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Myofasciitis in the Domestic Ferret

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Abstract. Since late 2003, an inflammatory disease of muscle and fascia has been diagnosed in several ferrets at Northwest ZooPath, and this report describes the condition in 17 ferrets. It is a disease of young ferrets, characterized by rapid onset of clinical signs, high fever, neutrophilic leukocytosis, treatment failure, and death (or euthanasia). Gross lesions include atrophy of skeletal muscle; red and white mottling and dilatation of the esophagus; and splenomegaly. Histologically, moderate to severe suppurative to pyogranulomatous inflammation is in the skeletal muscle and the fascia at multiple sites, including esophagus, heart, limbs, body wall, head, and lumbar regions. Myeloid hyperplasia of spleen and/or bone marrow also is a prominent feature. Ultrastructural lesions include mitochondrial swelling, intracellular edema, disruption of myofibrils and Z bands. Bacterial and viral cultures, electron microscopy, immunohistochemistry, and polymerase chain reaction were negative for a variety of infectious agents. The clinical presentation and distribution of lesions suggests that polymyositis in domestic ferrets is likely a distinct entity. The etiopathogenesis if this condition is not known.

Key words: Electron microscopy; ferrets; histopathology; immunohistochemistry; myofasciitis; PCR; polymyositis.

Spontaneous inflammatory polymyopathies are well documented, especially in humans[8,25] and dogs.[11,23] The most common myopathies occurring in humans include polymyositis (PM), inclusion body myositis (IBM), and dermatomyositis (DM).[8,25] Canine models of DM and PM also exist.[11,18,23] In addition, an immune-mediated form of masticatory myositis occurs in dogs.[11,34]

Experimentally or iatrogenically induced inflammatory myopathies also occur in animals. Localized myositis associated with the administration of vaccines has been described in cattle.[28] Lewis rats serve as an animal model for polymyositis associated with the feeding of L-tryptophan.[43] Experimental autoimmune myositis is an autoimmune disease induced in rabbits or guinea pigs after immunization with muscle homgenates.[43] Experimental infection of mice with Coxsackievirus B 1 can cause T-cell-mediated polymyositis.[43] Encephalomyocarditis virus can also cause polymyositis in adult mice under experimental condtions.[43]

The domestic ferret (*Mustela putorius*) is a popular house pet in the United States. During an 11-year span (1994–2005), Northwest ZooPath (NZP) has received 2040 domestic ferret biopsy or necropsy submissions, most were from privately owned pets. Since late 2003, a previously unrecognized inflammatory disease process involving muscle and fascia has been diagnosed in several ferrets at NZP. This report describes the clinicopathologic findings of this condition, tentatively termed myofasciitis (MF), in 17 ferrets.

Materials and Methods

Animals

The ferrets used in this study were obtained by private owners through the pet industry and were presented to veterinary practitioners for initial diagnostic workup and treatment when they became ill. For each animal, as much information as possible was obtained regarding the breeding facility and the vaccine history. Signalment, husbandry and medical history, clinical signs, blood and serum chemistry values, treatments, and manner of death were also recorded. Necropsies of case Nos. 1–12 were performed at referring clinics and case Nos. 14–17 at NZP.

Histopathology

Tissues obtained at necropsy were preserved in 10% neutral buffered formalin, processed routinely, sectioned at 5 μm, mounted on glass slides, and stained with hematoxylin and eosin (HE). Select sections of affected muscle from each ferret were also stained with Fite's acid fast, Warthin Starry, Brown and Brenn, Gomori's methylamine silver, and Gimenez techniques. Criteria for inclusion in the study were evidence of suppurative to pyogranulomatous myositis and fasciitis in esophagus, heart, and at least 1 section of skeletal muscle from leg or trunk regions. Lesions were graded based on the degree of inflammation as mild for small focal lesions, moderate if lesions were multifocal, and severe if lesions were coalescing or diffuse.

Electron microscopy

Formalin-fixed esophageal skeletal muscle from case No.1 was transferred to half strength Karnovsky's fixative,[21] followed by 2 washes in 0.2 M sodium cacodylate before postfixation in 2% osmium tetroxide reduced with 2.5% potassium ferrocyanide.[32] After osmification, the tissue was dehydrated in a graded ethanol series, was infiltrated, and subsequently was embedded in Spurr's epoxy resin.[37] Thick sections were cut, mounted on glass slides, and stained by toluidine blue O for light microscopic preview. Thin sections were mounted on 150-mesh copper grids, stained briefly with 6% methanolic uranyl acetate, followed by Reynold's lead citrate,[30] and examined in a Zeiss 906E transmission electron microscope (Zeiss Electron Microscopy, Thornwood, NY) at 60 kv accelerating voltage.

Immunohistochemistry

For immunohistochemical examination, deparaffinized sections of inflamed muscles from case Nos. 1–8 were incubated with polyclonal goat-antibodies against *S. neurona* (L. Mansfield, MSU, Lansing, MI), *T. gondii*, and *N. caninum* (both from VMRD, Inc. Pullman, WA) as previously described.[35] After deparaffinization, antigen retrieval was accomplished by incubation of slides in antigen retrieval solution (Dako, Carpinteria, CA) in a steamer (Black&Decker, Hampstead, MD) for 20 minutes. Endogenous peroxidases were blocked by using a 15-minute treatment with 3% hydrogen peroxide. Slides were also stained with the monoclonal antibody FCV3-70 (Custom Monoclonals International, West Sacramento, CA), which reacts with ferret coronavirus in paraffin embedded sections.[41] Nonspecific immunoglobulin binding was blocked by incubating the slides for 10 minutes in a protein-blocking agent (Dako). The primary antibodies were applied at a dilution of 1 : 100 and were allowed to react for 30 minutes at room temperature. Sections were stained in a Dako autostainer apparatus (Dako) after a routine protocol.[22] Briefly, a streptavidin-immunoperoxidase staining procedure (Dako) was used for immunolabeling. The reaction was visualized by application of the substrate-AEC chromogen AEC substrate (Dako). Sections were counterstained with Mayer's hematoxylin. Positive immunohistochemical controls included tissues that contained the different parasites to which the appropriate antisera were added. For negative controls the primary antibodies were replaced with homologous nonimmune sera.

