

## Gastroenteritis Associated with *Clostridium perfringens* Type A in Black-footed Ferrets (*Mustela nigripes*)

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*Key words:* Black-footed ferret; *Clostridium perfringens* type A; gastroenteritis.

A syndrome characterized by acute gastric distension, dyspnea, and cyanosis has been reported in weanling domestic ferrets (*Mustela putorius furo*).<sup>3,4</sup> The etiology of the condition is unknown but has been attributed to overeating and secondary overgrowth of *Clostridium perfringens* (*welchii*). A close relative of domestic ferrets, the black-footed ferret (*Mustela nigripes*), is among the world's most endangered mammals. In 1986, only 18 of these animals were alive in captivity and none were known to exist in the wild.<sup>8</sup> The survival of black-footed ferrets as a species depends largely on their care and management in captivity.

The National Zoological Park's Conservation Resource Center in Front Royal, Virginia, established an isolated colony of ten adult black-footed ferrets and their offspring in 1988. Starting around 28 days of age, kits were weaned using supplementation with a mixture of liver and diluted adult meat mix (rabbit meat and mink chow pellets) three times a day. Three weanling black-footed ferrets from this colony between 41 and 42 days of age died with marked abdominal

distension. One of these ferrets (ferret No. 1) was found dead with abdominal distension and no history of clinical disease. One year later, the other two ferrets (ferret Nos. 2 and 3) presented with gastric bloat and dyspnea. Ferret No. 2 was treated with subcutaneous saline, 5% dextrose solution, and dexamethasone followed by gastric trocharization. Gastric distension of ferret No. 3 was relieved by gastric trocharization and subsequent passage of an orogastric tube. Both treatments were unsuccessful and the animals died within 2 hours of presentation.

The ferret carcasses were immediately refrigerated and complete autopsies were performed within 16 hours of death. Imprints of gastric and small intestinal contents were stained with a modified Wright stain for cytologic examination. For light microscopy, tissue samples were fixed in 10% formaldehyde and processed by standard procedures; 6- $\mu$ m sections were embedded in paraffin and stained with hematoxylin and eosin. Sections of the gastrointestinal tract were also stained with Brown and Brenn, and Brown and Hopps stains.



**Fig. 1.** Gastrointestinal distension; 42-day-old black-footed ferret.

Heart blood, gastric contents, and small intestinal contents from all three ferrets and lung and large intestine from ferret No. 1 were cultured for bacteria in Schaedler's broth. Broth cultures were inoculated into prerduced liver-egg-brain medium, streaked on 5% bovine blood agar plates, and incubated anaerobically at 37 C. Five percent bovine blood agar plates were also streaked and incubated aerobically at 37 C. *Clostridium* sp. isolated from the gastric and small intestinal contents was identified based on cellular appearance, colony morphology, and biochemical characteristics. These cultures were examined for toxin production by injection 0.3 ml of filtered culture supernatant into the tail veins of two mice. The toxin was identified by the use of a mouse protection assay,<sup>7</sup> in which 1-ml aliquots of culture supernatant and specific diagnostic antisera (Wellcome Reagents, Wellcome Research Laboratories, Beckenham, England) were incubated for 30 minutes and then 0.3 ml of this mixture was injected into the tail veins of two mice.

Grossly, ferret Nos. 1 and 2 had markedly distended thin-walled stomachs containing a large amount of gas and a moderate amount of brown, semiliquid ingesta (Fig. 1). Because of gas and ingesta removal via trocharization and orogastric tube, ferret No. 3 had only a moderately distended stomach containing a moderate amount of gas, a small amount of brown particulate ingesta, and a few slivers of wood (bedding). This last ferret had regional transmural hyperemia of



**Fig. 2.** Gastric mucosa; black-footed ferret. Abundant gram-positive bacilli line denuded and superficially necrotic epithelium. Brown and Hopps Gram stain. Bar = 25  $\mu$ m.

**Fig. 3.** Small intestine; black-footed ferret. Many gram-positive bacilli line shortened and superficially necrotic villi. There are occasional neutrophils within the lamina propria (arrows). Brown and Hopps Gram stain. Bar = 25  $\mu$ m.

the greater curvature suggesting circulatory compromise. The intestines of all three ferrets were also thin-walled and moderately distended with a moderate amount of gas and a small amount of brown semisolid material. In addition, a 15-cm segment of small intestinal transmural hyperemia was present in ferret No. 1.

