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Gastric Colonization by *Campylobacter pylori* subsp. *mustelae* in Ferrets

J. G. FOX,* E. B. CABOT, N. S. TAYLOR, AND R. LARAWAY

Division of Comparative Medicine, Massachusetts Institute of Technology, 37 Vassar Street-45-104, Cambridge, Massachusetts 02139

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***Campylobacter pylori* subsp. *mustelae* was cultured from both normal and inflamed gastric mucosa of ferrets. Examination of neonatal, juvenile, and adult ferrets established that the gastric mucosa in the majority of preweanling (age, <6 weeks) ferrets sampled were not colonized with *C. pylori* subsp. *mustelae*, whereas the gastric mucosa of 100% of adult ferrets were colonized with this gastric organism. *C. pylori* subsp. *mustelae* was isolated from the gastric mucosa on a sequential basis from nine ferrets during a several-month period, inferring either persistent colonization or frequent reinfection with *C. pylori* subsp. *mustelae*.**

Historically, an infectious etiology as a cause of gastric disease was not considered seriously until reports from Australia in 1983 indicated a possible causal relationship of *Campylobacter pylori* infection with human gastritis and ulcers (8, 9, 15). These findings have been substantiated in several other studies conducted in different geographic regions (4–6, 10, 11). The pathogenesis of *C. pylori* and gastric disease, however, remains unresolved. Animal models are therefore needed to help establish the epidemiology and pathogenic significance of *C. pylori* colonization in gastric mucosae. In 1985, another *Campylobacter*-like organism (CLO) with many biochemical, molecular, and phenotypic similarities to *C. pylori* was isolated from normal and inflamed gastric mucosae of ferrets (3; J. G. Fox, P. Edmonds, N. S. Taylor, B. Pasteur, and F. DeWhirst, Proceedings of the IV International Workshop on Campylobacter Infections, Goteborg, Sweden, 1988, p. 50, abstract). The proposed name of the organism is *C. pylori* subsp. *mustelae* (J. G. Fox, N. S. Taylor, P. Edmonds, and D. J. Brenner, Int. J. Syst. Bacteriol., in press). Because of the common occurrence of gastritis and ulcers in ferrets, the isolation of CLO from ferret gastric mucosa, and the use of ferrets in our laboratory to study the role of bile reflux in gastric carcinogenesis, a survey was undertaken to ascertain at what age ferrets are colonized with gastric CLO and whether the CLO can be isolated repeatedly from gastric tissue from the same ferret over time.

Ferrets (*Mustela putorius furo*) of different ages raised commercially and used in biomedical research had samples from gastric mucosa cultured for *C. pylori* subsp. *mustelae* either via gastric biopsy or during necropsy. Adult animals were housed singly or in pairs in stainless steel cages (77 cm by 61 cm) and were fed commercial cat food and drinking water ad libitum. Three pregnant ferrets housed individually delivered 17 ferret kits (7, 6, and 4 kits from each litter); 3 kits died and 14 kits were euthanized with CO_2. Samples from each kit (ages, from 1 day through 6 months) to be used for culturing were obtained from the gastric antrum by sterile techniques at necropsy. Sixteen adult (age 1 to 2 years) control ferrets housed at another research institution were euthanized with CO_2. Stomachs from these animals were excised, and biopsy samples were obtained from the antrum and fundus and cultured for CLO. As part of a study on the role of bile reflux in gastric carcinogenesis, ferrets (age, 9 to 18 months) with maximal reflux were prepared by performing a pyloroplasty and a gastrojejunostomy. The proximal jejunum was anastomosed to the greater curvature of the stomach by a Billroth II anastomosis procedure, and a wide open pyloroplasty was performed. The procedure resulted in an intestinal loop in which bile was continually secreted into the stomach. A second group of ferrets of similar age with no bile reflux were prepared by performing a pylorectomy and a Roux-en-Y gastrojejunostomy without common bile duct resection. This procedure prevented bile reflux into the stomach. All surgery was performed aseptically under surgical anesthesia. After the ferrets recovered from gastric surgery, gastric biopsy samples were obtained from the antra from selected ferrets for culture at intervals that varied from 2 to 17 months between biopsies. This procedure was performed under surgical anesthesia. The fiberscope and biopsy forceps were disinfected after each use with 70% ethanol and rinsed in sterile water.

Stomachs examined at necropsy were opened along the greater curvature, and specimens of gastric mucosa were collected from the pyloric antrum and fundus. During gastric surgery and at endoscopy, gastric mucosa from the antrum was biopsied for culture. All specimens were processed (within 1 h) in a similar manner. A portion of the gastric tissue was homogenized; and the homogenate was cultured on a blood agar plate (Campy Blood Agar; Scott Laboratories, Inc., Frisksville, R.I.) or brucella blood agar with TVP (Remel, Lenexa, Kans.), placed in vented jars containing N_2, H_2, and CO_2 (80:10:10), and incubated for 7 days at 37°C. Positive identification of CLO was based on the isolation of a nonpigmented colony that was approximately 1 to 2 mm in diameter after 12 to 96 h of incubation at 37°C. The organism was a curved (3 by 0.5 μm) gram-negative rod; was strongly oxidase and catalase positive, weakly nitrate positive, sensitive to nalidixic acid, and resistant to cephalothin; and did not hydrolyze hippurate. By using Christensen urea agar, a positive urease reaction was seen within 5 min. By using further biochemical, ultrastructural, and DNA homology studies, these organisms were tentatively classified as *C. pylori* subsp. *mustelae* (Fox et al., in press).