Polymerase chain reaction assays

Formalin-fixed, paraffin-embedded samples of skeletal muscle from case Nos. 1–8 were processed for detection of deoxyribonucleic acid (DNA) from *Neospora caninum* and *Toxoplasma gondii*. Three sections, 20-μ thick, from each sample were placed in sterile microcentrifuge tubes. A 0.5% mixture of polyoxyethylene-sorbitan monolaurate (Tween 20, Sigma Chemical Co., St. Louis, MO) in DNase and RNase free molecular biology grade water was added to each microcentrifuge tube (200 μl per tube). The microcentrifuge tubes were then incubated at 100°C for 10 minutes, followed by centrifugation at 10,000 × *g* for 5 minutes at room temperature. The paraffin layer that hardened above the aqueous phase was removed with a sterile toothpick. DNA was extracted from the tissue pellet by using components of the QIAmp DNA Mini Kit following the protocol for paraffin-embedded fixed tissues provided in the handbook for the kit (Qiagen Inc., Valencia, CA). The extracted DNA was eluted in 50 μl of DNase and RNase free water. The polymerase chain reaction (PCR) primers used for detection of DNA from *Toxoplasma gondii* were derived from the 35-fold repetitive B1 gene.[6,7] The sequences of the primers were 5′-AACGGGCGAGTAGCACCTGAGGAGA-3′ and 5′-TGGGTCTACGTCGATGGCATGACAAC-3′. Those primers yield an amplicon of 115 base pairs. The PCR reaction mixture consisted of 2.5 μl of 10× PCR buffer (Qiagen); 0.2 mM each dATP, dGTP, dCTP, and dTTP; 1.5 mM $MgCl_2$, 0.3 pmol/μl each primer; 0.625 units HotStar Taq polymerase (Qiagen), and 5 μl sample DNA in a total reaction volume of 25 μl. The reaction conditions were 1 cycle of 95°C for 15 minutes; 40 cycles of 95°C for 30 seconds, 65°C for 30 seconds, 72°C for 30 seconds; followed by 1 cycle of 72°C for 5 minutes. Nested sets of PCR primers were used for the detection of DNA from *Neospora caninum.* The external set of primers was 5′-CCCAGTGCGTCCAATCCTGTAAC-3′ and 5′-CTCGCCAGTCAACCTACGTCTTCT-3′.[27] Those primers yield an amplicon of 337 base pairs. The internal set of primers was 5′-GGATACGTGGTTTGTGGTTAG-3′ and 5′-CAGTCAACCTACGTCTTCTG-3′ yield a product of 192 bases. The internal set of primers was used in a nested PCR and in a separate PCR, without using the external set of PCR primers. The PCR reaction mixture consisted of 2.5 μl of 10× PCR buffer (Qiagen); 0.2 mM each dATP, dGTP, dCTP, and dTTP; 1.5 mM $MgCl_2$; 0.3 pmol/μl each primer; 0.625 units HotStar Taq polymerase (Qiagen) and 2 μl sample DNA in a total reaction volume of 25 μl. The reaction conditions were 1 cycle of 95°C for 15 minutes; 40 cycles of 95°C for 40 seconds, 62°C

(55°C for the internal primers) for 40 seconds, 72°C for 40 seconds; followed by 1 cycle of 72 C for 5 minutes. Positive controls for the PCRs for *Toxoplasma gondii* and *Neospora canis* were DNA harvested from fresh and formalin-fixed, paraffin-embedded tissue from sheep or cattle infected with the organisms. Negative controls were PCR reaction mixtures containing PCR primers but not containing DNA template (no template control).

Cultures

Aerobic, anerobic, and viral cultures of pooled liver, lung, and kidney were performed for case Nos. 14–17. Fecal aerobic culture was performed for case No. 7. Aerobic culture of lung was performed for case No. 13. For viral culture, pooled tissue homogenates were prepared in minimal essential medium (MEM) (10% w/v), centrifuged, and inoculated onto preformed monolayers of Madin Darby Canine Kidney (MDCK) cells, Crandell Feline Kidney (CrFK) cells, and Vero cells. The cultures were observed daily for cytopathic effect and passaged at weekly intervals for 3 consecutive weeks.

Results

Clinical data

Clinical data are summarized in Table 1. The breeding facilities were known for 15 ferrets, and at least 3 different facilities were represented; two facilities were from the United States and one ferret was a hybrid of European and New Zealand origins. All ferrets had received at least 1 distemper vaccine as a kit, and many had also received booster distemper vaccines. Three different distemper vaccine products were used (Table 1), and 2 product (Fervac D, United Vaccines, Inc. Madison, WI) was used in all of the study ferrets. case Nos. 3 and 16 received 1 booster dose each of Purevax (Merial, Athans, GA), and Galaxy-D (Schering-Plough Animal Health Co, Omaha, NE) was administered as booster doses to ferret case Nos. 8 and 14. Rabies vaccine (Imrab-3, Merial, Athens, GA) was administered to 8 ferrets.

Signalment and history

Signalment, clinical signs, blood work, and vaccine history for ferrets with MF are summarized in Table 1. The ferrets ranged in age from 5 to 24 months of age and average age was 10 months. Ten were males, 8 females, and all but 1 male and 2 females were neutered. Clinical signs included fever (16); lethargy (14); recumbency, ataxia, posterior paresis, or pain when moving (12); bruxism, anorexia, or difficulty swallowing or drinking (6); and abnormal stools (3). Blood work revealed mild to marked leukocytosis, with mature neutrophilia (17); and mild to moderate, usually nonregenerative, anemia (11). Common serum abnormalities included mild to moderate elevation of serum alanine aminotransferase (6), mild hyperglycemia (9), and mild hypoalbuminemia (8). Treatment was attempted in 16 ferrets and included various antibiotics, nonsteroidal anti-inflammatory drugs, glucocorticoids, antipyretics, analgesics, interferon, and cyclophosphamide. Treatment was ultimately unsuccessful in all cases and the patients either died (6) or were humanely euthanized (12).

Gross lesions

Distribution and severity of lesions are summarized in Table 2. Necropsy at referring clinics usually revealed no gross lesions, although red and white mottling and dilatation of the esophagus, and white streaks in the heart, diaphragm, and intercostal muscles were seen in few ferrets. In case Nos. 13–17 necropsied by one of the authors (MMG), moderate to severe atrophy of muscle was noted in the limbs and the lumbar region (Fig. 1). Each of these ferrets had red-white mottling of the esophagus along its entire length, accompanied by mild atrophy of the tongue and some hyperkeratosis along the dorsal mucosal surface of the tongue (Fig. 2). The diaphragm also was thin and semitransparent (Fig. 3). Splenomegaly was observed in all of these ferrets (Fig. 4).

Histologic lesions

Histologic lesions are summarized in Table 2. Moderate to severe suppurative to pyogranulomatous inflammation was in the skeletal muscle and the fascia of the esophagus (18), the heart (18), and the muscles of the hind limbs or the lumbar region (11), and in front limbs, head, sternum, and abdominal wall of cases (case Nos. 13–17). In the esophagus, the inflammation was circumferential and primarily involved the muscular tunics but sometimes extended into the submucosal and the serosal tunics (Fig. 5). In case Nos. 13–17, inflammation extended the entire length of the esophagus. Suppurative inflammation also was seen in the smooth muscle tunics and associated submucosa of the small intestine (1), stomach (3), and urinary bladder (2).

Inflammation primarily was within the perimysium and the endomysium, and frequently extended into surrounding fascia or adipose tissue. Inflammation was composed predominantly of well-preserved neutrophils, with fewer macrophages, lymphocytes, and plasma cells (Fig. 6). Affected myofibers appeared to be randomly distributed within the fascicles; myofibers in affected areas were often widely separated by sheets of inflammatory cells and occasionally were swollen, hyali-

Table 1. Signalment, clinical signs, blood work and vaccine history for ferrets with myofasciitis.

