A Case of Sporadic Intestinal Cryptosporidiosis Diagnosed by Endoscopic Biopsy

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Sporadic intestinal cryptosporidiosis is not easily diagnosed and might be overlooked. We present here a case of this disease in a 23-year-old Japanese military man with 3 days of abdominal pain, watery diarrhea, and nausea. The frequency of his diarrhea was more than 10 times per day. After his diarrheal bowel symptoms subsided, a colonoscopy was performed because inflammatory bowel disease was suspected. Although the endoscopic findings indicated non-specific ileitis, intestinal cryptosporidiosis was suspected from the histology of ileal biopsy specimens, and this was confirmed ultrastructurally. At that time, however, the patient was on active duty, and thus it was not possible to confirm this as a definitive diagnosis by an adequate stool examination for \textit{Cryptosporidium}. Routine practitioners should be encouraged to carefully inspect patients for this disease, supported by detailed knowledge of it and its diagnosis. If stool-examination results are negative or are not obtained at first, histological diagnosis by endoscopic biopsy could be a useful way to screen for intestinal cryptosporidiosis. Furthermore, stool or histological examination should be performed in recovered patients because the oocysts may continue to be shed for 1 to 4 weeks after the symptoms disappear. Therefore, endoscopic and histological examinations may be useful tools for the early diagnosis of intestinal cryptosporidiosis, although admittedly they are invasive procedures.

Key words: intestinal cryptosporidiosis, histology

Intestinal cryptosporidiosis is an infectious disease caused by the coccidian parasite \textit{Cryptosporidium} \textsuperscript{[1]}. Previously considered a zoonotic disease, it can be caused in humans by \textit{Cryptosporidium hominis}, \textit{parvus}, and other species \textsuperscript{[2]}. The clinical manifestations are similar among \textit{Cryptosporidium species} \textsuperscript{[3]}: namely, after 2 to 10 days' incubation, the patient's chief symptom is severe, sometimes watery, diarrhea \textsuperscript{[1]}. The symptoms usually disappear within several days to 2 weeks, although in immunodeficient patients they are more severe and prolonged, and can be lethal \textsuperscript{[1]}.

These agents are transmitted by the fecal-oral route. Besides direct contact with infected animals or humans, contaminated water is considered an important source of human infection. The first human case was reported in 1976 \textsuperscript{[4]}, and since then water-borne diseases and outbreaks (WBDOs) by \textit{Cryptosporidium}
species have occasionally occurred throughout the world, even in developed countries [5]. The WBDOs reported to be caused by cryptosporidium infection have been associated with either drinking water (community or individual water supply systems) or recreational water (swimming pools or spas) [6]. Oocysts, which show strong infectivity, are resistant to disinfectants and chlorination, and thus are often found at low levels in treated, “safe” drinking water [7–9]. At present, cryptosporidium is one of the leading causes of reported recreational water-associated outbreaks of gastroenteritis [10], and the incidence of cryptosporidiosis is about 1 to 2 per 100,000 population in the United States [11]. Although intestinal cryptosporidiosis is considered an emerging infection, sporadic cases of intestinal cryptosporidiosis are often clinically and histopathologically overlooked. We report here a sporadic case of intestinal cryptosporidiosis in Japan, which has water supply systems similar to those found in the United States, and we discuss some potential diagnostic pitfalls.

Case Report

A 23-year-old Japanese man, a member of the Japan Self Defense Forces (JSDF), visited an outpatient clinic at the JSDF Hospital Kure (Hiroshima, Japan) complaining of abdominal pain, watery diarrhea, and nausea for the past 3 days. The diarrheal fluid was non-bloody and translucent yellow, and the frequency of diarrhea was more than 10 times per day. He had been otherwise healthy, but had experienced abdominal pain events once every 4 months or so in recent years. His family history was not remarkable, and he did not travel abroad. Moreover, he had no obvious recent history either of contact with animals or of drinking untreated water.

On his first visit, he exhibited low-grade fever, left abdominal tenderness, and increased bowel sounds. Laboratory data revealed a slight elevation in the serum C-reactive protein value. He showed no signs of being an immunocompromised host, and a serological test for human immunodeficiency virus proved negative. Without antibiotics, his symptoms subsided within a few days and his serum C-reactive protein value returned to within normal limits. Since an inflammatory bowel disease such as ulcerative colitis or Crohn’s disease was suspected, the first total colonoscopy was performed on the 8th day after his first visit. At the time of this total colonoscopy, however, inspection for parasitic oocysts in the stools was not performed because his diarrheal bowel symptoms had already subsided. Endoscopically, the terminal ileal mucosa showed evidence of non-specific ileitis, including slight lymphoid follicular hyperplasia and focal congestion (Fig. 1).

In the histological examination performed at the JSDF Hospital Yokosuka (Kanagawa, Japan), the terminal ileum displayed active ileitis (Fig. 1). Characteristic tiny round structures were observed on the luminal surface of the mucosal surface epithelium. They were stained purple with hematoxylin-eosin, deep purple with Giemsa, and red with Gram staining methods, compatible with the staining pattern exhibited by cryptosporidium [1]. These findings led to a suspicion of intestinal cryptosporidiosis. To confirm or rule out the diagnosis, an electron microscopic examination was performed using paraffin-embedded block samples in which intestinal cryptosporidiosis was suspected. Transmission electron microscopy (Hitachi H-7500, acceleration voltage 80 kV; Hitachi, Tokyo, Japan) revealed parasites about 5 micrometers in diameter on the mucosal surface. These represented various stages in the life cycle of the parasite, with features typical of Cryptosporidium species (Fig. 2). From these findings, we confirmed the diagnosis as intestinal cryptosporidiosis, although genetic confirmation was unfortunately not performed.

