SUBCUTANEOUS LIPOSARCOMA IN A FERRET (*MUSTELA PUTORIUS FURO*)

Dario D'Ovidio, Dr Med Vet, Giacomo Rossi, Dr Med Vet, Raffaele Melidone, Dr Med Vet, Dip. ACVP, Francesca Menna, Dr Med Vet, and Alessandro Fioretti, Dr Med Vet

Abstract

A 6-year-old spayed female ferret (*Mustela putorius furo*) exhibiting clinical signs of weakness, anorexia, lethargy, weight loss, and frequent urination was presented for a veterinary evaluation to determine the underlying cause of the aforementioned abnormal behavior and clinical condition. Physical examination revealed a large, firm, painless, movable subcutaneous mass at the base of the tail. Radiographic and ultrasound images confirmed the presence of a soft tissue mass compressing the sacrococcygeal vertebrae, but there was no evidence of metastatic lesions. Because of the poor prognosis and progressive deterioration of the animal's clinical condition, the ferret was humanely euthanized. Gross necropsy revealed a 4 × 4 × 3.5 cm, firm, yellow-tan, ovoid, subcutaneous mass wrapped around the rectum and the anus. The mass did not appear to breach the serosa. Evaluation of the abdominal cavity revealed a pale yellow liver, possibly associated with hepatic lipidosis with no gross evidence of metastasis in the body cavity. The histopathological features of the mass were consistent with a liposarcoma. To the best of the authors' knowledge, this is the first case of subcutaneous liposarcoma reported in a ferret.

Key words: ferret; liposarcoma; malignant; *Mustela putorius furo*; neoplasia

This case report describes the clinical signs, hematology, serum biochemical analysis, diagnostic imaging, macroscopic necropsy, histopathology, and immunohistochemistry of a subcutaneous liposarcoma in a ferret. Liposarcomas are malignant mesenchymal neoplasms that can originate from adipocytes and lipoblasts.[1] Based on the current literature, the overall prevalence of liposarcomas appears to be low in both companion animals and companion exotic species.[2] Liposarcomas have been described in reptiles,[1,3] birds,[4,5] and exotic companion mammals.[6,7] In ferrets (*Mustela putorius furo*), there has only been one case reported of liposarcoma, and in that case the tumor originated in the mandibular bone marrow.[8] To the authors' knowledge, this is the first reported case of a subcutaneous liposarcoma in a ferret.

CASE REPORT

A 6-year-old spayed female ferret was presented for clinical signs of weakness, anorexia, lethargy, weight loss, and frequent urination. The ferret was housed inside a home and was fed a commercial diet for cats. The animal had been regularly vaccinated against canine distemper (Eurican CH; Merial, Milan, Italy) and rabies (Rabisin, Merial), and there was no previous history of medical problems except for the clinical signs noted at the time of presentation. On physical examination, the ferret appeared to have a low body condition, weighing 600 g, and had been anorexic for 2 days before its examination at the veterinary clinic. It was slightly lethargic and showed signs of hind limb paresis. No fecal or urinary incontinence or loss of tail con-

From the Dipartimento di Patologia e Sanità Animale, Facoltà di Medicina Veterinaria, Università degli Studi di Napoli Federico II, Napoli, Italy; Dipartimento di Scienze Veterinarie (Rossi), Facoltà di Medicina Veterinaria, Università degli studi di Camerino, Matelica, Italy; and Toxikon Corporation, Bedford, MA USA.

Address correspondence to: Raffaele Melidone, Dr Med Vet, Dip. ACVP, Toxikon Corporation, 15 Wiggins Ave, Bedford, MA 01730. E-mail: rmelidone@gmail.com.

