Pathology in Practice

History

A 450-g (0.99-lb) adult female eastern box turtle (*Terrapene carolina carolina*) was found moribund by a landowner in July 2008, near the edge of a 1-acre pond in Ohio County, Ky. The landowner observed 7 additional dead box turtles in various stages of decomposition in the immediate vicinity and contacted the Kentucky Department of Fish and Wildlife Resources. The live turtle was subsequently collected by department personnel and was submitted to the Southeastern Cooperative Wildlife Disease Study, University of Georgia, for diagnostic evaluation.

Clinical and Gross Findings

Upon physical examination, the turtle had severe signs of depression and was dehydrated, lethargic, and poorly responsive to external stimuli. It had mild, bilateral, mucopurulent nasal discharge and slight respiratory difficulty. Given the severity of the clinical signs and grave prognosis, the moribund turtle was euthanized and a postmortem examination was performed.

Necropsy revealed that the right palpebral fissure was markedly distended and sealed shut. The entire right side of the face was moderately swollen and firm. The swelling extended from the dorsal midline ventrally along the face (involving the submandibular soft tissues) and from the cranial cervical region rostrally to the oral commissure. The skin overlying much of this area was yellow-tan and was partially sloughed. The entire hard palate was covered by a light-tan plaque with a rough and undulating surface, and the underlying mucosa was ulcerated (Figure 1). A similar lesion (0.75 X 1.5 cm) was present in the proximal portion of the esophagus. On cut surface, many muscles of the head were pale, dry, and firm. Numerous multifocal, discrete, 1- to 3-mm-diameter, raised, tan-white nodules were widely scattered over all coelomic and serosal surfaces. Many nodules had coalesced to form extensive white plaques, which were especially prominent on the mesentery, liver, spleen, and stomach. The nodules and plaques were tightly adhered to the surfaces of the viscera.

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

Sections of various tissues (including heart, trachea, lungs, liver, kidneys, spleen, pancreas, uterus, ovaries, esophagus, stomach, small and large intestines, head, and skin) were prepared for histologic examination. A common microscopic feature in multiple tissues was the infiltration of blood vessel walls by moderate to marked numbers of heterophils and fewer lymphocytes (Figure 2). In sections of skin and muscles of the head, lungs, intestines, mesentery, spleen, uterus, and ovaries, the walls of some arterioles were segmentally expanded by eosinophilic, amorphous material (fibrinoid change), and fibrin thrombi with admixed heterophils and karyorrhectic debris were common. In the skin and muscles of the head, expansile foci of coagulative necrosis and heterophilic inflammation often radiated from affected blood vessels and spread to adjacent soft tissues, with extensive involvement of the bones of the head. Similar expansile foci were also present in the intestinal mesentery and lungs.

Myonecrosis of the head muscles was characterized by myofiber swelling, loss of striations, fragmentation of sarcoplasm, and expansion of the interstitium by edema fluid, heterophils, fibroblasts, and karyorrhectic debris. The dermis and epidermis overlying affected muscles were typically effaced by multifocal to coalescing areas of coagulative necrosis and intense heterophilic inflammation that was centered on and extended from the vascular inflammation and necrosis. The bony trabeculae of the skull, meninges, and subdural space were multifocally infiltrated by expansile accumulations of heterophils and karyorrhectic debris.

The palatine and esophageal mucosae contained focally extensive ulcers that were lined by necrotic material and covered by dense serocellular crusts admixed with a few colonies of coccoid bacteria (Figure 3). The submucosa was markedly expanded by large numbers of heterophils, moderate numbers of lymphocytes, macrophages, and karyorrhectic debris that radiated from blood vessels.

The intestinal mesentery and intestinal serosa had variably sized expansile foci of necrosis radiating from blood vessels and were characterized by the accumulation of karyorrhectic debris, fibroblasts, and mixed cellular infiltrates. Rare aggregates of variably sized, typically small, dark pink, homogenous droplets (consistent with yolk) were present within the foci.

Pulmonary septae were multifocally expanded by accumulations of moderate numbers of heterophils, macrophages, lymphocytes, fibrin, and karyorrhectic debris. Thrombi of similar composition were frequently detected in blood vessels in the lungs, and small numbers of heterophils were accumulated within vessel walls. Occasionally, heterophils had infiltrated the overlying respiratory epithelium and accumulated within airways.

The walls of the splenic ellipsoids were frequently infiltrated by small numbers of heterophils. Occasionally, the ellipsoids were moderately expanded by homog-
enous to slightly fibrillar eosinophilic material (fibrin). Replacing the periellipsoid lymphocyte sheath were scant karyorrhectic debris and infiltrates of moderate numbers of heterophils with fewer macrophages and lymphocytes.

