

Acute Colitis Caused by *Helicobacter trogontum* in Immunocompetent Patient

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To the Editor: In industrialized countries, diarrhea and vomiting associated with acute gastrointestinal illness is estimated to occur at a rate of ≈ 1 episode per person per year; $\approx 0.3\%$ of patients are hospitalized because of severe symptoms associated with colitis or fever. The most commonly identified infectious agents of non-nosocomial diarrhea are calicivirus and *Salmonella*, *Campylobacter*, *Giardia*, and *Cryptosporidium* spp. However, for numerous cases, the causative agent is not identified. *Helicobacter* species other than *H. pylori*, but not *H. trogontum*, have emerged as causes of gastrointestinal and systemic disease, mainly in immunocompromised patients (1). We report a case of community-acquired colitis with bacteremia caused by *H. trogontum* in an immunocompetent patient and emphasize the diagnostic difficulties.

The patient was a 31-year-old woman with a history of recurrent epigastralgia, vomiting, diarrhea, and weight loss of 10 kg over an 8-year period. In April 2014, she was admitted to the Hôpital Européen Georges Pompidou emergency ward (Paris, France) because of abdominal pain, nonbloody diarrhea, fever, and chills, which had persisted for 3 days. Examination revealed a mildly tender abdomen without hepatosplenomegaly, signs of slight dehydration, and tachycardia. Leukocyte count was 13.2×10^9 cells/L (neutrophils, 11.4×10^9), and C-reactive protein level was 191 mg/L. Abdominal computed tomography images showed nonspecific right and transverse colitis (Figure, panel A).

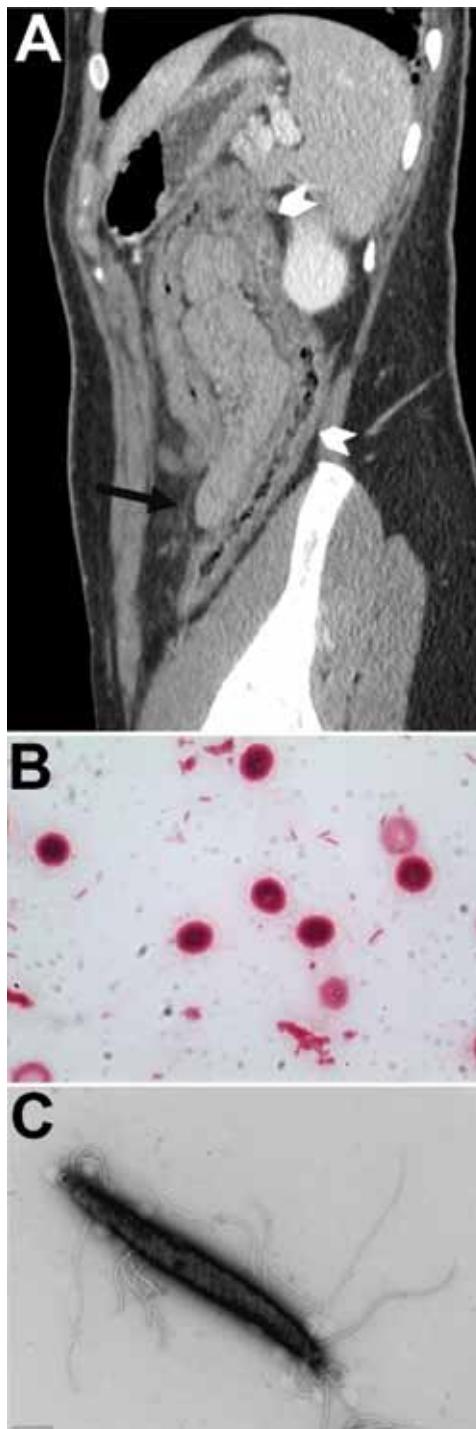


Figure. Computed tomographic image of patient with *Helicobacter trogontum* infection and micrographs of *H. trogontum*. A) Paramedian sagittal section of an abdominopelvic scan after injection of contrast medium in the portal phase, showing thickening of the transverse and right colon (white arrowheads) with tubular appearance and discrete thickening of the fat stranding (gray arrow). B) Gram-stained blood culture smear. Original magnification $\times 1,000$. C) Transmission electron micrograph of negatively stained *H. trogontum* showing bipolar flagella. Scale bar indicates 0.5 μm .

