

## Short Communication

# An Outbreak of Cryptosporidiosis Suspected to be Related to Contaminated Food, October 2006, Sakai City, Japan

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**SUMMARY:** On October 17, 2006, the Sakai City Public Health Center received a report of acute gastroenteritis among 4 members from the same company who had eaten raw meat dish called "Yukke: Korean-style beef tartar" and raw liver at a rotisserie in Sakai City on October 7. Based on information from interviews, the median incubation period was 5.5 (range, 5-7 days), and the median length of illness was 7 days (range, 4-10 days). The illness was characterized by a prolonged incubation period, non-bloody watery diarrhea, reduced vomiting, and light fever, which led us to suspect an enteric protozoan infection. Stool specimens obtained from 3 of the 4 symptomatic patients were positive for *Cryptosporidium* oocysts. They, along with 2 food workers, were negative for food poisoning bacteria or Norovirus. Genotyping of the *Cryptosporidium* isolates by direct sequencing of PCR products revealed that all the isolates were the *C. parvum* genotype II (bovine) and the subgenotype of IIa with 100% homology with respective 18S rRNA and *Cpgp40/15* genes. Positive implementation of tests for enteric protozoa including *Cryptosporidium* is necessary in the differential diagnosis of suspected foodborne gastroenteritis, particularly when it is characterized by a prolonged incubation period and severe watery diarrhea. In fact, we were able to diagnose the illness as cryptosporidiosis without waiting for the results of bacteriological and virological examinations, and thus prevented the possible occurrence of a secondary infection through an ill patient who works as cooking personnel in the company.

*Cryptosporidium*, an apicomplexan protozoan parasite, is a causative agent of human and animal gastrointestinal illness worldwide. This parasite causes a severe but self-limiting diarrhea in immunocompetent hosts, and the infection can be life-threatening in immunosuppressed individuals such as AIDS patients. Infection is via fecal-oral transmission, which can occur by consuming contaminated drinking water, raw or undercooked food handled by a person ill with *Cryptosporidium*, by direct contact with an infected animal, or by exposure to contaminated recreational water (1-4).

On October 17, 2006, the Sakai City Public Health Center received a report of acute gastroenteritis among 4 members of a group from the same company who ate a raw meat dish called "Yukke: Korean-style beef tartar" and raw liver at a rotisserie in Sakai City on October 7. Symptoms included non-bloody watery diarrhea, and the frequency of bowel movements varied from 8 to 15 times a day, and were accompanied by abdominal cramps, headache, light fever/chills, exhaustion, and nausea with/without vomiting. Based on information from interviews, the median incubation period was 5.5 days (5-7 days), and the median duration of illness was 7 days (4-10 days).

The illness was characterized by a prolonged incubation period, profuse non-bloody, watery diarrhea, and reduced vomiting, which led us to suspect an enteric protozoan infection. *Cryptosporidium* oocysts were detected in stool samples from 3 of the 4 ill patients collected on October 18 by the sucrose centrifugal flotation method followed by immunofluorescent staining and 4',6-diamino-2-phenylindole (DAPI). The remaining one ill person was also confirmed to be posi-