1557-5063/12/2103-$30.00

http://dx.doi.org/10.1053/j.jepm.2012.06.019

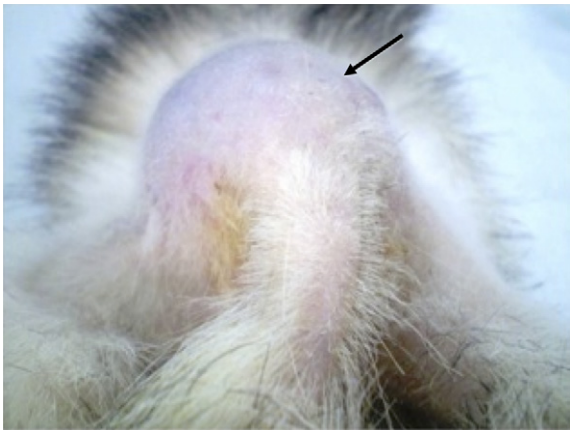

FIGURE 1. Ferret with liposarcoma. Caudodorsal view of the large, firm, roughly round, subcutaneous mass present at the base of the tail, immediately dorsal to the sacrococcygeal region (arrow).

trol were noted at the time of the physical examination or reported by the owner. A large, firm, painless, subcutaneous mass was identified at the base of the tail, dorsal to the sacrococcygeal region (Fig. 1).

Survey radiographs showed a large, soft-tissue opacity at the base of the tail and osteolysis of the sacrococcygeal vertebrae (Fig. 2). A blood sample was collected under manual restraint from the cranial vena cava for a complete blood count and serum biochemical analysis. The hemogram was within normal limits. Serum biochemical profile revealed slightly elevated alkaline phosphatase (130 IU/L; reference range: 30-120 IU/L) and a marked increased activity of alanine aminotransferase (898 IU/L; reference range: 82-289 IU/L).[9] A urine sample was also collected via cystocentesis for urinalysis. The laboratory results of the urinalysis revealed moderate leukocyturia. An abdominal ultrasound revealed a uniform thickening of the urinary bladder wall with no evidence of metastatic disease. A fine-needle aspirate of the mass was also obtained concurrent with the ultrasound examination and showed spindle cells and multinucleated giant cells with evidence of marked anisocytosis and anisokaryosis. A presumptive diagnosis of malignant mesenchymal neoplasia was established.

Seven days of supportive therapy was initiated and included enrofloxacin (5 mg/kg orally every 12 hours for 7 days, Baytril; Bayer Animal Health, Milan, Italy) for treatment of a suspected cystitis; meloxicam (0.2 mg/kg subcutaneously every 24 hours for 3 days, Metacam; Boehringer Ingelheim, Ingelheim, Germany) as an anti-inflammatory, ranitidine (3.5 mg/kg orally every 12 hours for 3 days, Zantac; GlaxoSmithKline, Verona, Italy) as an antacid to prevent possible gastric mucosal damage due to meloxicam, and syringe-feeding with Carnivore Care (Oxbow Animal Health, Murdock, NE USA).[6] Additional recommended diagnostic testing included computed tomography and biopsy of the mass, but were declined by the owner. Despite supportive treatment, the clinical condition of the ferret worsened. The mass rapidly increased in size and compressed the rectum, causing decreased fecal output and tenesmus. Because of the grave prognosis and size and invasiveness of the mass, the ferret was humanely euthanized.

The gross necropsy examination exposed a 4 × 4 × 3.5 cm, irregularly shaped, yellow-tan, firm, subcutaneous mass that wrapped around and compressed the rectum, without breaching the serosa. Evaluation of the abdominal cavity showed diffuse yellow-tan discoloration of the liver (commonly seen in ferrets diagnosed with hepatic lipidosis), but did not reveal any evidence of metastasis. The entire mass was removed and fixed in 10% neutral buffered formalin. A few samples of the same mass were collected and stored as frozen tissue. Samples fixed in formalin were routinely processed and embedded in paraffin according to accepted histologic technique. Five-μm-thick sections were stained with hematoxylin and eosin for microscopic examination. The histologic examination revealed a poorly delimited neoplastic proliferation of readily recognizable adipose cells and poorly differentiated neoplastic cells (Fig. 3), with pleomor-

