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CUTANEOUS EPITHELIOTROPIC T-CELL LYMPHOMA WITH METASTASES IN A VIRGINIA OPOSSUM (*DIDELPHIS VIRGINIANA*)

Christine T. Higbie, D.V.M., James W. Carpenter, M.S., D.V.M., Dipl. A.C.Z.M., Shambhunath Choudhary, D.V.M., Ph.D., Brad DeBey, D.V.M., Ph.D., Dipl. A.C.V.P., Mary Bagladi-Swanson, D.V.M., Dipl. A.C.V.D., and David Eshar, D.V.M., Dipl. A.B.V.P. (E.C.M.), Dipl. E.C.Z.M. (Small Mammal)

Abstract: A 2-yr-old, captive, intact female Virginia opossum (*Didelphis virginiana*) with a 7-mo history of ulcerative dermatitis and weight loss was euthanatized for progressive worsening of clinical signs. Initially the opossum was treated with several courses of antibiotics, both topically and systemically; systemic nonsteroidal anti-inflammatory medication; and, later, systemic glucocorticoids, with no improvement in clinical signs. Histopathologic samples of skin lesions taken 3 mo into the course of disease revealed no evidence of neoplasia; however, cytologic samples of a skin lesion taken 5 mo into the course of disease revealed mature lymphocytes, and were suggestive of cutaneous lymphoma. Postmortem histopathology revealed neoplastic cells consistent with lymphoma; these were found in the haired skin of the forearm, axilla, hind limb, face, and lateral body wall, as well as the liver, kidney, axillary lymph node, heart, and spleen. Multifocal neutrophilic and eosinophilic ulcerative and necrotizing dermatitis and folliculitis of the haired skin were also present. To the authors' knowledge, this is the first documented case of cutaneous lymphoma in a Virginia opossum and the first documented case with visceral metastases in a marsupial.

Key words: Cutaneous lymphoma, dermatitis, *Didelphis virginiana*, mycosis fungoides, opossum.

BRIEF COMMUNICATION

A 2-yr-old, intact female Virginia opossum (*Didelphis virginiana*) was first reported to show moist dermatitis of the skin in the left axillary region in late September 2013. The opossum reportedly licked at the area constantly, causing a 3 × 3-cm raised, erythematous, lichenified, and exudative lesion to form. The opossum was kept as an education animal at the Sunset Zoo, Manhattan, Kansas, since June 2011. Its annual examinations and clinical pathology testing prior to presenting skin lesions were within normal reference ranges.[9] The opossum was housed indoors in a large metal cage with supervised access to an entire room once daily.

In September 2013, a health examination was performed at the Kansas State University Veterinary Health Center. The opossum was placed under anesthesia using isoflurane (IsoFlo, Abbott Animal Health, North Chicago, Illinois 60064, USA) at 5% in 2 L/min of oxygen administered by face mask, and anesthesia was maintained using isoflurane at 1.5–2.5% in 2 L/min of oxygen administered by face mask. At that time, the opossum was significantly overweight at 4.23 kg (body condition score [BCS] = 4/5). Complete blood count (CBC) and serum biochemistry evaluation were within reference ranges.[9] Impression smears of the left axillary region showed multiple budding yeast. The lesion was cleaned with dilute chlorhexidine (Nolvasan S, Fort Dodge Animal Health, Pfizer Laboratories, Sandton 2196, South Africa) and then dried; this treatment was administered daily until the area was healed. Because the opossum was overweight with a largely sedentary lifestyle, recommendations were made to increase exercise opportunities and to limit bathing to reduce predisposition to moist dermatitis.

In mid-December 2013, the opossum was reported to have excoriations on the nasal planum and dorsal to the left eye. The lesions reportedly appeared to heal spontaneously, but would later recur without any noted trauma. On examination under manual restraint, the lesions were not apparently uncomfortable or pruritic when palpated and there was no change noted to the opossum's appetite or attitude. The opossum was rechecked 1 wk later because the facial lesions did not resolve on their own. The opossum weighed 4.0 kg, with recurrence of the moist dermatitis in a

From the Departments of Clinical Sciences (Higbie, Carpenter, Eshar, Bagladi-Swanson) and Diagnostic Medicine/Pathobiology (Choudhary, DeBey), College of Veterinary Medicine, Kansas State University, 1800 Denison Avenue, Manhattan, Kansas 66506, USA. Present address (Higbie): School of Veterinary Medicine, Louisiana State University, Skip Bertman Drive, Baton Rouge, Louisiana 70803, USA. Correspondence should be directed to Dr. Higbie (christinehigbie@yahoo.com).

