Two Cases of Campylobacter mucosalis Enteritis in Children

NATALE FIGURA,1* PAOLO GUGLIELMETTI,2 ALESSANDRA ZANCHI,2 NEDO PARTINI,2 DORETTA ARMELLINI,3 PIETRO F. BAYELLI,1 MASSIMO BUGNOLI,3 AND SILVANA VERDIA4

Istituto di Patologia Speciale Medica, University of Siena, Piazza Duomo, 2, I-53100 Siena,1 Istituto di Malattie Infettive, University of Siena, 2 I.R.I.S., Siena,3 and Diesse Diagnostica Senvista S.r.l., Monteriggioni (Siena),4 Italy

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Two cases of Campylobacter mucosalis enteritis in children are reported. The patients recovered without antimicrobial therapy. Strains were isolated only by the feces filtration technique. In one child, bactericidal antibodies to the homologous strain were detected in a convalescence-phase serum sample. C. mucosalis should be considered a primary intestinal pathogen.

Campylobacters are an important cause of enteritis worldwide. Campylobacter jejuni subsp. jejuni and C. coli are the most common species associated with cases of diarrhea; however, atypical or unusual campylobacters, especially C. upsaliensis, have been isolated on selective agar media (8). We describe two cases of C. mucosalis enteritis in children which occurred in Siena, Tuscany, Italy.

From January 1989 to December 1990, stool samples from 288 children with diarrhea, most of whom were admitted to the Institute of Infectious Diseases, University of Siena, were examined for campylobacteria by using the feces filtration method (5) with minor modifications. Feces also were streaked on a blood-free agar which contained charcoal and 32 μg of cefoperazone per ml. Plates were incubated at 37°C in a microaerobic environment obtained by evacuating an anaerobic jar to a negative pressure of 650 mm Hg (ca. 87 kPa) and refilling it with a gas mixture (70% N2-15% CO2-15% H2). The gas mixture used for testing the ability of strains to grow in the absence of H2 was composed of 85% N2 and 15% CO2. Plates were inspected daily for 5 days. Suspected colonies were tested by Gram stain and for oxidase, catalase, hippurate hydrolysis, nitrate reduction, rapid urease, indoxyl acetate, production of H2S in triple sugar iron, tolerance to 1% glucose and 1.5% NaCl, growth in a microaerobic environment at 25 and 42°C, and susceptibility to cephalothin and nalidixic acid (30-μg disks) (6). In doubtful cases, strains were identified by API 20 Campy strips (API, La Balme Les Grottes, France).

We also attempted to culture Salmonella spp. (feces were enriched in Selenite broth and subcultured on Hektoen enteric agar), and we attempted to culture Shigella spp., Yersinia enterocolitica, Aeromonas spp., and enteropathogenic Escherichia coli by using Hektoen enteric agar, salmonella-shigella agar with 2% sodium deoxycholate, DNase agar with 30 μg of ampicillin per ml and 0.001% toluidine blue, and MacConkey agar, respectively. Biochemical identification of suspected colonies was carried out by using API 20E and API 20 NE tests. Enteropathogenic E. coli strains were screened with sera provided by Behringwerke AG (Istituto Behring, Scoppito, L'Aquila, Italy). The presence of rotavirus and adenovirus was determined by using a latex assay (Rotalex and Adenolex; Orion Diagnostica, Espoo, Finland). In the two cases of C. mucosalis enteritis, feces were also examined for parasites, and for Blastocystis hominis by a formalin-ethyl acetate centrifugal sedimentation technique. Samples were examined unstained. Cryptosporidium oocysts were identified with acid-fast stain.

The two C. mucosalis isolates were tested for the presence of virulence factors. Strains were grown in diphasic medium (Mueller-Hinton agar and brucella broth [Difco]) in a microaerobic environment at 37°C for 4 days. Then cultures were centrifuged, and supernatants were filtered through 0.22-μm-pore-size filters and added at dilutions of 1:3, 1:5, 1:10, and 1:20 to CHO cells for determination of a cytotoxic-like effect and to Vero and CHO cells to detect cytotoxic and cytolytic distending effects (5). To test the ability of the two strains to adhere to cells in vitro, sedimented bacteria were washed three times with medium for cells and added to Intestine 407 cells in vitro at a ratio of 1:100. After 3 h of incubation at 37°C, cells were washed three times with phosphate-buffered saline, fixed with absolute methanol, stained with acidine orange for 2 min, and observed under a fluorescence microscope. As positive controls, we used C. jejuni 83-4182 (cytotoxic toxin producer), C. jejuni 83-3969 (cytotoxic toxin producer), and C. jejuni 83-4483 (cytotoxic distending toxin producer), kindly given by H. Lior (Reference Centre for Enterobacteriaeae, Ottawa, Ontario, Canada). An adhesitive E. coli clinical isolate was used as a control for the adhesiveness assay.