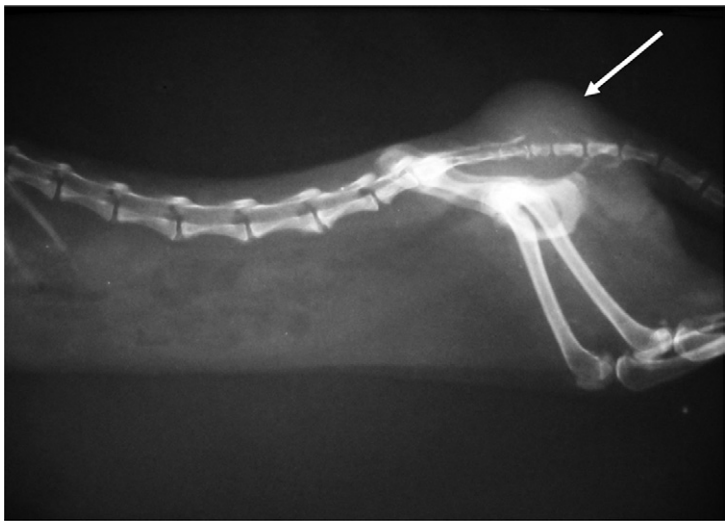

FIGURE 2. Left lateral radiographic view of ferret with a large, soft tissue opacity at the base of the tail (arrow).

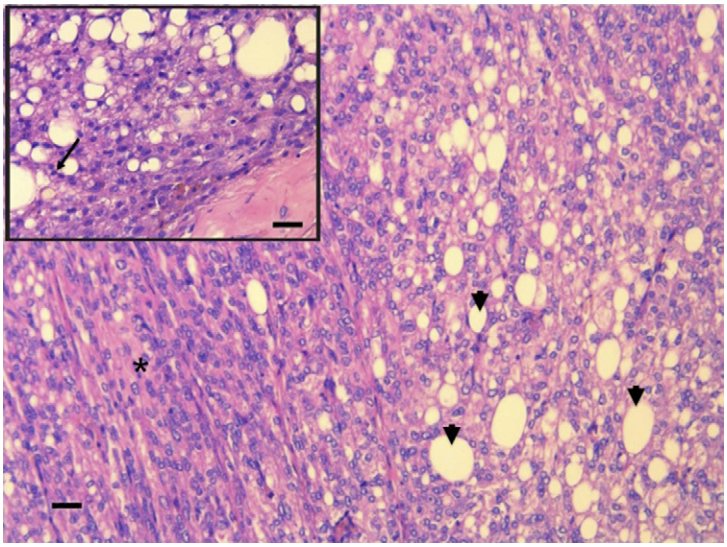

FIGURE 3. Photomicrograph of a histological section of the tail base mass composed of areas of round to polygonal cells, arranged in sheets, and alternating with areas of nonvacuolated to vacuolated spindle cells (asterisk). The neoplastic cells often contain a single, clear, round vacuole (arrowheads) within the cytoplasm, displacing the nucleus at the periphery. Occasionally, scattered binucleated and multinucleated (inset, arrow) neoplastic cells are also present. Hematoxylin and eosin stain. Bar = 200 μm (inset bar = 50 μm).

phic hyperchromatic nuclei and abundant cytoplasm containing lipid droplets, positively stained by Oil Red O method (Fig. 4) performed on frozen sections. Occasional mitotic figures were detected as well as an interstitial stroma that consisted of thin fibrous septa. A few multinucleated giant cells were also present in the examined tissue samples. Within the tumor, there were multifocal areas of necrosis accompanied by hemorrhage and mild to moderate neutrophilic and lymphoplasmacytic infiltrate. Paraffin blocks of the mass were sectioned and immunohistochemistry was performed on 5-μm-thick sections with commercially available primary antisera (DACO Corp., Carpinteria, CA USA) against vimentin, sarcomeric actin (AcSarco), smooth muscle actin (AcSmoothM), and factor VIII–related antigen. The standard avidin-biotin-peroxidase technique (Vectastain kit; Vector Laboratories, Burlingame, CA USA) was used. The tumor cells were positive for vimentin (Fig. 4), but they were uniformly negative to the AcSarco, AcSmoothM, and factor VIII–related antigen. On the basis of the histopathological special stain (Oil Red O) and immunohistochemical findings, the disease diagnosis was determined to be a low-grade liposarcoma. An undifferentiated hemangiosarcoma was considered as a possible differential diagnosis but was soon ruled out because of the negative stain for factor VIII–related antigen of the neoplastic cells. The cytoplasmic microvacuolization and thin stroma can also be observed in embryonal rhabdomyosarcomas[10]; however, the negative results of the immunohistochemistry for AcSarco and AcSmoothM ruled out the possibility of a muscle cell origin of the neoplastic cells.[11] On the basis of the histological appearance, in combination with the positive vimentin and presence of the lipid droplets, the final diagnosis was a well-differentiated liposarcoma of unknown origin, even though it is possible that the neoplasm originated from the mesenteric adipose tissue. Oil Red O staining was performed on frozen sections to demonstrate fat droplets in the tumor cells. Proposed classification of this tumor using the Tumor Node Metastasis classification scheme was IIb (Table 1).

