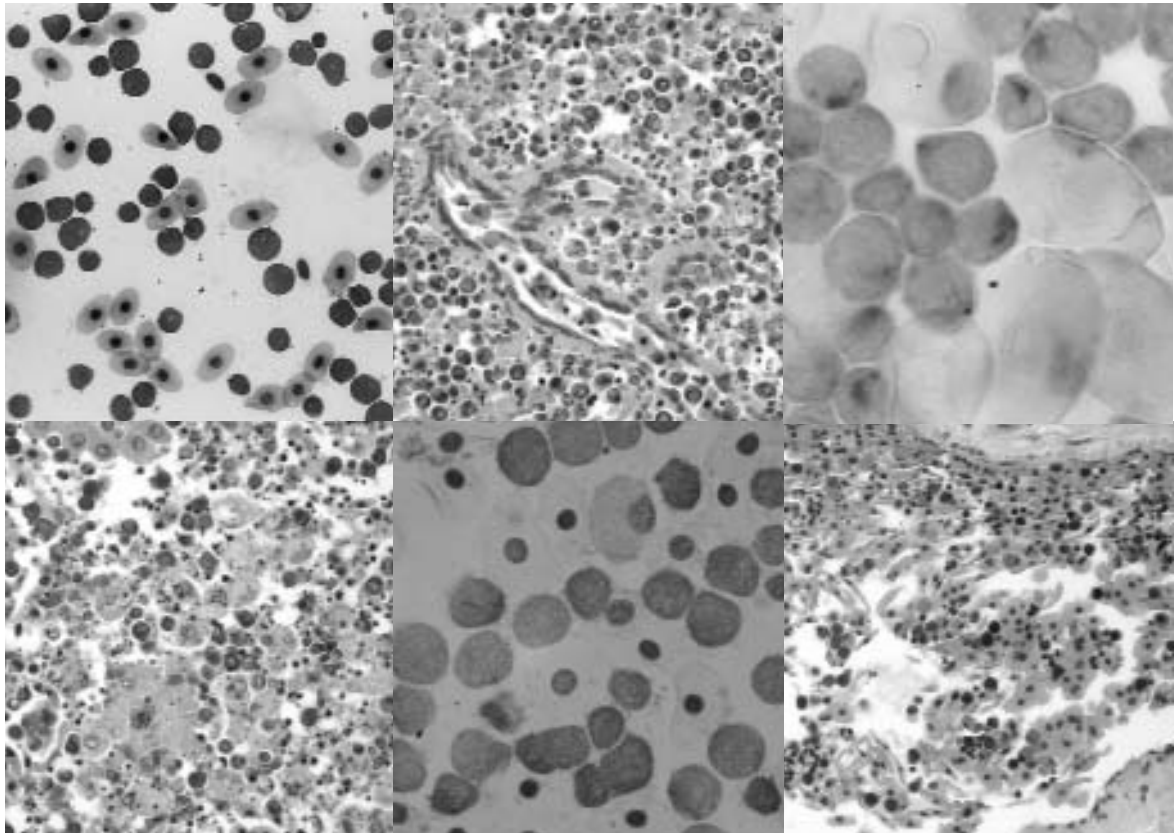
# Journal of Herpetological Medicine and Surgery