Ferret	Origin	Age	Sex*	Clinical Signs	Hemogram†
1	Marshall Farms	5 mo	n/m	Acutely moribund, recumbent, died	Neut. leukocytosis (46,000); HCT, 32%
2	Marshall Farms	10 mo	n/m	3 mo lethargy, generalized paresis, fever,died	Neut. leukocytosis (36,300); HCT, 30%
3	Marshall Farms	12.5 mo	n/f	5 days lethargy, fever, tachycardia, murmur, euthanized	Neut. leukocytosis (16,800); HCT, 15.9%; thrombocytopenia
4	Marshall Farms	12 mo	n/m	1 day fever, bruxism, vomiting, lethargy, euthanized	Neut. leukocytosis, (8,900); HCT, 30%
5	Marshall Farms	10 mo	n/m	Abnormal stool, posterior paresis, euthanized	Neut. leukocytosis, (29,000); HCT, 28.5%
6	M&P Farms	6 mo	m	Fever, pain, orange spots on skin, posterior paresis, inappetence, euthanized	Neut. leukocytosis (34,000); HCT, 29%
7	na	7 mo	f	Fever, lethargy, recumbency, pain, euthanized	Neut. leukocytosis (14,500); HCT, 26.4%
8	Hybrid European polecat × New Zealand	2 yr	n/m	Fever, lethargy, anorexia, pain, died	Neut. leukocytosis (24,500), HCT-31%
9	Marshall Farms	11 mo	m	Lethargy, weakness, died	Neut. leukocytosis (45,000); HCT, na
10	Marshall Farms	5 mo	f	Fever, lethargy posterior paresis, euthanized	Normal leukogram (6,500); HCT, 35%
11	Marshall Farms	6 mo	n/m	Anorexia, lethargy, fever, reluctant to drink, tachycardia, died	Neut. leukocytosis (16,700); HCT, 44%
12	Marshall Farms	6 mo	n/f	Lethargy, loose stools, seizures, fever, died	Neut. leukocytosis (28,700); HCT, 25.1%
13	Marshall Farms	12 mo	n/f	Paresis, bruxism, pain, fever, serous nasal discharge, tachycardia, wet breathing, panting, tarry stools, euthanized	Neut. leukocytosis (20,000); HCT, 19%
14	Marshall Farms	10 mo	n/f	Fever, lethargy, hind limb pain, euthanized	Leukocytosis (17,700) (57% neutros)
15	Marshall Farms	11 mo	n/f	Lethargy, fever, euthanized	Leukocytosis (30,000), Diff na
16	Path Valley	9 mo	n/f	Lethargy, fever, ataxia, paresis euthanized	Leukocytosis (19,100) (68% neutros); HCT, 32%
17	Path Valley	6 mo	n/m	Lethargy anorexia, fever, posterior pain and paresis, euthanized	Leukocytosis (79,500) (42% neutros); HCT, 24.6%

* m = male; n/m = neutered male; f = female; n/f = neutered female.

† Neut. leukocytosis = neutrophilic leukocytosis; HCT = hematocrit; na = not available; neutros. = neutrophils; Diff na = differential not available.

‡ El. CPK = elevated creatine kinase; AST = aspartate aminotransferase, ALT = alanine aminotransferase; alk phos = alkaline phosphatase; T. bili = total bilirubin; GGT = gamma glutamyl transferase; CK = creatine kinase.

§ nd = not done; neg. = negative; ng 1 = no growth aerobic culture; ng 2 = no growth anerobic culture; ng 3 = no growth viral culture.

‖ Fervac-D, United Vaccines, Inc, Madison, WI; Purvax and Imrab-3, Merial, Duluth, GA; Galaxy D, Schering-Plough, Kenilworth, NJ; mu = manufacturer unknown.

Mult. antibiotic = multiple antibiotics.

Table 1. Extended.

Chemistries‡	Cultures§	Vaccines‖	Treatments#
El. CK, AST, ALT, T. bili	nd	Fervac-D no. 1 (kit)	Mult. antibiotics
El. ALT, hyperglycemia	nd	Fervac-D no. 1 (kit)	Mult. antibiotics, banamine, prelone, dexamethazone, interferon
El. ALT, alk phos, GGT, T. bili, hyperglycemia	Liver, lung, kidney, *Ralstonia* sp. neg. anarobic, viral	Fervac-D no. 1 (kit) Purvax no. 2	Mult. antibiotics, banamine, meloxicam, alpha interferon, cyclophosphamide
Hypoalbuminemia	nd	Fervac-D no. 1 (kit)	Mult. antibiotics, dexamethazone, prednisolone
El. ALT	nd	Fervac-D no. 1 (kit),	Mult. antibiotics, interferon
Hypoalbuminemia	nd	Fervac-D ×3, Imrab-3 5.5 mo	Mult. antibiotics, rimadyl, digoxin, interferon, transfusion
Hypoalbuminemia, hyperglycemia	nd	Fervac-D	Mult. antibiotics, interferon
Hyperglycemia	nd	Fervac-D, Galaxy D ×3, Imrab-3; boostered both at 1.5 yr	Mult. antibiotics, prednisolone, cyclophosphamide
Hyperglycemia	nd	Fervac-D no. 1 (kit), Imrab-3 boosters	Mult. antibiotics, buprenorph, dexamethazone
Nd	nd	Fervac-D 4 mo, Imrab-3 4 mo	Mult. antibiotics, buprenorph, meloxicam
Hyperglycemia, hypoalbuminemia el. ALT, lipase	nd	Fervac-D no. 1 (kit) booster 2 mo, Imrab-3 3 mo	Mult. antibiotics, dexamethazone
Hypoalbuminemia	Light growth, *Bacillus* sp. lung	Fervac-D ×3 Imrab-3, 3 mo.	Mult. antibiotics, buprenorf, meloxicam
Hyperglycemia, hypoalbuminemia	ng 1, ng 2, ng 3, skeletal muscle, lung	Fervac-D, ×1 (kit)	Mult. antibiotics, acyclovir, furosemide, tumil-k, carafate, buprenorphine, pred, vitamin C, multivitamin w/iron, banamine
Hyperglycemia, increased CK	ng 1, ng 2	Fervac-D (no. 1) kit, Galaxy D no. 2, Imrab-3, Galaxy D no. 3	Amoxicillin w/clavulanic acid; fluids
na	Aerobic — normal flora/ contaminants, ng 2, ng 3	Fervac-D (no. 1) kit, no. 2 mu, rabies mu	None
Hyperglycemia	ng 1, ng 2, ng 3	Fervac-D (#1) kit, Purevax no. 2, Fervac-D no. 3, Imrab-3, (given twice accidentally)	Amoxicillin, enrofloxacin, tremadol, cimetidine, famotidine, prednisone, meloxicam, pet tinic, interferon, acyclovir, oseltamivir, fluids, goldenseal, pau d' arco; acupuncture
Hypoalbuminemia	ng 1, ng 2, ng 3	Fervac-D no. 1 (kit)	Enrofloxacin, amoxicillin, ampicillin, metronidazole, Pepto-Bismol

Table 2. Distribution and severity of histologic lesions and concurrent disease processes in ferrets with myofasciitis.*

