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 49–259 oocysts. Coverslips were removed after softening the seal with nail polish remover. Fixed material was resuspended in 3 dry ice/methanol freeze-thaw cycles, and DNA was extracted by using the QIAamp DNA Mini Kit (QIAGEN, Crawley, UK) and had onset dates consistent with consumed. Oocysts were separated from fecal debris by flotation, resuspended in reverse osmosis water (4), 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 rRNA 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. AY120901 and AY273771, respectively) (online Appendix Table, available from www.cdc.gov/EID/content/15/5/829-apt.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 and Vspl (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 AY120901 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.

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Sequences of the heat shock protein (HSP) 70 gene (HSP70) were homologous with each other, and 9 outbreak case isolates. All HSP70 sequences were homologous with AY273775 from a rabbit in the Czech Republic (online Appendix Table). One water sample, the rabbit genotype previously identified in the environment [thesis]. Cardiff (UK): University of Wales College of Medicine; 2005.

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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References


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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article’s publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.
### Appendix Table. Location of nucleotide differences in the partial small subunit rRNA and heat shock protein 70 genes between Cryptosporidium hominis and the rabbit genotype*

<table>
<thead>
<tr>
<th>Isolate (GenBank accession no.)</th>
<th>Location of nucleotide differences in the partial small subunit rRNA gene (nt 618–681)</th>
<th>Location of nucleotide differences in the partial heat shock protein gene (nt 716–792)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. hominis (AY204228)</td>
<td>TAAATTATATAAATTTATTTGTAATTTATTTCTAAATTTTTTTTTAGTTAT</td>
<td>GACTCGTGGAATTCTGTGTACAAGATTTCAAGAGAAAGAATAGAGGTATGGATTTAACTTCAAATGCTAGAGCTTTTA</td>
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<tr>
<td>Rabbit genotype (AY273771)</td>
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<tr>
<td>Rabbit genotype (AY120901)</td>
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<tr>
<td>Rabbit genotype from rabbit sample #17211</td>
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<tr>
<td>Rabbit genotype from water sample #17200</td>
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<tr>
<td>Rabbit genotype from human sample #17330</td>
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</table>

* Dots indicate nucleotide identity with the C. hominis sequence from GenBank, and dashes indicate nucleotide deletions.