Cryptosporidium sp. Rabbit Genotype, a Newly Identified Human Pathogen

To the Editor: Most human cases of cryptosporidiosis are caused by Cryptosporidium parvum or C. hominis, but pathogenicity of some unusual Cryptosporidium species/genotypes is uncertain (1). In July 2008, an outbreak caused by Cryptosporidium sp. rabbit genotype was linked to consumption of tap water in Northamptonshire, England (2). On June 23 and 24, Cryptosporidium oocysts were detected by operational monitoring of treated water at a surface water treatment works. A precautionary boil-water notice was implemented on June 25.

Enhanced surveillance for cases was established by the health protection team on June 25 in the affected area. Eight single-well immunofluorescent microscopy slides, on which oocysts were detected by water company sampling of the distribution system, were sent to the UK Cryptosporidium Reference Unit, Swansea, for typing. Slides contained 49–197 oocysts, and the rabbit isolate was from a rabbit carcass removed by the water company from a tank at the water treatment works. Oocysts were separated from fecal debris by flotation, resuspended in reverse osmosis water, and processed as above.

Cryptosporidium species were identified by bidirectional sequencing of PCR products generated by nested PCR for the small subunit (SSU) rRNA gene (3) from 4 DNA aliquots of each sample. SSU rDNA sequences from 7 water samples, containing 49–197 oocysts, and the rabbit isolate were homologous with isolates from rabbits in the People’s Republic of China (6) and the Czech Republic (7) (GenBank accession nos. AF120901 and AY273771, respectively) (online Appendix Table, available from www.cdc.gov/EID/content/15/5/829-620.htm). One sample from 1,391 L of water contained 259 oocysts but was not amplified. Other cryptosporidia were not identified.

Human stool samples from 34 local laboratory-identified cases of cryptosporidiosis in the affected area were sent to the UK Cryptosporidium Reference Unit for typing. To differentiate rabbit genotype from C. hominis (1), enhanced typing by SSU rRNA nested PCR–restriction fragment length polymorphism analysis with Sspl (1.5) was used for all isolates submitted to the UK Cryptosporidium Reference Unit during July and August. Samples from 23 cases (22 primary and 1 secondary) with rabbit genotype profiles were identified by visualization of 472-, 267-, and 109-bp bands generated by digestion with Sspl (1). All case-patients lived in the area affected by the water supply incident and had onset dates consistent with exposure by drinking water consumption or by person-to-person spread. All 23 samples were homologous to AF120901 and AY273771 (online Appendix Table). Of the other 11 samples, 6 were not confirmed by IFAT or PCR, 2 were C. hominis, 1 was C. parvum, and 2 were not typeable.
Sequences of the heat shock protein (HSP) 70 gene (HSP70) and, to identify subtype family, the 60-kDa glycoprotein (gp60) gene (HSP70) were determined for 7 water isolates and the rabbit and 9 outbreak case isolates. All HSP70 sequences were homologous with AY273775 from a rabbit in the Czech Republic (7) (online Appendix Table). One water sample, the rabbit sample, and 8 human samples amplified the gp60 gene. These sequences were homologous with each other, but distinct from those published for C. hominis (subtype family I), C. parvum (subtype family II), C. meleagridis (subtype family III), and C. fayeri (subtype family IV) (10). Each rabbit genotype isolate had 18 TCA (serine) tandem repeats in the gp60 microsatellite region. We propose subtype family Va, subtype A18 for these isolates. This subtype differs from the rabbit genotype previously identified in a human in the United Kingdom (1) (subtype VaA22) (GenBank accession no. EU437420) and from rabbits in the Czech Republic (subtype VbA19) and China (subtype VbA29). Sequences generated during this study have been deposited in GenBank under accession nos. FJ262724–FJ262734.

Six additional persons infected with Cryptosporidium sp. rabbit genotype were identified by testing 394 stool samples that were routinely submitted for typing from diarrheic patients in July and August from throughout the UK. All persons had onset dates inconsistent with the affected period and were from other regions of the UK. This finding may indicate a low background level of rabbit genotype cases; however, prevalence is currently unknown.

The Cryptosporidium rabbit genotype has been identified as the etiologic agent in an outbreak of diarrheal disease and should be considered a human pathogen. Further studies commissioned by the Drinking Water Inspectorate (England and Wales) and funded by the Department of Environment, Food and Rural Affairs UK are underway.

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