Volume 17, Number 3 • 2007

<b>Guidelines For Authors</b> .....	74
<b>Message To Members</b> .....	75
<b>Evaluating the Effect of Baquacil® and Sanosil® on <i>Salmonella</i> spp., in the Aquatic Habitat of the Red-Eared Slider Turtle, <i>Trachemys scripta elegans</i></b> .....	76
Trevor T. Zachariah, DVM, MS, Mark A. Mitchell, DVM, MS, PhD, Verna F. Serra, BA, Michael Walden, MS, DVM, Rudy W. Bauer, DVM, PhD, DACVP	
<b>Effects of Storage and Sample Type on Ionized Calcium, Sodium and Potassium Levels in Captive Tortoises, <i>Testudo</i> spp.</b> .....	84
Kevin Eatwell, BVSc (Hons), DZooMed (Reptilian), MRCVS RCVS Recognized Specialist in Zoo and Wildlife Medicine	
<b>Acute Lymphoblastic Leukemia in a Diamondback Terrapin, <i>Malaclemys terrapin</i></b> .....	92
Andrew M. Silverstone, DVM, Michael M. Garner, DVM, DACVP, John W. Wojcieszyn, PhD, C. Guillermo Couto, DVM, DACVIM, Rose E. Raskin, DVM PhD, DACVP	
<b>Systemic <i>Mycobacterium terrae</i> Infection in an Eastern Box Turtle, <i>Terrapene carolina carolina</i></b> .....	100
Hillary Noyes, DVM, Ellen Bronson, DVM, Sharon L. Deem, DVM, PhD, DACZM, Carlos Sanchez, DVM, Suzan Murray, DVM, DACZM	
<b>Preliminary Notes on Ivermectin to Control <i>Foleyella</i> Microfilaremia in Panther Chamaeleon, <i>Furcifer pardalis</i></b> .....	104
Mattia Bielli, Med.Vet	
<b>Book Review</b> .....	107
<b>Announcements</b> .....	108
<b>Conferences</b> .....	113

# Journal of Herpetological Medicine and Surgery



Volume 17, Number 3 • 2007

# 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.

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 con-

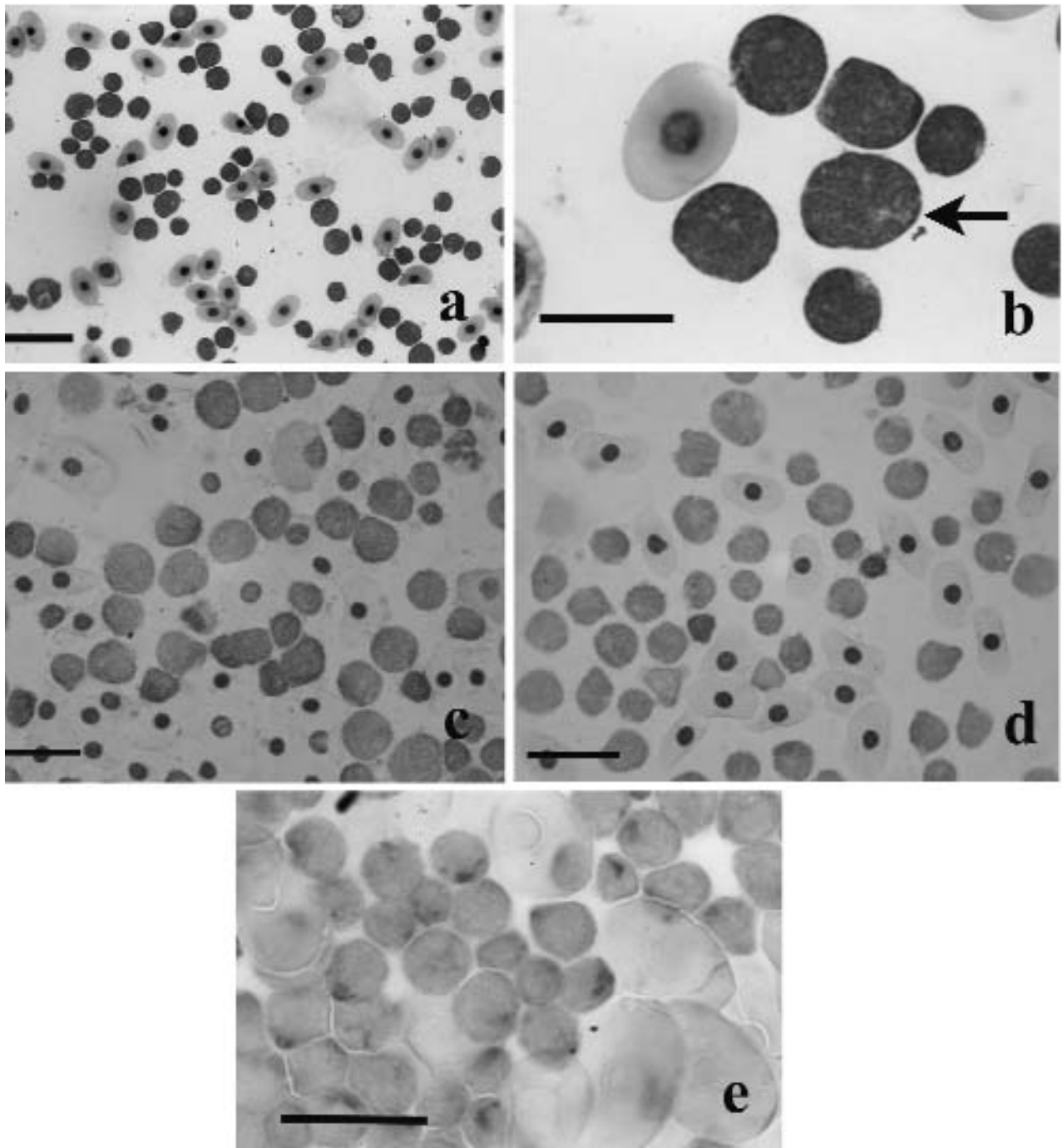
taining one or multiple prominent nucleoli, and rare bi-nucleation. Small well-differentiated lymphocytes, heterophils, basophils, and thrombocytes were also present in much fewer numbers. A marked lymphocytic leukocytosis was interpreted, making leukemia to be one of the suspected differential diagnoses. In addition to mature erythrocytes, occasional polychromatophils 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/, 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 cyanoacrylic 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

