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SPLENOPANCREATIC DUCTAL ADENOCARCINOMA WITH MULTIORGAN METASTASIS IN A NORTHERN WATER SNAKE (*NERODIA SIPEDON*)

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Abstract: A 16-yr-old northern water snake (*Nerodia sipedon*) presented with a large, firm midcoelomic swelling. A complete blood count, survey radiographs, coelomic ultrasound, and fine needle aspirate of the mass were performed. Survey radiographs revealed a soft tissue opacity mass. Ultrasonographic examination confirmed the presence of the mass but could not aid in its visceral localization. After 2 weeks, the snake presented again because of continued anorexia and poor quality of life. Euthanasia was performed. Gross necropsy revealed a multilobulated mass attached to and effacing the splenopancreas. Histologically, the mass was composed of cuboidal to columnar neoplastic epithelial cells forming tubules surrounded by variable amounts of fibrovascular stroma. Histological examination and immunohistochemical staining of other tissues revealed local invasion in the subserosa and tunica muscularis of the stomach, metastasis within the liver, in the mesovarium, and an intravascular metastasis within the ventricle of the heart surrounded by a thrombus.

Key words: Ductal adenocarcinoma, immunohistochemistry, *Nerodia sipedon*, northern water snake, splenopancreas.

BRIEF COMMUNICATION

Neoplasia is becoming more frequently reported in reptiles.[3,4,8,9] Retrospective studies have been performed to evaluate the incidence of common cancers in reptiles.[3,11] Common sites of neoplasia are the digestive system including liver, integument, hemolymphatic system, and soft tissues.[2,3,10] This report describes a case of a splenopancreatic ductal adenocarcinoma with multiorgan metastasis in a northern water snake (*Nerodia sipedon*), which to the best of the author's knowledge, has never before been reported in this species. This case also demonstrates the successful use of immunohistochemistry staining in the identification of metastases, a tool that is infrequently utilized despite having shown promise in the past.[7]

A captive 16-yr-old female northern water snake kept at a wildlife center presented for examination of a midcoelomic mass noted approximately 3 days prior to presentation to the Exotics and Zoological Medicine Service at The Veterinary Health Center, Kansas State University. The mass was oval shaped, immobile, and firm. It was located at approximately 30% of the distance between the head and the cloaca and measured approximately 7 cm long × 5 cm wide. The snake was thin (body condition score 2/5), lethargic, and unable to right itself after manipulation. The ribs were easily palpable. A healed rib fracture callus was palpated in the cranial quarter on the right side of the animal.

A blood sample was collected from the ventral tail vein and submitted for a complete blood count, which revealed a heterophilia (2.831×10^3/µl; reference interval $.285 \times 10^3 - 2.4 \times 10^3$/µl) and a monocytosis ($9.4 \times 10^3$/µl; reference interval $2.328 \times 10^3 - 6.468 \times 10^3$/µl) consistent with inflammation.[1] No blood biochemistry tests were performed. Whole-body two-view (lateral and dorsoventral) survey radiographs were performed and showed a large soft tissue opacity mass in the cranial coelom (Fig. 1). The mass was approximately 8 cm in length and caused significant distortion of the ventral body margin. There was a small amount of mineral opacity in the right dorsal aspect of the mass. A coelomic ultrasonographic examination confirmed the presence of the mass but could not aid in its specific visceral localization. A fine needle aspirate of the mass was performed. The aspirate revealed sheets of relatively uniform epithelial cells that had a small amount of basophilic cytoplasm and round to slightly oval nuclei with coarse chromatin patterns and poorly visible nucleoli. Obvious cyto-

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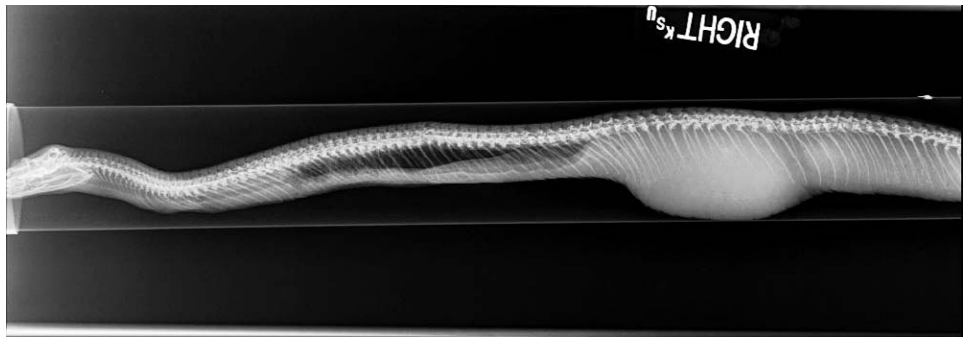

