JCM Journals.ASM.org

CASE REPORT

Human Cryptosporidiosis Caused by *Cryptosporidium tyzzeri* and *C. parvum* Isolates Presumably Transmitted from Wild Mice

Veronika Rašková,[a,b] Dana Květoňová,[b] Bohumil Sak,[b] John McEvoy,[c] Adam Edwinson,[c] Brianna Stenger,[c] Martin Kváč[a,b]

University of South Bohemia, Faculty of Agriculture, České Budějovice, Czech Republic[a]; Biology Centre Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic[b]; Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, North Dakota, USA[c]

We report a case of severe human cryptosporidiosis caused by *Cryptosporidium tyzzeri* and *C. parvum* with an unusually high frequency of liquid stools. Wild mice were the most likely source of infection, demonstrating the potential for wild-mouse-borne *Cryptosporidium* to infect humans and highlighting the health risks associated with synantropic rodents.

CASE REPORT

A 25-year-old female who was trapping wild rodents at field sites and working with rodents under laboratory conditions became ill and displayed typical clinical signs of cryptosporidiosis, including nonbloody profuse diarrhea and dehydration (Table 1). Following the commencement of diarrhea, stool samples were examined daily for the presence of *Cryptosporidium* using a standard aniline-methyl-violet staining method (1). Course of infection indicators, including stool consistency and color, frequency of defecation, and infection intensity, were examined. The diarrhea was classified according to Chappell et al. (2). *Cryptosporidium* oocysts with typical staining characteristics were identified in fecal smears. Diarrheal illness was characterized by the passage of 124 unformed stools in a 168-h period (Table 1). A high defecation frequency, which peaked at 32 per day, was recorded during the first week following commencement of disease symptoms. Oocyst shedding was detected at the commencement of diarrhea and subsequently for 14 days (from 2 to 15 days of observation); however, the prepatent period is not known. No shedding was detected after resolution of symptoms. Oocyst shedding intensity during the diarrheal period ranged from 2,000 to 70,000 per ml of stool. The combined diarrhea and anorexia resulted in a loss of 4.5 kg of body weight during a 20-day period. The individual was examined on two occasions for the presence of exogenous protozoan stages, helminth eggs, *Shigella*, *Salmonella*, *Campylobacter*, *Clostridium difficile*, amoebas, rotaviruses, and noroviruses. No other bacterial, viral, or parasitic pathogens were detected. Tests for HIV before and after the cryptosporidiosis were negative. The study was approved by the ethics committee of the Institute of Parasitology, Biology Centre Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic (protocol 1/2012).

To identify the *Cryptosporidium* spp. present, DNA was extracted and a nested PCR protocol was used to amplify a partial sequence of the *Cryptosporidium* 60-kDa glycoprotein (gp60) gene (3). PCR amplicons were either sequenced directly or were cloned (pGEM-T easy vector system; Promega, Madison, WI) before sequencing. Sequencing was carried out in both directions using an ABI 3130 sequencer analyzer (Applied Biosystems, Foster City, CA). The identity of obtained sequences was examined by a BLAST search (www.ncbi.nlm.nih.gov/blast). Partial gp60 sequences recovered from the patient have been submitted to GenBank under the accession numbers JX445924 (*C. tyzzeri* IXb subtype), JX445925 (*C. tyzzeri* IXa subtype), and JX445926 (*C. parvum*). The gp60 sequences were from *C. tyzzeri* (IXaA8 and IXbA6 subtypes) and *C. parvum* (IIaA13G1R1 subtype). These subtypes of *C. tyzzeri* and *C. parvum* were also detected in the wild mice that the patient was in contact with (data not shown).

