Human cryptosporidiosis diagnosed in Western Australia – a mixed infection with Cryptosporidium meleagridis, Cryptosporidium mink genotype and an unknown Cryptosporidium species.

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Abstract

This report describes a case of cryptosporidiosis from an immunocompetent patient from Perth, Western Australia suffering from diarrhoea and a spectrum of other symptoms. Molecular identification revealed that this patient was infected with three Cryptosporidium species - Cryptosporidium meleagridis, 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 E. coli, Yersinia spp., Plesiomonas spp. and rotavirus. The patient was interviewed using a 20 minute long questionnaire on his illness and known risk factors for Cryptosporidium. The case definition of diarrhoea was three or more loose stools in any 24 hour period and at the time of the interview, the patient had experienced intermittent diarrhoea for 12 days that was ongoing and had contained blood. The most number of stools the patient had in a 24 hour period was 6-10. 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 a HIV test was actually performed.

The faecal sample (C011) was submitted to Murdoch University for molecular analysis. Total faecal DNA was extracted using a QIAamp stool DNA extraction kit (Qiagen, Germany). PCR amplification and bi-directional sequencing of the Cryptosporidium 18S rRNA, actin and 60 kDa-glycoprotein (gp60) gene loci were carried out as previously described (1, 2). Nucleotide sequence analysis was carried out using ChromasPro version v2.3 (http://www.technelysium.com.au) and aligned with reference sequences from GenBank using Clustal W (http://www.genome.jp/tools/clustalw). Phylogenetic analysis, based on evolutionary distances calculated using Kimura-2 parameters and grouped using neighbour
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 analysis 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 (Figure 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 (Figure 2).

In the twelve days prior to onset of diarrhoea, the patient travelled to Cairns and Brisbane in Queensland and then overseas to Papua New Guinea (PNG). It is understood the patient travelled 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 diarrhoea 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. In 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.

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 faecal-oral route, usually through ingestion of contaminated water or food. The disease mainly manifests itself as watery diarrhoea with varying severity, abdominal cramps, loss of appetite, nausea, vomiting and low-grade fever (4). Duration of cryptosporidiosis in immunocompetent persons may last from days to weeks and 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 of human cryptosporidiosis with C. fayeri, C. andersoni and C. bovis respectively 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 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. tzyzzeri (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 with either the mink genotype or *C. meleagris*, it is likely that it corresponds to the unknown genotype identified at the actin locus, however this remains to be confirmed.
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Figure 1: Phylogenetic relationships of C011 with other Cryptosporidium spp. at the actin gene locus inferred by neighbour-joining analysis based on genetic distances calculated using Kimura-2 parameters. Bootstrap values from 1000 pseudoreplicates of >60% are shown.

Figure 2: Phylogenetic relationships of C011 and other Cryptosporidium spp. gp60 subtype families inferred by neighbour-joining (NJ) analysis based on genetic distances calculated using Kimura-2 parameters. C. fayeri gp60 sequence was used as an outgroup to generate the NJ tree. Bootstrap values from 1000 pseudoreplicates of >50% are shown.