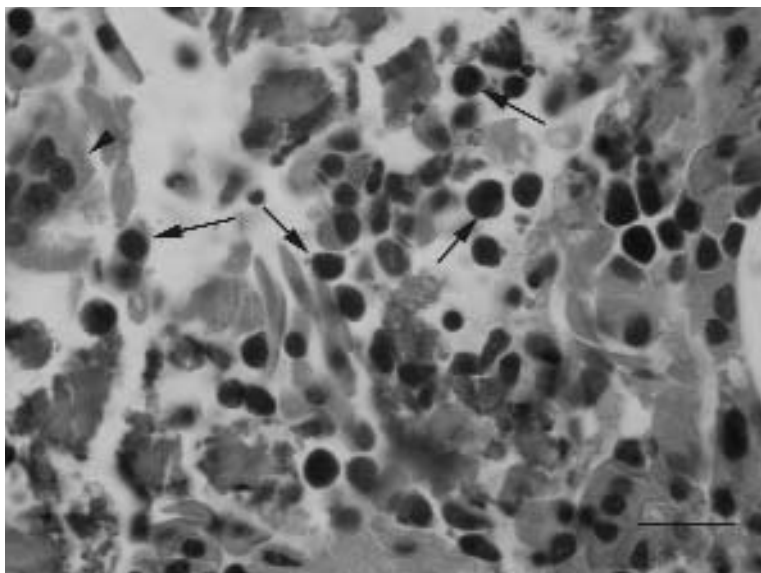
	Reference Interval (1)	First Presentation lymphatic dilution (results may be spurious)	Sixth Presentation	Seventh Presentation lipemia 2+	Eight Presentation	Ninth Presentation	Tenth Presentation	Eleventh Presentation	Twelfth Presentation
				T = 0	T = 7 d	T = 14 d	T = 24 d	T = 34 d	T = 46 d
		10/30/00	12/13/2000 a	2/17/01	2/24/01	3/3/01	3/13/01	3/23/01	4/4/01
Hematocrit	%	46	11	22	29	23	24	29	18
WBC Estimate	3-8 thds/cmm	154	1.5	200	65	33	10	16	28
Absolute Poly		10780	1020	8000	7800	6930	4800	6880	4480
Absolute Lymphs		140140	180	192000	57200	5940 (a)	2700	4800	22400
Absolute Monos		3080	300	0	0	20130	2500	3200	840
Absolute Basophils		0	0	0	0	0	0	0	280
Glucose	2.2-6.6 mmol/L or 40-120 mg/dL	9.6 174	15.4 279	1.7 31	2.6 47	9.0 163	7.3 131	9.8 177	1.3 23
Urea Nitrogen	3.5- 25 mmol/L or 10-70 mg/dL	0.4 1	5.4 15	31.8 89	49.6 139	64.6 181	37.8 106	31.4 88	24.6 69
Total Protein	33 – 55 g/L or 3.3-5.5 g/dL	52 5.2	18 1.8	33 3.3	32 3.2	32 3.2	35 3.5	38 3.8	24 2.4
Albumin	10-17 g/L or 1.0-1.7 g/dL	20 2	5 0.5	12 1.2	7 0.7	7 0.7	7 0.7	7 0.7	6 0.6
Globulin	1.7-2.9 g/dL	3.2	1.3	2.1	2.5	2.5	2.8	3.1	1.8
Calcium	2 - 3.2 mmol/L or 8-13 mg/dL	3.4 13.6	2.3 9.3	2.3 9.3	2.4 9.5	2.2 8.7	2.3 9.1	2.1 8.3	2.1 8.4
Phosphorus	1.6 – 2.5 mmol/L or 5-8 mg/dL	1.4 4.3	0.6 1.8	2.0 6.1	1.13 4	1.7 5.4	1.4 4.2	0.9 2.9	0.7 2.1
Sodium	130-150 mmol/L or 130-150 mEq/L	158 158	131 131	118 118	133 133	134 134	142 142	128 128	135 135
Potassium	2 – 5.5 mmol/L or 2.0-5.5 mEq/L	5.3 5.3	2.7 2.7	5.7 5.7	6.1 6.1	4.4 4.41.7	4.1 4.1	2.6 2.6	2.1 2.1
Chloride	90-120 mmol/L or 90-120 mEq/L	113 113	91 91	84 84	100 100	96 96	111 111	94 94	102 102
AST	10 - 80 U/L or 10 - 80 units/L	18 18	81 81	1396 1396	1948 1948	1332 1332	652 652	537 537	496 496
Creatine phosphokinase	none available	629	200	1916	8562	4480	764	950	3119
Uric Acid	119 – 416 μmol/L or 2-7 mg/dL	119 2	36.7 0.6	874 14.7	850 14.3	231 3.9	89.2 1.5	714 12	648 10.9