2 × 2 cm area in the left axilla. Two deep excoriations with an ulcerative appearance were present dorsal to the left eye and on the nasal planum, each measuring 1 cm in length by 0.5 cm in width. Dilute chlorhexidine and a thin layer of silver sulfadiazine cream (Thermazene cream, Kendall, Covidien Animal Health, Mansfield, Massachusetts 02048, USA) were applied once to twice daily on the lesions, and partial improvement was seen with this topical treatment.

Another recurrence of the facial and axillary skin lesions occurred in mid-January 2014 and topical treatment was performed as previously described. By mid-February 2014, necrotizing lesions appeared on the face, on the left axilla, and medially on the left thigh. The opossum's weight was reduced (3.66 kg); however, the CBC and biochemistry findings were again within reference ranges. The opossum was placed under anesthesia as previously described using isoflurane gas in oxygen administered by face mask. Impression smears and three 4-mm punch biopsies were taken from the skin of the face and from the left axilla. Buprenorphine (Buprenex injectable, Reckitt Benckiser Pharmaceuticals, Richmond, Virginia 23235, USA; 0.3 mg/ml, 0.03 mg/kg s.c.) was given prior to recovery from anesthesia. The cytology showed scant eosinophils and nonspecific inflammatory cells, and histopathology on the biopsies showed focally extensive inflammation within the dermis, composed of epithelioid macrophages, eosinophils, and occasional multinucleate cells. Several hair follicles contained intraluminal eosinophils, and a few multinucleate cells were present within the lumens of hair follicles as well. The epidermis was focally or completely ulcerated in all of the biopsies. Bacteria were present on the surface where the epidermis was ulcerated, although Wright-Giemsa stain and acid-fast stain did not reveal organisms in areas of deeper tissue inflammation. Because of the granulomatous nature of the lesions, infectious and parasitic causes could not be definitively ruled out; as such, antiparasitic treatment (selamectin, Revolution, Zoetis, Florham Park, New Jersey 07932, USA; 15 mg/kg topically), systemic antibiotics (amoxicillin–clavulanic acid suspension, Clavamox, Zoetis; 13.75 mg/kg p.o. b.i.d. for 10 days), and anti-inflammatory treatment (meloxicam oral suspension, Metacam, Boehringer Ingelheim Vetmedica, St. Joseph, Missouri 64506, USA; 0.1 mg/kg p.o. s.i.d. for 5 days) were initiated. Although no etiology was definitively determined, neoplasia was also considered as a differential diagnosis because of the animal's weight loss and the rapid progression of clinical signs.

No clinical response was noted following 10 days of treatment, and the opossum was reexamined. The animal's weight had decreased to 3.6 kg, with a BCS = 2/5. Selamectin was repeated topically, and cultures and impression smears of the lesions were obtained. Cytology of the axillary lesion revealed predominantly mature lymphocytes, suggestive of cutaneous lymphoma. Minimal bacteria were seen on the impression smears. Culture and sensitivity results from the axillary lesion showed a multidrug-resistant *Pseudomonas aeruginosa*, with sensitivity to third-generation cephalosporins. Because of ease of administration, cefovecin (Convenia, Zoetis; 10 mg/kg s.c., repeated in 10 days) was prescribed.

Despite these treatments, the skin lesions continued to worsen; however, the opossum had a consistently good appetite and normal behavior. The antibiotic therapy was changed to ciprofloxacin (Ciprofloxacin Granular Blend for Oral Suspension, Lupin Pharmaceuticals, Baltimore, Maryland 21202, USA; 10 mg/kg p.o. b.i.d. for 7 days). Cutaneous lymphoma was suspected because of the chronicity of the dermatitis, poor response to numerous medical treatments, and the cytologic presence of mature lymphocytes in the axilla. As a result, prednisolone therapy was initiated (PrednisoLONE Oral Solution, Hi-Tech Pharmacal, Amityville, New York 11701, USA; 0.2 mg/kg p.o. s.i.d.–b.i.d.).