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One day after admission, the patient was discharged with empirically prescribed ciprofloxacin and metronidazole for 7 days. After 4 days, aerobic blood culture was positive for motile, fusiform, gram-negative bacilli, suggestive of strictly aerobic bacteria that could not be identified directly (Figure, panels B, C). After 7 days of incubation under a microaerophilic atmosphere only, a blood subculture isolate was obtained; 23S and 16S rDNA sequencing (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/22/2/15-0287-Techapp1.pdf>) identified this isolate as *H. trogonum*. Of note, use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany) did not enable identification of the bacterium.

No common pathogens were detected in fecal samples. Upper and lower gastrointestinal endoscopic examinations conducted 1 month after discharge revealed no notable abnormalities. No immunocompromised condition was found. At most recent follow-up examination, the patient was free of symptoms.

The genus *Helicobacter* currently comprises 48 formally named species belonging to the gastric or enterohepatic group according to their ecologic niche. *H. trogonum* (enterohepatic group) has been isolated from apparently health animals (rat and piglet intestinal mucosa and swine feces), but its characteristics are typical of pathogenic bacteria ([2,3], online Technical Appendix). The apparent in vitro susceptibility of the isolate to metronidazole and the favorable patient outcome reported here are in agreement with the finding that metronidazole is an effective treatment for *H. trogonum* infection in rats ([4]; online Technical Appendix), but there are no antimicrobial drug susceptibility data for *H. trogonum* isolated from animals. We assume that the immunocompetent patient reported here had chronic colitis caused by *H. trogonum*, followed by an episode of acute colitis with bacteremia after several years of intermittent symptoms.

The rarity of reported *H. trogonum* infections might be linked to the difficulty associated with culturing and identifying the bacterium or to a low level of exposure to this pathogen. The mode of transmission, probably from animals to humans, remains unclear. Methods for isolation and rapid identification of *H. trogonum*, including the updating of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry databases, are needed for further elucidation of its pathogenic properties and the mode of contamination.

References

1. Fox JG. The non-*H. pylori* helicobacters: their expanding role in gastrointestinal and systemic diseases. *Gut*. 2002;50:273–83. <http://dx.doi.org/10.1136/gut.50.2.273>
2. Mendes EN, Queiroz DM, Dewhirst FE, Paster BJ, Moura SB, Fox JG. *Helicobacter trogonum* sp. nov., isolated from the rat

intestine. *Int J Syst Bacteriol*. 1996;46:916–21. <http://dx.doi.org/10.1099/00207713-46-4-916>

3. Kaakoush NO, Sirianni A, Raftery MJ, Mitchell HM. The secretome of *Helicobacter trogonum*. *Helicobacter*. 2013;18:316–20. <http://dx.doi.org/10.1111/hel.12045>
4. Nahidi L, Leach ST, Mitchell HM, Kaakoush NO, Lemberg DA, Munday JS et al. Inflammatory bowel disease therapies and gut function in a colitis mouse model. *Biomed Res Int*. 2013;2013:909613.

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Accuracy of Dengue Reporting by National Surveillance System, Brazil

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To the Editor: Dengue is an underreported disease globally. In 2010, the World Health Organization recorded 2.2 million dengue cases (1), but models projected that the number of symptomatic dengue cases might have been as high as 96 million (2). Brazil reports more cases of dengue than any other country (1); however, the degree of dengue underreporting in Brazil is unknown. We conducted a study to evaluate dengue underreporting by Brazil's Notifiable Diseases Information System (Sistema de Informação de Agravos de Notificação [SINAN]).

