Isolation of *Campylobacter jejuni* from an Appendix

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*Campylobacter jejuni* Skirrow biotype 1, Lior serotype 8 was isolated from the appendix of an 11-year-old boy who had a 6-h history of acute abdominal pain. Histological diagnosis on the appendix section was early acute appendicitis. Dilute carbol fuchsin stain and indirect fluorescent antibody test performed on the appendix section also revealed the presence of *Campylobacter* sp. The patient developed a significant bactericidal antibody titer of 1,024, providing substantial clinical evidence of the pathogenicity of the isolate. This case indicated that not only may abdominal pain caused by *Campylobacter* enteritis mimic appendicitis, but the organism may actually be recovered from the infected appendix.

It has been established over the last few years that *Campylobacter jejuni* is a common intestinal pathogen. The common features of *Campylobacter* enteritis are fever, bloody diarrhea, and abdominal pain (2). Some patients have abdominal pain as the only major symptom and may be admitted to a surgical ward because of suspected appendicitis (7). However, we were unable to find a report on the isolation of *C. jejuni* from the appendix itself. We describe here a case of acute appendicitis and the isolation of *C. jejuni* from the infected appendix of the patient.

A previously well 11-year-old boy was seen at the emergency department with a 6-h history of acute abdominal pain, located principally just to the right of the umbilicus and in the right lower quadrant. Apparently there had been some intermittent pain the day before, although not severe. He had nausea but no associated vomiting. He passed two to three loose stools on the night of the visit to the emergency department. The patient had not examined the stools. He had a mild fever the day before the visit. There had been no urinary symptoms and no exposure to contacts with patients with gastroenteritis.

Physical examination revealed a scaphoid abdomen with normal musculature. Bowel sounds were normal. The patient had diffuse abdominal tenderness, greater in the right lower quadrant and suprapubically. There was marked guarding and rebound in the right lower quadrant. On rectal examination, he was tender in the right side and stool was not present. The peripheral leukocyte count was 8,800/µL, and hemoglobin was 14.1 g/dL. The clinical diagnosis was acute appendicitis.

Appendectomy was performed for an acutely inflamed appendix. The patient tolerated the procedure well. There were no complications and after an uneventful recovery he was discharged home 5 days later.

A swab of the abdominal fluid and a swab from the appendix stump did not yield any aerobic or anaerobic bacterial growth on routine culture. The swab from the appendix stump was also set up for culture of *C. jejuni* on a Columbia agar plate (Armand-Frappier, Montreal, Quebec, Canada), containing 7% lyzed horse blood, 10 µg of vancomycin per ml, 5 µg of trimethoprim per ml, and 5 µg of polymyxin B per ml (3). The culture was incubated at 42°C in an atmosphere of 5% O₂-10% CO₂-85% N₂, and it yielded scanty growth of the organism. The isolate was subsequently identified as *C. jejuni* Skirrow (9) biotype 1 (H₂S negative and nalidixic acid sensitive [a 30-µg disk]), Lior serotype 8 (4, 5). It was susceptible to ampicillin, chloramphenicol, erythromycin, gentamicin, and neomycin but resistant to cefamandole and trimethoprim-sulfamethoxazole by the standardized single disk method described by Bauer et al. (1). The laboratory did not receive blood or stool samples for culture.

The excised appendix measured 9 cm in length and 0.7 to 0.9 cm in diameter. The serosa was mildly congested. Sections of the appendix showed focal mucosal ulceration with large clumps of neutrophils in the lumen. A few clumps of neutrophils were also seen infiltrating the mucosa. Histological diagnosis on the appendix section was early acute appendicitis. Because *C. jejuni* had been isolated from culture of the appendix, a dilute carbol fuchsin stain (carbol fuchsin stain diluted 1 in 20 with water) was performed on the appendix section. Organisms of *Campylobacter* spiral morphology were observed in the lumen. The red staining by dilute
carbol fuchsin, however, obscured the finding of *Campylobacter* sp. in the tissue. An indirect fluorescent antibody test was therefore carried out as follows.

