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The characterization and pathological significance of gastric campylobacter-like organisms in the ferret: a model for chronic gastritis?

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SUMMARY

Gastric campylobacter-like organisms (CLO) were isolated from gastric tissues removed at sacrifice from 17 mature ferrets; all animals were colonized, but no macroscopic mucosal lesions or histological features of chronic gastritis were seen. The isolates resembled *Campylobacter pylori* in many cultural and biochemical characteristics, and produced substantial urease activity, but there were sufficient differences from *C. pylori* to suggest that ferret gastric CLO represents a separate species. Comparison of human *C. pylori* and ferret gastric CLO may help to elucidate the pathogenicity of the former in patients with gastritis, and the ferret may serve as a useful animal model for the study of *C. pylori* infection.

INTRODUCTION

Campylobacter pylori has been isolated from human gastric mucosal biopsy specimens and its presence is strongly associated with non-autoimmune chronic gastritis (Goodwin, Armstrong & Marshall, 1986; Rathbone, Wyatt & Heatley, 1986). Spiral organisms have also been demonstrated on the gastric mucosa of several mammalian species (Doenges, 1939) and campylobacter-like organisms (CLO) have been isolated from the gastric mucosa of ferrets with, and without, gastric lesions (Fox *et al.* 1986; Cave *et al.* 1986). It was suggested in these studies that the ferret could be a useful animal model for the study of gastric campylobacter infection.

The purpose of this investigation was to determine the incidence of colonization of ferrets by gastric CLO, to characterize the organisms isolated and to examine ferret gastric tissue histologically to ascertain the relationship of any pathological changes to colonization by CLO.

METHODS

The ferrets studied were mature animals housed singly under standard laboratory conditions, fed on a pelleted diet formulated for ferrets (SDS) and provided with water *ad libitum*. They were sacrificed as part of a study carried out by the Department of Pharmacology, University of Bradford; intravenous cisplatin was administered under halothane anaesthesia 30 min after a meal and

the ferrets were killed 4 h later with intraperitoneal thiopentone sodium. Some of the animals received metoclopramide or domperidone.

Histology

The stomachs were excised immediately after death of the ferrets, carefully examined in the fresh state for the presence of ulceration or other mucosal abnormality, and fixed in 10% formalin immediately after mucosal specimens for microbiology had been obtained. Representative blocks from proximal and distal stomach were processed conventionally, and sections stained with haematoxylin and eosin for assessment of histology, and by modified Giemsa (Gray, Wyatt & Rathbone, 1986) for detection of mucosal-associated bacteria.

Isolation of strains

Gastric tissue was placed in a transport medium of Brain-Heart Infusion broth (Oxoid) with 0·4% w/v yeast extract (Oxoid) and 10% v/v horse serum. The tissues were homogenized in the transport broth in Griffiths tubes and resulting suspensions inoculated on heated blood agar and a selective medium (VCAT) used for the isolation of campylobacters from faeces and of *Neisseria gonorrhoeae* (VCAT medium: GC agar base (Oxoid) with 10% saponin-lysed horse blood, dextrose (1 g/l), L-glutamine (100 mg/l), L-cysteine (11 mg/l), cocarboxylase (1 mg/l), ferric nitrate (0·2 mg/l), vancomycin (3 mg/l), colistin (100000 units/l), amphotericin (1 mg/l), trimethoprim (5 mg/l)), (Tompkins *et al.* 1981). Plates were incubated microaerobically (3 l jar with Oxoid Gas Kit BR56) at 37 °C for 5 days and examined for bacterial growth. Colonies resembling those of *C. pylori* were Gram-stained and tested for production of oxidase, catalase and pre-formed urease.

Strains of *C. pylori* were isolated from human gastric biopsy specimens by standard methods (Goodwin *et al.* 1985a). *C. pylori* NCTC 11637 was used as a control; this organism did not grow on the selective medium (VCAT) but in comparative studies was cultured on the same medium without antibiotics. All strains were stored in 1% (w/v) Proteose peptone No. 3 (Difco) with 15% (v/v) glycerol at −70 °C.

