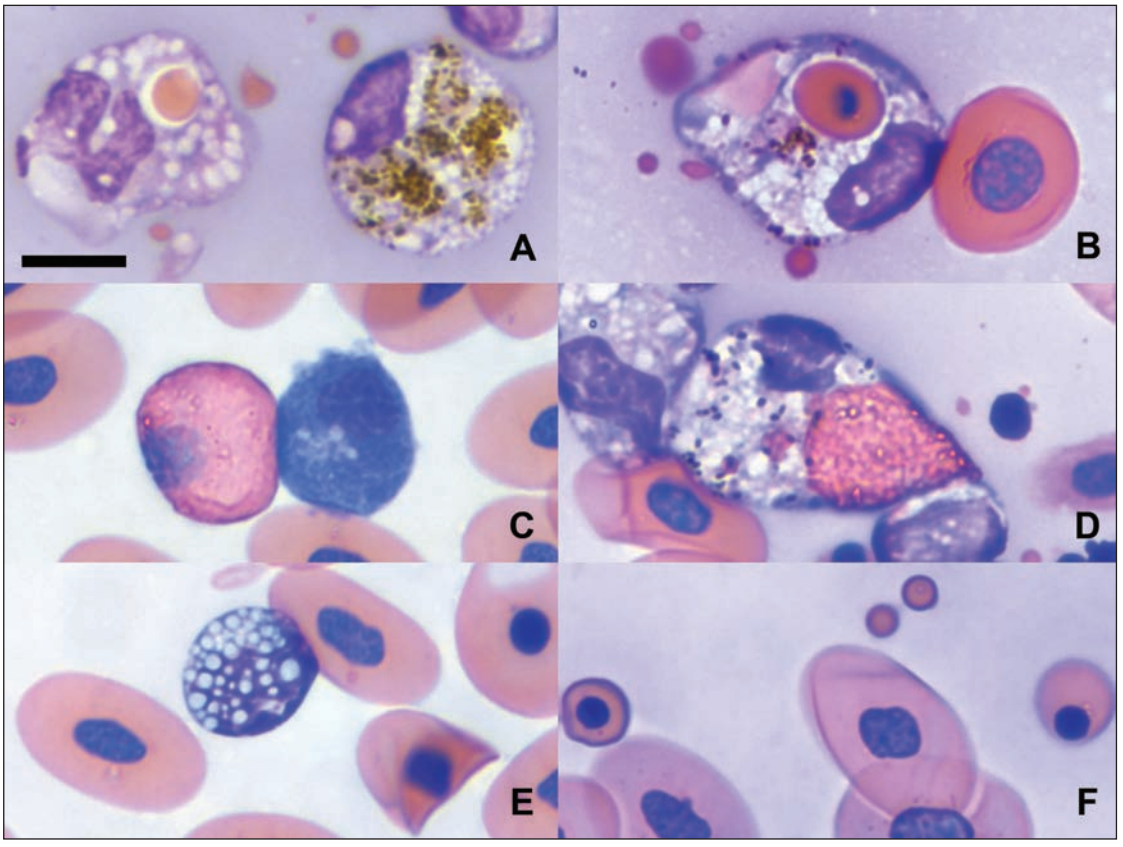

Figure 1—Representative photomicrographs of blood smear preparations obtained prior to euthanasia from an 8-year-old male diamond python *(Morelia spilota spilota*) that had a chronic respiratory tract infection with accompanying ulcerative stomatitis. Pertinent hematologic findings included an increased concentration of macrophages, some containing brown granules or evidence of erythrophagia (A and B); high concentrations of heterophils (left [C]) and plasma cells (right [C]); rare macrophages with leukophagia (eg, heterophils [D]), rare Mott cells (E); and fragmented and spherocytoid erythrocytes (F). Modified Wright-Giemsa stain; bar (applicable to all images) = 10 µm.

History, Clinical Findings, and Laboratory Data

An 8-year-old male diamond python (*Morelia spilota spilota*) developed a chronic respiratory tract infection with accompanying ulcerative stomatitis, which partially improved with repeated courses of antimicrobial treatment. Its overall health continued to deteriorate, with the development of diarrhea, regurgitation, and poor muscle tone, followed by severe mental dullness, signs of depression, marked weight loss, and dehydration. It was euthanized, and a final blood sample, obtained just prior to euthanasia, was prepared for hematologic assessment. Marked leukocytosis (86×10^9 leukocytes/L; reference interval,[1] 1×10^9 leukocytes/L to 29×10^9 leukocytes/L) was present, which consisted of predominant populations of heterophils (52×10^9 heterophils/L; reference interval, 1×10^9 heterophils/L to 22×10^9 heterophils/L) and monocytic cells (30×10^9 monocytes/L; reference interval, 1×10^9 monocytes/L to 13×10^9 monocytes/L). Many of the monocytic cells had intracytoplasmic, rod-shaped, refractile brown granules, and other clinically relevant hematologic findings were detected (**Figure 1**). The PCV was 0.25 (0.09 to 0.31).[1] In the blood smear, occasional thrombocyte clumps were noted and numbers of individual thrombocytes were moderately reduced, compared with findings expected for a healthy python.

