



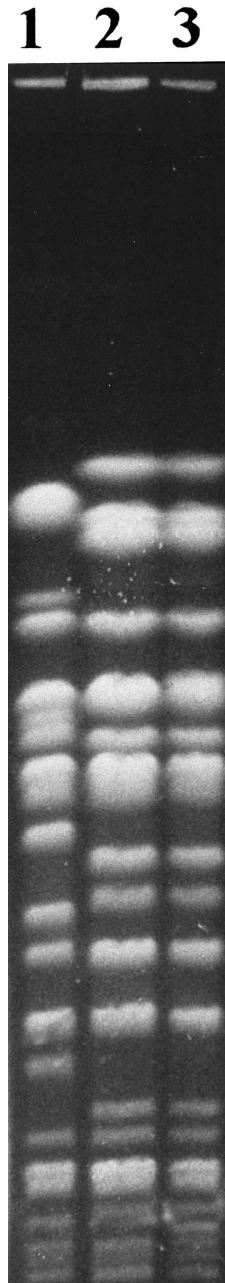
<b>Title</b>	<b>A unique strain of Escherichia coli O157:H7 that produces low verocytotoxin levels not detected by use of a commercial enzyme immunoassay kit</b>
<b>Author(s)</b>	<b>Yam, WC; Tsang, DNC; Que, TL; Peiris, M; Seto, WH; Yuen, KY</b>
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### A Unique Strain of *Escherichia coli* O157:H7 That Produces Low Verocytotoxin Levels Not Detected by Use of a Commercial Enzyme Immunoassay Kit

In June 1996, a food-borne outbreak due to *Escherichia coli* O157:H7 swept through Japan affecting more than 9,000 people [1]. This outbreak showed no sign of subsiding until late August 1996, and the government encountered major difficulties in controlling the outbreak. Because of the paucity of epidemiological information concerning this pathogen in Hong Kong, small outbreaks and sporadic cases of infection due to *E. coli* O157:H7 may go undiagnosed and unrecognized. To monitor the role of this pathogen in human disease in Hong Kong, including hemorrhagic colitis, uncomplicated diarrhea, and hemolytic uremic syndrome, screening for *E. coli* O157:H7 by use of MacConkey sorbitol agar was initiated in major hospitals in late 1996 [2]. We describe the first reported cases of infection due to *E. coli* O157:H7 in Hong Kong.

In July and October 1997, sorbitol-negative *E. coli* colonies were isolated from the fecal samples obtained from two patients with diarrhea at Queen Elizabeth Hospital and Tuen Muen Hospital. Serological evaluation of isolated colonies by use of latex agglutination assay (Remel, USA) confirmed *E. coli* O157:H7. However, strains from both patients were found to be nontoxicogenic by use of the Premier EIA for verotoxins I and II (Meridian Diagnostics, USA). Cytotoxicity on vero cells was demonstrated only when bacterial cells were treated with polymyxin B [3], and neutralization of cytotoxicity was also observed with use of verotoxin neutralizing antibodies (Meridian Diagnostics). Genes encoding verotoxin II were detected by use of a PCR assay, and subsequent DNA hybridization also identified both strains as verotoxin II producers [4]. In addition, both strains contained the *eae* gene, which is associated with the attaching-effacing ability evident on a fluorescent actin staining (FAS) assay and DNA hybridization using specific probe from recombinant plasmid pCVD434 as described previously [5]. Pulsed-field gel electrophoresis (PFGE) showed identical patterns for both strains when digested with restriction endonuclease *Xba*I (figure 1).

To our knowledge, we have described the first reported cases of infection due to *E. coli* O157:H7 in Hong Kong. Cytotoxicity is a major virulence factor of the pathogen, yet the commercial EIA kit failed to identify the verotoxins in the bacterial isolates from both patients. These false-negative results are probably attributable to the low level of extracellular toxins that are secreted by these two strains. Molecular diagnostic evaluation was a more effective approach for the identification of the pathogen in these two cases. Both strains possess the essential pathogenic attributes of *E. coli* O157:H7 that cause hemorrhagic colitis and hemolytic uremic syndrome. There was no epidemiological link between the two patients; the identical PFGE patterns may indicate the clonal



**Figure 1.** Pulsed-field gel electrophoresis (PFGE) of *Escherichia coli* O157:H7 strains with use of restriction *Xba*I endonuclease. Lane 1, control strain of *E. coli* O157:H7 from Public Health Laboratory Services (PHLS) (Colindale, UK). Lane 2, *E. coli* O157:H7 strain from Queen Elizabeth Hospital. Lane 3, *E. coli* O157:H7 strain from Tuen Muen Hospital.

spread of a unique strain of *E. coli* O157:H7 in our community, although no epidemics have been recorded.

W. C. Yam, D. N. C. Tsang, T. L. Que, M. Peiris,  
W. H. Seto, and K. Y. Yuen

Department of Microbiology, Queen Mary Hospital, The University of Hong Kong, Department of Pathology, Queen Elizabeth Hospital, and Department of Pathology, Tuen Muen Hospital, Hong Kong

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Reprints or correspondence: Dr. W. C. Yam, Department of Microbiology, The University of Hong Kong, Pokfulam, Hong Kong.