Characterization

Cultural and biochemical reactions

Growth on subculture was determined on VCAT plates incubated for 5 days at 37, 27 and 42 °C, in air, anaerobically and microaerobically. All subsequent incubations were at 37 °C for 5 days in a microaerobic atmosphere.

Production of catalase and oxidase was determined by standard methods (Cowan, 1974). The rapid test for pre-formed urease was performed as described by Owen, Martin & Borman (1985). Nitrate reduction and hippurate hydrolysis were tested by standard methods (Morris & Patton, 1985): indole-nitrate broth (BBL) supplemented with 0·4% (w/v) yeast extract and 10% (v/v) horse serum was used as the culture medium for the nitrate test. Growth was attempted on 5% (v/v) horse blood agar with and without an additional 1·5% NaCl. Sensitivities to 30 μg discs containing nalidixic acid or cephalothin were tested on heated blood agar plates.

Enzymatic reactions of 12 ferret isolates, five human isolates and *C. pylori*

NCTC 11637 were examined by the APIZYM system (API system, SA). A suspension of organisms, harvested after growth on heated blood agar for 5 days, was made in distilled water to give a turbidity equivalent to a McFarland No. 6 standard. The test strips were inoculated and incubated at 37 °C for 4 h as recommended by the manufacturer. After addition of reagents, colours of the cupules were compared with a chart supplied and graded negative, weekly positive (+) and strongly positive (++).

Soluble proteins of whole cell extracts of bacteria grown on heated blood agar, as above, were prepared by ultrasonication followed by centrifugation at 100000 g for 1 h and the supernatant analysed by polyacrylamide gel electrophoresis (PAGE) in a discontinuous buffer system (Laemmli, 1970).

Fatty acid methyl ester (FAME) profiles

Five ferret and five human gastric CLOs were cultured on VCAT, and *C. pylori* NCTC 11637 was cultured on the same medium without antibiotics; all plates were incubated microaerobically at 37 °C for 6 days. Bacteria were harvested and washed twice in phosphate-buffered saline, pH 7·3. FAMEs were prepared by the method of Goodwin *et al.* (1985*b*) and analysed on a Perkin-Elmer Sigma 3b Chromatograph with Sigma 15 data station, using a flame ionization detector. The chromatograph was equipped with a fused silica column (25 m × 0·22 mm internal diameter) coated with SGE-BP1 with a film thickness of 0·25 μm. A split/splitless injector with a split ratio of 40:1 was used. The injector and detector temperatures were maintained at 280 °C and a temperature programme was used with an initial temperature of 150 °C rising to 250 °C at 4 °C per min, and the nitrogen carrier gas flow rate was 1 ml per minute. Peaks from bacterial extracts were identified by comparison with relative retention times of a fatty acid standard (Supelco).

RESULTS

Histology

Morphologically, the gastric mucosa in the ferret was very similar to that in man (Fig. 1); in particular the body and antral regions could be readily distinguished, and showed the same distribution of specialized cells, with parietal and chief cells confined to the body region. Body-type mucosa appeared to occupy a greater proportion of the stomach, extending relatively more distally than in the human. The change from antral to duodenal type mucosa was also clear-cut, the proximal duodenum being characterized by sub-mucosal 'Brunner's glands', and the villous surface with absorptive and goblet cells, as in the human.

Surface bacteria were identified by the Giemsa stain in 14 of the 17 antral specimens, but in none of the 17 from the body. The antral bacteria occurred within the gastric pits, with a very patchy distribution, and were never as numerous as gastric campylobacters of human gastric mucosa. There was a tendency for mucosa-associated organisms to be more numerous distally in the antrum, but colonization ceased abruptly at the gastroduodenal junction. The bacteria appeared smaller than *C. pylori* in tissue sections, and were comma-shaped rather than spiral.