Given the lack of response to any of the treatments given, poor prognosis, poor quality of life, and continued progression of the skin lesions in both number and severity, the opossum was euthanatized in April 2014, 14 days after starting prednisolone therapy and 6.5 mo after initially presenting with a skin lesion.

At necropsy, the skin contained multifocal to coalescing erythematous, ulcerative, and crusty lesions throughout the body (up to 3 × 5 cm); the face, the ventral and lateral body wall, and the base of the tail were most severely affected (Fig. 1A, B). The left axillary lymph node was grossly enlarged (3 × 2 × 2 cm; Fig. 1A); upon sectioning, the parenchyma bulged and had a homogenous gray–white appearance. The liver contained multiple pinpoint white, firm nodules throughout the parenchyma, and the spleen contained multiple white, raised, firm nodules (up to 1 cm in diameter) on its capsular surface and parenchyma (Fig. 1C). There were no significant gross lesions in the other organs.

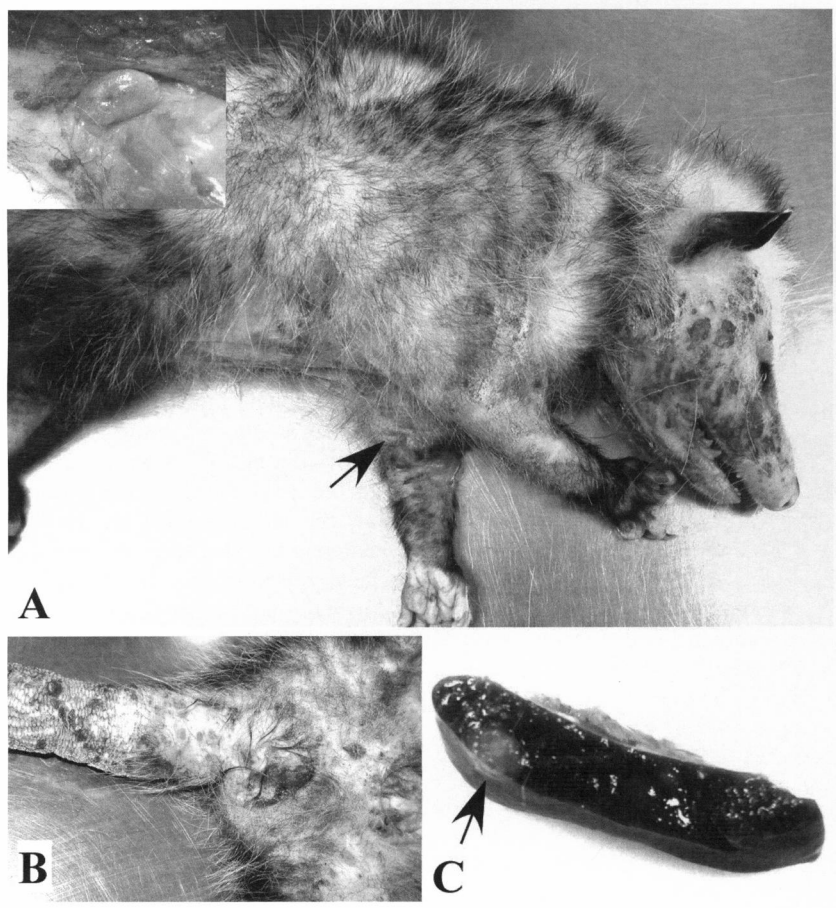

Figure 1. Gross appearance of cutaneous lymphoma in a 2-yr-old Virginia opossum. **A.** Erythematous, ulcerative, crusty skin lesions are present over the face and ventral and lateral body wall. An arrow points out the presence of an enlarged lymph node in the left axilla. Inset shows a closer view of the enlarged lymph node with skin and subcutaneous tissue removed. **B.** Erythematous, ulcerative, crusty skin lesions on the base of the tail and perianal region. **C.** Cut section of spleen showing one of the white, firm, raised nodules (arrow).