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### Meningitis in Adults Due to *Campylobacter fetus* Subspecies *fetus*

Campylobacteriosis is a zoonosis that occurs worldwide. Although the most common clinical manifestation is cryptogenic bacteremia, sites of localized infections have been described [1, 2]. Meningitis in adults due to *Campylobacter fetus* subspecies *fetus* is a rare entity and, to our knowledge, only seven cases have been reported in the French-, Spanish-, and English-language literature since 1983 [3-7]. We describe an additional case of meningitis due to *C. fetus* subspecies *fetus* in a patient with chronic alcoholism.

A previously healthy, 47-year-old man with chronic alcoholism (250 g alcohol per day) presented to the emergency department for evaluation of fever and pretibial cellulitis of 1 week's duration. Blood was drawn for two cultures. The patient was discharged on amoxicillin/clavulanate for 10 days, and his condition partially improved. Two weeks later (on 21 April 1997) the patient developed a high-grade fever (temperature, 39°C to 39.5°C), malaise, nausea, vomiting, photophobia, and a severe frontal headache. Findings on physical examination were notable for a temperature of 38°C, normal mentation, neck stiffness, and mild hepatomegaly. The patient indicated that he had two dogs and one cat, but he denied exposure to raw or undercooked meat or to unpasteurized milk.

The patient's WBC count was 9,700/mm<sup>3</sup> with a normal differential. Serologies for antibodies to HIV and hepatitis C virus were negative; however, there was evidence of previous infection with hepatitis B, given that serologies for antibodies to hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) were positive. A contrast-enhanced CT of the head was normal. Specimens for two additional blood cultures were obtained. The patient underwent a lumbar puncture, and evaluation of CSF revealed the following values: glucose, 3.2 mmol/L (7.2 mmol/L in blood); protein, 0.85 g/L; WBCs, 300/μL (80% neutrophils); and no RBCs. Evaluation of the CSF was negative for microorganisms. Empirical antibiotic therapy with a combination of cefotaxime, for 14 days, and vancomycin, for 3 days, was instituted. Cultures of blood (four specimens, the first and last of which were drawn 13 days apart) yielded *C. fetus* subspecies *fetus*. A culture of CSF remained negative. The patient's condition gradually improved, and he remained afebrile and was discharged.

Subsequently, the patient developed malaise, low-grade fever, and a mild frontal headache. A fourth lumbar puncture was performed, and a CSF specimen inoculated in two blood culture bottles (in a microaerobic environment at 25°C and 35°C) yielded pure growth of *C. fetus* subspecies *fetus* identified by use of API-Campy (bioMérieux, Cedex, France; API code no. 2400714) and PCR assay (Centers for Disease Control and Prevention [CDC], Atlanta). The MICs of the following antibiotics for the isolate were: penicillin, 94 μg/mL; cefotaxime, >32 μg/mL; ceftazidime, 128 μg/mL; nalidixic acid, >256 μg/mL; erythromycin, 0.75 μg/mL; ofloxacin, 1 μg/mL; gentamicin, 1 μg/mL; amoxicillin/clavulanate, 1 μg/mL; and imipenem, 0.064 μg/mL. Therapy with ofloxacin (400 mg t.i.d. for 4 weeks) and gentamicin (240 mg q.d. for 3 weeks) was instituted. On day 21 of therapy, the following levels of antibiotics were measured in blood/CSF 2 hours after administration: ofloxacin, 3.9/2.3 μg/mL; gentamicin, 5.68/0.36 μg/mL. Endocarditis was ruled out. The patient's condition gradually improved; there were no neurological sequelae, and results of all laboratory evaluations were normal. Nine months after treatment the patient continues to be healthy and asymptomatic.

A MEDLINE search (from 1983 to July 1997) for reports of meningitis in adults due to *C. fetus* subspecies *fetus* identified only seven cases (table 1) [3-7]. All patients had underlying predisposing conditions, chronic alcoholism and alcoholic cirrhosis being the two most common (six cases). Six patients had positive CSF cultures and five of eight patients had associated bacteremia. A history of ingestion of raw food or exposure to domestic or farm animals may help determine the diagnosis. One Moroccan patient had a cat, although *Campylobacter* species were not recovered from the animal's feces [4]. Another patient had traveled to Mexico to receive nutritional therapy, and this travel history might have contributed to the clinical illness [5]. The patient we described had two dogs and one cat, and they were not examined for carriage of fecal microorganisms, but they were the most likely origin of infection. This information was not available for the remaining five cases.

Community-acquired bacterial meningitis due to gram-negative bacilli is rare. *C. fetus* subspecies *fetus* is an uncommon human pathogen, particularly in association with meningitis. Some investigators have pointed out the paucity of *C. fetus* isolates recovered from humans; only 22 of 394 *Campylobacter* species isolates were identified in blood cultures during an 11-year period [2]. None of the isolates were identified in CSF. Classically, *C. fetus* has been differentiated from *Campylobacter jejuni* and other species on the basis of its sensitivity to cephalotin, resistance to nalidixic acid, and inability to grow at 42°C; however, it is currently accepted that a few strains may grow at 42°C and may be resistant to cephalotin [8, 9]. Kwon et al. [9], in a study of the antimicrobial susceptibility of 25 isolates of *C. fetus* subspecies *fetus* recovered from blood and synovial fluid samples, reported that a significant proportion of isolates were interpreted as intermediate or resistant

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Reprints or correspondence: Dr. Fernando Dronda, Enfermedades Infecciosas (Servicio de Microbiología Clínica), Hospital Ramón y Cajal, 28034 Madrid, Spain.

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