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Eosinophilic Gastroenteritis with Splendore-Hoeppli Material in the Ferret *(Mustela putorius furo)*

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Abstract. Eosinophilic gastroenteritis, focal or diffuse with eosinophilic infiltrations of the stomach or intestine, has been described in human beings, cats, dogs, and horses. In this paper, we describe infiltration of the gastrointestinal tract with eosinophils accompanied by a circulating eosinophilia in six ferrets *(Mustela putorius furoi.* Clinical signs included chronic weight loss, anorexia, and diarrhea. The small intestines from five ferrets had diffuse infiltrates of eosinophils. This resulted in focal or multifocal loss of the muscular tunic in three ferrets. Two of these ferrets also had eosinophilic gastritis. Eosinophilic granulomas with Splendore-Hoeppli material were present in mesenteric lymph nodes in four ferrets. Two ferrets had multiple organ involvement; one had eosinophilic granulomas in the liver, mesentery, and choroid plexus as well as moderate parapancreatic segmental arteritis with infiltration of eosinophils and mural thrombosis. The second ferret had, in addition to moderate diffuse gastric and small intestinal eosinophilic mucosal infiltrations, interstitial eosinophilic pulmonary infiltrates. Examination of all tissues failed to reveal an infectious agent.

Key words: Eosinophilic gastroenteritis; ferrets; Splendore-Hoeppli phenomenon.

Eosinophils kill invading helminths and are mediators of tissue damage in certain allergic conditions.[1,5,16] Increased numbers of circulating eosinophils, as well as tissue eosinophils, occur most commonly in response to allergy and parasitic infection.[1,5,16] Eosinophilia may also be observed in association with a wide variety of other diseases. In human beings, eosinophilia occurs in chronic bacterial diseases (brucellosis, leprosy), viral diseases (infectious lymphocytosis and erythema infectiosum), fungal diseases (bronchopulmonary aspergillosis), neoplasms (lymphoblastic leukemia), and selected immunodeficiencies and connective tissue disorders.v-" Infrequently, the underlying cause of circulating and tissue eosinophilia in human beings and animals is unknown. Examples of idiopathic conditions in human beings include hypereosinophilic syndrome and eosinophilic gastroenteritis.v'{'> In animals, the etiologies of feline eosinophilic granuloma complex and both canine and feline eosinophilic enteritis remain speculative.[2,6, 10, 14,21]

We recently observed eosinophilic gastroenteritis in ferrets. The purpose of this report is to describe the spectrum of lesions seen in six ferrets, two of which had evidence of disseminated organ involvement.

Materials and Methods

Histories, tissues, hemograms, and chemistry profile results from three of the ferrets (Nos. 1-3) in this study were submitted by veterinary practitioners located in Connecticut, New York, and California. The origins of these ferrets were not known by the referring veterinarians, Three ferrets (Nos. 4-6) originated from a commercial vendor located in New York. One ferret (No.4) was a control ferret on a gastric carcinogenesis research project. The other two ferrets (Nos. 5,6) were referred to our laboratory for diagnostic evaluation.

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for routine evaluation. Selected tissues were stained using Warthin-Starry, Gram, and acid-fast stains to reveal bacteria. Giemsa, Gomori's methenamine-silver, and periodic acid-Schiff were used to stain fungi or parasitic remnants.

Results

History, clinical signs, and laboratory findings

Five (Nos. 1-4,6) of the six ferrets had a history of chronic weight loss, anorexia, and bloody and/or mucoid diarrhea. Two of the ferrets (Nos. 1, 3) had episodic vomiting (Table 1). Five ferrets (Nos. 1,2,4-6) had palpably enlarged mesenteric lymph nodes, and three (Nos. 1, 2, 4) had intestinal thickening. Examination of the hemograms performed on five of the ferrets (Nos. 1, 2, 4-6) revealed eosinophilia (Table 1). All four ferrets (Nos. 1, 4-6) whose total protein and albumin were measured were hypoalbuminemic (Table I). Fecal examination for parasitic ova was negative by NaNO} flotation in five ferrets (Nos. 1, 2, 4-6). Results using the zinc sulfate centrifugal flotation technique on the feces of ferret No.2 were also negative.

