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CASE REPORT

Human Cryptosporidiosis Diagnosed in Western Australia: a Mixed Infection with *Cryptosporidium meleagridis*, the *Cryptosporidium* Mink Genotype, and an Unknown *Cryptosporidium* Species

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This report describes a case of cryptosporidiosis from an immunocompetent patient from Perth, Western Australia, suffering from diarrhea and a spectrum of other symptoms. Molecular identification revealed that this patient was infected with three *Cryptosporidium* species—*Cryptosporidium meleagridis*, the *Cryptosporidium* mink genotype, and an unknown *Cryptosporidium* species.

CASE REPORT

As part of a *Cryptosporidium* case control study currently being conducted by the Western Australia Department of Health, a 24-year-old male resident tested positive for *Cryptosporidium* and was enrolled in the study. Screening for the presence of other gastroenteric pathogens carried out showed that the patient was negative for the presence of *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., *Vibrio* spp., verotoxigenic *Escherichia coli*, *Yersinia* spp., *Plesiomonas* spp., and rotavirus. The patient was interviewed using a 20-min long questionnaire on his illness and known risk factors for *Cryptosporidium*. The case definition of diarrhea was three or more loose stools in any 24-h period, and at the time of the interview, the patient had experienced intermittent diarrhea for 12 days that was ongoing and had contained blood. The highest number of stools the patient had in a 24-h period was 6 to 10. Other symptoms included fever, chills, vomiting, stomach cramps, nausea, headaches, and muscle body aches. The patient reported no history of heart diseases, high blood pressure, asthma, cancer, arthritis, or any other chronic illness that may weaken the immune system and was not on any medications (i.e., antibiotics or antacids), suggesting that the patient was immunocompetent. The patient also reported being HIV negative, but it was unknown if an HIV test was actually performed.

The fecal sample (C011) was submitted to Murdoch University for molecular analysis. Total fecal DNA was extracted using a QIAamp stool DNA extraction kit (Qiagen, Germany). PCR amplification and bidirectional sequencing of the *Cryptosporidium* 18S rRNA, actin, and 60-kDa glycoprotein gene (*gp60*) loci were carried out as previously described (1, 2). Nucleotide sequence analysis was carried out using ChromasPro version 2.3 (Technelysium, South Brisbane, Australia) and aligned with reference sequences from GenBank using ClustalW. Phylogenetic analysis, based on evolutionary distances calculated using the Kimura two-parameter model and grouped using neighbor joining, was conducted using MEGA version 5.05 (3).

Sequence and phylogenetic analysis of the 18S rRNA gene locus, showed 100% similarity to the *Cryptosporidium* mink genotype (GenBank accession no. EF428191) (data not shown). However, sequence and phylogenetic analyses at the actin locus identified a mixed infection: *C. meleagridis* and an unknown *Cryptosporidium* species, which formed a group with *C. parvum*, the *Cryptosporidium* hedgehog genotype, *C. hominis*, *C. cuniculus*, and *C. tyzzeri*, with genetic similarities of 97.7%, 98.1%, 97.7%, 97.7%, and 98.5%, respectively (Fig. 1). Sequence and phylogenetic analysis at the *gp60* gene locus identified a single novel sequence that exhibited 92.7% similarity to the *C. parvum* IId subtype (Fig. 2).

In the 12 days prior to onset of diarrhea, the patient traveled to Cairns and Brisbane in Queensland, Australia, and then overseas to Papua New Guinea (PNG). It is understood the patient traveled alone, and no secondary cases were reported. In PNG, he trekked in a remote highland region, where he drank the river water. During the PNG trip, he also stayed on a rural property where he drank unboiled water, which was sourced from the local town's main water supply. At the time he was not aware of anyone else with diarrhea living in the same residence but did share a toilet with 25 other people. He did not report any contact with domestic, farm, or wild animals. During his exposure period, he ate raw fruits, fruit juices, and raw vegetables, and at least some of these were home grown. He also swam in public and commercially operated swimming pools. Human ethics approval for this project was provided by the Human Research Ethics Committee of the Western Australian Department of Health (DOHWA HREC), project number 2009/48.

