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Ruling out nosocomial transmission of Cryptosporidium in a renal transplantation unit: case report

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Abstract

Background: Cryptosporidium spp. is a ubiquitous parasite affecting humans as well as domestic and wild vertebrates, causing diarrhea in both immunocompetent and immunocompromised hosts worldwide. Its transmission occurs primarily by the fecal-oral route. In humans, C. parvum and C. hominis are the most prevalent species, whereas immunocompetent and immunocompromised individuals can also be infected by other zoonotic species. Renal transplant patients are prone to develop cryptosporidiosis, which can induce severe and life-threatening diarrhea.

Case presentation: We report here a series of nearly concomitant cases of acute symptomatic cryptosporidiosis in three renal transplant patients attending the Strasbourg University Hospital Nephrology Unit. The clinical presentation was persistent diarrhea and acute renal failure. The diagnosis was confirmed by microscopic stool examination using a modified Ziehl-Neelsen staining method and species identification by molecular tools. All patients were treated with nitazoxanide and recovered from diarrhea after 14 days of therapy.

Conclusion: Genotypic species identification was not consistent with an epidemic context, thus underlining the need for genotyping to monitor at risk patients.

Keywords: Cryptosporidium, Renal transplant, Zoonotic species, Genotypic species identification, Case report

Background

The coccidian protozoan Cryptosporidium spp. is an intestinal parasite and a significant cause of enteric disease in humans and numerous other vertebrates worldwide. Cryptosporidiosis is the most common zoonotic cause of human parasitic diarrhea (i.e., 60 % of epidemic cases linked to waterborne and 2–6 % of cases involving severe diarrhea worldwide), especially in immunocompromised individuals and young children [1]. The latter is notably the case in developing countries, where this parasite ranks second in the causes of death in children under 2 years [1, 2]. The prevalence of Cryptosporidium in stools of immucompetent persons was found to be lower in high-income countries than in developing regions [3]. Infection occurs by oocyst-stage ingestion via contaminated drinking water, food or recreational waters, as well as by direct or indirect human-to-human or animal-to-human contact [4]. In France, 407 cases of cryptosporidiosis were diagnosed between 2006 and 2009 [5]. A study conducted by the Strasbourg University Hospital between 2011 and 2013 detected Cryptosporidium spp. in 2.4 % of stools in which parasites have been detected out of a total of 6515 analyzed stools [6]. Over the past 20 years, three cryptosporidiosis outbreaks have been reported in France [5]. Other documented cases are linked to outdoor activity, swimming pools, day-care centers, and travel [3, 7]. Cryptosporidiosis can spread also among hospitalized patients and hospital staff and nosocomial outbreak of Cryptosporidium have been described. Source of infection could be...
contaminated water or contact with the hands of infected people [8, 9].

The increasing frequency of human cryptosporidiosis outbreaks raises relevant public health and economic concerns [10–12]. Human cases are commonly due to two species: C. hominis, which primarily infects humans, and C. parvum, which infects both humans and animals. Occasional infections by other species/genotypes, such as C. felis, C. meleagris, C. canis or chipmunk and rabbit genotypes have been primarily reported in immunodeficient patients [5]. C. hominis and the zoonotic Cryptosporidium species are associated with a variety of clinical manifestations in humans [13].

The severity and duration of human Cryptosporidium infections are linked to the host’s immune status [14]. Immunocompetent patients experience self-limiting disease, while in immunosuppressed patients, especially those with T-cell deficiency, cryptosporidiosis is often chronic and severe with risks of extra-intestinal disease development [15].

In renal transplant patients, post-transplant cryptosporidiosis with diarrhea is a frequent complication [16]. In France, 69.3% of clinically apparent cryptosporidiosis cases reported from 2006 to 2009 involved immunocompromised patients and 16.5% of them were reported in patients who had received solid-organ or stem-cell transplants [5]. One report from a pediatric renal transplant unit demonstrated that infections were the primary cause of diarrhea, with Cryptosporidium spp. diagnosed in 11% of 199 cases [3].