Ferret	Esophagus	Heart	Skeletal	Diaphragm	Tongue	Stomach	Intestine	Hepatitis†
1	+++	++ to +++	++	na	na	–	–	++
2	+++	+++	na	na	na	na	Na	na
3	+++	++	+++	+++	na	++ to +++	++ to +++	++ to +++
4	+++	+++	+++	na	+++	–	–	–
5	++ to +++	++ to +++	++ to +++	na	na	na	–	–
6	+++	+++	+++	na	na	na	Na	–
7	+++	+	++	+	na	+	–	–
8	+++	+	++	na	na	na	Na	+
9	+++	++	++ to +++	na	na	na	–	++
10	+++	+++	+++	na	na	–	–	+
11	+++	++	++ to +++	++	+++	–	–	–
12	++ to +++	+	++ to +++	na	na	–	–	++
13	++ to +++	+	++ to +++	++ to +++	++ to +++	–	–	–
14	++ to +++	+ to ++	++ to +++	++ to +++	–	–	–	+
15	+	+	+	+	–	–	–	+
16	+++	+	+++	+++	+++	+++	–	+
17	+++	+	++ to +++	+	++	–	–	–

* + = mild; ++ = moderate; +++ severe; – = tissue present but not affected; na = tissue not available.
† Suppurative hepatitis.
‡ spl = spleen; bm = bone marrow.
§ acute neutrophilic interstitial pneumonia.
‖ lymphoplasmacytic enteritis, considered unrelated to the polymyositis.
DIC = disseminated intravascular coagulation.

nized, fragmented, or had loss of cross-striation detail. Inflammatory cells occasionally infiltrated the sarcoplasm of the viable or degenerative myofibers (Fig. 7). In lesions interpreted as chronic, inflammation was often interspersed with areas of myofiber atrophy and fibrosis (Fig. 8).

Small arteries, small veins, and capillaries in inflamed foci occasionally had endothelial cell hypertrophy, fibrinoid degeneration of the muscular tunics, neutrophil margination and transmigration, and associated perivascular edema or fibrin deposition. Rarely, affected vessels contained partially occlusive fibrin thrombi. Random neutrophilic hepatitis (9), multifocal neutrophilic interstitial pneumonia (7), suppurative mediastinitis (7), and suppurative panniculitis (3) were also seen. The mediastinal and subcutaneous lesions appeared to represent an extension of inflammation from the esophagus and the regional skeletal muscles, respectively. Myeloid hyperplasia of spleen (15) and/or bone marrow (6) was also seen. The hyperplasia was primarily composed of myeloid precursors

Table 2. Extended.

Myeloid Hyperplasia‡	Pneumonia§	Other Lesions
na	+	Enteritis,‖ vasculitis/DIC,# renal mineralization
+++ (bm)	+	DIC, peritonitis, synovitis, mediastinitis
+++ (bm)	na	Splenitis, lymphadenitis, vasculitis, peritonitis, biliary cystadenoma, synovitis
+++ (spl)	–	Mineralization renal and muscle
+++ (spl)	na	Enteritis, mediastinitis
+++ (spl)	+	Enteritis, islet hyperplasia, hepatocellular vacuolar change, ependymitis
+++ (spl)	–	Portal hepatitis, enteritis, pulmonary congestion
+++ (spl)	+	Portal hepatitis, hepatic lipidosis, renal tubular necrosis
+++ (spl)	–	Mediastinitis, aspiration pneumonia, enteritis
+++ (spl)	+	Urocystitis
+++ (spl)	–	Hepatic lipidosis, pulmonary congestion, root ganglioneuritis, mediastinitis
+++ (spl, bm)	na	Centrilobular necrosis, enteritis
+++ (spl)	–	Enteritis, renal infarct
+++ (spl)	+	Panniculitis, steatitis, meningitis enteritis
+++ (spl, bm)	–	Rhabdomyolysis, enteritis
+++ (spl, bm)	+	Steatitis, mediastinitis urocystitis
+++ (spl, bm)	+	Steatitis mediastinitis urocystitis

with orderly maturation to a slight left shift (Fig. 9). Additional histologic changes considered secondary, incidental, or unrelated to the myositis, were seen in few ferrets and are listed in Table 2.

Electron microscopy

Neutrophils and macrophages within the lesions generally were well preserved and viable. Some mild vacuolar change was noted in few neutrophils. Macrophages rarely contained membrane bound whirls of material interpreted as phagolysosomes containing cellular debris. Blood vessels had

Fig. 1. Hind limb, ferret, No. 14. Note severe generalized atrophy of musculature of the hind limb of ferret with polymyositis (left), compared with age matched ferret that died of unrelated disease (right).

hypertrophied endothelial cells and some perivascular edema or fibrinoid degeneration (Fig. 10). Affected myofibers had mild to severe sarcoplasmic and mitochondrial swelling, and disruption of myofibrils by edema. Z bands were disrupted or completed destroyed (Fig. 11). No infectious agents were seen.

Immunohistochemistry

No immunohistochemical staining for *Toxoplasma gondii, Sarcocystis neurona, Neospora caninum,*

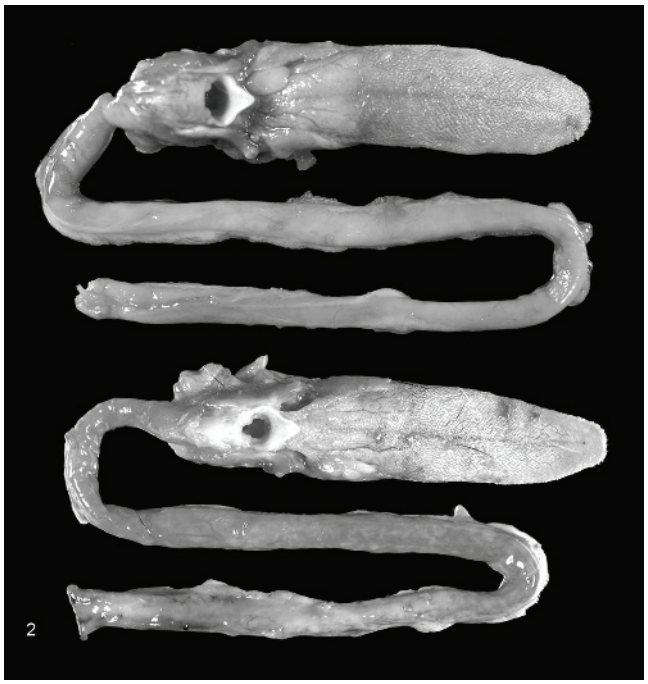

Fig. 2. Esophagus, ferret, No. 14. Note full length mottling of the esophagus, shrinkage of the tongue and lingual hyperkeratosis of ferret with polymyositis (bottom) compared with age matched ferret that died of unrelated disease (top).