Table 1. History, clinical signs, and laboratory findings in six ferrets with eosinophilic gastroenteritis,

History	Ferret No.					
	1	2	3	4	5	6
Clinical signs						
Vomiting	+	0*	+	0	0	0
Diarrhea	+	+	+	+	0	+
Weight loss	+	+	+	+	0	+
Anorexia	+	+	+	+	0	0
Physical exam						
Thickened intestine	+	+	0	+	0	0
Enlarged mesenteric lymph node	+	+	NAt	+	+	+
Complete blood count						
Eosinophilia	+	+	NA	+	+	+
Percent eosinophils:j:	19	22	NA	29	10	35
Absolute eosinophil count§	2,166	1,298	NA	2,465	1,190	3,290
Chemistry profile						
Hypoalbuminemia	+	NA	NA	+	+	+
Final deposition	IRII	IR	Euthanatized	Asymptomatic after mesenteric lymph node removed	Euthanatized	Euthanatized

* 0 = not observed.
t NA = not available.
‡ Normal percentage values: males (4-8 mos) 0-7%, WBC cts range 7,700-15,400; females (4-8 mos) 1-9%, WBC cts range 2,500-8,600. (Data from Fox JG. Normal clinical and biologic parameters. In: *Biology and Diseases of the Ferret,* Fox JG, ed. Philadelphia, Lea & Febiger, 1988.)
§ Absolute cell count = percentage x total white blood cell count.
‖ IR = in remission: responsive to steroid therapy.

Gross lesions

Tissues of three ferrets (Nos. 1-3) were mailed to us as biopsy specimens. Contributors' descriptions accompanying the specimens stated that lesions ranged from "substantial enlargement of gastric and mesenteric lymph nodes, [but] intestines/stomach [were] visually and palpably normal" (No.1) to "slightly enlarged mesenteric lymph node with [the] serosal surface of small intestine covered with varying sized 1-5 mm fluid-filled cysts" (No.2). No description of the gross examination of tissues from ferret No.3 was received. Multiple biopsies were taken at laparotomy from ferret No.4; the surgeon noted "enlarged mesenteric lymph nodes, one [of which] appeared cystic and measured approximately 1.5 cm in diameter. The colonic lymph node was prominent. In the proximal jejunum a focal area measuring approximately 0.4 em in length was palpably thickened and pale tan in color." Complete necropsies were performed on ferret Nos. 5-6. The gross findings in ferret No.5 were unremarkable. Multiple well-demarcated randomly distributed white foci 1.0 mm in diameter were visible both on the capsular surface and within the parenchyma in the liver of ferret No.6. Dense connective tissue around bile ducts was prominent.

Microscopic lesions

Lesions were most prominent in the gastrointestinal tract and associated lymph nodes (Figs. 1–4). Small intestine was available for examination from all ferrets except ferret No.1. In the remaining five cases, a moderate diffuse infiltrate of eosinophils was found in the small intestine (Table 2). In two of the ferrets (Nos. 4, 5), this infiltrate was limited to the lamina propria and submucosa (Fig. 2). In the other three ferrets (Nos. 2, 3, 6), a subserosal infiltrate consisting of eosinophils was also present. In its mild form, the subserosal infiltrate was moderate focal or multifocal and resulted in elevation of reactive mesothelial cells (Fig. 3). Occasionally the eosinophil-containing infiltrate extended into the muscular tunic and was associated with thinning of the muscular tunic in the small intestine of ferret Nos. 3 and 6, whereas in ferret No.2 there was a severe eosinophilic granulomatous reaction with marked multifocal interruption of the outer muscular tunic. The colon of ferret No.3 had a moderate diffuse

