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CLINICAL CHALLENGE

Michael A. Linden, M.D., Ph.D.,

Division of Hematopathology, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Mayo Mail Code 609, 420 Delaware Street S.E., Minneapolis, Minnesota 55455, USA

Sarah A. Williams, M.D.,

Division of Hematopathology, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Mayo Mail Code 609, 420 Delaware Street S.E., Minneapolis, Minnesota 55455, USA

Colleen Forster, B.S.,

BioNet, University of Minnesota Medical School, B173, Phillips Wangenstein Building, University of Minnesota, 516 Delaware Street S.E., Minneapolis, Minnesota 55455, USA

Paula Croonquist, Ph.D.,

Department of Biology, Anoka Ramsey Community College, 11200 Mississippi Boulevard N.W., Coon Rapids, Minnesota 55433, USA

Jennifer Braido, M.S.,

Department of Biology, Anoka Ramsey Community College, 11200 Mississippi Boulevard N.W., Coon Rapids, Minnesota 55433, USA

Scott Danneman, M.S.,

Department of Biology, Anoka Ramsey Community College, 11200 Mississippi Boulevard N.W., Coon Rapids, Minnesota 55433, USA

Mark Beckel, S.R.S.,

Wildlife Science Center, 5463 West Broadway Avenue, Columbus, Minnesota 55025, USA

Margaret Callahan, M.S., and

Wildlife Science Center, 5463 West Broadway Avenue, Columbus, Minnesota 55025, USA

Davis Seelig, D.V.M., Ph.D., Dipl. A.C.V.P.

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, 1352 Boyd Avenue, St. Paul, Minnesota 55108, USA

HISTORY

On routine examination of a captive-born 9-yr-old female Mexican gray wolf (*Canis lupus baileyi*), a subcutaneous mass ($4.7 \times 4.1 \times 3.1$ cm) was identified on the medial aspect of its left forelimb. The mass was discrete, firm, and tightly adhered to the surrounding tissues. Skin and hair coat appeared normal, and the remainder of the physical examination was

unremarkable. The animal was followed clinically for the next 7 mo, during which time the mass grew in size (6.7×5.8×3.9 cm) and presented with overlying dermal ulceration (Fig. 1A). Surgical excision for diagnosis and cure subsequently was performed. The mass grossly was rubbery in texture, thinly encapsulated, and multilobulated on the cut surface (Fig. 1B). Impression smears of the mass were prepared for cytologic evaluation (Fig. 2). The mass was fixed in 10% neutral buffered formalin and submitted for routine histopathologic examination (Fig. 3). Review the cytologic photomicrographs and provide a diagnosis.

INTERPRETATION AND OUTCOME

Impression smear cytology slides stained with Wright-Giemsa contained large numbers of atypical and pleomorphic mesenchymal cells. These cells were present both individually and clustered, and in association with few tendrils of pink matrix material (Fig. 2A). The atypical cells were ovoid to elliptical with indistinct to tapering cell borders and a small to moderate amount of basophilic cytoplasm. Small numbers of cells were occasionally rarified by small numbers of punctate, round vacuoles (Fig. 2B). Nuclei were round to ovoid with rosy chromatin and contained one to three variably sized nucleoli. Occasional bi- and trinucleate forms were identified. The atypical cells demonstrated moderate anisocytosis and anisokaryosis. Dispersed through the lightly basophilic background were small numbers of vacuolated macrophages and red blood cells. The cytologic diagnosis was sarcoma.

Histologically, the neoplasm was multilobulated, located within the subcutis, and both compressed and infiltrated the surrounding subcutaneous and cutaneous structures (Fig. 3). The neoplastic mesenchymal cells were arranged in lobules comprised of palisading bundles (Fig. 3A) and occasional whorls (Fig. 3B) separated by a small amount of dense fibrous stroma. Cells were elliptical to elongate with indistinct cell borders and a moderate amount of eosinophilic cytoplasm (Fig. 3C). Nuclei were oval to reniform with finely granular chromatin and one or two prominent nucleoli. Moderate anisocytosis and anisokaryosis with occasional binucleated cells and zero to one mitotic figures per high-power field (×400) were observed.

