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CASE REPORT

Ultrastructure of Another Spiral Organism Associated with Human Gastritis

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KEY WORDS: spirillum; gastritis; peptic ulcer; *Campylobacter pylori;* urease; cats.

C. pylori is the most common of a variety of spiral, urease-producing organisms that may infect the gastric mucosa. Other spiral organisms, morphologically distinct from *C. pylori,* are known to exist in the stomachs of cats, dogs, monkeys, and other mammals, but their significance has not been delineated. These organisms are morphologically similar to each other, but they are easily distinguished from *C. pylori,* being larger with 5–10 tightly coiled spirals and numerous flagella. According to Weber et al (1) the organism present in the stomach of the cat was described initially in 1881 by Rappin. Accordingly, this bacterium has been referred to as *Spirillum rappini.* It may be a single species or one of a family of closely related bacteria. The organism has not been cultured or well characterized, but it appears to be the source of urease present in the gastric mucosa of cats and dogs. Recently, Dent et al (2) described large spiral organisms that resembled *Spirillum rappini,* present in human stomachs. This organism was associated with active chronic gastritis in each of the three cases.

In this report we describe two patients with nonulcer dyspepsia and active chronic gastritis due to a spiral organism identical to that described by Dent et al. Six months after antibacterial therapy, the spiral organisms were no longer present, and the gastritis had resolved.

CASE REPORTS

Case 1. A 36-year-old white female was evaluated for nausea, bloating, and chronic epigastric pain. The pain was described as burning and partially relieved by antacids and food. Previous medical evaluations had revealed no specific cause for her complaints and at times she had been diagnosed as having irritable bowel syndrome and nonulcer dyspepsia. Her frustration became evident when she was told by one physician that she had a "Librax personality." The patient was fond of animals and as a child her family owned 14 cats. The cats were cuddled and kissed on many occasions. Her symptoms of dyspepsia had been present since childhood.

Physical examination revealed mild epigastric tenderness but was otherwise normal. Upper endoscopy revealed slight erythema of the antrum. Samples of the antrum and body mucosa were obtained for urease testing, light microscopy, and culture for *Campylobacter pylori.* A rapid urease test (CLOtest) (3) performed on body mucosa was positive at 3 hr. The specimen from the antrum remained negative at 24 hr. Hematoxylin and eosin-stained sections revealed a patchy active chronic gastritis with moderate polymorphonuclear leukocyte infiltration, lymphocytes, and plasma cells (Figure 1). The gastritis was more severe in the body than in the antrum. A Giemsa-stained section showed spiral bacteria, 0.5 μm in diameter, 3–7 μm in length, and with four to eight spirals (Figure 2). The organisms were present in groups or were single. They were seen on the surface of the mucosa, in the lumina of glands, and within parietal cells (Figure 3).

Separate specimens from the body and antrum of the stomach for electron microscopy were fixed in 2% glutaraldehyde (w/v) in 0.1 M sodium phosphate buffer pH 7.4, and postfixed for 1 hr at 24° C in a 2% aqueous solution of 2% osmium tetroxide at 4° C. Following dehydration in graded ethanol, tissues were *en bloc* stained overnight at 4° C in a solution containing 10% uranyl acetate in absolute methanol. The blocks were embedded in Epon via routine infiltration with propylene oxide. Ultrathin sections (60–70 nm) were further stained in 10% uranyl acetate in methanol for 15 min at 60° C.

Multiple spiral organisms were seen in the mucus layer but were not adherent to the gastric epithelial cells. The wavelength of each spiral was approximately 800 nm. The organisms had smooth cell walls and at least four terminal flagella. Axial filaments were not seen (Figure 4).

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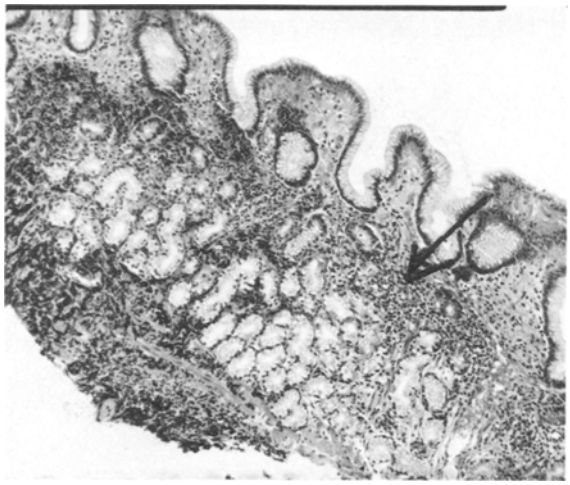

Fig 1. Antral mucosa from patient 1. The lamina propria contains an excess of lymphocytes, plasma cells, and neutrophils (arrow). Mucus-secreting epithelial cells appear normal (H&E, ×125).