DISCUSSION

This report describes the clinical presentation and histopathological and immunohistochemical features of a subcutaneous liposarcoma in a ferret. In domestic animals, such as dogs and cats, cutaneous swellings or masses are very commonly reported by veterinarians.[12] The underlying etiologic origins of non-neoplastic cutaneous masses in dogs and cats include hematomas, abscesses, granulomas, hernias, and cysts,[12] and the same can be expected in ferrets. Of neoplasms, cutaneous mast cell tumors, basal cell tumors, squa-

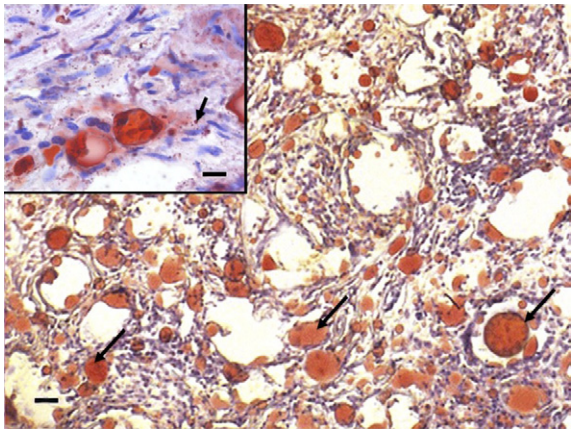

FIGURE 4. The cytoplasm of neoplastic cells often contains a large amount of lipid (bright red stain, arrows). A variable number of small lipid droplets are also present within the cytoplasm of the spindle-shaped neoplastic cells (inset, arrow). Oil Red O staining. Bar = 200 μm (inset bar = 25 μm). The neoplastic cells show intense intracytoplasmic positivity for vimentin (brown stain, arrows). Avidin-biotin-peroxidase complex method, hematoxylin counterstain. Bar = 25 μm.

TABLE 1. Modified Tumor Nodes Metastasis staging of soft tissue sarcomas[17]

T Primary Tumor
- T1: < than 5 cm
- T2: > than 5 cm

N Regional Lymph Nodes
- N0: no lymphnodal metastasis detected
- N1: lymphnodal metastasis present

M Distant Metastases
- M0: no distant metastases
- M1: distant metastases are present

Stage 1
- 1a: T1 N0 M0 (grade 1 or 2, < than 5 cm in diameter)
- 1b: T2 N0 M0 (grade 1 or 2, > than 5 cm in diameter)

Stage 2
- 2a: T1 N0 M0 (grade 3, < than 5 cm in diameter)
- 1b: T2 N0 M0 (grade 3, > than 5 cm in diameter)

Stage 3
- 3a: all T N1 M0 (tumors of any grade with a verified lymphnodal status)
- 3b: all T, all N M1