Gastric biopsies were performed on a longitudinal basis on a total of 11 ferrets (8 with maximal reflux, 3 with no bile reflux) (Table 1). Of the 11 ferrets, *C. pylori* subsp. *mustelae*

* Corresponding author.

TABLE 1. Gastric colonization of *C. pylori* subsp. *mustelae* in ferrets

Ferret no.	Age (mo)[a]	Gastric surgery[b]	Initial CLO culture	Interval (mo) between cultures	Second CLO culture	Interval (mo) between cultures	Third CLO culture
1	9	NR	+	10	−	12	−
2	15	NR	−	3	−		ND[c]
3	18	NR	+	7	+	2	+
4	15	MR	−	7	+	15	+
5	15	MR	−	9	+	8	+
6	15	MR	+	7	−	3	+
7	15	MR	+	17	−	6	+
8	22	MR	+	8	+		ND
9	22	MR	+	8	+		ND
10	12			+	11	+	ND
11	18	MR	+	10	+	7	−

[a] Approximate age at first biopsy.
[b] NR, No bile reflux gastric surgery; MR, maximum bile reflux gastric surgery.
[c] ND, Not done.

was isolated from 8 of them on initial culture. Of the three ferrets that were negative for the organism on initial biopsies, two were positive for gastric CLO on the second and third biopsies, while the third ferret remained negative for gastric CLO on the second biopsy. Of the 11 ferrets, 9 had gastric cultures positive for CLO on sequential biopsies at intervals that varied from 2 to 15 months. Two ferrets that were initially positive for CLO were negative for CLO on subsequent biopsies taken 8 to 10 months later. One of these was also negative for CLO on the third biopsy taken 22 months after the initial gastric biopsy. Of the 17 neonatal and juvenile ferrets from which samples for culture were taken, only 3 (18%) were positive for CLO. From days 1 to 17, eight ferrets were negative for *C. pylori* subsp. *mustelae*, whereas in the remaining nine ferrets surveyed from day 24 through month 6, 1 ferret each was positive for the organism on day 24, day 31, and month 6. The remaining six ferrets (from 2 litters) sampled at days 38 to 44 had negative gastric cultures. In all 16 adult ferrets from which samples were obtained at necropsy, *C. pylori* subsp. *mustelae* was isolated from both the antrum and the fundus.

All bacteria isolated from the gastric mucosa from ferrets of various ages met the criteria for *C. pylori* subsp. *mustelae*. These bacteria appeared to have similar colony and microscopic morphologies. Biochemically, there was some minor variability, but they all rapidly hydrolyzed urea and were catalase and oxidase positive (3). Almost 100% of the adult ferrets surveyed in our population were colonized with *C. pylori* subsp. *mustelae*. This is consistent with preliminary data reported in ferrets surveyed in both Canada and England (J. A. Hollingsworth, unpublished data) (13). There was a much lower isolation rate (3 of 17 [18%]) from neonatal and juvenile ferrets, implying that colonization probably occurs after weaning (age, ~6 weeks). At birth ferrets have nearly undetectable immunoglobulin levels; but by day 9 they have 77, 29, and 13% of the mean adult levels of immunoglobulin G (IgG), IgA, and IgM in serum, respectively (14). The postpartum transfer of immunoglobulin occurs across the intestinal mucosa via the products of lactation. The presence of immunoglobulins in ferret milk (with some presumably directed toward *C. pylori* subsp. *mustelae*) may afford protection of the gastric mucosa from colonization with the organism until after weaning in most cases. Similar to our findings in ferrets, data on *C. pylori* infection in humans indicate that the prevalence of the organism increases with age (1).

After initial colonization, *C. pylori* subsp. *mustelae* appears to establish a persistent infection of the gastric mucosa in ferrets. *C. pylori* subsp. *mustelae* was isolated from gastric mucosa on repeated biopsies from several of the ferrets with surgically altered bile refluxes. This infers that the colonizing strains are persistent. Reinfection is also possible, given that these ferrets were maintained in a confined space. However, in a select number of ferrets, the organism was not isolated on rebiopsy of the gastric mucosae. Sampling and culturing techniques may account for the negative cultures; alternatively, select ferrets may be colonized with *C. pylori* subsp. *mustelae* for finite periods. By using restriction endonuclease DNA analysis, *C. pylori* from a group of patients was studied to determine the persistence of colonizing strains (7). By using *Hin*dIII enzyme digestion, all the patients had isolates with different digestion patterns. In two patients, the *C. pylori* isolates recovered after an interval of 2 years were identical to those isolated originally, indicating that the same strain of *C. pylori* persisted during the 2-year period. In four patients, the digestion patterns of *C. pylori* cultured prior to antimicrobial agent therapy were identical to those of strains of the organism isolated after relapses, which occurred in each of the four patients several months after the cessation of therapy. Similar restriction enzyme studies are warranted in *C. pylori* subsp. *mustelae* isolated from ferrets on a longitudinal basis.

