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Enterotoxigenic *Escherichia coli* Infection in Captive Black-footed Ferrets

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ABSTRACT: Enterotoxigenic *Escherichia coli* with genes for heat stabile toxins Sta and STb was isolated from the gastrointestinal tract and multiple visceral organs of three adult and three juvenile black-footed ferrets (*Mustela nigripes*) that died in a captive breeding colony between 24 May 1998 and 2 July 1998. Similar isolates were obtained from rectal swabs of one adult and one juvenile that were clinically ill. All were fed a diet composed of mink chow, raw rabbit meat, beef liver powder, blood meal and lard. *Escherichia coli* of the same toxin genotype was isolated from the mixed ration. Clinical signs included sudden death, dehydration, anorexia and diarrhea. Necropsy lesions included acute enteritis with large numbers of rod shaped bacteria microscopically visible on intestinal villi.

Key words: Black-footed ferret, enterotoxigenic, *Escherichia coli,* heat stabile toxin, *Mustela nigripes*, STa, STb, polymerase chain reaction, gastroenteritis.

The literature contains few reports of bacterial enteric disease in the black-footed ferret (*Mustela nigripes*) (Schulman et al., 1993). Domestic ferrets (*Mustela putorius*) are reportedly susceptible to a variety of bacterial infections of the gastrointestinal tract including *Campylobacter jejuni, Salmonella* sp., *Clostridium perfringens* type A, *Lawsonia intracellularis, Helicobacter mustelae,* and *Mycobacterium* sp. (Fox, 1998). Enteric disease caused by *E. coli* infection has not been reported in either domestic or wild species of ferrets. However, hemolytic *E. coli* have been isolated from rectal swabs of clinically normal domestic ferrets and domestic ferrets with mastitis (Liberson et al., 1983). Here we report the isolation of enterotoxigenic *E. coli* from black-footed ferrets from a breeding colony with enteric disease.

Seventy-eight black-footed ferrets including 30 adults and 28 kits were kept in a captive breeding colony at the Phoenix Zoo (USA; 33°30′N, 112°10′W) as part of the United States Fish and Wildlife Service's Black-footed Ferret Recovery Program. Between 24 May 1998 and 2 July 1998 five adults and three kits died suddenly or exhibited a 12 to 24 hr history of anorexia and loose mucoid feces prior to death. Four of the adults and all of the kits were housed in separate cages in the breeding compound. The other adult animal was housed in an exhibit and had no recent contact with the others. All received the same prepared diet, which included mink chow, raw rabbit meat, beef liver powder, lard and blood meal. The same caretakers cared for all of the animals.

Gross necropsy examinations were performed on four of five dead adults and the three juvenile ferrets. Gross lesions were limited to diffuse reddening of the gastric mucosa in one adult and of the gastric and intestinal mucosa in a second adult animal. No gross lesions were seen in the three kits and the remaining adults. Small and large intestine, stomach, liver, lung, kidney, heart, spleen, and pancreas collected at the necropsy examinations were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin for microscopic examination. In addition, sections of the stomach and small intestine were stained with the method of Brown and Hopps (Brown and Hopps, 1973) and examined microscopically for gram-positive and gram-negative bacteria. Tissues or swabs of one or more tissues including stomach, small intestine, liver, kidney and spleen of the dead ferrets and rectal swabs from two

TABLE 1. Serologic and genotypic characterization of *E. coli* isolates from black-footed ferrets.

Individual ferret	Age	Sex[a]	Culture source[b]	*E. coli* isolate typing									
				Serotype[c]		Genotype[d]							
				H	O	LT	STa	STb	EAE	SLTI	SLTII	CNF1	CNF2
6502/497	Adult	M	L	NM	−	−	+	+	−	−	−	ND[e]	ND
7660/1255	Adult	M	SI	NM	−	−	+	+	−	−	−	−	−
7426/1493	Adult	F	K, L, Sp, St	NM	−	−	+	+	−	−	−	−	−
8565	Kit	F	K, L	NM	−	−	+	+	−	−	−	−	−
8564	Kit	M	K, L	A	R	−	−	−	−	−	−	+	−
8563	Kit	F	K, L, Sp, St	NM	−	−	+	+	−	−	−	−	−
7431	Adult	M	Re	NM	−	−	+	+	−	−	−	−	−
2089	Kit	F	Re	NM	−	−	+	+	−	−	−	−	−
NA[f]	NA	NA	F	NM	−	−	+	+	−	−	−	−	−

[a] M = male, F = female.

[b] L = liver, SI = small intestine, K = kidney, Sp = spleen, St = stomach, Re = rectal swab, F = food retrieved from the cage of animal 7426/1483.

[c] H = H antigen (flagellar), O = O antigen (somatic), NM = non-motile, A = autoagglutination, R = rough after heat treatment; untypeable, (−) = negative reactions.

[d] LT = labile toxin, St = stabile toxin, EAE = *E. coli* attaching and effacing, SLTI = shiga-like toxin I, SLTII = shiga-like toxin II, CNF1 = cytotoxic necrotizing factor type I, CNF2 = cytotoxic necrotizing factor type II, (+) = positive for the gene, (−) = negative for the gene.

[e] ND = test not performed.