Cryptosporidiosis, a gastroenteric disease in humans and animals, is caused by infection with protozoan parasites of the genus *Cryptosporidium*. Transmission of this parasite is via the fecal-oral route, usually through ingestion of contaminated water or food. The disease mainly manifests itself as watery diarrhea with varying severity, abdominal cramps, loss of appetite, nausea, vomiting,

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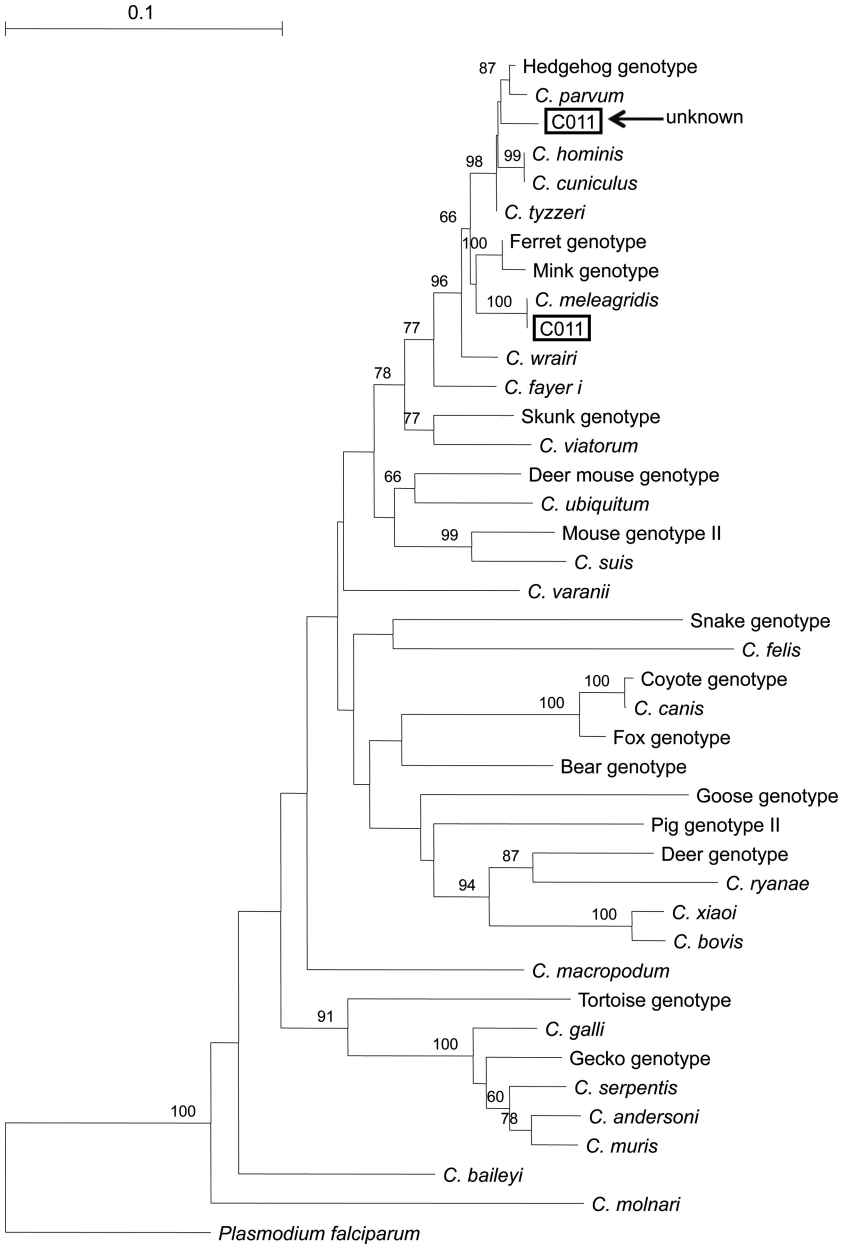

FIG 1 Phylogenetic relationships of C011 with other *Cryptosporidium* spp. at the actin gene locus inferred by neighbor-joining analysis based on genetic distances calculated using the Kimura two-parameter model. Bootstrap values from 1,000 pseudoreplicates of >60% are shown.

and low-grade fever (4). The duration of cryptosporidiosis in immunocompetent persons may last from days to weeks, and the disease is eventually eliminated by a combined cellular and humoral immune response. For children <5 years old, the elderly, or persons with compromised immune systems, the disease can be chronic and debilitating and can lead to severe dehydration and malnourishment (4). Most *Cryptosporidium* infections are caused by *C. hominis* and *C. parvum* and, to a lesser extent, *C. meleagridis*, *C. felis*, and *C. canis*, with recent reports of *C. cuniculus* as an emerging human pathogen (4, 5). Other *Cryptosporidium* species and genotypes that have also been detected in humans include *C. viatorum*, *C. fayeri*, *C. muris*, *C. andersoni*, *C. suis*, *C. bovis*, *C. ubiquitum*, the chipmunk genotype I, the skunk genotype, and the horse genotype (4, 6).