The amount of bile reflux did not appear to reduce the ability of ferret gastric mucosa to colonize *C. pylori* subsp. *mustelae*, which was isolated routinely over time from ferrets with and without bile reflux. This is contrary to findings reported in patients who underwent Billroth I partial gastrectomy, Billroth II partial gastrectomy, or truncal vagotomy and gastroenterostomy. The number of biopsy samples positive for *C. pylori* was significantly lower ($P < 0.001$) in patients who underwent these procedures compared with the number in patients with active duodenal ulcerations (12). The investigators suggested that bile reflux may disrupt mucus and thus cause the death of *C. pylori* living beneath the protective mucous layer.

The ferret stomach has many anatomical and physiological similarities to that of humans (2). In addition, it is readily available commercially, economic to purchase, easy to maintain, and amenable to gastric manipulation. Furthermore, the ferret has naturally occurring gastritis and ulcer disease which may be associated with the presence of *C. pylori* subsp. *mustelae* (3). The ferret, therefore, appears to be an ideal model for the study of *C. pylori* gastric colonization, persistence, pathogenesis, and response to antimicrobial agent therapy (both short-term and long-term eradication).

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ADDENDUM IN PROOF

DNA hybridization data obtained after the completion of this study indicate that the ferret gastric *Campylobacter* isolate may be a separate species rather than a subspecies of *C. pylori* (P. Edmonds, personal communication).

LITERATURE CITED

1. **Blaser, M. J.** 1987. Gastric *Campylobacter*-like organisms, gastritis, and peptic ulcer disease. Gastroenterology **93:**371–383.
2. **Fox, J. G. (ed.).** 1988. The biology and diseases of the ferret. Lea and Febiger, Philadelphia.
3. **Fox, J. G., B. M. Edrise, E. B. Cabot, C. Beaucage, J. C. Murphy, and K. S. Prostak.** 1986. *Campylobacter*-like organisms isolated from gastric mucosa of ferrets. Am. J. Vet. Res. **47:**236–239.
4. **Jones, D. M., A. Curry, and A. J. Fox.** 1984. An ultrastructural study of the gastric *Campylobacter*-like organism '*Campylobacter pyloridis*.' J. Gen. Microbiol. **131:**2335–2341.
5. **Kasper, G., and N. Dickgieber.** 1984. Isolation of *Campylobacter*-like bacteria from gastric epithelium. Infection **12:**179–180.
6. **Langenberg, M. L., G. N. J. Tygat, M. E. I. Schipper, P. J. G. M. Rietta, and H. C. Zanen.** 1984. *Campylobacter*-like organisms in the stomach of patients and healthy individuals. Lancet **i:**1348–1349.
7. **Langenberg, W., E. A. J. Rauws, A. Widjojokusumo, G. N. J. Tytgat, and H. C. Zanen.** 1986. Identification of *Campylobacter pyloridis* isolates by restriction endonuclease DNA analysis. J. Clin. Microbiol. **24:**414–417.
8. **Marshall, B. J., H. Royce, D. I. Annear, et al.** 1984. Original isolation of *Campylobacter pyloridis* from human gastric mucosa. Microbios **25:**83–88.
9. **Marshall, B. J., and J. R. Warren.** 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **i:**1311–1314.
10. **McGraud, F., F. Bonnet, M. Garnier, and H. Lamouliatte.** 1985. Characterization of "*Campylobacter pyloridis*" by culture, enzymatic profile, and protein content. J. Clin. Microbiol. **22:** 1007–1010.
11. **McNulty, C. A. M., and D. M. Watson.** 1984. Spiral bacteria of the gastric antrum. Lancet **i:**1068–1069.
12. **O'Connor, H. J., M. F. Dixon, J. I. Wyatt, et al.** 1986. Effect of duodenal ulcer surgery on enterogastric reflux on *Campylobacter pyloridis*. Lancet **ii:**1178–1181.
13. **Rathbone, B. J., A. P. West, J. I. Wyatt, A. W. Johnson, D. D. Tompkins, and R. V. Heatley.** 1986. *Campylobacter pyloridis*, urease, and gastric ulcers. Lancet **i:**400–401.
14. **Suffin, S. C., G. A. Prince, K. B. Muck, and D. D. Porter.** 1979. Ontogeny of the ferret humoral immune response. J. Immunol. **123:**6–9.
15. **Warren, J. R., and B. Marshall.** 1983. Unidentified curved bacilli in gastric epithelium in active chronic gastritis. Lancet **i:** 1273–1275.