Biopsy specimens from the antrum and body mucosa were minced and inoculated onto 7% horse blood agar plates with GCHI enrichment (Remel). They were then placed in a *Campylobacter* atmosphere at 37° C for 10 days. No urease-positive or spiral organisms were isolated.

The patient was treated with 21 days of bismuth subsalicylate (BSS, Pepto-Bismol) 30 ml four times a day, with the addition of amoxicillin 500 mg four times a day during days 7–21 of treatment. She was also given metronidazole 500 mg twice a day during days 18–21 of therapy. Endoscopy, performed one month after completion of treatment showed normal gastric mucosa. Histological examination revealed complete resolution of the gastritis and absence of the spiral organisms. At six-month follow-up, endoscopy was again normal and the patient was asymptomatic.

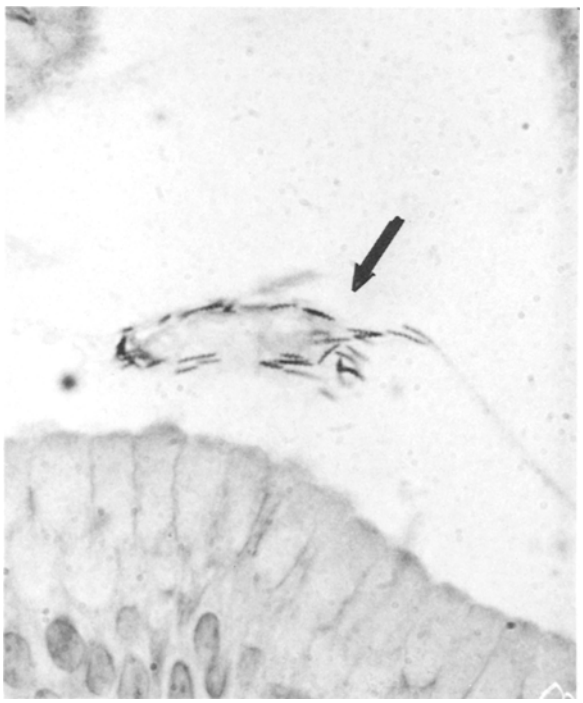

Fig 2. Spiral organisms within mucus adjacent to the gastric epithelium (Giemsa stain, ×1250).

Fig 3. Spiral organisms within a parietal cell (Giemsa stain, ×1250). In electron micrograph sections the organisms were seen to be within the acid secreting coraliculi rather than in the cytoplasm.

Case 2. A 77-year-old white male presented for evaluation of episodic prolonged postprandial hiccoughing, diarrhea, and "gas" that had been present for at least 10 years. The patient had two Irish setters as house pets and related having dogs living in the house for most of his adult life. An upper endoscopy revealed mild esophagitis and antral erythema. The rapid urease test (CLOtest) from an antral biopsy was positive. A small-bowel aspirate and biopsy were negative for *Giardia* and bacterial overgrowth. Antral biopsy revealed active chronic gastritis and numerous large spiral organisms distinct from *C. pylori* but identical to those seen in case 1. Electron microscopy also revealed spiral organisms identical to those in case 1. The patient was treated with 14 days of bismuth subsalicylate liquid (Pepto-Bismol) 30 ml four times a day a.c. with concurrent amoxycillin 2 g daily and metronidazole 1 g daily from day 4 to day 14. After therapy, the hiccoughs did not recur. There was a 50% reduction in the diarrhea and "gas" symptoms.