Skin samples were taken from the face, the ventral and lateral body wall, and the base of the tail. Histologic examination of the lesions revealed a densely cellular neoplasm composed of a monomorphic population of pleomorphic neoplastic lymphocytes replacing the dermal and subcutaneous tissue, and occasionally infiltrating the overlying epidermis (Fig. 2A). The neoplastic cells had distinct cellular borders, a scant to moderate amount of eosinophilic cytoplasm, irregularly round to oval hyperchromatic nuclei with stippled chromatin, and one to three nucleoli. There was marked anisocytosis and anisokaryosis with 36 mitotic figures per 10 high-power fields (×400; Fig. 2B). The overlying hyperplastic epidermis was multifocally ulcerated and was covered with a thick serocellular crust composed of keratin, hemorrhage, serum, and cellular debris, admixed with large numbers of degenerate viable neutrophils, eosinophils, and few bacterial colonies (Fig. 2A). Multifocally, the superficial dermis was moderately expanded by low to moderate numbers of neutrophils, macrophages, and eosinophils admixed with moderate numbers of neoplastic lymphocytes. Frequently, hair follicles were filled with numerous degenerate and viable neutrophils, eosinophils, and eosinophilic necrotic debris. In the spleen, liver, kidneys, axillary lymph node, and heart there were neoplastic cells similar to those previously described in the skin.

The microscopic lesions seen are characteristic of lymphoma, with the origin of the lymphoma most likely the skin (cutaneous lymphoma).

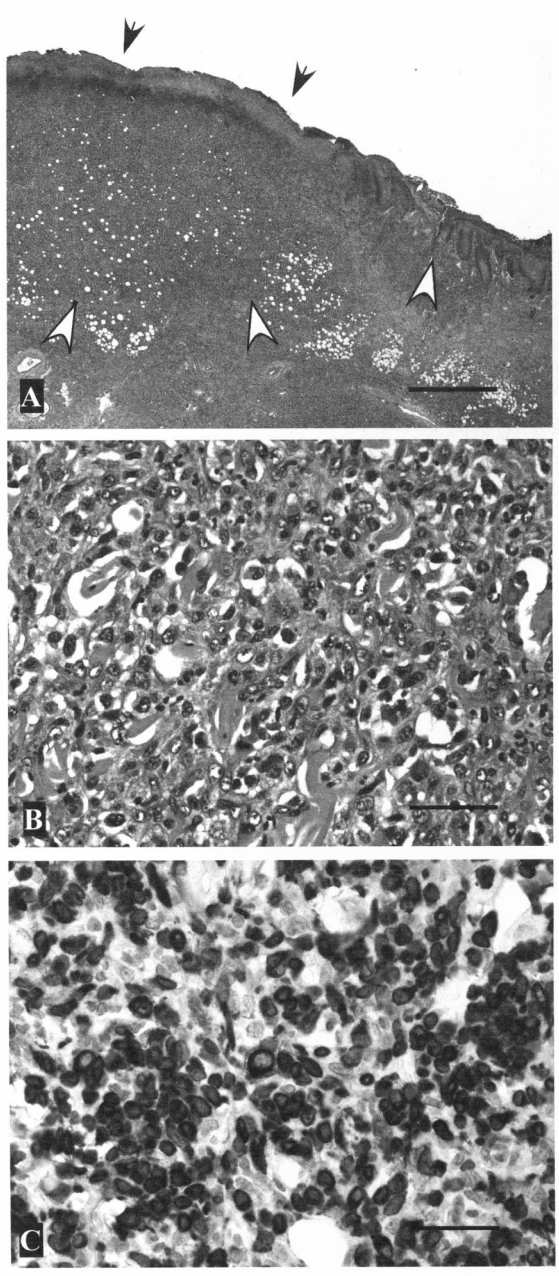

Figure 2. Photomicrograph of cutaneous lymphoma in a 2-yr-old Virginia opossum. **A.** The neoplasm is composed of a monomorphic population of pleomorphic neoplastic lymphocytes that replaces dermal and subcutaneous tissue and occasionally infiltrates the overlying epidermis (white arrowheads). The overlying epidermis is multifocally ulcerated and covered with thick serocellular crust (black arrowheads). Hematoxylin and eosin stain; bar = 1 mm. **B.** Neoplastic cells are highly pleomorphic with marked anisocytosis and anisokaryosis. Hematoxylin and eosin; bar = 50 μm. **C.** Immunohistochemical staining for CD3 shows strong cytoplasmic staining in the neoplastic cells; bar = 50 μm.