None of the ferret mucosae showed a mononuclear cell infiltrate in the lamina

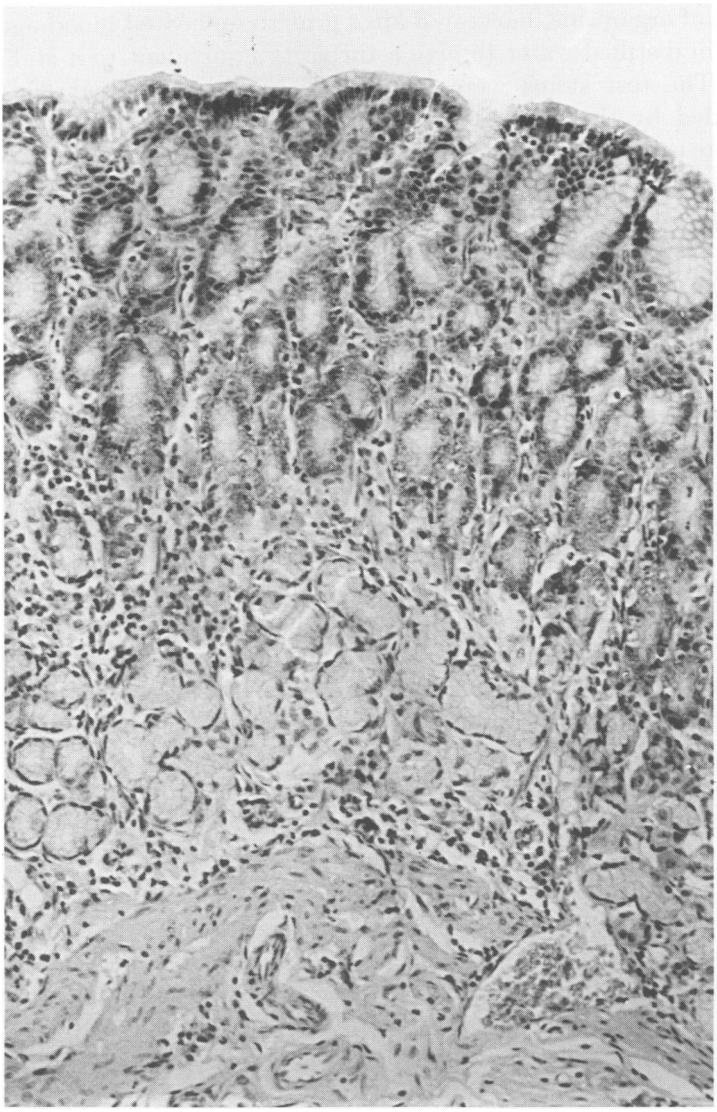

Fig. 1. Ferret gastric antral mucosa (haematoxylin and eosin), ×48.

propria, or evidence of epithelial damage, or glandular atrophy, which are characteristic of chronic gastritis associated with *C. pylori* colonization in the human stomach. Seven of 14 colonized antral specimens showed occasional cell debris within gastric pits, with adjacent intra-epithelial neutrophils (Fig. 2); these slight changes were not observed in body sections, or in uncolonized antral tissue. There was no epithelial mucin depletion, a feature common in *C. pylori*-associated gastritis, and surface bacteria were not accompanied by vascular congestion or oedema.

Bacteriology

Campylobacter-like organisms were isolated from the gastric tissue of all 17 ferrets examined, growing as flat brown colonies of varied size on VCAT medium