Formulate differential diagnoses from the history, clinical findings, and Figure 1—then turn the page →

This report was submitted by Graham J. Stock, BVMS, DACVP; Yedra Feltrer, DVM, MSc; Edmund J. Flach, MA, VetMB, MSc, DZooMed; and Balazs Szladovits, DVM, DACVP; from the Department of Pathology and Infectious Diseases, Royal Veterinary College, North Mymms, Hatfield, Hertfordshire, AL9 7TA, England (Stock, Szladovits); and the Veterinary Department, Zoological Society of London, Regent's Park, London, NW1 4RY, England (Feltrer, Flach).

Address correspondence to Dr. Stock (graham.stock@googlemail.com).

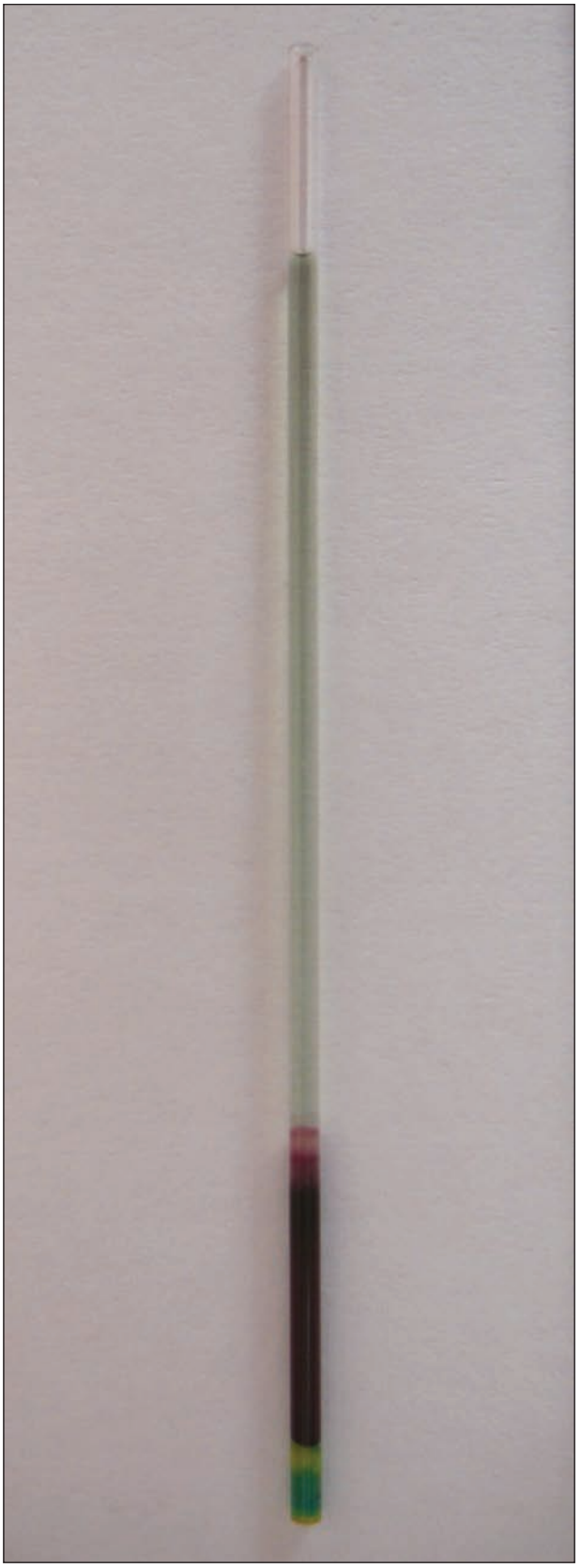

Figure 2—Photograph of a microhematocrit capillary tube containing blood obtained from the diamond python in Figure 1 after centrifugation. Notice the green color of the plasma.

Additional Clinicopathologic Findings

Special stains were applied to blood smears or buffy coat preparations derived from the final blood sample. The rod-shaped granules in many of the monocytic cells reacted with Masson Fontana stain, confirming the presence of melanin. Reticulocytes were not identified in the new methylene blue–stained smears. Granular intracytoplasmic material within rare macrophages contained iron, as indicated by Prussian blue staining.

The main serum biochemical findings for the final blood sample included marked hyperuricemia (uric acid concentration, 6,061 µmol/L; reference interval,[1] 47 to 301 µmol/L), which was likely attributable to dehydration and possibly renal functional compromise. Moderately high creatine kinase activity (7,561 U/L; reference interval, 379 to 4,767 U/L) reflected muscle damage.

The plasma in the microhematocrit capillary tube was found to be green (**Figure 2**), whereas that obtained from a healthy female cagemate of the same species was straw colored. Given the unusual color of the plasma, serum from the last blood sample was kept frozen at –20°C and analyzed 2 months following collection for biliverdin concentration at the Infectious Diseases Laboratory, University of Georgia, Athens, Ga. The serum biliverdin concentration was 45 µmol/L (no reference interval).