A rabbit was immunized, using a suspension of the patient's strain of *C. jejuni* (10⁸ organisms per ml in 0.04% formaldehyde in phosphate-buffered saline [pH 7.2]) mixed with an equal volume of Freund complete adjuvant (Difco Laboratories). Intramuscular injections (0.5 ml of vaccine into each leg) were given twice with a 10-day interval. Serum taken 10 days after the second injections had an agglutination titer of 2,048 against the *C. jejuni* strain. An indirect fluorescent antibody test was used to demonstrate the presence of *C. jejuni* in appendix sections from our patient, using rabbit hyperimmune serum at a dilution of 1 in 20 and a fluorescent anti-rabbit globulin (Wellcome Reagents Ltd., Beckenham, England), also at a dilution of 1 in 20. Fluorescent organisms of appropriate *Campylobacter* morphology were found in the appendix sections from the patient (Fig. 1). The specificity of the test was demonstrated by the following controls: (i) a smear of *Escherichia coli* (ATCC 25922) stained with hyperimmune rabbit serum; (ii) sections of the appendix of three other patients with early acute appendicitis and no known *Campylobacter* infections; and (iii) sections of the appendix of our patient stained with rabbit preimmunization serum. All these controls were negative.

The patient was followed up at 2 weeks and at 8 weeks after the appendectomy. He had loose stools at about 6 weeks. However, a stool culture at 8 weeks did not yield *C. jejuni* or any other potential enteric pathogens. A serum sample taken at 8 weeks showed a high bactericidal antibody titer (3) of 1,024 using the patient's own strain of *C. jejuni* as the test organism. Serum samples from 11 laboratory workers who did not have diarrhea at the time gave bactericidal antibody titers ranging from <4 to 256 to this particular strain of *C. jejuni*. Apart from a mild

FIG. 1. Section of appendix showing *Campylobacter* organisms (indicated by arrows) by indirect fluorescent antibody test (for details see text). Magnification, ×2,200.
upper respiratory tract infection, the patient did not have any other complications seen at 8 weeks.

It has been described that the abdominal pain caused by Campylobacter enteritis can lead to hospital admission and appendectomy. In one report (11), appendectomy was performed on an 11-year-old girl, but no abnormalities were found and her appendix was histologically normal. Similarly, in another report (6), appendectomy was performed on a patient with abdominal pain, but the appendix was found to be normal. Pearson et al. (7) described four children with abdominal pain, of whom two were found at appendectomy to have acutely inflamed appendices and two were found to have acute mesenteric adenitis. However, confirmation of Campylobacter infection on these four cases was based on positive stool cultures, and it was not mentioned whether Campylobacter sp. was looked for in the appendix specimens.

In this particular case, the patient had an acutely inflamed appendix. Blood and stool samples were not taken for culture, and the diagnosis of Campylobacter infection would have been missed if the appendix specimen had not been cultured specifically for C. jejuni.

We initiated procedures to culture for C. jejuni from appendix specimens 2 years ago, and we have cultured 103 appendices. To date, this has been the only isolate, which indicates that appendicitis due to Campylobacter sp. is not common. This case suggests, however, that Campylobacter sp. can be a cause of appendicitis, as demonstrated by (i) histopathology of the appendix, (ii) failure to isolate other organisms on culture, (iii) presence of Campylobacter sp. in the appendix tissue, (iv) successful isolation of the organism from the appendix by culture, and (v) a high serum bactericidal antibody titer against the patient’s homologous strain of C. jejuni compared with 11 asymptomatic subjects.

Since the development of a selective medium by Skirrow (8) and the realization of the necessity to culture under special temperature and atmospheric conditions (10), C. jejuni has been found to be a common cause of enteritis in many parts of the world. Considering that the rise of Campylobacter enteritis from obscurity to notoriety was due to the employment of selective culture techniques rather than to the sudden prevalence of the disease, it is possible that there may be an increase in appendicular Campylobacter isolates once clinical laboratories routinely culture appendix specimens for C. jejuni.

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ADDITIONAL
During the review process of this paper, the first report on isolation of C. jejuni from an appendix appeared (F. Megraud, C. Tachoire, J. Latrille, and J. M. Bondy, Br. Med. J. 285:1165–1166, 1982). We recently recovered a second appendicular isolate of C. jejuni from a 12-year-old boy with early acute appendicitis.

LITERATURE CITED