**Morphologic Diagnosis**

Severe, chronic, locally extensive, ulcerative and necrotizing pharyngitis and esophagitis with multicentric heterophilic vasculitis, thrombosis, and fibrinoid change; severe, chronic, multifocal to coalescing, necrotizing, fibrinoheterophilic myositis and osteomyelitis of the head; mild, chronic, multifocal, heterophilic menigitis with vacuoles; severe, subacute, diffuse, fibrinoheterophilic, necrotizing splenitis; moderate, multifocal, subacute, heterophilic, necrotizing multinodular pneumonia with vasculitis and thrombosis; and severe, diffuse, chronic, heterophilic, lymphohistiocytic, necrotizing coelomitis (peritonitis) with occasional egg yolk globules.

**Comments**

On the basis of the gross and histologic findings, differential diagnoses for the turtle of this report included ranavirus infection, herpesvirus infection, and septicemia, each with or without concurrent mycoplasmosis. A ranavirus was isolated subsequently from samples of the turtle's lungs, liver, spleen, and kidneys cultured on *Terrapene carolina* heart cells. Sequencing of a 495-bp fragment of the major capsid protein of the isolate revealed a 100% nucleotide identity with Frog virus-3 (FV-3). Furthermore, PCR assays were performed on an oropharyngeal swab sample and results were positive for FV-3 DNA but negative for herpesvirus DNA. Aerobic bacterial culture of liver tissue and an oropharyngeal swab yielded no growth. Results of mycoplasmal culture and PCR assay of nasal flush and oropharyngeal swab samples were negative. The isolation of FV-3 from various tissues along with gross and histologic findings that were consistent with previous reports was indicative of a ranaviral infection in this case. However, given that these findings pertained to 1 turtle, it cannot be definitively concluded that FV-3 infection was the cause of the remaining turtle deaths at the pond in Ohio County, Ky.

Commonly reported clinical signs of ranavirus infection in chelonians include signs of depression, dyspnea, palpebral edema, ocularonasal discharge, and death. Gross necropsy often reveals conjunctivitis and yellow-tan casoude plaques adhered to the tip of the tongue, palate, pharynx, and esophagus. Characteristic microscopic lesions include necrocerulear glosstis, stomatitis, pharyngitis, and esophagitis. Splenitis and multicentric vasculitis with fibrinoid change are also typical. Multifocal, necrotizing tracheitis and pneumonia and subcutaneous abscesses have also been reported. Basophilic intracytoplasmic inclusion bodies are sometimes present in affected tissues. The gross and histologic findings in the turtle of this report were consistent with those previously reported with a few exceptions. The most notable difference was the extensive coagulative necrosis of soft tissues of the head and severe necrotizing coelomitis. The cranial myonecrosis appeared to be secondary to severe vasculitis and expanding soft tissue inflammation. The coelomic lesions were complicated, and at least partially originated from a multifocal egg yolk coelomitis. Intracytoplasmic inclusion bodies were not detected histologically in any tissue from the turtle, but that finding is consistent with other reports.

Iridoviruses in the genus *Ranavirus* are known for their ability to cause mass mortality events in many amphibian and fish species, and there is increasing evidence of their ability to infect reptiles. Ranaviral infections in free-ranging box turtles in Maryland, Tennessee, Florida, Georgia, Pennsylvania, and New York state have been previously reported. Other than box turtles, the only other reported ranaviral infection in a free-ranging North American chelonid was identified in a gopher tortoise (*Gopherus polyphemus*) from Florida. To our knowledge, the turtle of this report represents the first reported case of a ranavirus infection in a free-ranging chelonid in Kentucky.

The medical treatment, rehabilitation, and release of free-ranging chelonians and amphibians that have a suspected or confirmed ranaviral infection should not be attempted. Because of the broad host range of this pathogen and the potential population-level consequences of ranaviral infection on free-ranging amphibians and chelonians, infected animals should be euthanatized. When treating wildlife for suspected or confirmed infectious and contagious processes, one must consider the health of the entire population ahead of that of the individual patient. Proper diagnostic evaluation is essential in this process.

**References**

a. ATCC No. CCL-50, TH-1, Subline B1, American Type Culture Collection, Manassas, Va.
b. Veterinary Diagnostic and Investigational Laboratory, University of Georgia, Tifton, Ga.
c. Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, Ga.


**Ranavirus**