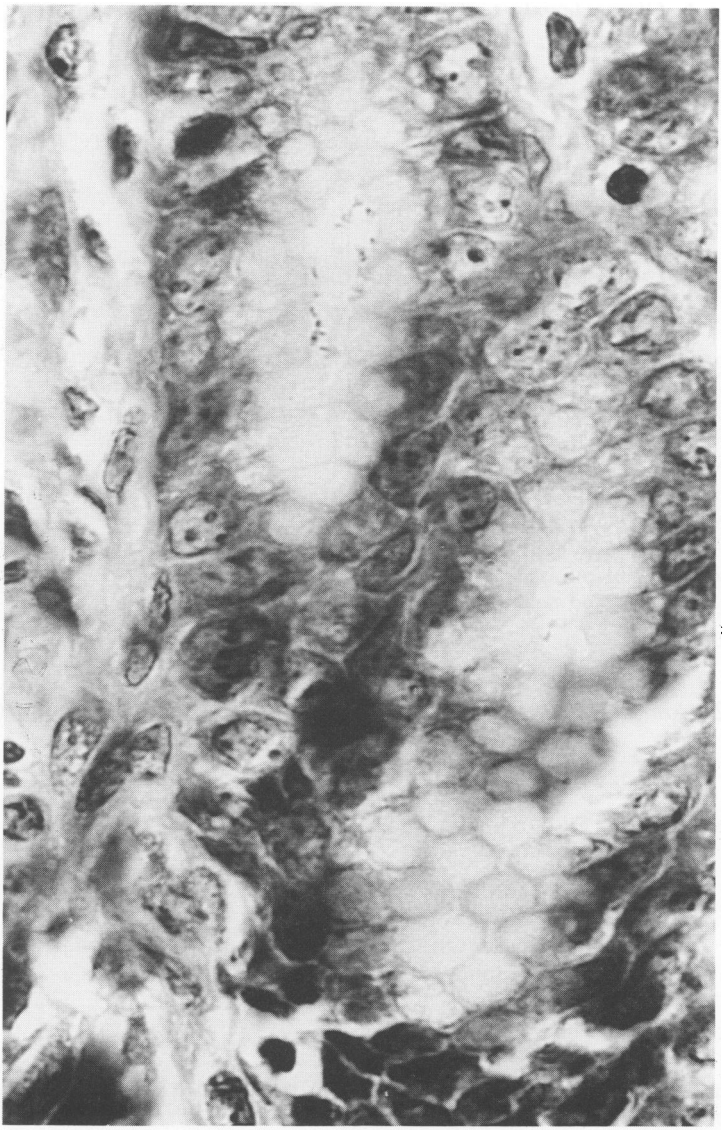

Fig. 2. Ferret gastric CLO in gastric pits (modified Giemsa), ×320.

and heated blood agar. Larger colonies were produced on the selective medium and occasional overgrowth by other bacteria was seen on the heated blood agar. All isolates were Gram-negative curved bacilli, positive for catalase, oxidase and rapid-urease production.

Results of cultural and biochemical tests are shown in Table 1. Enzymatic profiles are shown in Table 2; positive results were obtained with 6 of the 19 substrates tested. The test is semi-quantitative but was reproducible when results were graded as described. Human isolates consistently produced large amounts of leucine arylamidase which was absent or present in trace amounts only in the ferret isolates. Soluble protein profiles of six *C. pylori* isolates and four ferret

Table 1. *Characteristics of ferret gastric CLO compared with* C. pylori

	Isolates from ferrets		
	Tested	Positive	*C. pylori* NCTC 11637
Growth on heated blood agar for 5 days			
37 °C Microaerobic	17	17	+
27 °C Microaerobic	16	0	−
42 °C Microaerobic	16	13	−
37 °C Aerobic	17	0	−
37 °C Anaerobic	17	0	−
Gram-negative curved bacilli	17	17	+
Oxidase	17	17	+
Catalase	17	17	+
Rapid-urease	17	17	+
Nitrate reduction	13	13	−
Hippurate hydrolysis	13	0	−
Nalidixic acid (30 μg) sensitive	13	13	−
Cephalothin (30 μg) sensitive	13	0	+
Growth – blood agar	15	15	+
Growth – blood agar + additional 1·5% NaCl	15	0	−

Table 2. *Comparative enzymatic profiles of ferret gastric CLO and* C. pylori *(human isolates)*

Apizyme strip no.	Enzyme	Ferret GCLO ($n = 12$)	Human *C. pylori* ($n = 5$)	C. pylori NCTC 11637
2	Alkaline phosphatase	++	++	++
3	Esterase (C4)	+	+	+
4	Esterase lipase (C8)	+	+	+
6	Leucine arylamidase	+/−	++	++
11	Acid phosphatase	++	++	++
12	Naphthol-AS-B1-phosphohydralase	++	++	++

++, strongly positive reaction; +, weakly positive reaction; Other enzymes tested gave negative reactions.

gastric CLOs are shown in Fig. 3. The profiles of the human isolates are similar to each other and differ from the ferret isolates which are also similar to each other.

Five FAMEs were predominant in the profiles of both human and ferret gastric CLOs, and results of one representative experiment are shown in Table 3. The concentration of individual fatty acids varied on repetitive extraction, as has been described for other campylobacters (Curtis, 1983), but in all experiments hexadecanoic acid (16:0) was present as a high percentage of total esters in ferret isolates but as a much lower percentage in human isolates.