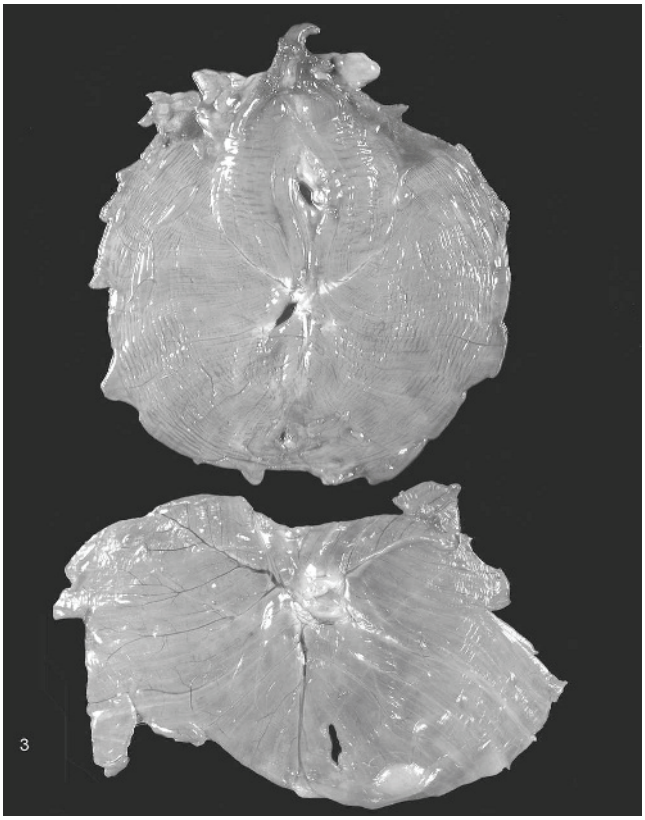

Fig. 3. Diaphragm, ferret, No. 14. Note diffuse thinning of the diaphragm and few white linear foci (bottom), compared to age matched ferret that died of unrelated disease (top).

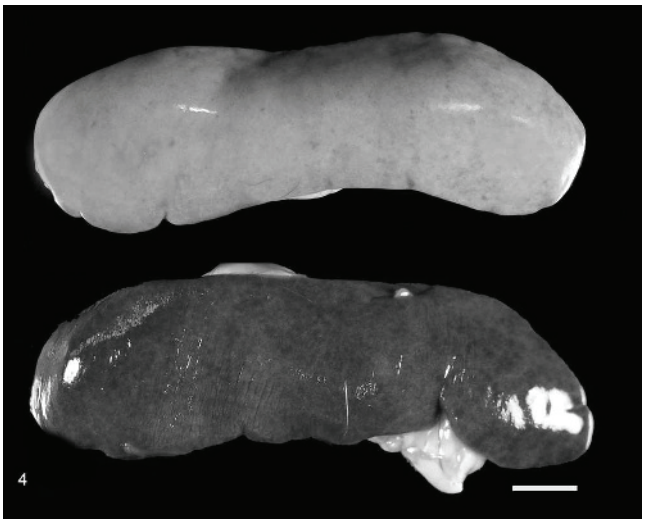

Fig. 4. Spleen, ferret, No. 14. Note tan discoloration of spleen in ferret with myositis (top), compared with purple-red spleen of old ferret that died of unrelated disease (bottom). Both spleens are enlarged to approximately 10 times normal size. Bar = 1 cm.

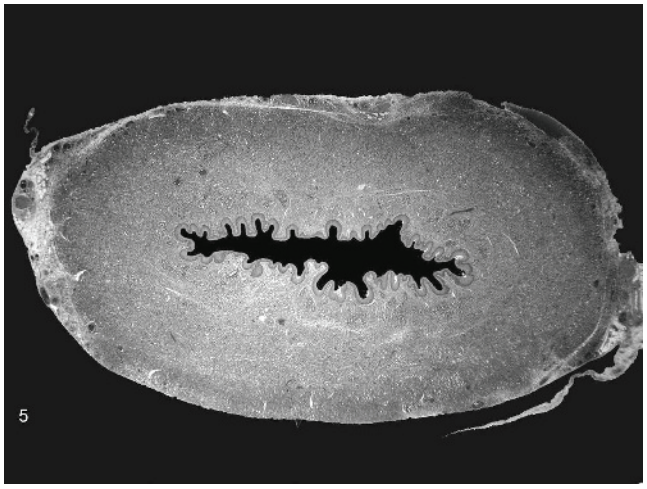

Fig. 5. Esophagus, ferret, No. 18. Note severe circumferential inflammation in the muscular, submucosal, and serosal tunics, and sparing of the mucosal surface in this Subgross photomicrograph. HE.

or feline or ferret coronavirus antigen, was detected in the 8 ferrets examined. No background staining was noted in the controls.

Polymerase chain reaction

The PCR assay for DNA from *Toxoplasma gondii* was positive in skeletal muscle from 3 of 8 ferrets, and results from positive and negative control reactions were as expected (data not shown). The PCR assays for DNA from *Neospora caninum*, either as nested or as separate assays, did not produce any amplicons from any of the tissue samples from ferrets (data not shown). The PCR assays (nested and separate) for *Neospora caninum*

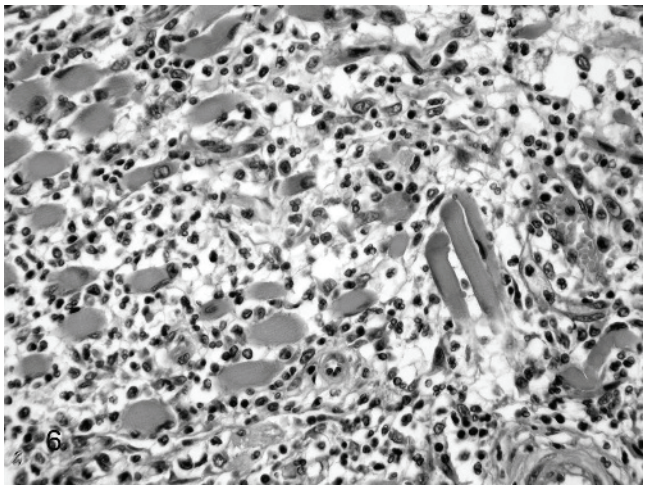

Fig. 6. Esophagus, ferret, No. 14. Note severe inflammation in the muscular tunic, with a loss of myofibers and disorganization of remaining fibers. Inflammation is predominantly neutrophils. HE.

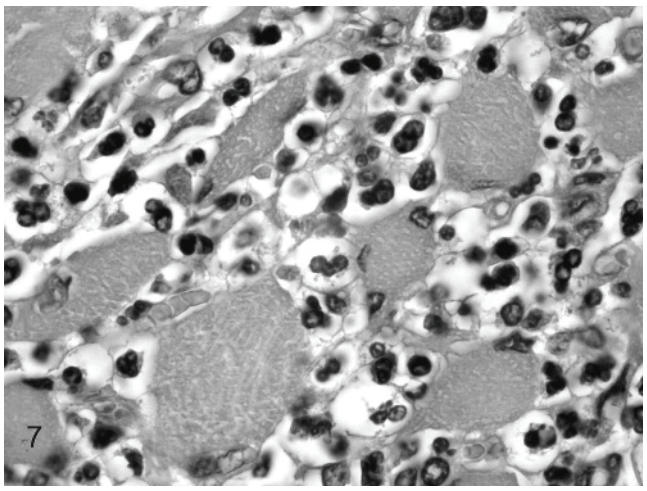

Fig. 7. Biceps femoris muscle, ferret, No. 16. Note inflammation is in the endomysium. Extension of inflammation into the myofiber is not present in the image and was only rarely observed in the study. HE.