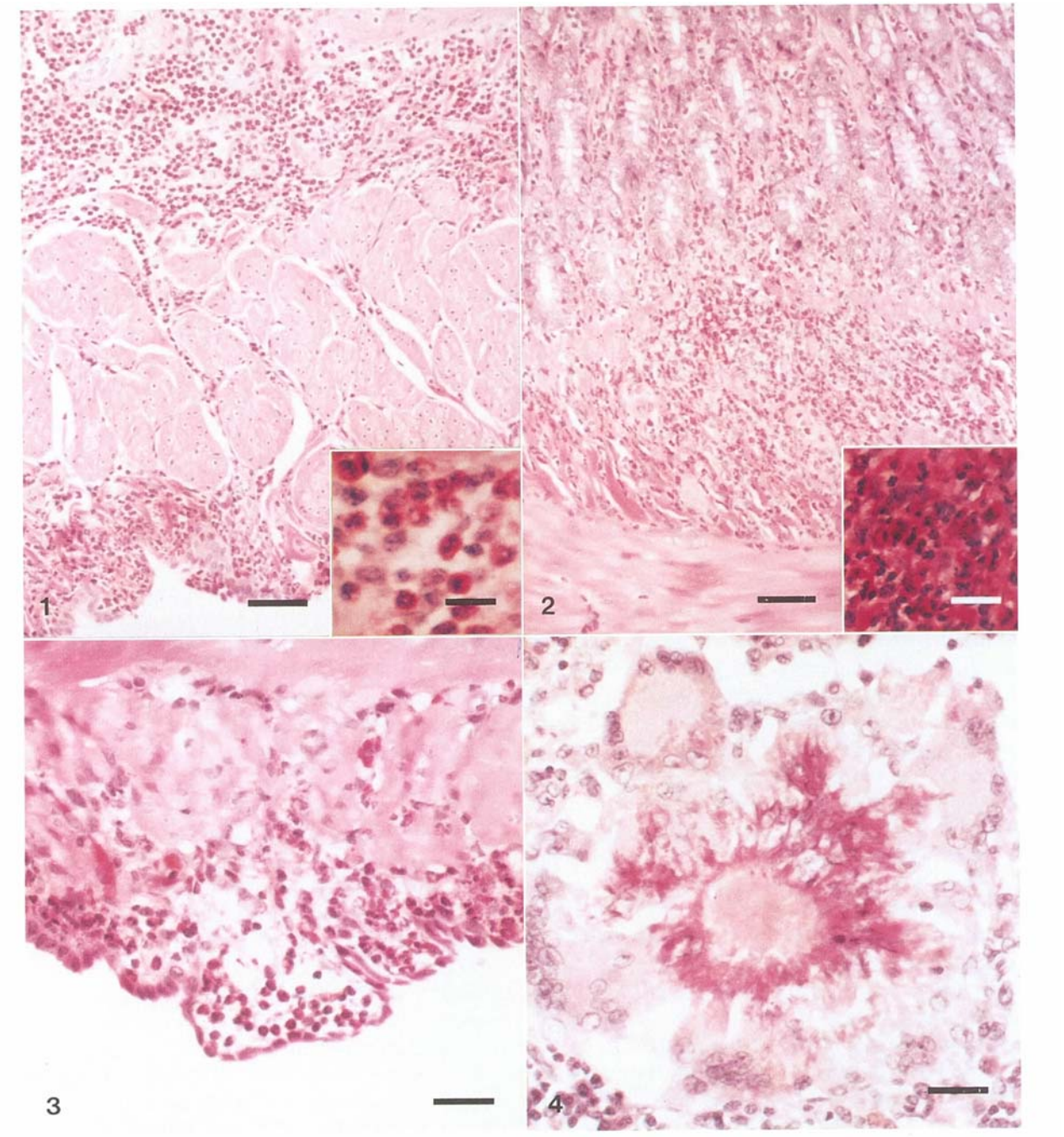

Fig. 1. Stomach; ferret No.6. Eosinophil infiltration of subserosa, muscular tunic, and submucosa. HE. Bar = 50 μm. *Inset:* Infiltration of eosinophils into the tunica muscularis. Giemsa. Bar = 25 μm.