Figure 1. Radiographic image of this water snake, in a right lateral view, revealing a large soft tissue opacity measuring approximately 8 cm in length and causing distortion of the ventral body wall.

logic features of malignancy were not seen on the examined preparations. Rare to occasional spindle cells consistent with reactive fibroblasts were also scattered throughout the smear. The cytologic diagnosis was epithelial neoplasia with concurrent reactive fibroplasia. Histopathologic examination of the tissue was recommended to establish a definitive diagnosis.

Based on physical, radiographic, and cytological test results, a presumptive diagnosis of neoplasia was presented to the caretakers of the snake. The snake was returned to its habitat and monitored.

Two weeks from the initial visit, the snake presented again for examination of its mass. At presentation, the snake was emaciated (body condition score of 1/5) and had small discolored red cutaneous ulcerations corresponding to the edges of the mass. Given the deterioration in its condition, the snake was humanely euthanized and the body submitted for necropsy.

At necropsy, the snake was emaciated. The body of the snake was distorted by a firm bulge approximately one third of the distance from the head to the cloaca. The coelomic cavity was filled with clear gelatinous visceral adipose tissue consistent with serous atrophy of fat. A firm, multilobulated mass approximately $7.5 \times 5 \times 3.5$ cm was attached to the stomach by a fibrous stalk. The gall bladder and spleen could not be identified on gross examination, and were hypothesized to have been replaced by the mass in the area. On cut section, the mass was fibrous, tan/yellow with multifocal red areas and contained small cysts filled with thin watery greenish fluid. A second oval mass measuring 2×1 cm was present posterior to the larger mass located adjacent to the posterior kidney and mesovarium.

Tissue samples were fixed in 10% neutral buffered formalin before being trimmed, routinely processed, embedded in paraffin, sectioned at 4–5 μm, stained with hematoxylin and eosin, and examined. Selected tissue sections were also immunostained for reactivity with a monoclonal anti-human vimentin antibody (mouse monoclonal SRL33, Leica Biosystems, Newcastle Upon Tyne NE12 8EW, United Kingdom) and a monoclonal cocktail anti-human multicytokeratin (AE1/AE3 Multi-Cytokeratin, Leica Biosystems, Newcastle Upon Tyne NE12 8EW, United Kingdom), both using a polymer detection method (Powervision Poly-AP α-mouse IgG, Leica Biosystems, Newcastle Upon Tyne NE12 8EW, United Kingdom). These immunostains have not been validated in snakes. Positive controls consisted of various mammalian tissues.

Histologically, the splenopancreas was infiltrated and partially effaced by a moderately cellular, unencapsulated neoplasm that infiltrated into adjacent peripancreatic adipose tissue, the spleen, and the serosa and tunica muscularis of the stomach. The neoplasm was composed of cuboidal to columnar epithelial cells arranged in variably sized and shaped tubules suspended in fibrovascular stroma that varied from dense collagenous stroma to loosely arranged fibrovascular stroma arranged in a swirling pattern surrounding the tubules (Fig. 2). Epithelial cells lining the tubules were arranged in a simple cuboidal to simple columnar to pseudostratified columnar pattern with two–four layers of nuclei, and occasionally formed blunt papillae that projected into tubular lumens. The neoplastic cells had indistinct borders, moderate to abundant amounts of eosinophilic cytoplasm, basal nuclei with finely stippled chromatin, and contained one–three small nucleoli. There was mild anisocytosis and anisokaryosis and no mitotic figures. In some areas, scattered individual and small nests of polygonal neoplastic epithelial cells