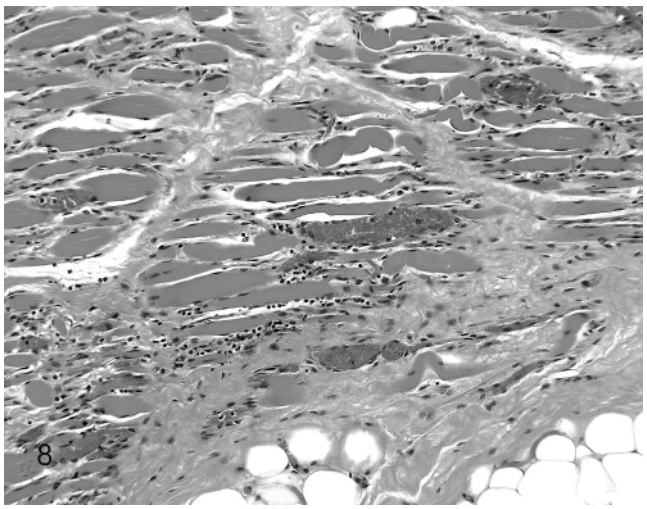

Fig. 8. Biceps femoris muscle, ferret, No. 14. Note extensive endomyseal and perimyseal fibrosis, disorganization, and variability in size and shape of myofibers. A small focus of inflammation is also present. HE.

produced amplicons of the expected size from the positive controls, by using fresh tissue. By using formalin-fixed, paraffin-embedded tissue, only the internal set of PCR primers produced an amplicon of expected size from positive control tissue.

Cultures

Aerobic culture of pooled liver, kidney, and lung from case No. 3 revealed *Ralstonia* sp. bacteria. Anerobic and viral culture of pooled liver, kidney, and lung from this ferret revealed no growth. Aerobic culture of feces from case No. 7 revealed no growth of known pathogenic bacteria. Aerobic culture of lung from case No. 12 revealed light growth of *Bacillus* sp. Aerobic and anerobic bacterial cultures and viral cultures of pooled skeletal muscle, liver, and lung from case Nos. 13–17 revealed no growth. Pooled tissue homogenates for viral culture have been negative to date on CrFK and Vero cells. Passages are continuing on MDCK cells and will the subject of further investigation.

Discussion

Myofasciitis in ferrets is a relentlessly progressive fatal disease in spite of the use of a variety of antibacterial, antiviral, antifungal, and anti-inflammatory or immunomodulating drugs. It appears to be a disease of young adult animals, characterized by rapid onset of clinical signs, high fever, neutrophilic leukocytosis, treatment failure and death (or euthanasia). Because the condition is disseminated and muscle is the predominant target tissue of the inflammatory process, the terms ''polymyositis'' or disseminated idiopathic myositis have also been used for this condition; however, inflammation also is seen in the fascia around muscle bundles and in adjacent adipose tissue of some animals, so MF seems a more appropriate morphologic term. Also, the use of the term ''polymyositis'' may cause confusion with the human PM, which is a distinctly different inflammatory condition.

Antemortem diagnosis of MF is based on the presence of suppurative to pyogranulomatous inflammation within biopsy samples of muscle fascicles, in conjunction with clinical signs of weakness, muscle atrophy or lethargy, unresponsive fever, neutrophilic leukocytosis, and failure to respond to various treatments. Serum chemistries do not appear to be a very useful aid in establishing an antemortem diagnosis. The alanine aminotransferase (ALT) was mildly elevated in few ferrets, possibly because of hepatic or muscle damage,[3] but this was not a consistent finding and was seen in some ferrets that did not have hepatic lesions. Also, some ferrets had hepatic lesions and no elevation in ALT, suggesting that elevation of this enzyme was not specific for hepatitis in these ferrets. Perhaps the most interesting and enigmatic serum chemistry observation is the absence of an elevation in creatine kinase (CK) or aspartate aminotransferase (AST), generally considered indicators of muscle damage.[3] The cause for this is not known, but unlike the known myopathies in other species, elevated CK or AST do not appear to be useful screening tools for polymyositis in ferrets; paradoxically, a lack of elevation in muscle enzymes in

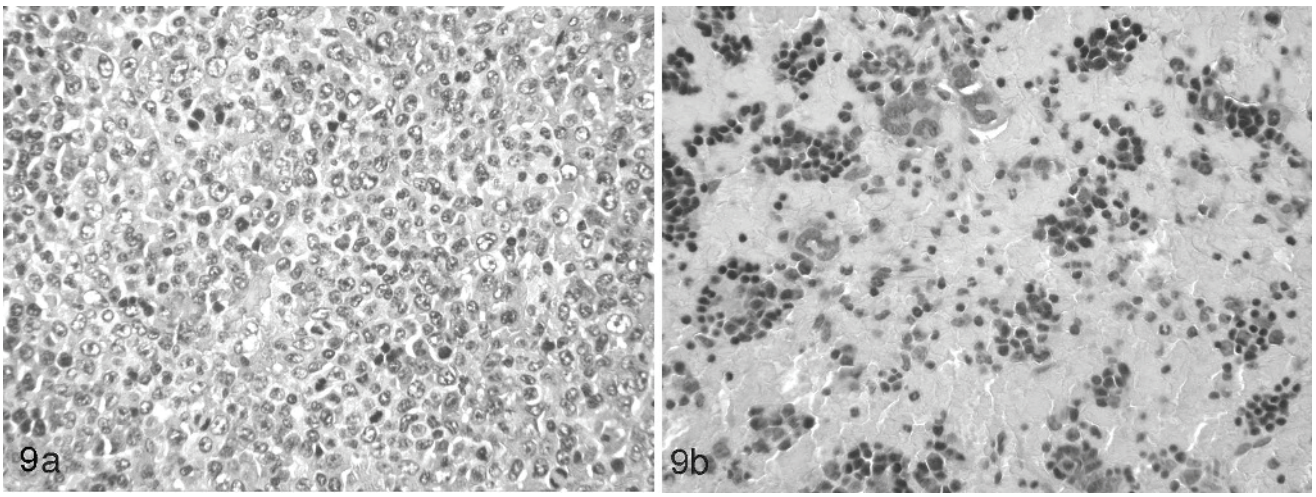

Fig. 9. Spleen, ferret, No. 14. **Fig. 9a**. Note that the red pulp was infiltrated by large numbers of myeloid precursors with a slight left shift in the ferret with myositis. **Fig. 9b**. Spleen of an old ferret that did not have polymyositis was enlarged primarily from congestion and hematopoiesis, representing all cell lines with orderly maturation. HE.

conjunction with other clinical and laboratory values may be helpful in establishing an antemortem diagnosis. It is considered possible that the CK and AST are not significantly altered because, depending on the stage of the disease, muscle is mostly displaced by inflammation or atrophied rather than necrotic.

