Subtype Analysis of Cryptosporidium Specimens from Sporadic Cases in Colorado, Idaho, New Mexico, and Iowa in 2007: Widespread Occurrence of One Cryptosporidium hominis Subtype and Case History of an Infection with the Cryptosporidium Horse Genotype

Lihua Xiao,1* Michele C. Hlavsa,1 Jonathan Yoder,1 Christina Ewers,2 Theresa Dearen,1 Wenli Yang,1 Randall Nett,1,3 Stephanie Harris,4 Sarah M. Brend,3 Meghan Harris,5 Lisa Onischuk,2 Amy L. Valderrama,1 Shaun Cosgrove,6 Karen Xavier,6 Nancy Hall,5 Sylvia Romero,7 Stephen Young,7 Stephanie P. Johnston,1 Michael Arrowood,1 Sharon Roy,1 and Michael J. Beach1

Centers for Disease Control and Prevention, Atlanta, Georgia 303411; New Mexico Department of Health, Santa Fe, New Mexico 875022; Idaho Department of Health and Welfare, Boise, Idaho 837203; EPA Region 10 Laboratory, Port Orchard, Washington 983664; Iowa Department of Public Health, Des Moines, Iowa 503195; Colorado Department of Public Health and Environment, Denver, Colorado 802466; and Tricore Reference Laboratories, Albuquerque, New Mexico 871027

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Subtyping was conducted in late 2007 on 57 Cryptosporidium specimens from sporadic cases in Colorado, Idaho, New Mexico, and Iowa. One previously rare Cryptosporidium hominis subtype was indentified in 40 cases (70%) from all four states, and the Cryptosporidium horse genotype was identified in a pet shop employee with severe clinical symptoms.

The protozoan pathogen Cryptosporidium continues to be an important cause of waterborne disease outbreaks and gastrointestinal illness in the United States (4, 6, 21–23). While few recent cryptosporidiosis outbreaks have been associated with drinking water, the incidence of recreational water-associated outbreaks is increasing. In the United States, specimens from patients with outbreak-related cases have been extensively genotyped and subtyped specimens from sporadic cases in Colorado, Idaho, New Mexico, and Iowa revealed no epidemiologic links between cases (13). Nevertheless, investigations of the sporadic Colorado, Idaho, New Mexico, and Iowa cases revealed no epidemiologic links with any known outbreaks of cryptosporidiosis. These states, and the United States as a whole, reported increased numbers of sporadic cryptosporidiosis cases during 2007 (2). We also report on a symptomatic case of human infection with the Cryptosporidium horse genotype.

Specimens and laboratory and epidemiological investigations. A total of 57 Cryptosporidium-positive stool specimens were collected from patients with sporadic cryptosporidiosis, including 5, 10, 11, and 31 specimens from New Mexico, Iowa, Colorado, and Idaho residents, respectively. During 2007, totals of 125, 610, 211, and 464 laboratory-confirmed cases were reported in New Mexico, Iowa, Colorado, and Idaho, respectively, and 11,170 cases were reported in the country (2). Only one specimen per patient was used in the study. Specimens were confirmed to be Cryptosporidium positive by submitting laboratories using direct immunofluorescence microscopy or enzymatic immunoassays.

Cryptosporidium DNA was extracted from 0.2 ml of each stool specimen, as described previously (7). Parasite DNA was genotyped by nested PCR-restriction fragment length polymorphism (RFLP) analysis of the small-subunit (SSU) rRNA gene (8). PCR products of the Cryptosporidium horse genotype were sequenced to verify the identification. All Cryptosporidium spp. were further subtyped by DNA sequencing of the 60-kDa glycoprotein (gp60) gene amplified by a nested PCR (1), using modified primary PCR primers LX0374 (5'-TTA CTC TCC GTT ATA GTC TTC-3') and LX0375 (5'-GGA AGG AAC GAT GTA TCT GA-3'), which reduced the overlapping sequence shared between primary and secondary forward primers. To confirm the results, analysis at each locus was repeated at least three times using 2 µl of DNA per PCR. The established subtype nomenclature was used in identifying subtype families and subtypes (15).

The patient infected with the Cryptosporidium horse geno-
type was interviewed via telephone by a state-based epidemiologist to collect clinical and exposure data, using a structured questionnaire. 2.5 months after the initial cryptosporidiosis diagnosis. Informed consent was obtained from the adult patient before the interview.

**Distribution of Cryptosporidium species/genotypes and Cryptosporidium hominis and C. parvum subtypes.** PCR-RFLP analysis of the SSU rRNA gene identified *C. hominis* in 51 stool specimens and *C. parvum* in 5. PCR amplicons from a specimen obtained in New Mexico showed an RFLP banding pattern nearly identical to that of the previously reported Cryptosporidium horse genotype (14). DNA sequencing of the gp60 gene confirmed the identification of *C. hominis* and *C. parvum* in the stool specimens (Table 1). The *C. hominis* specimens belonged to five subtypes in two subtype families, whereas the *C. parvum* specimens belonged to four subtypes in the well-recognized zoonotic subtype family Ila. The most commonly identified subtype was the *C. hominis* subtype IlaA25R4, which was found in 40 specimens (70%) from all four states sampled. Other *C. hominis* and *C. parvum* subtypes were each found only in one or two specimens (Table 1).

**Characteristics of the Cryptosporidium horse genotype.** The partial SSU rRNA and gp60 genes of the Cryptosporidium horse genotype from the human case were sequenced, and the partial SSU rRNA and gp60 genes of the *Cryptosporidium* identified subtype was the recognized zoonotic subtype family IIa. The most commonly reported subtype was the *C. parvum* subtype IaA25R4, which was found in 40 specimens (70%) from all four states sampled. Other *C. hominis* and *C. parvum* subtypes were each found only in one or two specimens (Table 1).