Postmortem Examination and Histopathologic Findings

A postmortem examination was performed, during which tissue samples from various organs were collected for microscopic examination. The python was found to be thin, and the dorsal left region of its pharynx and left tonsil were hyperemic with the presence of slight purulent material. Twenty milliliters of clear, green-gray fluid filled the coelom, which clotted in the sample pot. The liver was enlarged, light brown-gray, and friable when sectioned.

The most important histopathologic findings included focal necrotizing and heterophilic myocarditis, mild diffuse chronic-active interstitial pneumonia with intralesional rod-shaped bacteria, focally extensive necrotizing and heterophilic inflammation with intralesional gram-negative rod-shaped bacteria in the gallbladder, ulcerative and heterophilic stomatitis in the buccal mucosa, and mild to moderate hepatic lipidosis. On assessment of bone marrow from a rib, hematopoietic tissue appeared normo- to hypercellular, with a predominance of late heterophil precursors and a smaller proportion of late erythroid precursors.

Microbiological, Molecular, and Immunohistochemical Findings

Culture of swab specimens obtained at the time of the postmortem examination from liver, lungs, and blood from the heart failed to yield bacterial growth. Swab specimens from the dorsal aspect of the pharynx and tonsils had been obtained twice during treatment, yielding cultures of nonhemolytic *Staphylococcus* spp

and *Morganella morganii*. Culture of a swab specimen obtained from the same site during the postmortem examination yielded *Sphingomonas paucimobilis*. Results of a reverse transcriptase PCR assay and cDNA:RNA in situ hybridization to detect ophidian paramyxovirus on formalin-fixed, paraffin-embedded lung and heart tissue were negative, as were results of immunohistochemical testing for *Chlamydophila* spp on the same tissues.

Interpretation and Case Summary

Interpretation: marked heterophilia and monocytosis, along with considerable numbers of phagocytic macrophages, melanomacrophages, and reactive lymphocytes, indicative of a severe inflammatory response; moderate erythrocyte fragmentation and mild erythrophagocytosis consistent with hemolysis; and possible thrombocytopenia.

Case summary: marked leukocytosis as a result of end-stage bacteremia or septicemia in a diamond python.

Comments

Melanomacrophages are a major component of the mononuclear phagocytic system of fish, amphibians, and reptiles.[2] Because they have enhanced function, compared with mammalian macrophages, at low temperatures in cell cultures, they may play an important role in maintaining effective immune function during periods of reduced body core temperature (eg, hibernation).[3]

Melanin granules (or melanosomes) are thought to be synthesized by the macrophages, although it is possible for the cells to acquire granules through phagocytosis.[2] Melanin is known to neutralize free radicals (eg, iron compounds from heme) and toxic agents and may have antibacterial properties.[4] The presence of melanomacrophages in reptilian peripheral blood is not necessarily an abnormal finding, although the high concentration of these cells in the final blood sample obtained from the snake of the present report was likely attributable to the influence of septic inflammatory disease and hemolysis.[5]

For the case described in this report, considerable erythrocyte poikilocytosis (including erythrocyte fragmentation) and erythrophagocytosis in the blood smears may have represented any of a number of hemolytic mechanisms (eg, shear trauma or immune-mediated hemolysis), which remain open to speculation. Prominent erythrophagocytosis in reptilian blood has previously been documented and attributed to hemophagocytic syndrome and recirculation of erythrophagocytic macrophages from a site of hemorrhage.[6,7] Erythrophagocytosis has also been documented as an incidental finding in healthy reptiles.[5] The exact reason why this process occurs in reptiles more often than it does in mammals is uncertain.

Green plasma evident in the pre-euthanasia blood sample obtained from the diamond python of the present report was also remarkable. The plasma of most reptiles is straw colored, although it can be greenish yellow in pythons because of the presence of carotenoids and riboflavin.[8] Also, some reptilian species may have high physiologic concentrations of biliverdin that will color the plasma green.[8] Considering that plasma from a healthy female cagemate of the same species was straw colored, it was likely that the green plasma was due to hyperbiliverdinemia as a result of hemolysis.

End-stage bacteremia or septicemia remains a likely cause for the hematologic findings in the snake of this report. A primary bacterial pathogen from the septic ulcerative stomatitis may have been aspirated into the lower airways, resulting in further dissemination.[9] *Morganella morganii* and *Staphylococcus* spp, isolated from the python's oral cavity lesions, were considered commensals at this site. *Sphingomonas paucimobilis*, which exists in land and water habitats, was also isolated from an oral lesion.[10–12] Alternative pathogens that may have predisposed the snake to secondary bacterial infections include ophidian paramyxovirus, *Chlamydophila* spp, and inclusion body disease virus. We cannot completely rule out infection with any of these pathogens on the basis of results of the diagnostic tests performed. Hematologic findings aided in establishment of the presence of inflammatory disease in the reptile of this report. The most notable findings indicative of inflammation included marked leukocytosis characterized by heterophilia and monocytosis; a high concentration of phagocytic macrophages, including melanomacrophages; and large numbers of reactive lymphocytes and plasma cells. Concern for a hemolytic process was prompted by prominent erythrocyte poikilocytosis with accompanying erythrophagocytosis.

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