Cryptosporidium Pig Genotype II in Immunocompetent Man

To the Editor: Protozoan parasites from the genus Cryptosporidium have been described as a cause of diarrheal disease in immunodeficient and immunocompetent humans worldwide. Although C. hominis and C. parvum (cattle genotype) cause most infections, humans can be infected by several other Cryptosporidium species or genotypes: C. meleagridis; C. felis; C. canis; C. suis; C. muris; C. andersoni; C. hominis monkey genotype; C. parvum (mouse genotype); and Cryptosporidium rabbit genotype, deer genotype, skunk genotype, horse genotype, and chipmunk genotype I (1–4). Wild and domestic animals are sources of infection for humans (and other animals) and important contributors to contamination of food and drinking water; many nonhuman Cryptosporidium species or genotypes are detected in untreated water (5). We examined the diversity of Cryptosporidium spp. in immunocompetent persons in South Bohemia in the Czech Republic.

Diarrheal fecal samples (n = 457) from 203 anonymous immunocompetent patients ≤69 years of age with suspected cryptosporidiosis (at least 2 samples/patient/3-day period) were obtained from local health departments and public hospitals in South Bohemia during 2005–2007. Samples were examined for Cryptosporidium oocysts by using aniline-carbol-methylene blue staining and light microscopy at × 1,000 magnification (6). The microscopically positive samples were confirmed by DNA sequencing of the small subunit (SSU) rRNA gene. Total DNA was extracted from 200–300 mg stool by using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer’s instructions, after previous homogenization and disruption of oocysts with the Mini-BeadBeater (Biospec Products, Bartlesville, OK, USA). An 830-bp fragment of the SSU rRNA gene was amplified by nested PCR according to Jiang et al. (7). Purified PCR products were sequenced in both directions on an ABI3130 sequencer analyzer (Applied Biosystems, Foster City, CA, USA) by using the secondary PCR primers and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were assembled by using Chromas Pro (www.techneleyum.com/au/chromas.html) and aligned with reference sequences using ClustalX (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX). The BLAST server (www.ncbi.nlm.nih.gov/BLAST) was used for DNA database searches. The SSU rRNA gene partial sequences of the 7 patient isolates have been submitted to GenBank (Table).

Of the 203 patients, 7 (3.4%) (6 children and 1 adult) had positive results for Cryptosporidium spp. Moreover, all samples obtained from these persons during the 3-day period were Cryptosporidium spp. positive; partial sequences of the Cryptosporidium SSU rRNA gene were obtained from all positive samples identifying 3 different species or genotypes of Cryptosporidium. Five were C. parvum (bovine genotype), 1 was C. hominis, and 1 contained the Cryptosporidium pig genotype II (Table). Cryptosporidium pig genotype II was found in stool samples from a 29-year-old man who also was infected with Giardia intestinalis (assemblage A) (data not shown).

Only C. parvum (bovine genotype), C. hominis, and Cryptosporidium rabbit genotype have been implicated in waterborne outbreaks of cryptosporidiosis in humans. Further studies are needed to determine the potential of other cryptosporidia of animal origin. Recent genetic and biologic characterization studies have identified 2 distinct host-adapted cryptosporidia in pigs, C. suis and Cryptosporidium pig genotype II. Furthermore, both above-mentioned cryptosporidia have been identified in untreated water (8). Pigs could be sources of Cryptosporidium water and food pollution and a consequent risk to public health.

Although human infection with C. suis has been previously described (9), human infection with Cryptosporidium pig genotype II has been never reported. This genotype was found in diarrheal stool of 1 adult patient in this study. However, onset of diarrhea could have been caused by co-infection with G. intestinalis (assemblage A), which recently also has been described in pigs (10). Contact with infected animals and ingestion of contaminated food or water could be
## Table. Cryptosporidium genotypes identified by using sequencing of partial sequences of the small subunit rRNA gene in the stool samples of immunocompetent humans, Czech Republic

| Patient no. | Age, y/sex | Examination year | Cryptosporidium species/genotype | Infection intensity* | GenBank accession no. |
|-------------|------------|------------------|---------------------------------|---------------------|----------------------|
| H15         | 9/M        | 2005             | C. parvum†                      | 56                  | EU331237             |
| H23         | 10/M       | 2005             | C. hominis                       | 77                  | EU331242             |
| H98         | 10/F       | 2005             | C. parvum†                      | 43                  | EU331238             |
| H101        | 11/M       | 2006             | C. parvum†                      | 11                  | EU331239             |
| H132        | 8/M        | 2006             | C. parvum†                      | 150                 | EU331240             |
| H158        | 11/M       | 2007             | C. parvum†                      | 26                  | EU331241             |
| H199        | 29/M       | 2007             | Cryptosporidium pig genotype II | 38‡                 | EU331243             |

*Numbers of oocysts per 30 fields at ×1,000 magnification, unless otherwise indicated.
†Bovine genotype.
‡Numbers of oocysts per whole slide at ×1,000 magnification.

the source of both Cryptosporidium and Giardia infection in the Cryptosporidium pig genotype II–positive patient. The passage of oocysts can be excluded because of the number of oocysts detected in repeat samples (Table). Moreover, identification of the infection in an immunocompetent patient underlines the zoonotic potential of this pig genotype and possible presence of risk factors in rural areas with poor water treatment or inadequate biosecurity in pig units. Further evidence of the zoonotic potential of this Cryptosporidium genotype is needed to show its pathogenic potential in immunocompetent patients as a cause of gastroenteritis (in the absence of Giardia spp. and other established enteropathogens) and to demonstrate invasive tissue stages. The use of molecular techniques to identify Cryptosporidium spp. probably will show more zoonotic species or genotypes in humans.

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## Crimean-Congo Hemorrhagic Fever, Southwestern Bulgaria

To the Editor: Crimean-Congo hemorrhagic fever virus (CCHFV) causes a severe multisystem disease characterized by profuse bleeding with a case-fatality rate as high as 30%. The infection is endemic to the Balkans (1,2). In Bulgaria, most cases are reported from the central and eastern parts of the country (3,4). We report a cluster of cases observed in early spring 2008 in southwestern Bulgaria, an area considered at low risk for CCHF outbreaks.