We report here three Cryptosporidium spp. infections with acute diarrhea and abdominal pain, observed almost simultaneously in three renal transplant patients who were subject to species genotyping in order to investigate a potential epidemic context in an outpatient nephrology unit.

**Case presentation**

The three cases were diagnosed in the outpatient unit of the Nephrology Department at Strasbourg University Hospital, France.

**Clinical histories**

Patient #1 was a 60-year-old man who underwent transplantation at the age of 52 for chronic renal failure due to polycystic kidney disease. He initially received immunosuppressive treatment, consisting of tacrolimus (4 mg/day), mycophenolate mofetil (MMF) (1 g x 2/day), and prednisone (7.5 mg/day). Eight years after renal transplantation, he presented with watery diarrhea, nausea and vomiting starting 15 days before consulting (September 25th, 2014). Physical examination revealed asthenia, weight loss (6Kg), hypotension, dry mouth, and acute renal failure (glomerular filtration rate (GFR): 30 ml/min/1.73 m²). The patient reported no recent travel or contact with swimming pool water, non-drinking water or farm animals, but admitted to own a dog. No other family member experienced diarrhea.

Patient #2 was a 64-year-old man of Malian origin who had lived in France for 40 years and undergone transplantation at the age of 62 for chronic renal failure secondary to glomerulonephritis. Immunosuppressive treatment consisted of tacrolimus (7 mg x 2/day), MMF (750 mg x 2/day), and prednisone (10 mg/day). Two years and 4 months following renal transplantation, he presented with watery diarrhea and abdominal pain lasting for 15–20 days (starting September 26th, 2014). Physical examination revealed weight loss (13Kg), esophageal pain, and acute renal failure (GFR: 36 mL/min/1.73 m²). The patient also presented leucopenia and neutropenia, initially attributed to an overdose of tacrolimus. He reported no previous contact with non-drinking water, swimming pool water or farm animals, but had travelled to Mali for 2 months shortly before the onset of diarrhea. No other person of his family experienced diarrhea.

Patient #3 was a 34-year-old man of Kosovar origin who underwent transplantation aged 24-year-old for an undetermined reason. Acute transplant rejection 2 years later led to a second transplantation in September, 2014. Immunosuppressive treatment consisted of tacrolimus (6 mg x 2/day), MMF (750 mg x 2/day), and prednisone (25 mg/day). Ten days following the second renal transplantation (September 21th, 2014), the patient exhibited watery diarrhea and abdominal pain. Physical examination indicated weight loss (10Kg) and acute renal failure (GFR: 16 ml/min/1.73 m²). The patient, who reported no contact with non-drinking water, swimming pool water or farm animals, had travelled to Kosovo for 1 month before transplantation. His 2-year-old daughter also presented with diarrhea from an unknown cause that lasted for 3 days. No stool analyses were done for the daughter.

**Parasitological investigations**

For all three patients, stool examinations performed at the first consultation revealed the presence of Cryptosporidium oocysts, using a modified Ziehl-Neelsen staining method (5–10 oocysts/slide, >100 oocysts/slide, and 1–5 oocysts/slide for cases #1, #2, and #3, respectively). All stool samples were negative for the bacteria Clostridium difficile, Salmonella, Shigella but also for rotavirus and norovirus, and for parasites Giardia and microsporidia.

DNA was extracted from the stool samples using a NucliSENS easyMAG device (bioMérieux, Marcy l’Étoile, France) [17]. Briefly, it consisted of adding 400 mg of stool samples to 1 mL of NucliSENS lysis buffer in a tube containing ceramic beads (lysing matrix D; MP Biomedicals,
For Patient #3, the *C. hominis* were identified based on their melting curve analysis, with both species exhibiting the same DNA sequence at the hybridization probe locus, all isolates identified as *C. hominis* were then sequenced to differentiate *C. hominis* from *C. cuniculus*.