Smears of the gastric and intestinal contents of all animals contained abundant extracellular gram-positive bacilli, approximately  $1 \times 3\text{--}6 \mu\text{m}$ , admixed with a much smaller number of extracellular mixed bacteria, occasional epithelial cells, erythrocytes, and rare to small numbers of neutrophils.

Histologically, the ferrets had similar gastrointestinal lesions that differed only in severity. There was diffuse superficial to full-thickness mucosal necrosis with sloughing of epithelium. Small numbers of neutrophils infiltrated the lamina propria and submucosa multifocally. Numerous  $1\text{--}3\text{--}6\text{-}\mu\text{m}$  gram-positive bacilli lined the denuded mucosal surface, multifocally extended into the gastric glands and intestinal crypts, and occasionally were observed in the lamina propria (Figs. 2, 3). Ferret No. 1 had severe submucosal congestion accounting for the hyperemia seen grossly.

Additional abnormal histologic findings common to all three ferrets included lymphoid necrosis of lymph nodes (3/3), spleen (3/3), and thymus (2/2); mild to moderate dilation of central hepatic sinusoids with mild acute centrilobular hepatocellular dissociation; and multifocal aggregates of small numbers of often necrotic neutrophils within portal areas.

*Clostridium perfringens* was isolated from the stomachs and intestinal tracts of all three ferrets. The isolates were further identified as *C. perfringens* type A based on the presence of  $\alpha$  toxin and absence of  $\beta$ ,  $\epsilon$ , and  $\iota$  toxins in culture supernatant. The  $\alpha$  toxin produced demonstrable lethal activity of approximately 10 mouse minimum lethal doses (MLD) per milliliter. No other primary pathogens were cultured from the gastrointestinal tracts or heart blood of ferret Nos. 2 and 3. However, ferret No. 1 had multiple other potential pathogens identified in several organs including *Enterococcus* sp. cultured from the heart blood; *Enterococcus* sp., *Proteus* sp., *Klebsiella pneumoniae*, and *Escherichia coli* from the stomach and lung; and *Klebsiella pneumoniae* from the small and large intestine.

The pathogenesis of *C. perfringens* type A enterotoxemia is not well defined, but it is believed to be analogous to enterotoxemia caused by *C. perfringens* type D.<sup>1</sup> Under certain circumstances, such as intestinal hypomotility or increased concentration of carbohydrates in the gastrointestinal tract (overeating), *C. perfringens* multiplies rapidly, producing overwhelming amounts of toxins. The enterotoxin attacks the villous epithelial cells and leads to sloughing, death, and lysis of these cells.<sup>5</sup> Although most enteropathogenic strains of *C. perfringens* produce a relatively small amount of  $\alpha$  toxin, its lecithinase activity can destroy cell membranes and lead to cell necrosis.<sup>1,6</sup> Gas production by the proliferating bacteria may result in gastrointestinal distension.

The change in diet and/or overeating may have predisposed these weanlings to *C. perfringens* overgrowth. Grossly, abdominal distension is consistent with excessive gas production during rapid bacterial proliferation; histologically, the layer of gram-positive bacilli lining the superficially necrotic gastrointestinal mucosa is characteristic of colonization and overgrowth of *C. perfringens*, with production of a necrotizing toxin; and cytologically, abundant gram-positive bacilli within the gastrointestinal tract of these animals in the absence of obvious putrefaction supports a potential causal relationship. These pathologic changes in conjunction with isolation of *C. perfringens* type A with readily measurable

and lethal toxin production from all three ferrets strongly suggest that *C. perfringens* type A was a major factor in the deaths of these animals.

Hepatic lesions may be secondary to shock, toxemia, and/or bacterial emboli from compromised intestinal tracts. Lymphoid necrosis can be a sequela to physiologic stress.<sup>2</sup>

Other bacteria cultured from multiple organs in ferret No. 1 may have participated in the pathogenesis of the gastroenteritis in this case. However, absence of histologic evidence of infection in the other tissues from which identical bacteria were cultured (i.e., lungs and heart blood) suggests that these bacteria were postmortem contaminants.

Prevention and treatment of this disease are difficult because of the ubiquitous nature of the organism, the weak antigenicity of the toxin, and the short course of the disease.<sup>6</sup> Management is the primary means of prevention. Because of the association of overfeeding with *C. perfringens* overgrowth, weanlings are now supplemented only twice a day. Since this change in management, no more incidents of the abdominal distension syndrome in black-footed ferrets have occurred.

Weanling black-footed ferrets are susceptible to an abdominal distension syndrome associated with *C. perfringens* type A that can apparently be controlled by feeding management.

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