**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  $\mu\text{m}$ . b) Note Giemsa-stained atypical lymphocytes have scant deeply basophilic cytoplasm, and frequent cleaved or lobed nuclei with prominent nucleoli (arrow). Bar = 20  $\mu\text{m}$  c) Atypical lymphoid cells have positive cytoplasmic staining for BLA36, a mammalian B cell marker. Bar = 20  $\mu\text{m}$  d) Normal circulating lymphocytes have positive cytoplasmic staining for CD3, a mammalian T cell marker. Bar = 20  $\mu\text{m}$  e) Atypical lymphocytes stain focally and diffusely positive for acid phosphatase. Bar = 20  $\mu\text{m}$ .

glue (Nexaband, Veterinary Products Laboratories, Phoenix, AZ). Postoperative doses of cefotaxime sodium (Claforan, Hoechst/Roussel, Kansas City, MS; 18 mg/kg SQ) and silver sulfadiazine (Silvadene Cream, Hoechst Marion Roussel, Kansas City, MS; 1% topical) were administered. The biopsy specimens were processed routinely (without decalcification), embedded in paraffin wax, sectioned routinely at 5  $\mu\text{m}$ , and stained with hematoxylin and eosin.

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



**Figure 2.** Bone marrow biopsy, gular bridge, diamondback terrapin. *Malaclemys terrapin*. Note atypical round cells (arrows) consistent with neoplastic lymphocytes admixed with bone fragments, erythrocytes and an osteoclast (arrowhead). HE, bar = 25  $\mu$ m.