In Australia, sporadic cases of human cryptosporidiosis are mainly due to infection with *C. hominis*, *C. parvum*, and to a lesser extent, *C. meleagridis* (1, 7). There has been one case each of human cryptosporidiosis with *C. fayeri*, *C. andersoni*, and *C. bovis* in Australia (6, 8). It is likely that this patient was infected with *Cryptosporidium* during his travels to PNG through ingestion of contaminated water; however, this cannot be confirmed. As three species of *Cryptosporidium* were identified in this patient, it is difficult to determine if the clinical symptoms were due solely to infection with *C. meleagridis*, which is a known human pathogen, or if the *Cryptosporidium* mink genotype, the unknown *Cryptosporidium* species, or other unidentified pathogens were also contributing to the spectrum of symptoms reported. This is the first report of the *Cryptosporidium* mink genotype in a human as previously it had only been

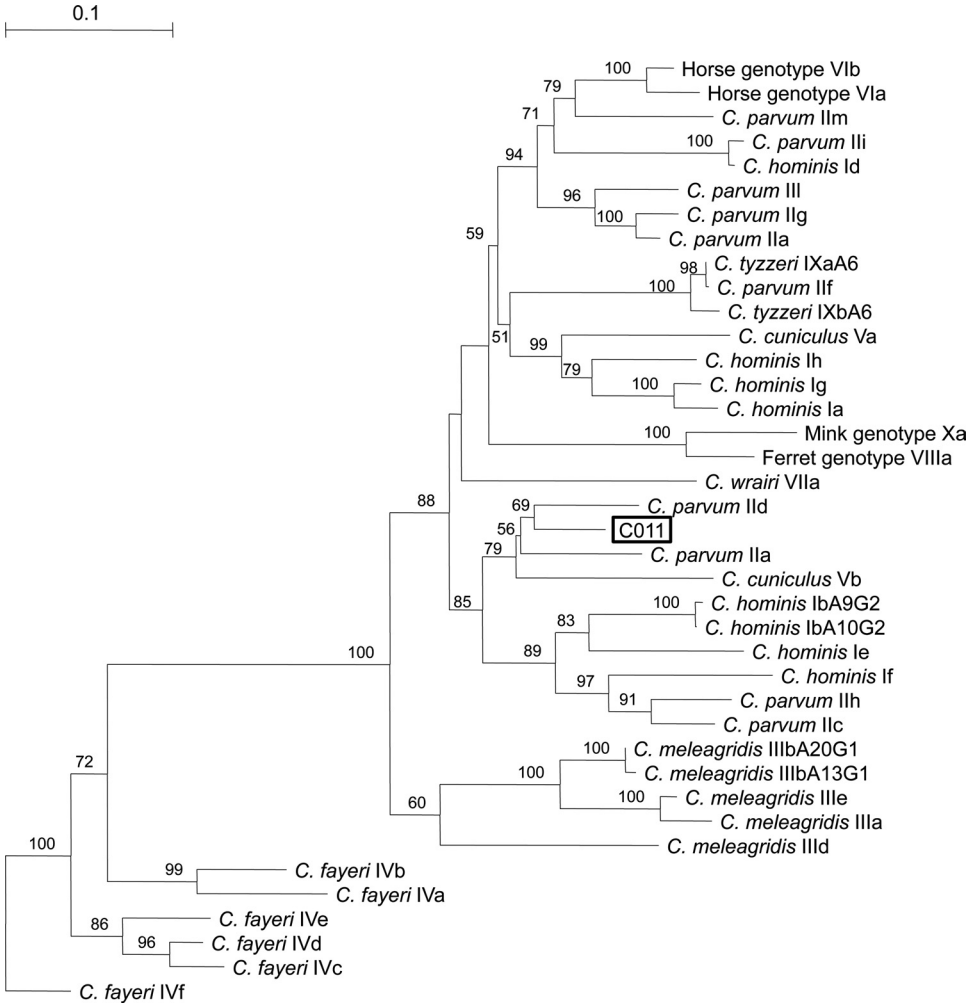

FIG 2 Phylogenetic relationships of C011 and other *Cryptosporidium gp60* subtype families inferred by neighbor-joining (NJ) analysis based on genetic distances calculated using the Kimura two-parameter model. The *C. fayeri gp60* sequence was used as an outgroup to generate the NJ tree. Bootstrap values from 1,000 pseudoreplicates of >50% are shown.

identified in mink (*Mustela vison*) (9). The unknown *Cryptosporidium* species identified at the actin gene locus in the present study was genetically closest to *C. tyzzeri* (syn. *Cryptosporidium* mouse genotype I), which mainly infects domestic mice and small rodents (10). Further studies are required to elucidate the identity of this unknown *Cryptosporidium* species and to determine if it is capable of causing disease in humans. As the *gp60* sequence obtained did not match either the mink genotype or *C. meleagridis*, it is likely that it corresponds to the unknown genotype identified at the actin locus; however, this remains to be confirmed.

Nucleotide sequence accession numbers. Sequences generated from the present study have been submitted to GenBank under accession no. JX471002 to JX471005.

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