**Public health significance of the Cryptosporidium horse genotype.** The Cryptosporidium horse genotype has been identified in only one person (a recently reported case), a 30-year-old immunocompetent woman with diarrhea in a rural area of southwest England (3, 12). Findings of unusual Cryptosporidium species/genotypes in humans have been reported occasionally, such as *C. andersoni*, *C. muris*, *C. suis*, and Cryptosporidium of the cervine, skunk, and chipmunk I genotype families (10, 12, 20). Recent reviews have identified at least 85 cases of *C. felis*, 24 cases of *C. canis*, and 21 cases of the Cryptosporidium cervine genotype infections in immunocompetent and immunocompromised persons around the world, in addition to the three common Cryptosporidium spp., as follows: *C. hominis*, *C. parvum*, and *C. suis*.
parvum, and C. meleagridis (17, 19). Reports of human infections caused by other Cryptosporidium spp. were identified in only one to a few cases. The Cryptosporidium horse genotype represents another among the increasing number of unusual Cryptosporidium species and genotypes identified in human stool specimens.

The pathogenicity of the unusual Cryptosporidium spp. infecting humans is generally unclear, as most reports lack detailed data on the clinical history of the affected patients. Detailed clinical manifestations of C. muris and C. suis infections have been reported for only two persons, both human immunodeficiency virus-positive adults in Lima, Peru (11, 18). The patient infected with the Cryptosporidium horse genotype had no recognized immunocompromising conditions. Nevertheless, she experienced severe clinical symptoms consistent with cryptosporidiosis and required emergency room care, including intravenous fluids, repeated antimicrobial prescriptions, and sick leave from work. No detailed clinical data are available on the human case of infection with the Cryptosporidium horse genotype in England, although the patient was assumed to have diarrhea (12).

The source of the Cryptosporidium horse genotype infection is not clear, although results from the traditional epidemiologic investigation indicate that it was probably of animal origin. Because the patient had no contact with horses and was in close contact with various animals at work and home, it was impossible to identify the animal species involved in this probable case of zoonotic transmission of Cryptosporidium. Few studies have investigated the infection source associated with unusual Cryptosporidium species and genotypes in humans. The Peruvian infected with C. suis had no exposure to pigs or other farm animals. In that case, anthroponotic rather than

FIG. 1. Phylogenetic relationship among major Cryptosporidium gp60 subtype families inferred by a neighbor-joining analysis of nucleotide sequences, using a sequence alignment generated by the ClustalX 1.81 package and the Kimura two-parameter genetic distances calculated by the Treecon W program. I, C. hominis subtype families; II, C. parvum subtype families; III, C. meleagridis subtype families; V, rabbit genotype subtype families; VI, horse genotype subtype families. The equine and bovine specimens of the Cryptosporidium horse genotype belong to subtype family VIa, while the human specimen of that genotype belongs to subtype family VIb.

FIG. 2. Oocysts of C. parvum (A), C. hominis (B), and the Cryptosporidium horse genotype (C) under differential interference contrast (magnification of ×1,000) on a Zeiss Axiophot microscope (Carl Zeiss MicroImaging Inc., Thornwood, NY).
zoonotic transmission was a strong possibility, as the patient was a homosexual man with multiple sex partners and participated in anal intercourse (18). The person infected with the Cryptosporidium horse genotype in England reported swimming and foreign travel but no contact with animals (12).

RARE C. HOMINIS SUBTYPES AND CRYPTOSPORIDIOSIS OUTBREAKS. Another unique finding of this study is the predominance of the IA28R4 subtype among the tested specimens. Prior to 2007, IA28R4 was a rare subtype, having been implicated only in two swimming pool-associated outbreaks of cryptosporidiosis in Ohio (2005) and South Carolina (2006) amid the 28 waterborne and food-borne cryptosporidiosis outbreaks in the United States, whose investigation included genotyping (20). This subtype was found in 40 of the 57 specimens (70%) from sporadic cases detected in late 2007 from all four states sampled and was also responsible for two of the seven cryptosporidiosis outbreaks investigated in the summer and fall of 2007, as follows: a swimming pool-associated outbreak in Pennsylvania (n = 730) and an interactive splash park-associated outbreak in Idaho (n = 51). In contrast, the C. hominis subtype IbA10G2, commonly implicated in cryptosporidiosis outbreaks, especially major ones in North America and Europe (20), was seen only in 8/57 cases. It might have been a more frequently identified subtype if the sample size were bigger and other states participated in this study. Nevertheless, results of this small-scale study with limited sampling indicate that some “sporadic” cases might have been part of larger multistate outbreaks caused by IA28R4. This is a unique but expected finding in the context of increased cryptosporidiosis reporting in the United States in 2007 and coincides with a large state-wide outbreak in Utah (13), a state that borders three of the four states sampled in this study. Unfortunately, no specimens from Utah were available for Cryptosporidium typing, despite numerous attempts to acquire positive stool specimens. The number of sporadic cryptosporidiosis cases increased dramatically in the United States during 2007, and without molecular characterization, it is impossible to know whether this increase represented large, unrecognized cryptosporidiosis outbreaks. To better understand the transmission of Cryptosporidium in the United States, a national system is needed to systematically characterize Cryptosporidium in sporadic and outbreak-related cases over an extended period of time.

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS. The partial SSU rRNA and gp60 gene sequences of the Cryptosporidium genotype were deposited in the GenBank database under accession numbers FJ435960 to FJ435964.

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REFERENCES