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 and gross swellings observed at the left stifle and the distal phalanges of all the limbs. Sclerotic bony changes were present radiographically in both femurs and tibias, and extensive bony lysis was present in the stifles and both tarsi. There was extensive disruption of the bone density in the tarsal region. A radiological mixed intensity was interpreted at the stifle, consistent with mineralization associated with tophaceous gout. A fine needle aspirate was obtained from swelling around the left stifle. A diagnosis of gout was made based on the fine needle aspirate of the swelling. The stifles and tarsi were palpably enlarged due to urate deposits resulting from gout.

At the sixth presentation (nine weeks after the first presentation), 14 unstained, air-dried blood smears were obtained and four were submitted for immunocytochemistry and ten for cytochemistry. For immunocytochemistry, air-dried blood smears were fixed in cold acetone for three minutes and then allowed to air-dry for 15 min. Each slide was washed in Tris buffer (pH 7.6), excess buffer was removed, and the smears were incubated for 30 min with a protein-blocking reagent. All incubations were performed at room temperature. Following a brief wash in Tris buffer, endogenous biotin binding sites were blocked using a commercial Avidin/Biotin blocking kit (Signet Pathology Systems, Dedham, MA). An additional protein block was applied using SuperBlock (Pierce, Rockford, IL) for five minutes. Antibodies to BLA36, at a dilution of 1:10 and CD3, at a dilution of 1:1000, were applied to separate slides and incubated for one hour.

Detection of the bound antibodies was accomplished using commercially available biotinylated mouse or rabbit antibodies and streptavidin linked alkaline phosphatase kits (Signet Pathology Systems). The final colored product was developed with a New Fuchsin substrate kit (KPL, Gaithersburg, MD). Negative controls were performed by substitution of the primary antibody with irrelevant antisera.

Results of immunocytochemical staining of peripheral blood cells obtained at week nine revealed that the neoplastic cells were positive for the BLA36 antigen, demonstrating a 3-4+ membrane stain (Figure 1c). The neoplastic cells were negative for CD3 antigen, although few circulating small well-differentiated lymphocytes were positive for this marker (Figure 1d).

Cytochemical staining of blood films was performed using techniques described previously (Raskin and Valencio, 2000). Positive controls were run concurrently that involved blood and bone marrow smears from domestic animals. The neoplastic cells in this case did not stain with benzidine peroxidase, chloroacetate esterase, or alpha naphthyl butyrate esterase. Acid phosphatase (Figure 1e) demonstrated both diffuse and focal positive staining of the neoplastic cells.