tive for *Cryptosporidium* by other diagnostic laboratory tests. All samples were negative for bacterial pathogens and Norovirus by the routine tests conducted thereafter due to suspicion of food poisoning. For further identification, genotyping of the isolates by direct sequencing of PCR products were made for the 18S rRNA gene and *Cpgp40/15* gene. Briefly, oocysts were isolated from fecal specimens by the sucrose centrifugal flotation method. After washing in PBS twice, each of 40  $\mu$ l of concentrated oocyst suspension was transferred to 100  $\mu$ l of the lysis buffer (10 mM Tris-HCl, 50 mM KCl, 0.5% Tween 80). The suspensions were frozen (-80°C) and thawed (37°C) 15 times, and incubated at 100°C for 15 min to release DNA from the oocysts. They were then treated with a GENECLEAN KIT (Q-BIOgene, Carlsbad, Calif., USA) to obtain the template DNAs for PCR. A pair of primers (5'-AAG CTC GTA GTT GGA TTT CTG-3' and 5'-TAA GGT GCT GAAGGA GTA AGG-3') designed by other workers was used for amplification of the 18S rRNA gene (5). The condition used for the DNA amplification was 98°C 5 min, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. This was followed by extension at 72°C for 7 min. The *Cpgp40/15* gene was amplified by PCR using the primers (5'-ATG AGA TTG TCG CTC ATT-3' and 5'-TTA CAA GAA TAA GGC-3') previously published (6). The conditions used for DNA amplification were 95°C for 2 min, followed by 45 cycles of 95°C for 40 s, 49°C for 50 s, and 72°C for 1 min. This was followed by extension at 72°C for 10 min. If PCR products were insufficient for sequencing using the first set of primers, a second PCR was performed using primers (5'-CCG TTA TAG TCT CCG CTG TA-3' and 5'-AAA GCA GAG GAA CCG GCA T-3') according to the method by Wu et al. (7). The PCR products were purified using NucleoSpin Extract II (MACHERY-NAGEL, Düren, Germany) and directly sequenced by the dye-terminator

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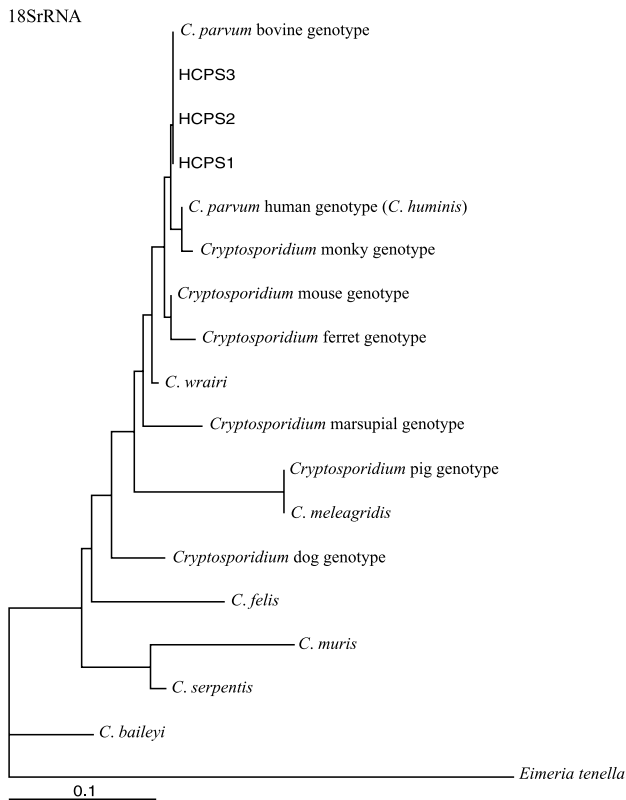


Fig. 1. Phylogenetic relationship of the 18SrRNA gene of *Cryptosporidium* parasites found in Sakai City outbreak (HCPS1, HCPS2, HCPS3). The GenBank accession number for the genotypes and *Cryptosporidium* spp. are follows : bovine genotype (AF108864), human genotype (AF108865), monkey genotype (AF112569), mouse genotype (AF112571), ferret genotype (AF112572), marsupial genotype (AF112570), pig genotype (AF115377), dog genotype (AF112576), *C. meleagridis* (AF112574), *C. wrairi* (AF115378), *C. baileyi* (L19068), *C. felis* (AF108862), *C. serpentis* (AF108866), *C. muris* (X64342).

method using an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer, Foster City, Calif., USA). The PCR products of the *Cpgp40/15* gene were sequenced at the National Institute of Infectious Diseases, Japan. Homology searching of the nucleotide database was carried out using the BLAST program, and phylogenetic analysis was made using the ClustalW program at the DNA Data Bank of Japan.

All of the present *Cryptosporidium* isolates were identified as the *Cryptosporidium parvum* genotype II (GenBank accession number AF108864) by genetic analysis for the 18S rRNA gene and as genotype IIa (GenBank accession number AY167592) for the *Cpgp40/15* gene (6,7), respectively. They showed 100% homology among samples, suggesting that this outbreak was due to *C. parvum* of a single origin (Figs. 1, 2).