Fig. 2. Small intestine; ferret No.2. Infiltration of eosinophils into the lamina propria, submucosa, and muscularis muscosa. HE. Bar = 50 μm. *Inset:* Infiltration of eosinophils into the submucosa. Giemsa. Bar = 50 urn.

Fig. 3. Small intestine; ferret No.2. Serosal elevation produced by subserosal infiltrating eosinophils and hyperplastic mesothelial cells. HE. Bar = 25 μm.

Fig. 4. Mesenteric lymph node; ferret No.6. Splendore-Heoppli phenomenon within eosinophilic granuloma. Note the radiating eosinophilic deposits from a central core surrounded by multinucleate giant cells and eosinophils. HE. Bar = 25 μm.

Table 2. Tissue targets for eosinophil accumulation in eosinophilic gastroenteritis of six ferrets.

Ferret No.	Stomach	Intestine	Mesenteric Lymph Nodes (granulomas)	Lung	Eosinophilic Granuloma in Mesentery	Other
1	NA*	NA	+t	NA	NA	NA
2	NA	+‡	+	NA	NA	NA
3	NA	+‡	+t	NA	NA	NA
4	0	+	+t	NA	NA	NA
5	+	+	0	+	+	0
6	+	+‡	+t	+	+	Multiple tissues§

* NA = tissue not available; + = accumulation present; 0 = accumulation absent.
t Splendore-Hoeppli phenomenon in granulomas.
‡ Erosion of muscular tunic.
§ Eosinophilic granuloma in liver; vasculitis with thrombosis; choroid plexus.

eosinophilic hypercellularity of both the superficial and the deeper mucosa.

Eosinophilic lymphadenitis was present in mesenteric lymph nodes from five ferrets (Nos. 1–4, 6, Table 2). **In** four ferrets (Nos. 1, 3, 4, 6), eosinophilic granulomas in the lymph nodes, predominantly in medullary cords and sinuses, consisted of variable numbers of eosinophils, macrophages, lymphocytes, plasma cells, and multinucleate giant cells. The giant cells were frequently oriented around eosinophilic bodies 1-2 mm in diameter made of granular and fibrillar extracellular eosinophilic material arranged in a radiating pattern. These multiple structures are compatible with Splendore-Hoeppli material (Fig. 4). When sections of lymph nodes were stained with Giemsa, periodic acid-Schiff, and Gomori's methenamine-silver, the central areas of the eosinophilic bodies were pale, but the peripheral areas were densely stained (with Giemsa, the peripheral areas were intensely pink). Organisms were not visible in acid-fast, Giemsa, Gram, or Warthin-Starry stained sections. Giemsa stain did not reveal mast cells in significant numbers.

Two ferrets (Nos. 5, 6) had eosinophilic cellular infiltration of the stomach and lungs and eosinophilic granulomas in the mesentery. The eosinophilic gastritis was predominantly within the lamina propria but occasionally extended into the submucosa (Fig. 1). Eosinophils were often accompanied by lymphocytes and macrophages. **In** ferret No.5, there was a multifocal, mild to moderate infiltrate containing eosinophils in alveolar septa. The pulmonary infiltrates in ferret No. 6 had a bronchiolar with a mild multifocal eosinophilic cellular infiltration beneath peribronchiolar epithelium.