Table 3. *Predominant FAMEs of human isolates of* C. pylori (*H*) *and ferret gastric CLO* (*F*)

Strain	FAME as percentage of total esters				
	14:0	16:0	18:1	18:0	19:0*
H1	46	3	2	6	36
H3	50	6	5	11	27
H4	44	3	0	9	43
H6	41	4	2	9	38
H7	36	5	7	8	37
F2	22	41	3	4	28
F5	15	45	4	5	28
F6	14	37	10	5	34
F7	20	36	4	13	27
F8	12	43	7	8	29
C. pylori NCTC 11637	40	5	3	11	36
Mean *C. pylori*	43·4	4·2	3·2	8·6	36·2
Mean Ferret GCLO	16·6	40·4	5·6	7·0	29·2

* indicates cyclopropane ring. Minor peaks (< 2%) of other esters occurring in some isolates are not recorded.

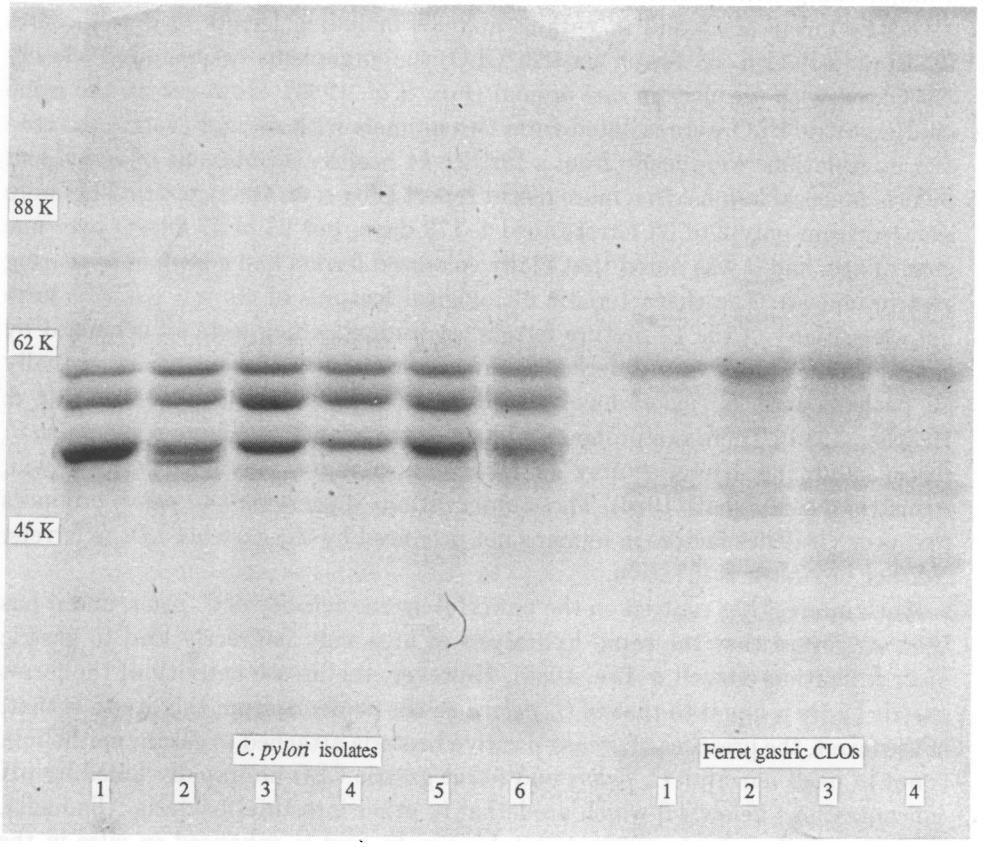

Fig. 3. Protein profiles of six human *C. pylori* isolates and four ferret gastric CLO.