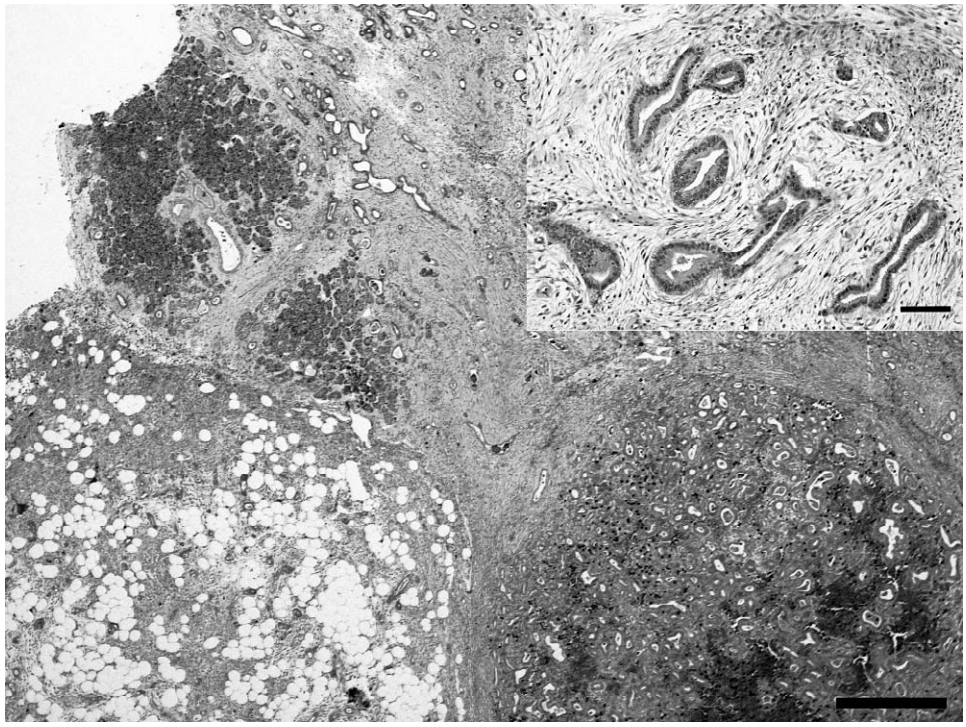

Figure 2. Photomicrograph of ductal adenocarcinoma composed of variably sized and shaped tubules suspended in a fibrovascular stroma in the splenopancreatic region of the snake. Pancreas is to the upper left, peripancreatic adipose tissue is lower left, and spleen is lower right. H&E stain. Bar = 500 μm. Inset: Higher magnification of another area showing tubules lined by single to multilayered cuboidal to columnar epithelial cells with basally located nuclei suspended in a loose swirling fibrovascular stroma. H&E stain. Bar = 100 μm.

without formation of tubules were present within the fibrovascular stroma. There were also small numbers of plasma cells and lymphocytes scattered within the fibrous tissue, and a focal area of stromal osseous metaplasia. The gall bladder and extrahepatic bile duct were not found. Immunostaining with the vimentin antibody failed to label any normal or neoplastic snake tissue. The AE1/AE3 antibody strongly labeled epithelial cells of the normal pancreatic intralobular and interlobular ducts, intrahepatic bile duct epithelium, and epithelium of the neoplastic tubules. Pancreatic acinar epithelial cells and hepatocytes did not label with the AE1/AE3 antibody.

Histologic examination of other tissues revealed subcapsular metastasis within the posterior aspect of the liver, within the mesovarium, and an intravascular metastasis within the ventricle of the heart attached to the endocardium and surrounded by a thrombus. Findings considered incidental included a chronic hematoma on the posterior end of the liver and the mass adjacent to the posterior kidney, which histologically was identified as a granuloma and was negative for acid-fast bacteria. A diagnosis of ductal adenocarcinoma of either pancreas, extrahepatic bile duct, or gall bladder, with metastasis to the liver, mesovarium, and heart, was made.

There have been several reports of primary pancreatic and biliary adenocarcinomas in snakes.[3] The recently reported case of metastatic ductal adenocarcinoma in a Hognose snake was the first reported case of metastasis in ductal adenocarcinomas.[9]

Although determining the existence of metastasis may be difficult, recommended methods include radiographs with contrast media, coelomic ultrasound, and exploratory coeliotomy. The success of immunostaining in this case also suggests that immunostaining may be a useful tool in the diagnosis of metastatic neoplastic processes in snakes where metastasis is suspected. To the best of the authors' knowledge, this is the first report of a case of splenopancreatic ductal adenocarcinoma with metastasis to the mesovarium and heart reported in a snake. In addition, in this case staining with AE3/AE1 was successful in labeling both neoplastic and normal ductal pan-

creatic cells, establishing the role that immunohistochemistry can play in diagnosis of neoplasms in snakes.

A potential route of treatment for this tumor could have been surgical excision, but with the presence of metastasis, adjuvant chemotherapy would likely also have been indicated.[6] However, because of the paucity of knowledge and clinical experience in treating this malignancy in snakes, the outcome of any potential treatment is speculative at best.

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