Following his next visit (which was not until 5 weeks after the first colonoscopy because he was on active duty), a stool examination for the oocysts of parasites, not specifically for cryptosporidium oocysts, was performed by a commercial laboratory. However, none was found. Re-biopsy specimens taken during follow-up colonoscopy revealed no parasites. The immunochromatographic test for cryptosporidium was not performed. The patient made a full recovery and finished by visiting a follow-up outpatient clinic. We found no definite source of infection and no evidence of other diarrheal patients in his vicinity.

Discussion

In routine practice, the diagnosis of sporadic intestinal cryptosporidiosis is difficult. On the basis of the present case, three major pitfalls can be identified
in the making of such a diagnosis. First, physicians seldom encounter intestinal cryptosporidiosis; in Japan, for example, only a small number of cases are reported annually (excluding the occasional outbreaks) [12, 13]. Although watery diarrhea is characteristic of intestinal cryptosporidiosis, immunocompetent patients may show only mild, self-limiting abdominal symptoms. The second pitfall is that the usual stool examination is not particularly good for detecting small cryptosporidium oocysts, like those in the present case. Even if physicians suspect the disease, detection of cryptosporidium oocysts in the stools is
not easy for inexperienced technicians. Therefore, special oocyst-collecting methods, such as the sucrose-gradient-collecting flotation method, may be necessary [14]. The third pitfall is that a total colonoscopy for histological examination is not necessarily performed for patients whose only symptom is diarrhea. Even if adequate biopsy samples are obtained, diagnostic pathologists may not notice the characteristic morphological features of intestinal cryptosporidiosis in materials stained with hematoxylin and eosin, as the oocysts are easily mistaken for stain droplets, concreted mucus, or epithelial fragments. This problem is related to the first pitfall (viz. the rarity of this infection). When cryptosporidiosis is included within a differential diagnosis histologically, special stains, such as Giemsa, should be added. Furthermore, immunohistochemical or ultrastructural studies are also useful for confirming the diagnosis [1], as in the present case. This discussion indicates that careful inspection, supported by an awareness and detailed knowledge of this disease and how to diagnose it, is a necessity for routine practitioners. However, further species-discrimination for cryptosporidial subtypes is difficult from morphology alone. Indeed, it requires genetic analysis [15], which was unfortunately not performed in the present case.

Detection of parasitic oocysts by stool inspection is the gold standard for the diagnosis of diarrheal diseases caused by protozoan organisms. Adequate oocyst collection and immunofluorescent detection of cryptosporidium oocysts are required for the diagnosis of cryptosporidiosis by stool examination. This is a non-invasive test and should be recommended as the first approach. Without such a test, establishing the diagnosis of sporadic cases is especially difficult, because typical manifestations are easily masked by other symptoms. Therefore, many cryptosporidial cases with mild symptoms may be missed as non-specific diarrheal illness. The scenario is different when a patient is suspected of having an inflammatory bowel disease (e.g., ulcerative colitis or Crohn’s disease), because then an invasive examination such as colonoscopy would be performed as the first approach. Fortunately, this would provide us with a second way of finding cryptosporidiosis, as in the present case. In cases with negative or no stool-examination results, endoscopic and histological examinations may be useful tools for the early diagnosis of intestinal cryptosporidiosis. In immunocompromised patients, including patients with acquired immunodeficiency syndrome, intestinal cryptosporidiosis may sometimes be lethal, and its diagnosis will therefore be critically important [1]. In such cases, endoscopy should be considered as a second-line diagnostic tool if the patient’s general condition allows. Pathological examination of biopsy materials may detect not only cryptosporidium, but also isospora, cyclospora, or other gastrointestinal pathogens responsible for opportunistic infections. Isospora or cyclospora, either of which causes similar watery diarrhea, can be discriminated from cryptosporidiosis because these pathogens are found deeply located within the cytoplasm of the mucosal surface epithelium [1, 16, 17].

In Japan, cryptosporidium oocysts are actually found in rivers and recreational waters [18–19]. From the viewpoint of public health and preventive medicine, it is important that even rare diseases are included in a differential diagnosis of diarrheal illness. Although the source of infection could not be elucidated in the present case, hidden sources, such as contaminated water or infected animals or humans, may exist. Recovered patients may still be shedding oocysts, as in the present case (in which active infection was confirmed even after the diarrheal bowel symptoms had subsided). In general, oocysts are thought to continue shedding for up to 1 week, or even 4 weeks, after the symptoms have subsided [20]. Even if the symptoms have subsided, any patient giving cause for suspicion should undergo a stool examination (inspection for parasitic oocysts) or histological examination. Furthermore, suspect patients should avoid recreational waters, not only during the period for which they have a diarrheal bowel symptoms, but also for a few months after recovery (because recovered patients are a potential source of WBDOs).

In conclusion, knowledge of the methods necessary to establish a diagnosis of intestinal cryptosporidiosis, together with an awareness of its clinical manifestations, should be mandatory for routine practitioners. If stool-examination results are negative or not obtained, histological diagnosis by endoscopic biopsy is a useful alternative for detecting intestinal cryptosporidiosis. Furthermore, stool examination or histological examination should be performed in apparently recovered, but still possibly infected, patients to
determine if they are a potential source of future outbreaks.

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References