Endoscopy was repeated six months later and was normal apart from antral erythema. Biopsy specimens from antrum and body were histologically normal and the

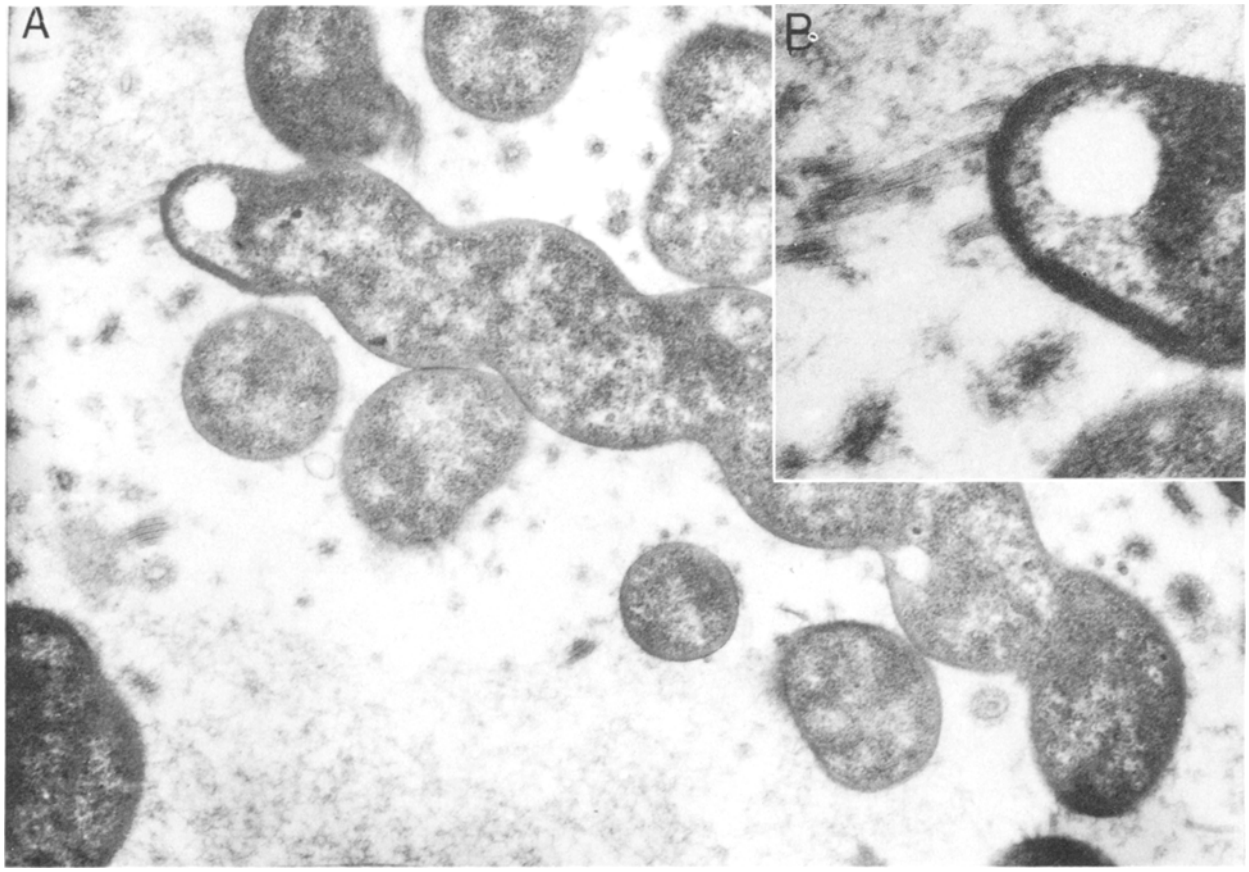

Fig 4. (A) Electron micrograph from patient 2 showing a spirillum cut in longitudinal section with adjacent transverse cuts of other spirilla. Note the smooth cell wall. We could not see axial filaments. (B) Further enlargement reveals at least four sheathed flagella emerging from the end of the organism.

spiral bacteria could not be detected. CLOtests of antrum and body mucosa were also negative.

DISCUSSION

The spiral organisms present in our two patients were easily distinguished from *Campylobacter pylori* and resembled the spirilla we have seen in the stomachs of cats, dogs, and nonhuman primates (Figure 5A).[5] Unlike *C. pylori* (Figure 5B), the veterinary organisms are usually seen within the lumina of the gastric glands and within parietal cells. They do not appear to be adherent to the gastric mucus cells. Histological changes are not seen in cats and dogs infected with these spirilla.