For sub-genotyping analysis, DNA samples were subjected to amplification of an 850-bp fragment of the gp60 gene using a nested PCR method [19]. The total volume of PCR mixture was 50 μL, containing 5 μL of DNA for the primary PCR or 5 μL of the primary PCR products, primers (outer primers: AL3531 and AL3535; inner primers: AL3432 and AL3534) at a concentration of 0.4 μM, 0.2 mM deoxyribonucleotide triphosphate mix, and 1.25U of DreamTaq DNA polymerase. Each PCR reaction was subjected to 40 cycles of 30s denaturation at 95 °C, 60s annealing at 55 °C, and 60s extension at 72 °C, with an initial 5 min denaturation at 95 °C and a final 10 min extension at 72 °C. PCR products were visualized by electrophoresis on an ethidium bromide stained 2 % agarose gel electrophoresis. Amplicons were purified and sequenced in both directions with the forward and reverse primers used in the secondary PCR at a concentration of 0.32 μM. Sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and an ABI PRISM 3100 Genetic Analyzer® (Applied Biosystems, USA). We analyzed the quality of the generated electrophoregrams from each strand using 4Peaks software and compared them with those available in the GenBank database using the basic local alignment search tool. Subtype assignment was based on the number of trinucleotide repeats (TCA, TCG or TCT) in the coding for serine [20].

Results

For Patient #1, genotyping revealed a *C. felis* infection. For Patient #2, genotyping revealed *C. hominis* sub-genotype IaA13. For Patient #3, the *C. parvum* sub-genotype IIaA13G1R2 was identified.

Treatment

All three patients were treated with nitazoxanide (500 mg x 2/day for 14 days). For Patient #1, the stools tested negative 2 weeks after treatment initiation, with no recurrence of diarrhea observed 4 months after the first episode. For Patient #2, a reduction of tacrolimus was initiated and the diarrhea regressed 8 days after treatment initiation, although 3 months after therapy was started, his stools still tested positive. A second administration of nitazoxanide was thus prescribed and we requested a stool sample from his daughter for testing. One month after the second treatment course, his stools were tested negative for *Cryptosporidium* oocysts. For Patient #3, tacrolimus was also reduced and his diarrhea
was regressing 1 month after treatment initiation. Four months after the second treatment course, his stools were tested negative for Cryptosporidium oocysts.

Conclusions
We report here a series of nearly concomitant cases of acute symptomatic cryptosporidiosis in three renal transplant patients attending the same outpatient unit of a Nephrology Department. The patients’ consultations records in the Nephrology department showed that they could have been in contact in July 2014. This possibility of contact before the onset of symptoms suggesting a possible nosocomial infection requires a genotyping to explore this hypothesis. In these patients, Cryptosporidium species and gp60 genotypes, which were determined to document a possible outbreak, provided no evidence of nosocomial transmission.

As our report demonstrates, the detection of three different Cryptosporidium species in three cryptosporidiosis patients excluded the possibility of nosocomial transmission in the Nephrology unit, where renal transplant patients frequently consult and come into contact with each other. Our findings highlight the risk of symptomatic cryptosporidiosis in immunosuppressed renal transplant patients. In 2014, nine out of ten patients with cryptosporidiosis diagnosed by the medical Parasitology and Mycology laboratory of the Strasbourg University Hospital were renal transplant patients (unpublished data). In a pediatric renal transplantation unit, Cryptosporidium spp. was confirmed as the principal cause of diarrhea in patients between 6 months and 12 years of age following transplantation. In Poland and India, the prevalence of Cryptosporidium spp. in renal transplant patients was reported to be 18.8 and 20%, respectively [21, 22]. Cryptosporidium spp. infections were more commonly associated with profuse watery diarrhea in solid-organ recipients than in immunocompetent patients [21, 23, 24]. Our patients undergoing combined immunosuppressive therapies exhibited watery diarrhea for several weeks before consulting, suggesting that the prevalence of Cryptosporidium spp. infections is probably underestimated in renal transplant units where screening of patients with diarrhea is not routinely performed. In all three of our patients, the symptoms completely resolved within 8 days to 1 month, in line with previous reports of slower recovery duration compared to immunocompetent patients in whom diarrhea symptoms usually cease after 10 to 15 days without treatment [3, 16]. Considering the role of immunosuppression in the appearance and persistence of cryptosporidiosis, we opted to reduce the immunosuppressive regimen in two of our patients, which, in association with the anticryptosporidial agent, could prove an effective method in reducing both duration and severity of symptoms [3, 12, 25].