mous cell carcinomas, and sebaceous epitheliomas are commonly diagnosed in ferrets of all ages, whereas fibromas, fibrosarcomas, sebaceous gland adenomas, hemangiomas, and benign cystic adenomas are frequently identified in middle-aged to older ferrets.[13-15] The most common location of the tumors listed above includes the cranial aspect of the body (e.g., head, neck, shoulders, flanks) as well as the hind limbs. Chordomas are also commonly reported neoplasms in ferrets that arise from remnants of the primitive notochord in the spinal column and are mainly observed at the tail tip; these neoplasms are rarely found in the cervical region or other locations of the spinal cord.[16] Liposarcomas are soft tissue sarcomas, a group of malignant neoplasms that include fibrosarcomas, hemangiopericytomas, liposarcomas, rhabdomyosarcomas, leiomyosarcomas, malignant peripheral nerve sheath tumors, myxosarcomas, and mesenchymomas.[17] Soft tissue sarcomas are slow-growing malignant neoplasms (usually well delimited), which rarely metastasize, even though they tend to invade the surrounding tissues. Liposarcomas are rare malignant tumors of white adipose cell lipoblasts.[1] Literature concerning liposarcomas mentions 3 variants of the neoplasm: well differentiated, pleomorphic, and myxoid.[11] In the well-differentiated variant, the majority of neoplastic cells resemble normal adipocytes with a single, clear fat vacuole and a peripheral nucleus.[11] In the pleomorphic variant, neoplastic cells have highly variable morphology and are mixed with multinucleated giant cells.[11] The diagnostic intracytoplasmic fat vacuoles are usually present in a small percentage of cells.[11] In the myxoid variant, there are scattered spindle cells, lipocytes, and lipoblasts loosely arranged in a mucoid stroma.[11] In the ferret case described here, the histological appearance of the neoplastic cells fell into the category of a well-differentiated liposarcoma. Most reports indicate that liposarcomas tend to be aggressive and may metastasize to the lungs, liver, and bone.[11] Liposarcomas are considered soft tissue neoplasms because of their mesenchymal or connective tissue cell origin and often arise from the skin and subcutis.[12] The specific etiology of this neoplasm is unknown, although a few recently reported cases in domestic animals seemed to be related to the introduction of a foreign material (e.g., glass or microchip) into tissue.[18,19] However, in this case, because of the absence of foreign bodies and lack of previous injections or known trauma at the site of neoplasm development, the tumor was considered of unknown origin. In soft tissue sarcomas, fine-needle aspirate biopsies are often nondiagnostic, because the tumors do not readily exfoliate when compared with epithelial and round cell tumors.[20] Moreover, with lipomas and liposarcomas, the fine-needle aspirates usually cannot provide a definitive diagnosis, because the lipid droplets within the adipocytes (lipomas) or neoplastic cells (liposarcomas) will be dissolved by the alcohol associated with the staining procedure.[2] However, cytology is helpful in distinguishing between lipomas, abscesses, or hematomas, and it is helpful in identifying adipocytes, if these cells are present.[12] Histopathologic evaluation is essential in the diagnosis of liposarcomas, and biopsy and histopathology should always be performed on all cutaneous masses to determine the most effective treatment protocol and establish a prognosis.[15] Histologically, liposarcomas are composed of lipoblasts that exhibit moderate anisocytosis and anisokaryosis with variable degrees of cell differentiation.[2] Special stains such as Sudan, Oil Red O, and osmium may be used to demonstrate the presence of lipid in frozen tissue sections if necessary.[2] In our case, Oil Red O special stain confirmed the presence of the lipid within the cytoplasm of the neoplastic cells. Clinical staging is strongly recommended to establish a prognosis and should include the results of the physical examination, blood work,

survey radiographs, ultrasound, and 3-dimensional images techniques (e.g., computed tomography scan, magnetic resonance imaging). In our case, based on the Tumor Node Metastasis staging system (Table 1), which evaluates the size and extent of primary tumor, the extent of spread to the lymph nodes, and the presence of distant metastases,[21] we propose the ferret as stage IIb (Table 1). Treatment and prognosis of malignant cutaneous tumors will depend on the extent of the local disease, and long-term outcome is related to the size and site of the lesion, histological grade, and whether the neoplasm is invading the adjacent tissues.[11] Multimodal treatment is currently considered the best choice in all species.[22] Surgical excision of the neoplasm, without the ability to achieve wide surgical margins,[23] should be performed in association with radiation therapy to prevent or delay the tumor regrowth, and chemotherapy to help control potential metastases.[22] The locally aggressive nature of liposarcomas requires aggressive surgical resection; therefore, the location of the tumor is a crucial prognostic factor.[12] In this case, the rapid progression of the neoplasm, difficulty of obtaining wide surgical margins, and the rapid clinical decline of the patient's health led toward the decision of euthanasia.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Joerg Mayer, Dr Med Vet, MSc, Dip. ABVP (Exotic Companion Mammal), for contributions to this manuscript.