Ferret No.6 was the most severely affected animal. **In** addition to the changes in the intestine, lymph node, stomach, lung, and mesentery, there were lesions in the liver. These consisted of periportal eosinophilic granulomas, random multifocal abscesses of eosinophils, and a proliferative vasculopathy of portal triad arteries (characterized by thickening of the tunica media with distortion of the lumen). **In** arteries in peripancreatic fat, a mild to moderate segmental, sometimes necrotizing, vasculitis with mural thrombosis and an eosinophilic cellular infiltrate was present. Eosinophils in these arteries were usually located just beneath the tunica intima. Eosinophils were found in the adventitia of small veins in the subserosal connective tissue attached to the urinary bladder. A perivascular eosinophilic granulomatous infiltrate was found in the choroid plexus.

Discussion

Eosinophilic gastroenteritis in these six ferrets shares many similarities with idiopathic eosinophilic gastroenteritides reported in human beings and other species. The disease is characterized by infiltration of the gastrointestinal tract with eosinophils, a peripheral blood eosinophilia, clinical signs such as vomiting, diarrhea, and weight loss (depending on the site of gastrointestinal tract involvement), and lack of evidence of parasitic or allergic disease.

Five of the ferrets (Nos. 2-6) had a diffuse form of eosinophilic gastroenteritis similar to that described in human beings, dogs, cats, and horses. This was characterized by transmucosal infiltration of eosinophils with resultant thickening of the affected portion of the stomach or intestine; however, reactive fibrous tissue (replacing intestinal tissue) seen in cats with a similar disease was not present in the disease **in** ferrets.[6,10–12,14,19] The disseminated nature of the disease **in** two ferrets (Nos. 5, 6) was similar to that seen in human beings and some cats with hypereosinophilic syndrome.t-v' **In** contrast to the histopathologic presentation of the eosinophilic gastroenteritides and hypereosinophilic syndromes is the presence of Splendore-Hoeppli material in the mesenteric lymph nodes of four ferrets.

The eosinophilic granulomas in the ferrets' lymph nodes (Nos, I, 3, 4, 6) bear a resemblance to eosinophilic granulomas in subcutis or oral cavity of dogs and cats.[2,13,20,21] It was suggested recently that in feline cutaneous eosinophilic granulomas and in similar lesions of other species, the acidophilic granular material may be eosinophil products (degranulation or disintegration products) that incite a granulomatous response.' In the ferret granulomas, none of the adjacent collagen had lesions of collagen necrobiosis. The multiple eosinophilic bodies with radiating granular and fibrillar components found in the eosinophilic granulomas in the lymph nodes in four ferrets more closely resemble the material found in a variety of infectious and non-infectious conditions that has been given the name "Splendore-Hoeppli phenomenon."[9,24] It is defined as having an eosinophilic, usually radiating, substance that surrounds helminths, bacteria, fungi, or inanimate objects.">' Special stains for bacteria and fungi of ferret tissues with Splendore-Hoeppli material were unrewarding. The pathogenesis of the Splendore-Hoeppli phenomenon has had little investigation and is poorly understood. Eosinophils are frequently present. An example of this is the Meyers-Kouwenaar bodies that are remnants of microfilaria covered by acidophilic deposits. These Meyers-Kouwenaar bodies are located in focal lesions in multiple organs of naturally occurring occult filariasis in human beings and in ferrets experimentally infected with *Brugia malayi*.[8,23] The Splendore-Hoeppli material present in the center of the abscesses of experimentally infected ferrets is very similar morphologically to the Splendore-Hoeppli deposits observed in the granulomatous lymph nodes of ferrets in this study. In addition, ferrets experimentally infected with *Brugia* sp. had peripheral eosinophilia."

Factors incriminated in the pathogenesis of eosinophilic gastroenteritis in human beings, dogs, and cats include atopic and dietary allergies and collagen disorders.[1D-12,22] The fact that all five ferrets in which complete blood counts were performed had eosinophilia initially suggested either an allergic or parasitic condition; however, no evidence of larval or adult parasites was found histopathologically or by fecal analysis. Because we were unable to evaluate serum immunoglobulin E concentration or skin test ferrets, we were unable to rule out an allergic etiology. Further studies of the naturally occurring eosinophilic syndrome in ferrets are required.

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