Case Report: Diagnosis of Cryptosporidiosis in Renal Transplantation in a Low-Prevalence Setting

Shuwei Zheng,^{1*} Kwan Ki Karrie Ko,² Kian Sing Chan,³ and Indumathi Venkatachalam¹

¹Department of Infectious Diseases, Singapore General Hospital, Singapore; ²Department of Microbiology, Singapore General Hospital, Singapore; ³Department of Molecular Pathology, Singapore General Hospital, Singapore

Abstract. In high prevalence settings, cryptosporidiosis is commonly implicated as a cause of a gastroenteritis syndrome in the organ transplant population. Stool microscopy is predominant diagnostic modality. Therapeutic options in this group of patients are limited, making their management exceptionally challenging. We describe a case of a renal transplant recipient with cryptosporidiosis confirmed by the stool FilmArray gastrointestinal panel (GIP) nucleic acid-based assay and stool microscopy, describe our institutional experience in diagnosing cryptosporidiosis in a low-prevalence setting, and review the available literature on management of this condition in the organ transplant population. In a low-prevalence setting, the GIP can serve as a rapid screening tool in the diagnosis of cryptosporidiosis.

INTRODUCTION

Cryptosporidiosis causes a predominantly gastrointestinal illness, transmitted largely through the consumption of contaminated food and water. *Cryptosporidium* spp. is ubiquitously distributed in the environment, although prevalence of the disease is much more common in developing countries than in developed countries.^{1,2} Although the disease is largely self-limiting in the immunocompetent host, treatment in the immunocompromised population such as in the organ transplant recipients is particularly challenging, because of its propensity to cause significant morbidity and a lack of antiparasitic therapeutic options. Diagnosis of the disease in the susceptible population is dependent on having a low index of suspicion, especially in a low-prevalence setting, because routine stool sampling for ova, cysts, and parasites is not diagnostic. Herein, we describe a case of cryptosporidiosis in a renal transplant recipient proven by both stool microscopy and stool FilmArray gastrointestinal panel (GIP) nucleic acid-based assay, describe our institutional experience in diagnosing cryptosporidiosis in a low-prevalence setting, and review the literature about management options in the organ transplant population.

CASE REPORT

A 37-year-old man presented with a 2-day history of severe acute Bristol 7 diarrhea, up to 10 episodes a day, associated with generalized abdominal discomfort and coryzal symptoms. He did not have fever, jaundice, or travel outside the country in the preceding 2 weeks. He was clinically dehydrated, but apart from the transplanted kidney in the right iliac fossa, there were no other significant findings on examination.

The patient had a past history of end-stage renal failure secondary to chronic glomerulonephritis, morbid obesity, hypertension, and hyperlipidemia. He underwent an ABO-incompatible living-related renal transplant 2 years before his presentation. This was complicated by acute rejection requiring plasmapheresis, anti-thymocyte globulin, and intravenous immunoglobulins with good response. At

presentation, he was maintained on moderate dose prednisolone, mycophenolic acid, and tacrolimus. Previous opportunistic infections include cytomegalovirus colitis and chronic hepatitis E infection.

When he presented with acute diarrheal illness, he was in acute renal failure, with a serum creatinine of 244 $\mu\text{mol/L}$ (pre-transplant serum creatinine of 146–198 $\mu\text{mol/L}$). Full blood count, liver function test, and C-reactive protein were unremarkable. Tacrolimus level was in the target therapeutic range. Stool specimens for *Campylobacter*, *Shigella*, and *Salmonella* culture and ova, cysts, and parasites were negative. Out of 22 gastrointestinal pathogens tested on the stool GIP, only *Cryptosporidium* spp. was positive. Eventually, multiple oocysts consistent with cryptosporidiosis were confirmed in stool specimens stained with modified acid-fast bacilli (mAFB) and immunofluorescent (MERIFLUOR® *Cryptosporidium*/*Giardia*; Meridian Bioscience®, Inc., Cincinnati, OH) stains (Figure 1). Serologic testing for human immunodeficiency virus was negative. He was treated with combination oral paromomycin 1 g twice daily and azithromycin 500 mg daily with prompt resolution of symptoms. Stool microscopy repeated 1 week after treatment was still positive for *Cryptosporidium* spp. Hence, combination drug therapy was continued for 4 weeks with clinical and microbiological resolution. He remained asymptomatic 4 weeks following antimicrobial cessation.

Singapore General Hospital, the largest acute-care, restructured, tertiary hospital in Singapore, has 1,785 inpatient beds and offers a wide variety of clinical services including solid organ and hematopoietic stem cell transplant. Periodically, our microbiological laboratories receive specimen requests from other restructured and private hospitals. Over the course of 10.5 years, our bacteriology laboratory received a total of 1,211 requests for *Cryptosporidium* microscopy; of which, only 15 specimens (1.24%) were found to be positive. In the 18-month period following the introduction of GIP testing by our molecular laboratory, 2,590 stool specimens have been tested. Twenty-two of 2,590 (0.85%) samples from 21 patients returned positive for *Cryptosporidium* spp. As part of the initial validation process for GIP testing in our laboratory, 10 clinical specimens, of which two were positive for *Cryptosporidium* spp. and eight were negative on the GIP, were compared with stool microscopy, and shown to have 100% concordance. After the official launch of GIP testing,

* Address correspondence to Shuwei Zheng, Department of Infectious Diseases, Singapore General Hospital, Outram Rd., Singapore 169608. E-mail: zheng.shuwei@singhealth.com.sg

FIGURE 1. (A) Modified acid-fast bacilli stains, magnified at $\times 1,000$, showing oocysts of *Cryptosporidium* spp. measuring approximately 4.62 μm . (B) Immunofluorescence staining, magnified at $\times 400$, revealing oocysts of *Cryptosporidium* spp. measuring 4.40 μm . This figure appears in color at www.ajtmh.org.

the first two specimens positive by nucleic acid testing were also positive by microscopy (excluding the current case).