Since Rappin's initial discovery, many investigators have confirmed the presence of spiral organisms in the stomachs of animals (4). In 1893, Bizzozero described a spirochete in the parietal cells of dogs (5) and Salomon, in 1896, detected the same spirillum in the stomachs of dogs, cats, and rats (6). Salomon was successful in transmitting the organisms into the fundic glands of white mice, but failed to infect birds and frogs. He described three separate forms of the organism that had minor morphologic variations. The organism was observed to be actively motile, and it had terminal flagella. Even though large numbers of spirilla were present, there was no inflammation. In 1906, Krienitz discovered spirochetes in the stomach of a human suffering from stomach cancer (7). Lucet in 1910 detected spirochetes in lesions of a dog suffering from hemorrhagic gastroenteritis (8). In 1912, Dubosq and Lebailly reported spiral organisms in the stomach of a fox (9). Unaware of Rappin's work, these authors credited Bizzozero with the original discovery and named the organism *Spirilla canis*. Kasai and Kobayashi (1919) reported the spirillum in 43 of 49 dogs, eight of 13 cats, one of 38 wild rats, and each of 13 monkeys. The organism was absent in 20 rabbits, 15 guinea pigs, 10 white

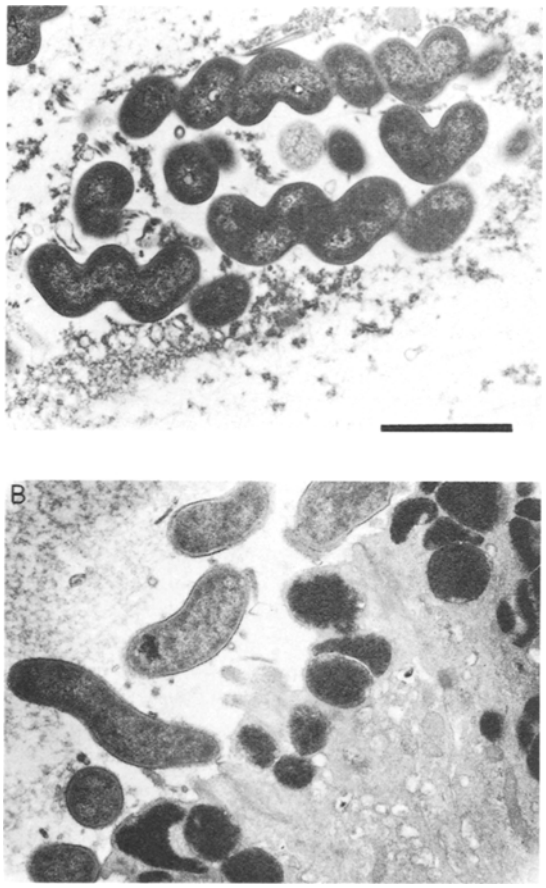

Fig 5. (A) Electron micrograph of the gastric spirillum of the cat. Again note the smooth cell walls. Sectioned sheathed flagella in groups of seven or eight can be seen (arrow). (B) For comparison, an electron micrography of *C. pylori* adjacent to gastric epithelial cells. Same scale as A.

rats, and 15 mice. If the host was otherwise normal, the organism was believed to be nonpathogenic, but if the host was compromised by infection with measles, scarlet fever, or rabies, a hemorrhagic gastritis could be induced upon inoculation with the spirillum (10). In 1958, Weber et al (1) wrote that the American literature was devoid of discussion regarding these organisms despite their ubiquitous nature in adult dogs, cats, and many other mammals. He recognized that the bacterium might be a factor in causing gastritis in these animals and might represent a public health interest. In 1987, Curry et al reported a spiral bacterium in the baboon stomach. This bacterium was similar to the previously described organisms, except that it had an unusual outer membranelike wall that was not closely adherent to the body spirals (11). This feature has not been described in spirilla from other mammals. Our own work in animals has revealed large spiral organisms in the stomachs of each of four dogs, four adult cats, and six eight-week-old kittens. The organisms were not associated with light microscopic evidence of inflammation. We did not detect gastric spirilla or gastric urease activity in two opossums that we examined.

Patients with gastric spirilla similar to those we describe were reported by Dent et al (2); their patients also reacted serologically with a spirillum isolated from cats by Lee et al (12). The cultured cat spirillum described by Lee et al is structurally different from that seen in our patients because it has an axial filament, a feature present in spirochaetes and some spirilla, but not seen in the organisms we have detected in patients or in our cats. A spirillum called *Flexispirillum rappini,* isolated from humans and animals by Archer et al (13), has multiple periplasmic fibrils and therefore is unlike the gastric spirillum we describe here.