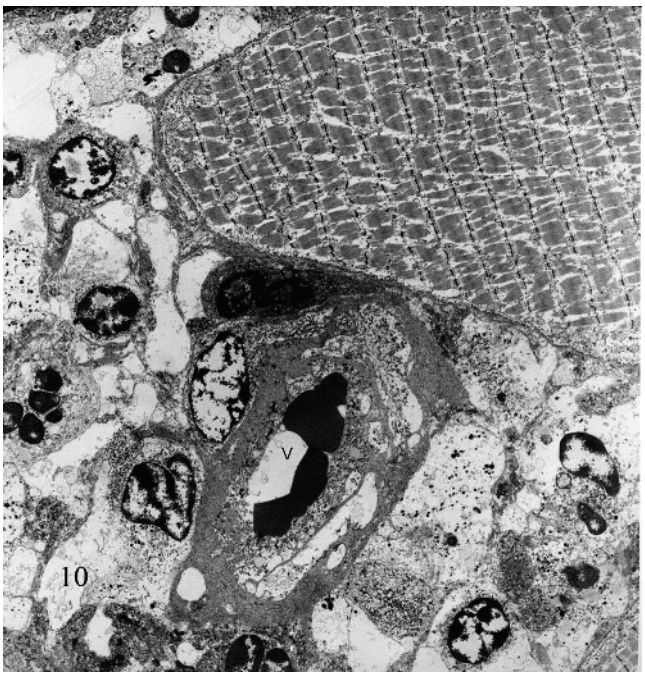

Fig. 10. Esophagus, ferret, No. 1. Note edema and fibrinoid degeneration in the wall of venule (v), and predominant infiltrate of neutrophils with fewer lymphocytes in the endomysium. Early Z band lesions in myofiber (upper right) are barely visible at this magnification. Uranyl acetate and lead citrate.

Ferrets with MF present with a variety of ambulatory problems, primarily recognized clinically in the hind limbs. Some of the ferrets also present with various alimentary tract problems. All muscle groups appear to be involved in the inflammatory process, including alimentary smooth muscle, and it seems likely that the clinical signs in these animals are specifically from the pain

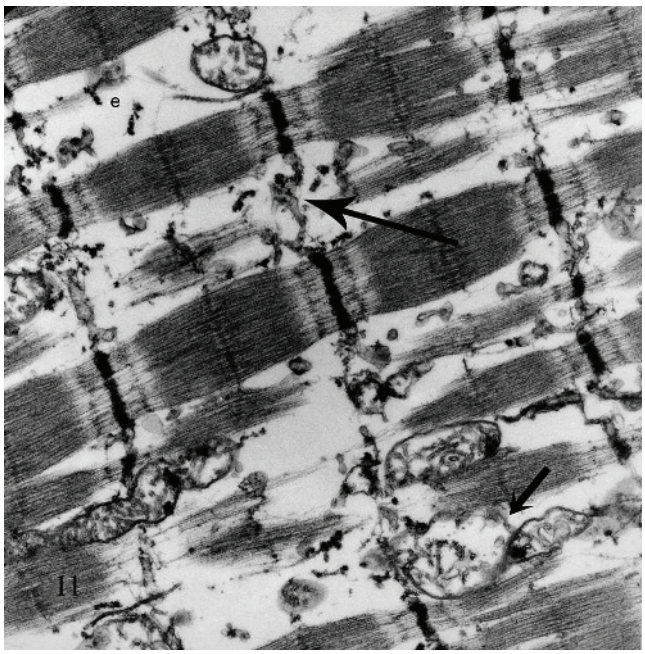

Fig. 11. Esophagus, ferret, No. 1 Note edema separating myofibrils (e), mitochondrial vacuolar swelling (small arrow), and destruction of z bands (large arrow). Uranyl acetate and lead citrate.

and the dysfunction that accompany the inflammation in this condition. Although some ferrets were described as ataxic, this disease does not appear to primarily target the nervous system, and the ataxia described clinically was likely because of muscle dysfunction.

The distinct clinical presentation and the distribution of histologic lesions, particularly in the esophagus, suggest that this is likely a single distinct entity. The authors are not aware of any other reported pathologic condition characterized by suppurative to pyogranulomatous MF, with a propensity for severe diffuse circumferential involvement of the entire length esophagus and propose that the esophageal lesion described in this report may be unique. Megaesophagus has been described in ferrets but is generally a disease of older animals, and, although inflammation is a minor component of megaesophagus in some cases, the distribution and cellular constituents of the inflammatory response differ from those of ferrets with MF.[4] In addition, ferrets with MF do not have dilatation of the esophageal lumen. Esophagitis is a component of DM and PM in humans, but the esophagus does not appear to be as severely affected in these disorders as in the ferret condition, and the inflammatory cell infiltrates of these conditions differ from those of ferrets with MF.[13,25,38]

Splenomegaly is a common but nonspecific feature of MF in ferrets. The spleen in affected ferrets is enlarged because of a nearly homogeneous infiltrate of myeloid precursor cells in the red pulp. Maturation of these cells generally is orderly. Myeloid hyperplasia also is seen in the bone marrow, and this likely is a compensatory response for the tissue inflammation in these animals. Splenomegaly also is seen frequently in old ferrets,[19] and, in most of these animals, it is because of a combination of congestion and extramedullary hematopoietic in the red pulp, rather than a compensatory response for anemia or inflammation. Aside from the microscopic differences, the spleens differ in gross appearance as well: the spleens of old ferrets typically are dark red to purple, spongy, and ooze much blood on cut surface, whereas the spleens of ferrets with MF are tan and firm, and do not ooze much blood on cut surface.

The febrile nature of the disease, leukocytosis (often marked), suppurative inflammatory features of the muscle lesions, and endotoxin-like affects on vessels suggest a bacterial etiology;[5] however, the uniform failure of treated patients to respond to a broad variety of antibiotics and the inability to detect bacteria in histologic sections of treated and untreated ferrets suggested that bacterial infection was not likely. Although bacterial cultures were not obtained for all ferrets in this study, a failure to isolate significant bacterial pathogens from some of these animals also suggested that bacterial infection was not the cause. Pyomyositis or tropical pyomyositis is a human condition characterized by suppurative inflammation in a number of different skeletal muscle groups and has some morphologic features similar to MF.[1,12] Pyomyositis has been associated with infection by *Staphylococcus aureus* and has been linked to a number of underlying viral conditions, including human immunodeficiency virus. [1,12] Pyomyositis typically responds to antibiotic therapy.[1,12] The absence of infectious agents and the lack of response to treatment distinguish MF from pyomyositis of humans. It is likely that the leukocytosis is compensatory for the inflammation in the muscles. The fever is likely from mediators associated with the inflammatory process. The lesions in vessel walls may be because of complement or antibody deposition, as has been described for other myopathies.[11,25]

Viruses have been associated with myositis in humans[12] and mice.[43] Although ferret cell lines were not available, viral culture was attempted in 4 ferrets, by using pooled skeletal muscle, lung, and liver; however, pooled tissue homogenates for viral culture have been negative to date on CrFK and Vero cells. No viral inclusions were seen in histologic sections, and no viral particles were seen by electron microscopy. The sometimes pyogranulomatous nature of the infiltrate and the occasional vascular degeneration seen in this condition vaguely resemble feline infectious peritonitis,[2] an immune complex disorder of felids mediated by a variant of feline enteric coronavirus. An enteric coronavirus has been implicated as a cause of enteritis in the domestic ferret,[41] and enteritis was seen in some of the study ferrets. For these reasons, immunohistochemistry was performed to screen for feline and ferret coronavirus antigen in the lesions, but no antigen was detected. Based on our findings, it seems unlikely that coronavirus infection is a factor in the development of MF. Passages are continuing on MDCK cells and will the subject of further investigation.