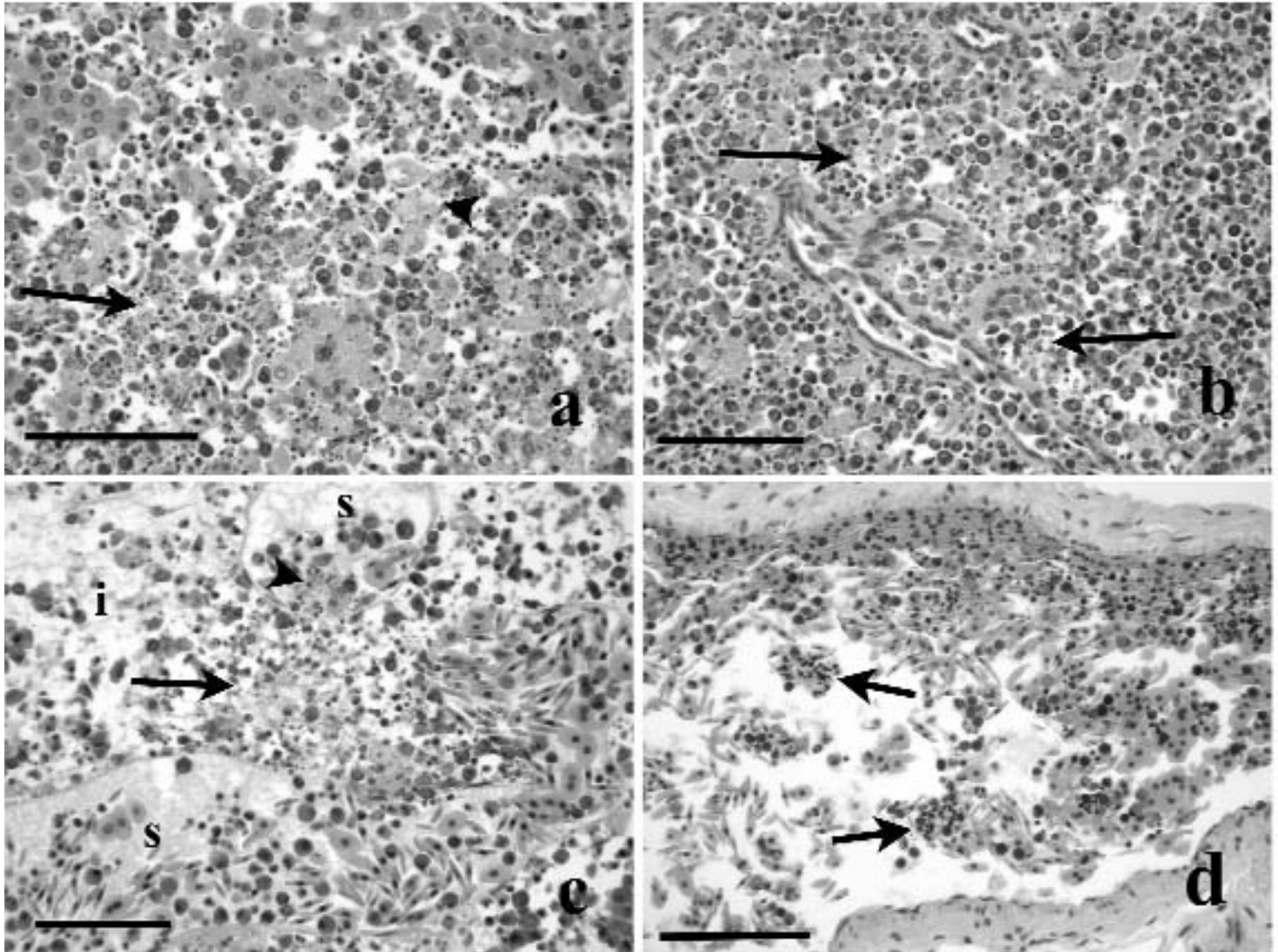
A CBC and serum chemistry were performed, but the results were deemed to be spurious due to lymphatic dilution.

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, Research Triangle Park, NC; 20 mg/kg PO q 48 hr). The anticancer therapy consisted of prednisone (Deltasone, Pharmacia 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 (ninth 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 diamond-back terrapin, *Malaclemys terrapin*. a) Note extensive necrosis of neoplastic lymphoid cells (arrow) and necrotic hepatocytes (arrowhead) in liver, bar = 115  $\mu$ m. b) Note necrosis of lymphoid cells (arrows) in spleen, bar = 100  $\mu$ m. 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  $\mu$ m. d) Note necrosis of neoplastic cells in lumen of large pulmonary vein, bar = 100  $\mu$ m.

## DISCUSSION

Neoplasms were not included in the differential diagnoses at the time of presentation. Chelonian tumors involving the integumentary (Billips and Harshbarger, 1976, Jackson, *et al*, 1981, Machotka, 1984, Norton, *et al*, 1990, hematopoietic (Frye and Carney, 1972, Harshbarger, 1974, Harshbarger, 1976, Machotka, 1984), endocrine (Cowan, 1968, Frye and Carney, 1975 Frye, 1991, Machotka, 1984), respiratory (Machotka, 1984, Smith, *et al*, 1941), gastrointestinal (Cowan, 1968, Frye, 1991, Smith, *et al*, 1941), reproductive (Frye, *et al*, 1988), urinary (Machotka, 1984, Norton, *et al*, 1990), and musculoskeletal systems (Cooper, *et al*, 1983) have been reported. There are only nine reported cases of hematopoietic tumors in chelonians reported to the Registry of Tumors in Lower Animals (Cooper, *et al*, 1983), (Frye and Carney, 1972, Harshbarger, 1974, Harshbarger, 1976, Machotka, 1984, Roskopf, *et al*, 1981). Of these nine cases, four were leukemic (Cooper, *et al*, 1983, Frye and Carney, 1972, Harshbarger, 1976, Roskopf, *et al*, 1981). The other cases involving hematopoietic tumors were 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 a bone marrow biopsy, CT, MRI, as well as cytochemical 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 projection of the plastron or the bridge of the plastron and carapace where bone marrow and cancellous bone are present between the thick cortices (Garner, *et al*, 1996, Jenkins, 1996). Although a diagnostic sample was obtained using a 14 ga hypodermic needle, the sinusoidal architecture was fragmented in our sample. Biopsy samples may be better preserved if obtained 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 monocytic cells or atypical lymphoid cells. A certain human B-cell leukemia termed hairy cell leukemia is frequently associated with diffuse acid phosphatase staining.