The neoplasm was further characterized by immunohistochemistry. The cells had uniform weak to moderate vimentin immunostaining (Fig. 3D), and a low to moderate Ki-67 proliferative index. Neoplastic cells lacked immunoreactivity to CD3, CD79a, CD31, CD68, myeloperoxidase, smooth muscle actin, and S100 antibodies, which are markers of cells of T lymphocyte, B lymphocyte, endothelial, macrophage, myeloid, smooth muscle, or neural or melanocytic origin, respectively. Mast cells were rare based on a toluidine blue stain. The final histologic diagnosis was soft tissue sarcoma (STS).

DISCUSSION

The Mexican gray wolf is a subspecies historically found within the southwestern United States and central Mexico.¹ Although this wolf was listed as an endangered subspecies in 1976 and considered extinct in the wild by the 1980s, modern conservation efforts have restored wild populations of approximately 109 animals with another 321 maintained in captivity.⁹ Published reports of natural disease in the species are rare, consisting of a single

report describing the species' susceptibility to both canine distemper virus and parvovirus, and a single case of mammary carcinoma;^{6,7} although the taxon-specific manual has reported frequent occurrence of nasal and brain neoplasms.²

STS has been reported across a number of vertebrate classes. In domestic mammals, STS is most common in the domestic dog (*Canis familiaris*), in which it comprises up to 15% of all skin and subcutaneous neoplasms.⁸ It is most commonly considered to be a sporadic disease, although the link between chronic inflammation, including reports associated with trauma, foreign bodies, orthopedic implants, and infection with the nematode *Spirocerca lupi*, has been reported in this species.⁸ In the presented case, no evidence of a potentially contributing condition was identified.

The initial diagnosis of STS was made based upon the cytologic findings, given the atypia and pleomorphism of the nearly singular population of spindloid nucleated cells. However, owing to poor cellular exfoliation and the morphologic overlap between benign and malignant mesenchymal processes, the cytologic interpretation of mesenchymal lesions is often more challenging. Although directed studies addressing the diagnostic utility of cytology in the gray wolf have not been performed, a 63% agreement rate between cytology and histology in the diagnosis of canine STS has been reported.³

Based upon their microscopic, ultrastructural, and immunochemical features, STSs can be subtyped according to their presumed tissue of origin. The six types include fibrosarcoma, perivascular wall tumor, peripheral nerve sheath tumor, myxosarcoma, liposarcoma, and malignant fibrous histiocytoma.⁵ The presented tumor demonstrated features most suggestive of a peripheral nerve sheath tumor. Routine diagnostic tumor subtyping can be challenging, as no cytochemical or immunohistochemical approach can reliably differentiate these types. Moreover, the often substantial histomorphologic overlap between subtypes and the nonuniform application of classification criteria by pathologists make the process potentially inaccurate. Finally, no definitive evidence exists to suggest that STS subtype offers useful prognostic or treatment information.^{4,8} Canine STSs often are assigned a histologic grade of 1, 2, or 3 based upon degree of differentiation, mitotic score, and tumor necrosis. Histologic grading provides important prognostic information as tumor grade is proportional to the likelihood of local recurrence and distant metastasis (i.e., grade 1 tumors have lower rates of local recurrence and distant metastasis than either grade 2 and 3 lesions). Although the histologic features in the present case were consistent with a grade 2 lesion, the authors were reluctant to apply a domestic dog grading scheme to a novel species. Additionally, no ancillary diagnostics, including survey thoracic radiographs, were performed to evaluate for metastatic disease. The wolf was ambulatory immediately after surgery and at 10 mo postoperatively and lacked clinical evidence of external neoplasm recurrence, thus illustrating the clinical benefits of mass extirpation.

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Figure 1.

Gross photos of a subcutaneous mass from a 9-yr-old, female Mexican gray wolf (*Canis lupus baileyi*) that had been present for 7 mo: external (**A**) and cut (**B**) surfaces.

Figure 2. Cytologic impression preparations of a subcutaneous mass impression from a 9-yr-old, female Mexican gray wolf (*Canis lupus baileyi*) that had been present for 7 mo: Wright, (A) ×20 objective; (B) ×100 objective.

Figure 3. Histologic photomicrographs from a subcutaneous mass from a 9-yr-old, female Mexican gray wolf (*Canis lupus baileyi*) that had been present for 7 mo: hematoxylin and eosin, (A and B) $\times 20$ objective; (C) $\times 40$ objective; and (D) 3,3'-diaminobenzidine chromogen, hematoxylin counterstain, $\times 50$ objective.