The *C. parvum* IIaA13G1R1subtype detected in this study was previously reported in immunocompetent humans, HIV/AIDS patients, and domestic animals in Malaysia (4). A single case of human cryptosporidiosis caused by *C. tyzzeri* (incorrectly reported as the *C. parvum* IIf family, A6 subtype) was reported previously in a Kuwaiti child (5); however, the source of the infection was not identified. In immunocompetent hosts, cryptosporidiosis is usually a self-limited disease lasting 10 to 14 days, while infection in immunocompromised hosts may cause severe and persistent disease. Experimental infections of healthy volunteers have been undertaken to address gaps in our understanding of the duration and intensity of infection and oocyst shedding patterns in immunocompetent hosts (2, 6, 7). Adding to these data, we report a well-characterized natural infection in a healthy individual. The duration, signs, and symptoms of illnesses are generally similar to previous reports. The median duration of diarrhea and/or gastrointestinal symptoms in this case study (144 h of liquid diarrhea) was similar to reports in volunteers infected with *C. parvum* (155 h; range, 41 to 336), *C. hominis* (137 h; range, 49 to 518), and *C. meleagridis* (77 h; range, 50 to 105) (2, 6, 7). However, the total number of unformed stools detected in this case study (124) is far greater than that detected in volunteers infected with *C. parvum* (mean, 10; range, 3 to 15), *C. hominis* (mean, 9; range, 2 to 19), and *C. meleagridis* (mean, 8; range, 3 to 15). This higher number of unformed stools in the present case may be due to the coinfection of *C. parvum* with *C. tyzzeri*.

This report demonstrates that transmission of *Cryptosporidium* spp. from synanthropic rodents to humans can occur. Our

Received 6 September 2012 **Returned for modification** 9 October 2012
Accepted 17 October 2012
Published ahead of print 24 October 2012
Address correspondence to Martin Kváč, kvac@paru.cas.cz.

doi:10.1128/JCM.02346-12

TABLE 1 Clinical symptoms during the course of a *C. tyzzeri* and *C. parvum* infection in an immunocompetent patient[a]

Day	Defecation frequency (stools per day)	Infection intensity (oocyst per ml)	Stool consistency	Stool color	Symptom
−3	0	ND	ND	ND	No symptoms
−2	1	ND	Pellets	Brown	No symptoms
−1	0	ND	ND	ND	No symptoms
1	2	ND	Liquid/paste	Brown-gray	Diarrhea, nausea, abdominal pain, sweat, trembling
2	32	2,000	Liquid	Green-gray	Diarrhea, nausea, abdominal pain, fatigue, vomiting
3	26	2,000	Liquid	Green-gray	Diarrhea, nausea, abdominal pain, fatigue
4	22	4,500	Liquid	Gray-green	Diarrhea, nausea, abdominal pain, fatigue
5	17	40,000	Liquid	Gray-green	Diarrhea, nausea, abdominal pain, fatigue
6	15	70,000	Liquid	Gray-green	Diarrhea, nausea, fatigue
7	10	40,000	Liquid/paste	Brown-gray	Diarrhea, nausea, fatigue
8	4	40,000	Paste	Brown-orange	Diarrhea, nausea, fatigue
9	5	50,000	Paste	Orange	Diarrhea, nausea, fatigue
10	6	25,000	Dense paste	Orange	Diarrhea, nausea, fatigue
11	4	15,000	Paste with pellets	Brown-orange	Diarrhea, nausea, fatigue
12	4	25,000	Paste with pellets	Brown-orange	Diarrhea, nausea, fatigue
13	3	18,000	Paste with pellets	Brown-orange	Nausea
14	4	10,000	Thinner pellets	Brown	Nausea, diarrhea
15	3	5,000	Thinner pellets	Brown	Nausea
16	2	0	Pellets	Brown	Nausea
17	1	0	Pellets	Brown	Nausea
18	1	0	Pellets	Brown	No symptoms
19	0	0	ND	ND	No symptoms
20	1	0	Pellets	Brown	No symptoms

[a] ND, not determined.

data suggest that *Cryptosporidium tyzzeri*, which is considered to have a narrow host range, can be zoonotic.

Cryptosporidium, a ubiquitous protozoan parasite of vertebrate species, causes cryptosporidiosis, a diarrheal disease that can become chronic and life threatening in the absence of a competent immune response. *Cryptosporidium hominis* and *C. parvum* are responsible for most human cases of cryptosporidiosis. However, at least 17 other *Cryptosporidium* spp. have been reported to be infectious to humans (8–10). Most human cryptosporidiosis is reported to be caused by a single species; however, it is evident that both humans and animals can have mixed species and genotypes. Although Cama et al. (11) showed that mixed *Cryptosporidium* infections are not uncommon, they noted that mixed infections may be underreported due to the preferential amplification of the predominant species or genotype by PCR.