The use of CT and MRI for a tortoise has been described (Raiti and Haramati, 1997, Silverman, 2006, Wyneken, 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 obsoleta*. 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*

*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, *Cordylus giganteus*. The patient survived 11 m following one whole body radiation treatment of 1 Gray (Martin, *et al*, 2003).

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 nasicornia*, 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 clinic 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 (Willete, *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 monocytosis 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, VMD, University of Wisconsin School of Veterinary Medicine for consultation; J.L. VanSteenhouse, DVM, PhD, Antech Diagnostics for diagnostic services; Helmunth Trieshmann, 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.

## REFERENCES

- Alleman AR, Jacobson ER, Raskin RE. 1992. Morphologic and cytochemical characteristics of blood cells from the desert tortoise (*Gopherus agassizii*). *Am J Vet Res*, 53:1645-1651.
- Billips LH, Harshbarger JC. 1976. Neoplasia: Reptiles. In Melby EC, Altman NH (eds): *Handbook of Laboratory Science*, Volume III. CRC Press, Cleveland, OH:343-56.
- Cooper JE, Jackson OF, Harshbarger JC. 1983. A neurilemmal sarcoma in a tortoise (*Testudo hermanni*). *J Comp Pathol*, 93:541.
- Cowan DF. 1968. Diseases of captive reptiles. *JAVMA*, 153:848.
- Frye FL. 1991. Common Pathological Lesions and Disease Processes: Neoplasia. In Frye FL (ed): *Biomedical and Surgical Aspects of Captive Reptile Husbandry*, Volume 2. Kieger Pub Co, Malabar, FL:576-609.
- Frye FL, Carney J. 1972. Myeloproliferative disease in a turtle. *JAVMA*, 161(6):595-9.
- Frye FL, Carney J. 1975. Parathyroid adenoma in a tortoise. *Vet Med Sm Anim Clin*, 70:582.
- Frye FL, Dybal NO, Harshbarger JC. 1988. Testicular interstitial tumor in a desert tortoise (*Gopherus agassizii*). *J Zoo Anim Med*, 19(1-2):55.
- Garner MM, Homer BL, Jacobson EB, Raskin RE, Hall BJ, Weis WA, Berry KH. 1996. Staining and morphologic features of bone marrow hematopoietic cells in desert tortoises (*Gopherus agassizii*). *Amer J Vet Res*, 57(11):1608-15.
- Harshbarger JC. 1974. *Activities Registry of Tumors in Lower Animals, 1965-1973*. Smithsonian Institution Press, Washington D.C.:53, 61-65, 69, 72, 80, 82.
- Harshbarger JC. 1976. *Activities Registry of Tumors in Lower Animals, 1975 Supplement*. Smithsonian Institution Press, Washington D.C.:14, 15, 19, 22.
- Jacobson E. 1981. Neoplastic Diseases. In Cooper JE and Jackson OF (eds): *Diseases of Reptilia*, Volume 2. Academic Press. San Diego, CA:429-68.
- Jacobson E, Calderwood MB, French TW, Iverson W, Page D, Raphael B. 1981. Lymphosarcoma in an eastern kingsnake and rhinoceros viper. *JAVMA*, 179:1231.