Immunophenotyping of the neoplastic lymphocytes by immunohistochemistry was positive for CD3 and negative for CD79a, confirming a T-lymphocyte lymphoma (Fig. 2C). The epitheliotropism seen in this case is typical of T-cell lymphoma (mycosis fungoides).[12,16] Metastasis of both epitheliotropic and nonepitheliotropic cutaneous lymphomas to the viscera has been well documented in many species,[16] and is represented in this case. The eosinophilic inflammation in the hair follicles is an unusual finding in cutaneous lymphoma, however, and is suspected to be present as a result of eosinophilopoietin secretion, especially interleukin-5, by T-lymphocytes.[5]

The previously submitted, premortem biopsy samples did not show evidence of lymphoma. It is possible that, because the samples were taken from the leading edges of the lesions, the neoplasm had not spread to the margin at that time. It is also possible that the biopsy samples didn't show evidence of lymphoma because of the small sample size.

Cutaneous epitheliotropic T-cell lymphoma (CETL) is a rare, malignant neoplasm that has been reported in a variety of mammals, including domestic dogs,[1–3,6,10–16] domestic cats,[2,3,16] horses,[16] and a variety of small mammals, including marsupials (a sugar glider,[7] a cuscus,[7] and two Tasmanian devils[15]). The etiology of CETL is unknown.[6] The dermatosis is characterized by infiltration of neoplastic T lymphocytes with a specific tropism for the epidermis and adnexal structures.[2,12] The lesions evolve from a patch–plaque stage with prominent epitheliotropism into a tumor stage in which distant metastasis is observed.[12] In dogs, the mean time between the first onset of skin lesions and the final diagnosis of CETL has been shown to be 5.5 mo.[1,3]

A possible predisposing factor for CETL in humans is chronic inflammation,[6] and a recent study performed in dogs has suggested a possible association between atopic dermatitis and CETL.[14] The odds of having CETL were 12 times higher in dogs with atopic dermatitis.[14] It is possible that previous dermatitis or dermatoses may have contributed to the development of disease in this case.

Following environmental or infectious injury, T cells are recruited from postcapillary venules into the skin, causing inflammation.[11] When there is a dysregulation in lymphocyte division, malignant T cells express skin-homing receptors such as cutaneous lymphocyte antigen or CC-chemokine receptor 4.[11] These receptors are crucial for the exocytosis of lymphocytes into the skin, binding

to epidermal keratinocytes, and Langerhans cells.[11] In CETL, T lymphocytes also express high levels of the β1-integrin intercellular adhesion molecule.[3,12] This expression may be important for the adhesion to surface receptors and thus for the epitheliotropism observed in the disease.[3]

In general, the prognosis for dogs with CETL is poor.[6] Natural death is rarely observed as a result of CETL, but the cause is usually generalization of the lymphoma or a secondary septicemia.[1,2] Many treatment protocols for CETL have been reported,[4] but none of them have shown much efficacy.[2,4] The most benign of the treatments reported is linoleic acid given at high concentrations;[10] however, larger studies confirming the efficacy of this treatment are lacking.[6] Retinoids and prednisolone have also been used for palliative care.[2] Currently, the most promising protocols in dogs include the use of lomustine.[13] Therapies for CETL in humans have included topical glucocorticoids,[17] photodynamic therapy,[8] and radiation,[4,6] as well as treating for secondary skin infections and pruritis.[6]

This case demonstrates that Virginia opossums may be at risk for developing cutaneous lymphoma. Practitioners should be aware that similar clinical signs in this species may be associated with cutaneous lymphoma.

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