From January 1, 2009, through December 31, 2011, we performed enhanced surveillance for acute febrile illness

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Technical Appendix

***Helicobacter trogontum* Culture, Identification, and Antibiotic Apparent Susceptibility**

A set of aerobic and anaerobic blood culture bottles was incubated at 35°C using the BactT/ALERT 3D DUAL-T system (bioMérieux, Marcy l'Étoile, France). Aerobic blood culture was positive after 4 days with motile, fusiform and slightly curved gram-negative bacilli (Figure B). Subculture of the aerobic sample was positive after 7 days of incubation on chocolate or sheep blood (5% v/v) agar only under microaerophilic conditions; for subsequent cultures, incubation for 4 days was sufficient. In the case of blood infection with *Helicobacter cinaedi*, Araoka et al. showed that the time required for the blood culture to become positive ranged from 2 to 12 days (1). If non-*H. pylori* *Helicobacter* species (NHPH) are searched for, the clinician and microbiologist must be aware that the duration of monitored blood cultures should be extended. Currently, there are no selective media or molecular methods for the rapid detection and identification of *H. trogontum*, as well as other NHPH, in clinical samples, including feces and digestive biopsies.

Growth occurred at 35°C and 42°C and appeared in the form of grayish colonies spreading across the agar surface. The isolate had catalase, oxidase and urease activities, as shown by Mendes et al. (ref. 2 cited in the article). Cultures older than ≈9 days consisted of coccoid forms that were nonviable. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Billerica, MA, USA) did not enable identification of the bacteria directly from cultured or subcultured blood samples and did not reveal any relationship with the genus *Helicobacter* although *H. pylori*, *H. canadensis*, *H. canis*, *H. cholecystus*, *H. cinaedi*, *H. fennelliae* and *H. mustelae* were represented in the Bruker MS database. Molecular identification of *H. trogontum* was made, after DNA purification using InstaGene Matrix (Bio-Rad, Hercules, CA, USA), using 16S and 23S rDNA-specific primers (2,3). Sequences were compared to those in the GeneBank database using BIBI^{PQP} and Sepsitest blast programs (<https://umr5558-bibiserv.univ-lyon1.fr/lebibi/lebibi.cgi>, <http://www.sepsitest-blast.de/en/index.html>). The 16S and 23S rDNA exhibited 98.4% identity with that of *H. trogontum*

strain ATCC700114 isolated from rat (GenBank accession number U65103). *H. trogontum* was originally classified as *Flexispira* Taxon 1, 4, 5, 6 and also comprised in the heterogeneous entity called *Helicobacter rappini* (4).

H. trogontum presents multiple bipolar active flagella (7 on the present isolate) and periplasmic fibers (Figure C, Supplemental Figure A, B). This finding explains its ability to move in mucus and to interact with enterocytes.

After 4 days of incubation on blood agar, the isolate analyzed here showed inhibition zone diameters of >50 mm for rifampin and tetracycline and 37 mm for metronidazole. The isolate also had MICs for amoxicillin, ciprofloxacin, and clarithromycin of respectively 2, 32 and 0.38 g/L.

Caco-2/TC7 Cell Experiments

H. trogontum induces subclinical inflammatory bowel disease (IBD) in germ-free mice and acute-to-chronic typhlocolitis with weight loss and diarrhea in B6-IL-10^{-/-} mice, in which it causes loss of microvilli and risk of sepsis. This risk is also linked to the dysbiosis caused by *H. trogontum* (5,6). In the present case, we assume that the immunocompetent patient had a chronic colitis due to *H. trogontum* and that she had an episode of acute colitis with bacteremia after several years of intermittent symptoms. The patient is further monitored for IBD, especially because preliminary experiments showed a Caco-2 cell cycle arrest after incubation with the *H. trogontum* isolate and this effect may be involved in the early stage of IBD.

Caco-2/TC7 cells were plated on glass coverslips and grown in Dulbecco modified Eagle medium containing 20% fetal calf serum (DMEM and fetal calf serum; GIBCO, Grand Island, NY, USA) with nonessential amino acids, at 37°C in a CO₂ (10%) incubator. Three days later, cells were challenged with *H. trogontum* overnight, and for 72 hours, in DMEM at 37°C; control cells were incubated in DMEM only or challenged with *H. trogontum* heated for 10 minutes at 100°C. Cells were washed once with phosphate-buffered saline (PBS; GIBCO) and fixed with 3.7% paraformaldehyde. Bacteria were permeabilized with ethanol for 5 min at -20°C, washed three times with PBS, blocked in PBS buffer containing 10% fetal calf serum for 15 min, and incubated with DAPI (4',6-diamidino-2-phenylindole) (1:100) and Alexa Fluor 488 Phalloidin (1:200). Z sections (200 nm) were acquired using spinning-disk confocal microscopy.