Protozoan agents such as *Toxoplasma gondii*, *Neospora caninum*, and *Sarcocystis* spp. can cause myositis in a number of different mammalian species[20]; however, no protozoa were seen histologically in any of the animals, and immunohistochemistry was negative for *T. gondii, N. caninum,* and *S. neurona* in the 8 ferrets that were tested for these agents. DNA to *Toxoplasma gondii* was

detected in 3 of 8 ferrets that were tested by PCR. Ferrets can contract clinical toxoplasmosis,[39] but, generally, organisms are easy to detect histologically, and lesions are not similar in morphology or distribution to those of MF. The positive DNA tests in this study may have been because of subclinical infection or laboratory contamination. It seems unlikely that *T. gondii* infection is the cause of MF. At 337 base pairs, the expected amplicon for *N. caninum* may have been too large to detect readily in formalin-fixed tissue. Failure to detect DNA from *Neospora caninum* in the formalin-fixed, paraffin-embedded tissue from the ferrets may indicate that the tissue was free of that organism or may reflect a technical problem attributable to the size of the expected amplicons. At 337 base pairs and 192 base pairs, the expected amplicons for *Neospora caninum* may have been too large to readily detect the target DNA in formalin-fixed tissue.

Although rare, a heritable predisposition has been documented for some forms of myositis in humans[16] and in dogs.[18] No controlled breeding trials have been conducted for ferrets, but a heritable basis for MF seems unlikely as affected ferrets are from different breeding facilities, and the disease has been seen in both the USA and in The Netherlands (NJS, personal communication).

An acquired immune mediated process is a possible cause for MF. Noninfectious immune-mediated polymyopathies are well documented. The myopathies occurring in humans include PM, DM, and inclusion body myositis.[8,25] Canine examples of DM and PM also have been documented.[11,18] Polymyositis is the most common of the human myopathies, and its generalized distribution and symptoms closely parallel those of polymyositis in ferrets. The human form of PM is caused by the development of antinuclear and anticytoplasmic antibodies such as antibyosyn and antimyoglobin antibody. The condition may be associated with a variety of autoimmune diseases, connective tissue diseases, or viral infections. Humans with PM have weakness of the shoulders and pelvic girdle, muscle pain, and dysphagia. Inflammation is within the fascicles, as seen with MF; however, inflammation is predominantly nonsuppurative, primarily T lymphocytes and macrophages, and this differs considerably from the suppurative to pyogranulomatous infiltrate seen in MF. Also, the CK is typically elevated in humans with PM but is not elevated in affected ferrets. Dermatomyositis involves skin and muscle and is characterized by perivascular infiltrates of CD4 + T lymphocytes and B lymphocytes associated with endothelial cell injury because of immunoglobulin and complement deposition in the vessel walls, with subsequent ischemic damage to the periphery of the muscle bundles and structures of the integument.[8] Although some vascular damage is seen in MF, the cell type and distribution of the inflammatory infiltrate and the nature of the muscle lesion in DM differ considerably from those of MF. Inclusion body myositis is a disease of older human patients, has a male predominance, clinical signs like PM, and is a predominantly T-lymphocyte inflammatory lesion of the endomysium and within necrotic myofibers. Affected myofibers have granular basophilic inclusions distributed around the edge of slit-like vacuoles in the sarcoplasm.[8,25] Aside from the difference in inflammatory cell type, affected ferrets do not appear to have a sex predilection, and inclusions are not seen in the myofibers.

For MF to be an acquired condition, then all affected ferrets would have to be exposed to a substance at some time after birth that precipitated the inflammatory event. Adverse reactions to vaccine constituents are common, and can be because of an immune-mediated event directed at a killed or modified infectious agent, adjuvant, residual materials, or contaminants.[26,36] The only known commonality among all of the ferrets of this report is the administration of at least 1 dose of a commercial canine distemper vaccine licensed for use in ferrets (Fervac-D). Ferrets seem prone to developing adverse reactions, particularly anaphylaxis, after administration of this vaccine.[15] Various forms of vaccine-related myositis are seen in humans,[9,10,17,26,29,31,40,42] but no form of myositis or fasciitis has not been described as an adverse affect of distemper vaccination in ferrets.[15] A considerable interval exists between the documented time of vaccine administration and the onset of clinical signs for some of these ferrets. If a vaccine is involved, then it is possible that there is a delayed mechanism in the development of the disease or that the disease exists subclinically up to a point, then progresses rapidly after initial clinical signs develop. It is interesting to note that one of the authors (NJS) inadvertently reproduced a disease identical to the cases of this report in an entire group of ferrets administered an experimental castration vaccine that contained an aluminium adjuvant.[33] Myopathies associated with administration of aluminium adjuvant vaccines have been shown to have aluminium hydroxide deposits in the foci of muscle damage.[14] Although conclusions that incriminate vaccines cannot be drawn from these preliminary studies, it may be prudent to further

investigate the role of vaccines in the development of MF.

A number of different molecular alterations occur in the inflammatory myopathies of humans. Interleukin (IL) 1α, IL-1β, and Transforming Growth Factor (TGF) β1-3 are dominant cytokines expressed in muscle tissue of patients with inflammatory myopathies.[24] There does not appear to be a qualitative difference in the cytokine expression patterns of PM, IBM, and DM. The predominant IL-1α expression in the blood vessels indicates an importance of endothelial cells in the inflammatory process.[24] Immunohistochemistry or reverse-transcriptase PCR has shown that muscle fibers in the inflammatory myopathies express various cytoplasmic and surface molecules that are not detectable in normal fibers. These molecules, likely induced by locally secreted cytokines, include histocompatibility antigen (HLA) class 1 antigens, heat-shock proteins, and adhesion molecules. Immunohistochemistry has demonstrated fiber type specific autoantibodies in dogs with inflammatory myopathies.[34] Identifying molecular alterations in the lesions of MF may be helpful in defining its pathogenesis.

In summary, MF in ferrets is an idiopathic disease characterized by suppurative to pyogranulomatous myositis and fasciitis affecting skeletal, smooth, and cardiac muscle, and associated fascia in multiple locations. The extensive suppurative esophagitis appears to be unique to this condition. The disease affects young adults of both sexes and has distinct clinical features, although biopsy of muscle is needed for definitive diagnosis. The disease appears to be uniformly fatal, and the lack of response to various treatments is not understood. Future studies regarding the pathogenesis of MF should include characterization of molecular events in muscle lesions, and a search for possible infectious or vaccine-related mechanisms of immune mediated disease.

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