C. felis cryptosporidiosis (patient #1) is rarely diagnosed in France (4.8% of all cases between 2006 and 2009 vs., 54% for C. parvum and 36% for C. hominis) [5]. No contact with cats was reported, in agreement with previous reports showing that cat ownership is not a significant risk factor for C. felis infection and that C. felis host specificity is not very strict, since it was observed in cats, cattle and humans, thus rendering it often difficult to determine the source of infection [5, 26, 27].

C. hominis infection observed in Patient #2, is prevalent worldwide, and especially in developing areas, with similar incidences to those of C. parvum infection in most European countries, but less frequently reported than C. parvum infection in France and the Middle-East area [20, 24–29]. Travel-related cryptosporidiosis and small family outbreaks have been frequently associated with C. hominis infection, consistent with the onset of symptoms during our patient’s trip to Africa [5]. To the best of our knowledge, human cases of the C. hominis IaA13 sub-genotype have only been reported in Australia, but reports of the C. hominis genotypes present in Africa are scarce [30].

C. parvum, the predominant species in French cryptosporidiosis patients, was detected in Patient #3 [5]. The C. parvum IIaA13G1R2 genotype had previously been published data). In a pediatric renal transplantation unit, Cryptosporidium National Network: Accoceberry I CHU Bordeaux, Aignamey P CHU Amiens, Angoulvant A CHU Bicêtre Paris, Aubert D CHU Amiens,
Belkhadi G CHU St Antoine, Paris, Berry A. CHU Toulouse, Blanchet D CHU Cayenne, Bonhomme J CHU Caen, Botterel F CHU Creteil, Bougnoux ME CHU Paris, Paris, Brunet J CHU Strasbourg, Buffet P CHU Pitié, Paris, Dalle F CHU Dijon, Dananaui E HEPG, Paris, Darde ML CHU Limoges, De Gentile LCHU Angers, Debourgogne A CHU Nancy, Debruyne M (Cerba, Paris), Degeilh B CHU Rennes, Demar M CHU Cayenne, Desbois N CHU Fort de France, Desobeaux G CHU Tours, Delanaye P CHU Nice, Flori P CHU St Etienne, Gargala G CHU Rouen, Goubard A Biomiris Paris, Grenouillet F CHU Besancon, Hamanner S CHU St Louis, Paris, Houze S CHU Bichat, Paris, Jamet D CHU Brest, Kapell N CHU Pitié, Paris, Labbe F CH Le Havre, Lennetermin D Lab. St Valéry en Caux, Magne D CHU St Antoine Paris, Marty P CHU Nice, Menotti J CHU St Louis, Paris, Million S CHU Besancon, Morelle C CHU Cayenne, Desbois N CHU Fort d’Enfer, Morio F CHU Nantes, Murat LB CHU Grenoble, Nevez G CHU Brest, Nicolas M CHU Guadeloupe, Poirier P CHU Clermont-Ferrand, Rabodonirina M CHU Lyon, Rosier MH CHU Poitiers, Sautour M CHU Dijon, Thellier M CHU Pitié, Paris, Totet A CHU Amiens, Valentin A CHU Toulouse, Villena I CHU Reims, and Yera H CHU Cochin, Paris.

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Availability of data and materials
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Authors’ contributions
JB, BP, LF, EC were responsible for data analysis, management of data and wrote the manuscript. JPL, MS, FD, AWP, RR, AA helped in manuscript revision. SV, CM, CB, SE, BM conducted clinical investigation and helped in manuscript revision. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Consent of each patient was obtained for the clinical data presented in this article.

Ethics approval and consent to participate
Not Applicable.

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