DISCUSSION

Our laboratory data suggest that cryptosporidiosis may account for 0.85–1.24% of clinical cases presenting with diarrheal illness. This appears consistent with published data suggesting a low prevalence for cryptosporidiosis in this region.^{2,3} The higher positivity rate for *Cryptosporidium* microscopy over the GIP is likely a result of a higher pretest probability when clinicians request specifically for the specialized mAFB microscopy test.

While stool microscopy has historically been the diagnostic method of choice in the evaluation of suspected parasitic gastrointestinal infections, nucleic acid detection methods are increasingly adopted for their supposedly increased sensitivity, specificity, and quick turnaround time. However, the correlation between these methods remain uncertain, given the wide variations in nucleic acid assays available and their differing protocols, coupled with the low number of positive clinical specimens for targeted comparisons.⁴ The latter is exceptionally true in low-prevalence settings, which are also often developed countries where such assays are more widely available. Indeed, nucleic acid–based assays can often detect pathogen DNA at low levels, or even that of nonviable microorganisms, casting doubts on clinical significance in such cases.⁵ Few studies, if any, offer direct clinical correlation in the concordance between various diagnostic methods. GIP contains two assays for the detection of approximately 23 different *Cryptosporidium* spp., including *Cryptosporidium hominis* and *Cryptosporidium parvum*, but does not differentiate between species. It fails to detect the very rare species of *Cryptosporidium bovis*, *Cryptosporidium ryanae*, and *Cryptosporidium xiaoi*.⁶ In one of the largest multicenter evaluation for GIP, its sensitivity and specificity for diagnosing cryptosporidiosis were 100% and 99.6%, respectively, but it was tested with only 18 positive clinical specimens. In this study, the comparator assay was real-time polymerase chain reaction (PCR).⁷

Our case demonstrates host susceptibility, with infection in a renal transplant recipient presenting with a gastroenteritis syndrome. He had nucleic acid positivity for cryptosporidiosis confirmed by stool microscopy, highlighting the potential for the use of GIP in immunocompromised patients as a rapid and broad screening tool, where the differential diagnoses for moderate to severe infective gastroenteritis are many, and empirical treatment may sometimes be inadequate. Any doubts arising from positive PCR results on any potentially treatable pathogens may then be independently confirmed by conventional methods. Our laboratory data, albeit limited, also suggest an excellent concordance between the two diagnostic methods available in our laboratory, in a low endemic setting. Larger studies with clinical correlation will be helpful in further evaluating the diagnostic utility of GIP for cryptosporidiosis.

After the acquired immune deficiency syndrome (AIDS), cryptosporidiosis is well-recognized in the solid organ transplant (SOT) population. However, most reports are restricted to case reports or small case series, largely in the renal transplant cohort. The disease incidence varies from 0.34% to 28.5%, reflecting environmental pathogen burden.^{2,7} In a retrospective, single-center study in India, Bhadauria et al.⁸ reported that 119/1,235 (28.5%) renal transplant recipients required hospitalization and evaluation for diarrheal illness. Of these, 34/119 (28.5%) patients had cryptosporidiosis diagnosed through mAFB staining of stool samples. Treatment using combination nitazoxanide and fluoroquinolone appeared superior in oocyst clearance and response when compared with nitazoxanide monotherapy. In another single center study in Argentina, 11/14 (78.6%) symptomatic and 11/26 (42.3%) asymptomatic renal transplant patients had positive stool mAFB for cryptosporidiosis.⁹ Raja et al.,¹⁰ in a single center study in Pakistan, identified 343/644 (53%) symptomatic renal transplant patients with *Cryptosporidium* oocysts identified in their stools using mAFB staining. In the TRANSCRYPTO nationwide retrospective French study, 47 solid-organ transplant patients (41 kidneys) with cryptosporidiosis were diagnosed by stool microscopy, from 2006 to 2010. Of 42 patients with available clinical data, three received symptomatic treatment only,

four had immunosuppression taper only, whereas 35 received nitazoxanide alone (25 patients) or in combination with azithromycin (10 patients). Thirteen of the 35 patients who received nitazoxanide also had immunosuppressants tapered or ceased.¹¹

In AIDS patients, immune reconstitution with highly active antiretroviral therapy has been consistently shown to be the cornerstone of cryptosporidiosis treatment. Likewise, it is widely believed that tapering immunosuppressive therapy may help in the case of SOT. Although various other therapeutic regimens including nitazoxanide,¹² and combination paromomycin and azithromycin¹³ have been used in AIDS patients with some response, therapeutic measures for SOT recipients remain largely anecdotal. Drugs including nitazoxanide, paromomycin, spiramycin, azithromycin, trimethoprim/sulfamethoxazole, rifampin, and fluoroquinolones have been used either as monotherapy or in combination, of varying duration, with response.¹ We illustrate a renal transplant recipient who responded well to a prolonged course of combination paromomycin and azithromycin, with clinical improvement and microbiological clearance.

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Authors' addresses: Shuwei Zheng and Indumathi Venkatachalam, Department of Infectious Diseases, Singapore General Hospital, Singapore, E-mails: zheng.shuwei@singhealth.com.sg and indumathi.venkatachalam@singhealth.com.sg. Kwan Ki Karrie Ko, Department of Microbiology, Singapore General Hospital, Singapore, E-mail: karrie.ko.k.k@sgh.com.sg. Kian Sing Chan, Department of Molecular Pathology, Singapore General Hospital, Singapore, E-mail: chan.kian.sing@singhealth.com.sg.

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