Three separate experiments were made with counts of 6,000 cells for each condition. The Caco-2 cell cycle arrest assays yielded similar results with overnight and 72-hour incubations, revealing,

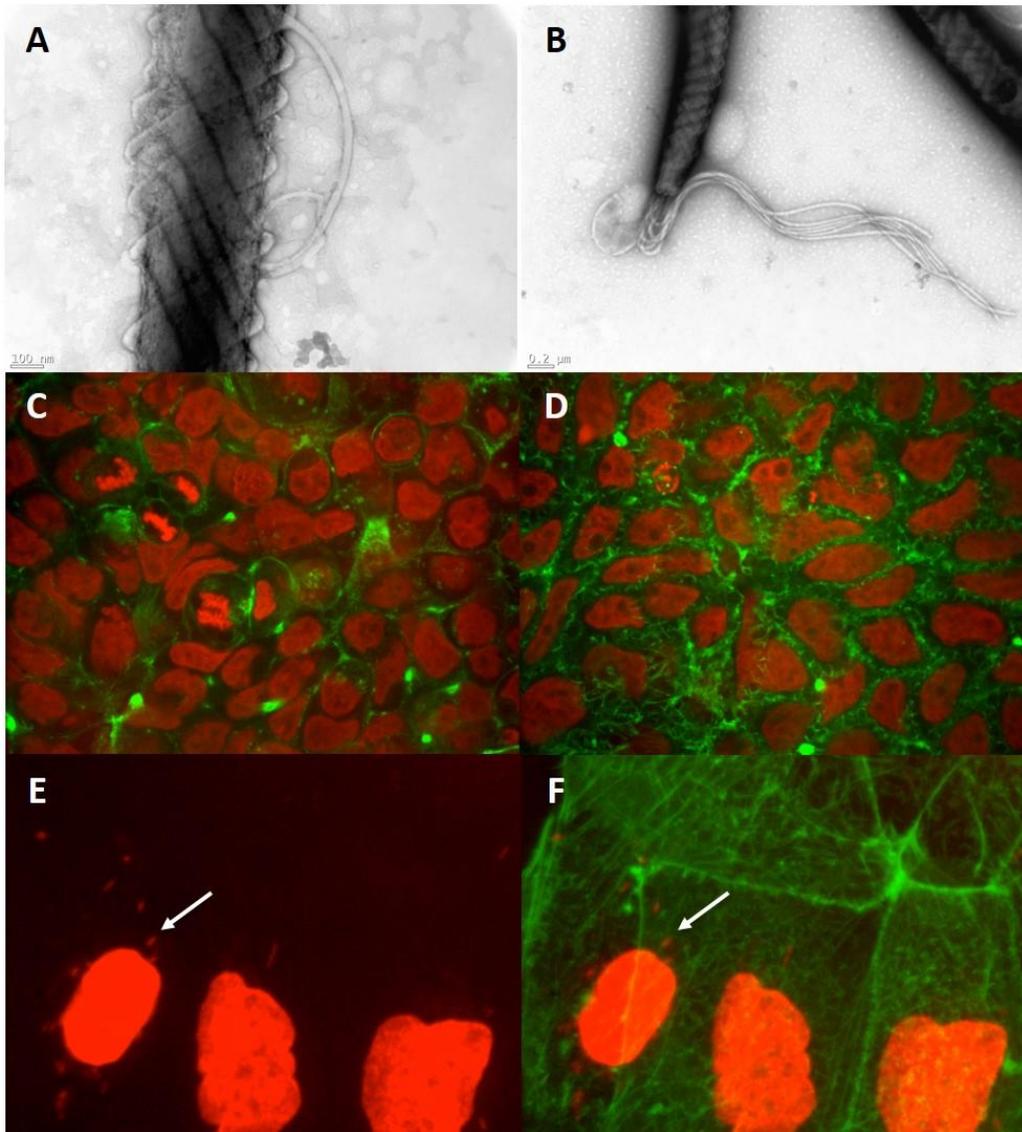
respectively, only 4 or 7 dividing cells/1,000 as opposed to 44 or 45/1,000 cells in the controls ($p < 0.001$, by χ^2 ; Supplemental Figure C, D). This effect, undescribed to date, may be involved during initiation or throughout the early stages of colitis (possibly preceding chronic IBD) as supported by the fact that Caco-2 cell cycle arrest was also observed with dextran sulfate sodium, an inducer of colitis in animal IBD models, and considering that *H. trogontum* is able to initiate IBD in a rodent model (7, ref. 3 cited in the article). Repeated intestinal surface damage and injury are considered to be prominent in diverse intestinal disorders, e.g., IBD. The intestinal epithelial cells have a high turnover rate, which could explain why cell cycle arrest may impede barrier functions and absorption (7). Extensive studies are necessary to further elucidate the role of NHPH in enteric diseases.