Cryptosporidium parvum and *C. tyzzeri* are reported to have different host ranges. While *C. tyzzeri* is primarily restricted to rodents and appears to be adapted to house mice (8), *C. parvum* is common in farm animals (12), is zoonotic, and is one of the most frequent causes of human cryptosporidiosis. Although adult mice are generally not susceptible to experimental infections with *C. parvum*, pet and wild rodents are reportedly susceptible to infection with this species (8). Although wildlife animals can be reservoirs for various zoonotic pathogens, including protozoan parasites, it has been suggested that they are not a significant source of human-pathogenic *Cryptosporidium* spp. (13). However, the commensal relationship that house mice have with humans distinguishes them from most other wildlife. The evidence from this study suggests that *Cryptosporidium* from house mice can cause disease in an immunocompetent human and shows that *C. tyzzeri*, a species considered to be restricted to rodents, can be zoonotic. Moreover, the detection of both IXa and IXb subtypes of *C. tyzzeri*, which appear to be geographically isolated and specific for Western house mice and Eastern house mice, respectively (unpublished data), suggests that multiple exposures to infection are possible.

ACKNOWLEDGMENTS

This study was funded by a project from the Ministry of Education, Youth and Sports of the Czech Republic (LH11061), by the Grant Agency of the University of South Bohemia (022/2010/Z), and by NIH grant 2P20 RR015566 from the National Center for Research Resources.

REFERENCES

1. **Miláček P, Vítovec J.** 1985. Differential staining of cryptosporidia by aniline-carbol-methyl violet and tartrazine in smears from feces and scrapings of intestinal mucosa. Folia Parasitol. (Praha) **32**:50.
2. **Chappell CL, Okhuysen PC, Sterling CR, Wang C, Jakubowski W, DuPont HL.** 1999. Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. Am. J. Trop. Med. Hyg. **60**:157–164.
3. **Alves M, Xiao L, Sulaiman I, Lal AA, Matos O, Antunes F.** 2003. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. J. Clin. Microbiol. **41**:2744–2747.
4. **Iqbal A, Lim YA, Surin J, Sim BL.** 2012. High diversity of *Cryptosporidium* subgenotypes identified in Malaysian HIV/AIDS individuals targeting gp60 gene. PLoS One **7**:e31139. doi:10.1371/journal.pone.0031139.

5. **Sulaiman IM, Hira PR, Zhou L, Al-Ali FM, Al-Shelahi FA, Shweiki HM, Iqbal J, Khalid N, Xiao L.** 2005. Unique endemicity of cryptosporidiosis in children in Kuwait. J. Clin. Microbiol. **43**:2805–2809.
6. **Chappell CL, Okhuysen PC, Langer-Curry R, Widmer G, Akiyoshi DE, Tanriverdi S, Tzipori S.** 2006. *Cryptosporidium hominis*: experimental challenge of healthy adults. Am. J. Trop. Med. Hyg. **75**:851–857.
7. **Chappell CL, Okhuysen PC, Langer-Curry RC, Akiyoshi DE, Widmer G, Tzipori S.** 2011. *Cryptosporidium meleagridis*: infectivity in healthy adult volunteers. Am. J. Trop. Med. Hyg. **85**:238–242.
8. **Elwin K, Hadfield SJ, Robinson G, Crouch ND, Chalmers RM.** 2012. *Cryptosporidium viatorum* n. sp. (Apicomplexa: Cryptosporidiidae) among travellers returning to Great Britain from the Indian subcontinent, 2007–2011. Int. J. Parasitol. **42**:675–682.
9. **Kváč M, Květoňová D, Sak B, Ditrich O.** 2009. *Cryptosporidium* pig genotype II in immunocompetent man. Emerg. Infect. Dis. **15**:982–983.
10. **Waldron LS, Cheung-Kwok-Sang C, Power ML.** 2010. Wildlife-associated *Cryptosporidium fayeri* in human, Australia. Emerg. Infect. Dis. **16**:2006–2007.
11. **Cama V, Gilman RH, Vivar A, Ticona E, Ortega Y, Bern C, Xiao L.** 2006. Mixed *Cryptosporidium* infections and HIV. Emerging Infect. Dis. **12**:1025–1028.
12. **Watanabe Y, Yang CH, Ooi HK.** 2005. *Cryptosporidium* infection in livestock and first identification of *Cryptosporidium parvum* genotype in cattle feces in Taiwan. Parasitol. Res. **97**:238–241.
13. **Simpson VR.** 2002. Wild animals as reservoirs of infectious diseases in the UK. Vet. J. **163**:128–146.