[f] NA = not applicable.

clinically ill animals were inoculated onto trypticase soy agar plates with 5% sheep blood (Hardy Diagnostics, Higley, Arizona) for bacterial culture. In addition, intestinal content and rectal swabs were inoculated into tetrathionate broth and brilliant green agar (Hardy Diagnostics) for isolation of *Salmonella* sp. Stomach and liver tissue from one adult (7426/1493) and one juvenile ferret (8563) were inoculated directly onto blood agar and incubated under anaerobic conditions. All cultures were incubated at 37 C for 48 hr and examined for pathogenic bacteria by the techniques of Carter and Cole (1990). Two different samples of the ration recovered from the cages of affected ferrets and lard, blood meal and liver powder used to formulate the ration fed to the affected ferrets were similarly cultured. Rabbit meat used to formulate the diet had been entirely mixed and fed and was not available for culture nor was ration that had not already been placed in cages with the ferrets. The mink chow was not cultured. Hemolytic *E. coli* were recovered from the rectal swabs and from stomach, liver, kidney, spleen and small intestine. Hemolytic *E. coli* were also isolated from a sample of the ration retrieved from the cage of one affected adult ferret (7426/1493). Cultures of the blood meal, liver powder and lard used to formulate the ration did not yield any significant isolates. *Escherichia coli* isolates were forwarded to the *E. coli* Reference Center (Wiley Laboratory, Orchard Road, The Pennsylvania State University, University Park, Pennsylvania, USA), for serotyping (Orskov et al., 1977) and for polymerase chain reaction (PCR) testing to identify genes for heat stable toxins A and B (STa and STb) (Ojeniyi et al., 1994), heat labile toxin (LT) (Schultsz et al., 1994), *E. coli* attaching and effacing (EAE) (Gannon et al., 1993), shiga-like toxins I and II (Gannon et al., 1992) and cytotoxic necrotizing factors 1 and 2 (Blanco et al., 1996). The results are presented in Table 1. Briefly, the hemolytic *E. coli* isolated from the tissues of five of the six ferrets, the two rectal swabs from clinically ill ferrets and one of the complete ration samples were positive by PCR for the heat stabile toxin genes A (STa) and B (STb).

The isolate from one ferret was negative for the heat stabile toxin gene but positive for the cytotoxic necrotizing factor gene. All of the enterotoxin producing isolates were non-motile (H antigens) and none reacted with standard antisera to O antigens. Microscopic lesions in affected black-footed ferrets varied from none to some mild congestion of gastric and intestinal mucosa with large numbers of gram-negative bacteria on the surface of intestinal villi. The lungs of the juvenile ferrets also had some mild, interstitial hypercellularity due to increased numbers of macrophages and neutrophils.

Enterotoxigenic strains of *E. coli* are well-recognized causes of enteric diseases of humans and animals including traveler's diarrhea, diarrhea in infants and children and colibacillosis in calves and piglets. Contaminated food and water and direct contact with an infected person/animal are the usual sources of infection (Sussman, 1997). The organisms produce a secretory type diarrhea without causing morphologic lesions. This is the result of bacterial attachment to the epithelium of the intestinal tract and production of a toxin that acts locally on enterocytes (Nagy and Fekete, 1999). The toxins are of two main types designated heat-labile (LT) and heat-stabile (ST) toxins (Gyles, 1993). There are two forms of heat stabile enterotoxin; STa and STb. Of these, STb is the least understood and has previously only been identified in porcine and human *E. coli* isolates (Nair and Takeda, 1997).

A food-born route of infection was considered most likely in our ferrets. This was based on the isolation of enterotoxigenic *E. coli* from one of two samples of the ration being fed and the fact that no new cases occurred once the original batch of rabbit meat was depleted and replaced with a new one. The component of the ration that was the source of the infection was not established. Enterotoxigenic *E. coli* was not isolated from the beef liver powder, blood meal or lard used to formulate the ration. However, the rabbit meat used in the ration formulation was not available for culture and was a potential source of the infection. Animal to animal transmission was considered unlikely in these cases because (1) The animals were in a closed population with no recent introductions; and (2) the animals were housed in separate cages without opportunity for contact. One of the adult ferrets was housed in an exhibit that was separate from the breeding facility. Mechanical transmission by the caretakers could not be entirely excluded as a possible mode of infection but seemed unlikely since the problem ceased with the aforementioned change in the diet formulation while all other husbandry practices remained the same.

One of the isolates did not type as an enterotoxigenic *E. coli* but instead contained the gene for cytotoxic necrotizing factor 1. This isolate was probably erroneously selected from the culture plate for typing. This may have occurred due to the fact that both this isolate and the enterotoxigenic isolates cultured were hemolytic and had similar colony morphology. Hemolytic *E. coli* has previously been isolated from rectal swabs of normal domestic ferrets (Liberson et al., 1983).

Escherichia coli has not previously been reported as a cause of enteritis/diarrhea in black-footed ferrets. However, concern has been expressed about possible food-borne transmission of disease in captive breeding populations of this endangered species. Preventative measures have been introduced in some facilities to limit exposure to food-borne pathogens. These include inspection of rabbit carcasses and the feeding of prepared rations to colonies of domestic ferrets to screen for potential problems prior to feeding to the black-footed ferrets (Williams et al., 1992).

The presence of septicemia was an unusual aspect of the disease in these ferrets. Enterotoxigenic *E. coli* infections typically do not exhibit invasiveness (Nagy and Fekete, 1999). However, one strain of enterotoxigenic *E. coli* has been identified that

demonstrates the ability to invade human intestinal epithelial cell lines (Elsinghorst and Kopecko, 1992) by triggering an actin polymerization-dependant uptake process. It remains unclear if this ability is relevant for enterotoxigenic disease but suggests the possibility that some strains may have the capacity for invasion in-vivo. It is also unusual that the infection in this colony involved both adult and young ferrets. Enteric infections with enterotoxigenic *E. coli* infections in animals are usually limited to the young (Gyles, 1993). However, adult humans are susceptible to infection (Sussman, 1997). The adult ferrets may have been naïve to the causative organism much like human travelers visiting developing countries.

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