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References

1. Araoka H, Baba M, Kimura M, Abe M, Inagawa H, Yoneyama A. Clinical characteristics of bacteremia caused by *Helicobacter cinaedi* and time required for blood cultures to become positive. J Clin Microbiol. 2014;52:1519–22. PubMed <http://dx.doi.org/10.1128/JCM.00265-14>
2. Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. N Engl J Med. 1992;327:293–301. PubMed <http://dx.doi.org/10.1056/NEJM199207303270501>
3. Dewhirst FE, Shen Z, Scimeca MS, Stokes LN, Boumenna T, Chen T, et al. Discordant 16S and 23S rRNA gene phylogenies for the genus *Helicobacter*: implications for phylogenetic inference and systematics. J Bacteriol. 2005;187:6106–18. PubMed <http://dx.doi.org/10.1128/JB.187.17.6106-6118.2005>
4. Hänninen ML, Utriainen M, Happonen I, Dewhirst FE. *Helicobacter* sp. flexispira 16S rDNA taxa 1, 4 and 5 and Finnish porcine *Helicobacter* isolates are members of the species *Helicobacter trogontum* (taxon 6). Int J Syst Evol Microbiol. 2003;53:425–33. PubMed <http://dx.doi.org/10.1099/ijs.0.02389-0>
5. Whary MT, Danon SJ, Feng Y, Ge Z, Sundina N, Ng V, et al. Rapid onset of ulcerative typhlocolitis in B6.129P2–IL10tm1Cgn (IL-10–/–) mice infected with *Helicobacter trogontum* is associated with decreased colonization by altered Schaedler's flora. Infect Immun. 2006;74:6615–23. PubMed <http://dx.doi.org/10.1128/IAI.01091-06>
6. Hansen R, Thomson JM, Fox JG, El-Omar EM, Hold GL. Could *Helicobacter* organisms cause inflammatory bowel disease? FEMS Immunol Med Microbiol. 2011;61:1–14. PubMed <http://dx.doi.org/10.1111/j.1574-695X.2010.00744.x>

7. Araki Y, Sugihara H, Hattori T. In vitro effects of dextran sulfate sodium on a Caco-2 cell line and plausible mechanisms for dextran sulfate sodium-induced colitis. *Oncol Rep.* 2006;16:1357–62. [PubMed](#)



Technical Appendix Figure. Transmission electron micrograph of *Helicobacter trogontum* and Caco-2 cells challenged with *H. trogontum*. Transmission electron micrograph of a negatively stained preparation showing periplasmic fibers coiled around the protoplasmic cylinder (A) and 7 bipolar flagella (B). Representative image of Caco-2 cells with DNA (red, DAPI [4',6-diamidino-2-phenylindole] and actin (green, alexa488-phalloidin) staining. Control cells showing 3 dividing cells (C); Cells challenged with *H. trogontum* showing no division after 12 hours (D). Representative image showing the presence of *H. trogontum* (arrows) in Caco-2 cells. DNA (red, DAPI) staining (E); DNA and actin (green, alexa488-phalloidin) staining (F). Original magnification for panels C–F is $\times 60$.