We believe that the large spiral organisms seen in our patients are *Spirillum rappini* or a very closely related species. At present, this organism has not been cultured from man so we cannot be certain that it is identical to the spirillum we observe in cats. The new spirillum is uncommon in human gastric mucosa, as the three cases of human infection described by Dent et al (2) were detected in a series of over 1300 consecutive patients who had dyspepsia. Our patients represented two of almost 400 patients who have been biopsied to exclude *C. pylori* infection. So far, all human reports of this new organism have associated it with gastritis. The resolution of gastritis in our patient is evidence that *Spirillum rappini,* like *C. pylori,* is a cause of active chronic gastritis in humans. Eradication of the organism was also associated with complete resolution of symptoms, suggesting that the clinical syndrome was related in some way to the infection or the gastritis.

SUMMARY

Campylobacter pylori may not be the only organism that causes active chronic gastritis in man. We report two cases of gastric infection with a spiral organism distinct from *C. pylori.* The first patient is a 36-year-old female who presented with epigastric pain and abdominal colic present since childhood and who had 14 cats. Endoscopy was normal. The second patient kept two dogs. Histology of gastric mucosal biopsy specimens in both patients revealed active chronic gastritis, most severe in body mu-

cosa. Giemsa stain revealed bacteria with four to eight spirals, 0.5 μm in diameter and 3–7 μm in length. The organisms had multiple sheathed flagella at the pole and smooth cell walls without axial filaments. The organisms resembled the gastric spirillum that has been seen in cats, dogs, and nonhuman primates. After antibacterial therapy with bismuth subsalicylate, amoxicillin, and metronidazole, the organisms disappeared in both patients and the gastritis healed.

Unlike *C. pylori,* this new spirillum prefers to colonize gastric mucosa containing parietal cells. Whereas this type of organism is a common commensal in other mammals, it appears to be associated with and a possible cause of gastritis in humans.

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REFERENCES

1. Weber AF, Hasa O, Sautter JH: Some observations concerning the presence of spirilla in the fundic glands of dogs and cats. Am J Vet Res 19(72):677–680, 1958
2. Dent JC, McNulty CAM, Uff JC, et al: Spiral organisms in the gastric antrum. Lancet 2:96, 1987 (letter)
3. Marshall BJ, Warren JR, Francis GJ, et al: Rapid urease test in the management of *Campylobacter pyloridis* associated gastritis. Am J Gastroenterol 82:200–216, 1987
4. Lim RKS: A parasitic spiral organism in the stomach of the cat. Parasitology 12:108–112, 1920
5. Bizzozero G: Sulle ghiandole tubulari del tube gastroenterico e sui rapporti del loro coll'epitelio de rivestimento della mucosa. Atti R Accad Sci Torino 28:233–251, 1892
6. Salomon H: Ueber das Spirillum des Saugetiermagens und sein Verhalten zu den Belegzellen. Centralbl Bakteriol 19:433–442, 1896
7. Krienitz W: Ueber das Auftreten von Spirochaeten verschiedener Form im Mageninhalt bei Carcinoma ventriculi. Dtsch Med Wochenschr 32:872, 1906
8. Lucet M: Sur la presence de spirochetes dans un cas de gastro-enterite hemorrhagique chez le chien. C R Acad Sci 151:260–262, 1910
9. Dubosq O, Lebailly C: *Spirilla canis,* n.g., n.sp., spirille de l'estomac du chien. C R Acad Sci 154:835–837, 1912
10. Kasai K, Kobayashi R: The stomach spirochete occurring in mammals. J Parasitol 1(6):1–10, 1919
11. Curry A, Jones DM, Eldridge J: Spiral organisms in the baboon stomach. Lancet 2:634, 1987 (letter)
12. Lee A, Dent J, Hazell S, McNulty C: Origin of spiral organisms in human gastric antrum. Lancet 1:300–301, 1988 (letter)
13. Archer JR, Romero S, Ritchie AE, Hamacher ME, Steiner BM, Bryner JH, Schell RF: Characterization of an unclassified microaerophilic bacterium associated with gastroenteritis. J Clin Microbiol. 26(1):101–105, 1988