REFERENCES

1. Schmid BN, Simova CS, Kaufmann BM, et al: Liposarcoma in a veiled chameleon, *Chamaeleo calyptratus*. J Herp Med Surg 17:132-134, 2008
2. Haag KM, Hernandez-Divers S, Latimer KS, et al: Infiltrative lipoma in a black rat snake, *Elaphe obsoleta*. J Herp Med Surg 17:129-131, 2008
3. Reavill DR, Dahlausen B, Zaffarano B, et al: Multiple cutaneous liposarcomas in a red-tailed boa, *boa constrictor*, and chameleon. Proc Conference Assoc Rep Amph Vet, Reno, NV, pp 5-6, 2002
4. Tully TN, Morris JM, Veazey R, et al: Liposarcomas in a monk parakeet, *Myiopsitta monachus*. J Assoc Avian Vet 8:120-124, 1994
5. Reavill DR: Tumors of pet birds. Vet Clin North Am Exot Anim Pract 7:537-560, 2004
6. Von Bomhard W, Goldschmidt MH, Shofer FS, et al: Cutaneous neoplasms in pet rabbits: a retrospective study. Vet Pathol 44:579-588, 2007
7. Jelinek F: Spontaneous tumors in guinea pigs. Acta Vet Brno 72:221-228, 2003
8. Fuentealba C, Blue-McLendon A: Liposarcoma arising from the mandibular bone marrow in a ferret. Can Vet J 36:779-780, 1995
9. Gamble C, Morrisey JK: Ferret, in Carpenter JW (ed): Exotic Animal Formulary. St Louis, MO, Elsevier/W. B. Saunders (ed 3), pp 447-476, 2005
10. Willen H, Akerman M, Carlen B: Fine needle aspiration (FNA) in the diagnosis of soft tissue tumours: a review of 22 years experience. Cytopathology 6:236-247, 1995
11. Withrow SJ, MacEwen EG: Small Animal Clinical Oncology (ed 3). Philadelphia, PA, W. B. Saunders, pp 286-295, 2001
12. Berger BG, Latimer KS, LeRoy BE, et al: Liposarcomas in the dog and cat: Clinical Pathology Clerkship Program. College of Veterinary Medicine, The University of Georgia, Athens, GA, Class of 2004
13. Kelleher SA: Skin diseases of ferrets. Vet Clin North Am Exot Anim Pract 4:136-140, 2001
14. Hoppman E, Barron HW: Ferret and rabbit dermatology. J Exot Pet Med 16:225-237, 2007
15. Mitchell MA, Tully TN: Manual of Exotic Pet Practice. St. Louis, MO, Elsevier W. B. Saunders, 2009
16. Dunn DG, Harris RK, Meis JM, et al: A histomorphologic and immunohistochemical study of chordoma in twenty ferrets (*Mustela putorius furo*). Vet Pathol 28:467-473, 1991
17. Romanelli G: Sarcomi dei tessuti molli: presentazione clinica, diagnosi e stadiazione. Proceedings of the 62nd Italian Companion Animal Veterinary Association International Congress, Rimini, Italy, pp 249-250, 2009
18. McCarthy PE, Hedlund CS, Veazy RS, et al: Liposarcoma associated with a glass foreign body in a dog. J Am Vet Med Assoc 209:612-614, 1996
19. Elcock LE, Stuart BP, Wahle BS, et al: Tumors in long-term rat studies associated with microchip animal identification devices. Exp Toxicol Pathol 52:483-491, 2001
20. Liptak JM, Forrest LJ: Soft tissue sarcomas: overview and surgical management, in Withrow and MacEwen's Small Animal Clinical Oncology. St. Louis, MO, Elsevier W. B. Saunders, p 435, 2007
21. Owen LN, editor. TNM Classifications Tumors in Domestic Animals. Geneva, Switzerland, World Health Organization, pp 46-47, 1980
22. Morello E: Principi di trattamento multimodale dei sarcoma dei tessuti molli: Proceedings of the 62nd Italian Companion Animal Veterinary Association Int Cong, Rimini, Italy, pp 181-182, 2009
23. Mehler SJ, Bennett RA: Surgical oncology of exotic animals. Vet Clin North Am Exot Anim Pract 7:783-805, 2004