Considering the incubation period of 5-7 days (Table 1) of this disease, either the Yukke or raw liver consumed on October 7 were suspected to be the possible source of infection, although available data were inadequate to conclusively implicate either food in this infection. The patients had no history of overseas trips. The rotisserie concerned had been using piped water from a public water service, and has no well water facility. Gastrointestinal illness was not reported among 19 of other 10 groups of restaurant visitors who ate at the same rotisserie on the same day. No complaint was registered against 3 other rotisseries that purchased raw liver from the same meat supplier as the rotisserie concerned. Other common food sources of this outbreak could have been from

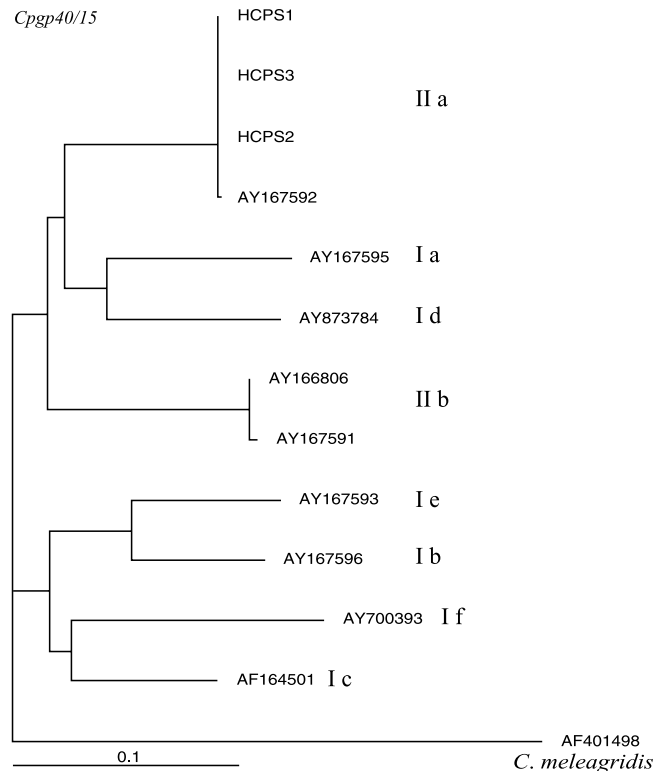


Fig. 2. Phylogenetic relationship of the *Cpgp40/15* gene of *Cryptosporidium* parasites found in the Sakai City outbreak (HCPS1, HCPS2, HCPS3).

Table 1. Patient information

	Patient no. 1	2	3	4
Sex	female	male	female	male
Age	32	25	29	26
Onset date	12-Oct	13-Oct	14-Oct	12-Oct
Onset time	19:00	6:00	22:00	23:00
Length of illness (days)	7	4	10	7
Abdominal pain	○	○	○	○
Diarrhea	watery	watery	watery	watery
Frequency of diarrhea	10	8	15	10
Nausea	○	○	×	×
Frequency of vomiting	1	×	×	×
Fever (°C)	37.2	37	×	38
Headache	×	○	×	○
Chill	×	○	×	×
Fatigue	○	×	○	○
Exhaustion	○	○	○	○
Paralysis	×	×	×	○
Convulsion	○	×	×	○
Incubation period (days)	5	5.4	7.1	5.1

the lunch delivered by a food deliveries service. The same lunch was consumed by around 100 employees at the same workplace on October 7, including the 4 patients, but there was no evidence of gastrointestinal illness among the other workers, providing that the lunch had not been the source of the infection.

Some food-borne outbreaks of cryptosporidiosis have been reported in the United States (4). Thus, positive implementation of stool examination for protozoan pathogens should be considered in the differential diagnosis of suspected foodborne gastroenteritis, particularly when it is characterized by a prolonged incubation period and severe watery

diarrhea. In fact, we were able to diagnose the illness as cryptosporidiosis without delay, and thus prevented the possible occurrence of a secondary infection via raw or undercooked food handled by one of the patients, who works as cooking personnel in the company.

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