DISCUSSION

The ferret gastric CLOs are similar to *C. pylori* isolates from human gastric tissue morphologically and biochemically. In particular, all isolates produce large amounts of urease. They do not grow in the presence of 1·5% NaCl, which differentiates the gastric CLOs from the urease-positive campylobacters isolated from water and shellfish (Bolton, Holt & Hutchinson, 1985). Fox *et al.* 1986, have shown that the structure of the ferret isolates is similar to that of *C. pylori*, with multiple sheathed polar flagella. We have confirmed these observations (data not shown), and their findings that, unlike human isolates of *C. pylori*, ferret gastric CLOs reduce nitrate, are sensitive to nalidixic acid and resistant to cephalothin. In this study we have also shown differences in enzyme, FAME and protein profiles between *C. pylori* and ferret gastric CLO. The enzyme profiles of *C. pylori* shown in Table 2 are in agreement with the results of Megraud *et al.* (1985) and McNulty & Dent (1987) who found *C. pylori* strains to be a homogeneous group, with all of the 162 strains tested producing leucine aminopeptidase (arylamidase). Activity of this enzyme was weak or absent in 12 ferret gastric CLOs, but other reactions in the APIZYME profiles were identical to those of *C. pylori* (Table 2). The FAME profile of ferret gastric CLOs is closer than the *C. pylori* profile to the pattern shown by other campylobacters, which have hexadecanoic acid (C16:0) as a major component (Curtis, 1983).

Gastric ulcers are found in various animals including ferrets, and in the first reported isolation of ferret gastric CLO the organisms were found closely associated with an ulcer in one animal (Fox *et al.* 1986). However, in the same study, gastric CLO were isolated from two animals with normal gastric mucosae and no isolations were made from a further 14 healthy ferrets, one of which had pyloric mucosal lesions. In a more recent report (Fox *et al.* 1988) gastric CLO were isolated from only 2 of 33 ferrets aged 1–173 days, but 25 of 27 ferrets over one year of age, and it was noted that many colonized ferrets had a normal appearing gastric mucosa. The characteristic histological features of chronic gastritis were not seen in any of the 17 mature ferrets we studied, which were all colonized by gastric CLO. This is very different from the situation in humans where virtually all patients with *C. pylori* have active chronic gastritis (Rathbone, Wyatt & Heatley, 1986). There is a pronounced local and systemic antibody response to *C. pylori*, and the bacteria may attract and activate polymorphs (Goodwin, Armstrong & Marshall, 1986). These observations suggest that *C. pylori* produces toxins or virulence factors in humans not produced by the gastric CLOs in ferrets. Neither organism is invasive.

Much interest has centred on the powerful urease activity of *C. pylori* and it has been suggested that the rapid hydrolysis of urea may indirectly lead to gastric ulcer formation (Hazell & Lee, 1986). However, the urease activity of the ferret gastric CLOs is equal to that of *C. pylori*, so the evidence from this study is that, in the ferret, the presence of urease-positive bacteria attached to gastric epithelium is not in itself harmful. *C. pylori* and ferret gastric CLO are rapidly killed by pH concentrations below 4·0 which are lethal to other intestinal bacteria (Giannella, Broitman & Zamcheck, 1972), but tolerance to acid is enhanced *in vitro* in the presence of physiological amounts of urea (Tompkins & West, 1987).

Gastric CLO have now been isolated from pigs, baboons and macaques (Jones & Eldridge, 1988; Bronsdon & Schoenknecht, 1988; Newell, Hudson & Baskerville, 1987). Isolates from these mammals appear to be closely similar or identical to *C. pylori* isolates from humans, and in rhesus monkeys are associated with gastritis. The ferret gastric CLO share the same ecological niche and many of the other characteristics of the *C. pylori* strains. Our observations indicate that ferrets are commonly colonized by gastric CLO which usually do not cause any major pathological effects in the host. In a preliminary experiment, 1 of 5 weanling ferrets inoculated with a human strain of *C. pylori* developed haemorrhagic erosions in the gastric mucosa, but a minimal inflammatory response (Fox *et al.* 1988).

The ferret may prove to be a useful model for the study of infection with *C. pylori* and the development of gastritis if human strains can be established in ferret stomachs. Study of differences between *C. pylori* and ferret gastric CLO may reveal the virulence factors which lead to the inflammatory response seen in the human stomach colonized by *C. pylori*.

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