- Jenkins JR. 1996. Diagnostic and Clinical Techniques. In Mader DR (ed): *Reptile Medicine and Surgery*. WB Saunders Co, Philadelphia, PA:264-276.
- Leach MW, Nichols DK, Hartsell W. 1991. Radiation therapy of a malignant chromatophoroma in a yellow rat snake (*Elaphe obsoleta quadrivittata*). *J Zoo Wild Med*, 22(2):241.
- Machotka SV. 1984. Neoplasia in Reptiles. In Hoff GL, Frye FL, Jacobson ER (eds): *Diseases of Amphibians and Reptiles*. Plenum Press, New York, NY:519-80.
- Martin JC, Moore AS, Ruslander D, Mayer J, Raymond J, Garner M, Knoll J, James S, Bengtson A. 2003. Successful radiation treatment of leukemia in a sun gazer lizard. *Proc ARAV*, 8.
- Monzon-Mayor M, Yanes C, DeBarry J, Capdevilla-Carbonell C, Renau-Piqueras J, Tholey G, Gombos G. 1998. Heterogenous immunoreactivity of glial cells in the mesencephalon of a lizard: a double immunohistochemical study. *J Morphology*, 235(2): 109-19
- Mouldin GN, Done LB. 2006. Oncology. In Mader DR (ed): *Reptile Medicine and Surgery*. WB Saunders Company, Philadelphia, PA:299-322.
- Norton TM, Jacobson ER, Sundberg JP. 1990. Cutaneous fibropapillomas and renal myxofibroma in a green turtle, *Chelonia mydas*. *J Wildl Dis*, 26(2):265.
- Raiti P, Haramati N. 1997. Magnetic resonance imaging and computed tomography of a gravid tortoise (*Geochelone pardalis pardalis*) with metabolic bone disease. *J Zoo Wildl Med*, 28(2):189-97.
- Ramsay EC, Fowler M. 1992. Reptile neoplasms at the Sacramento Zoo, 1981-1991. *Proc Joint Conf AAZV and AAWV*. Oakland, CA:153-5.
- Raskin RE, Valenciano A. 2000. Cytochemistry of Normal Leukocytes. In Feldman BF, Zinkl JG, Jain NC (eds). *Schalm's Veterinary Hematology*. 5<sup>th</sup> Ed., Lippincott, Williams & Wilkins, Philadelphia, PA:337-346.
- Roberts WG, Klein MK, Loomis M, Weldy S, Berns MW. 1991. Photodynamic therapy of spontaneous cancers in felines, canines, and snakes with chloroaluminum sulfonated phthalocyanine. *J Natl Cancer Inst*, 83(1):18.
- Rosenthal K. 1994. Chemotherapeutic treatment of a sarcoma in a corn snake. *Proc ARAV*, 46.
- Roskopf WJ, Howard EB, Gendron AP. 1981. Granulocytic leukemia in a tortoise. *Mod Vet Pr*, 701-2.
- Silverman S. 2006. Diagnostic Imaging. In Mader DR (ed): *Reptile Medicine and Surgery*. WB Saunders Company, Philadelphia, PA:471-489.
- Smith GM, Coates C, Nigrelli R. 1941. A papillomatous disease of the gallbladder associated with infection by flukes, occurring in the marine turtle, *Chelonia mydas*. *Zoologica*, 26:13.
- Willette MM, Garner MM, Drew M. 2001. Chemotherapeutic treatment of lymphoma in a king cobra. *Proc AAZV*, 20.
- Work TM, Raskin RE, Balazs GH, Whittaker SD. 1998. Morphologic and cytochemical characteristics of blood cells from Hawaiian green turtles. *Am J Vet Res*, 59:1252-1257.
- Wyneken J. 2006. Computed Tomography and Magnetic Resonance Imaging Anatomy of Reptiles. In Mader DR (ed): *Reptile Medicine and Surgery*. WB Saunders Company, Philadelphia, PA:1088-1096.