A convalescence-phase serum sample was available in one of the two cases of C. mucosalis enteritis. The serum bactericidal assay (4) was used to detect bactericidal antibodies to the homologous and the heterologous strains.

C. mucosalis strains were isolated in two cases (0.7%). Organisms were isolated only by using the feces filtration technique. The two fecal samples were negative for the other intestinal pathogens. The prevalence values of the other pathogens were as follows: C. jejuni subsp. jejuni, 6.9%; C. coli, 2%; C. upsaliensis, 0.7%; C. jejuni subsp. doylei, 0.7%; Salmonella spp., 14.5%; Aeromonas spp., 2.4%; Shigella spp.; and Y. enterocolitica, 0%; rotavirus, 19.4%; adenovirus, 4.5%.

Both children with C. mucosalis enteritis were male (12 and 18 months old). Clinical findings included mild diarrhea with loose stools and mucus but no gross blood in the feces. Duration of diarrhea was 4 days in one patient and 6 days in the other. Mild fever was present. There was no vomiting, and no antimicrobial therapy was given. Both children made a full recovery.
The two *C. mucosalis* organisms were microaerobic, gram-negative, curved rods, which were motile, oxidase and nitrate positive, catalase, hippurate, indoxyl acetate, and rapid urease negative, and susceptible to cephalothin and nalidixic acid used as 30-µg disks. The bacteria grew in a microaerobic environment at 30 and 42°C, but not at 25°C, and in normal air at 37°C. Both strains required hydrogen for growth and produced H₂S detectable with lead acetate strips and in triple sugar iron stabbed with the bacteria (weak H₂S production in triple sugar iron was observed with one strain). Both strains tolerated 1% glycine, but not 1.5% NaCl (1, 2, 7). Colonies were yellowish, circular, smooth, shining, opaque, and butyrous. Final identification was obtained with the API 20 Campy system. The species identification of the strain isolated from the child whose serum sample was available was confirmed in the United Kingdom by R. Owen (National Collection of Type Cultures, London) by the morphological study of colonies and bacteria and by metabolic, biochemical, tolerance, and susceptibility tests. Strains were tested for in vitro virulence characteristics. Broth cultures of both organisms induced elongation of CHO cells cultured in vitro (cytotoxic-like response). Strains did not produce lytic or cytolethal distending toxins (3) and did not adhere to Intestine 407 cells in vitro.

The convalescence-phase serum taken from one of the two children contained bactericidal antibodies (4) to the homologous strain up to a dilution of 1:64, which indicated an ongoing or recent infection (personal observation on serum samples from 30 children with campylobacter enteritis). No bactericidal activity was detected against a *C. jejuni* subsp. *jejuni* serogroup LIO 1 strain. Serum samples from three children without diarrhea did not possess bactericidal antibodies to either strain. This indicated that the antibody response was homologous strain specific.

This is the first report of human *C. mucosalis*-induced diarrhea of which we are aware. On the basis of data from 288 children with enteritis, this organism represented about 6% of all campylobacteria isolated in this area of Italy when the filtration technique was used. *C. mucosalis* has been associated with proliferative enteropathies in pigs (5). However, in the two cases of *C. mucosalis* diarrhea, the children did not have contact with any animal.

The scientific literature is controversial with regard to the results of some tests for *C. mucosalis* identification. Our strains were susceptible to nalidixic acid and did not grow at 25°C. In the work of Roop et al. (7), only one *C. mucosalis* organism of eight strains tested was susceptible to nalidixic acid, and all strains grew at 25°C. However, the type strain *C. mucosalis* NCTC 11000 in a report by Edmonds et al. and the two *C. mucosalis* isolates tested in a report by Barrett et al. were susceptible to nalidixic acid and unable to grow at 25°C (1, 2). Thus, the results of these two tests should be considered variable.

Our observations suggest that *C. mucosalis* should be considered a potential pathogen for humans, and an etiologic association should be considered when the organism is isolated from patients with diarrhea.

REFERENCES