Acute Blastocystis-Associated Appendicular Peritonitis in a Child, Casablanca, Morocco

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Despite increasing reports that Blastocystis infection is associated with digestive symptoms, its pathogenicity remains controversial. We report appendicular peritonitis in a 9-year-old girl returning to France from Morocco. Only Blastocystis parasites were detected in stools, appendix, peritoneal liquid, and recto-uterine pouch. Simultaneous gastroenteritis in 26 members of the child’s family suggested an outbreak.

Blastocystis is a genus of anaerobic protozoan parasites that infect humans and a vast range of animal species. Prevalence in humans varies from 0.5%–24% in industrialized countries to 30%–76% in developing countries (1,2). Classic clinical features of infection include gastrointestinal symptoms such as nausea, anorexia, flatulence, and acute or chronic diarrhea. Fever is usually absent. An association with irritable bowel syndrome and extraintestinal manifestations such as urticaria has been suggested (2). Reports about invasive infection or disseminated diseases are rare (3). Here, we report the case of a pediatric patient infected with Blastocystis that was manifested by gastroenteritis associated with supplicative appendicitis and peritonitis.

The Study
In August 2013, a 9-year-old girl who was returning to France after a 1-month stay with her family in Casablanca, Morocco, was admitted to Lille University Hospital in Lille. Symptoms started in Casablanca 3 days before hospital admission and included fever, severe diarrhea (>10 liquid defecations/day), vomiting, and abdominal pain in the hypogastric area and in the right and left lower quadrants associated with bilateral dorsal pain, anorexia, and weakness.

Blood count showed 13,850/mm³ leukocytes (75.4% neutrophils, 15.9% lymphocytes, 8.5% monocytes). C-reactive protein level was increased at 266 mg/L (Low risk: <1.0 mg/L; average risk: 1.0–3.0 mg/L; high risk >3.0 mg/L). Traveler’s gastroenteritis was diagnosed, and symptomatic treatment with acetaminophen, phosphogluconol glucoside, and acetorphan was prescribed. However, abdominal pain increased, and total food intolerance occurred in the following hours.

An abdominal ultrasound was performed, revealing appendicitis with suppuration in the recto-uterine pouch and a reflex ileus. Parasitologic examination of fecal matter revealed only abundant Blastocystis vacuolar forms, with >5 parasites per field. We further confirmed absence of Cryptosporidium spp. using glycerin assay and real-time PCR. Yeasts and a multimicrobial flora were present in the fecal material, but other infectious agents such as Salmonella spp., Shigella spp., Campylobacter spp., Yersinia enterocolitica, adenovirus, and rotavirus were not detected. Similarly, multimicrobial flora, but no pathogenic bacteria, were detected in the peritoneal liquid and recto-uterine pouch (Table). Histopathologic observation revealed acute suppurative appendicitis with ulcerations extending deep into the muscularis, which was covered with a supplicative and fibrinous exudate. We observed infiltration by numerous neutrophils, eosinophils, plasma cells, and lymphocytes through all layers and into the serous membrane (Figure, panel A).

After hematoxylin-eosin staining and immunofluorescence labeling by using the anti-Blastocystis Paraflo B monoclonal antibody (Boulder Diagnostics, Marlborough, MA, USA), we detected parasitic forms in the lumen and in the lamina propria of the mucosa (Figure, panels B, C). We used real-time PCR for Blastocystis parasite detection as described (4), using DNA extracted from stools, appendix, peritoneal liquid, and the recto-uterine pouch, which all tested positive. We subsequently performed small subunit rDNA (SSU rDNA) amplification, then cloned the PCR product and sequenced 5 clones from all the DNA samples to subtype (ST) Blastocystis isolates and detect mixed infections (5). We identified ST3 in all the analyzed compartments. Mixed infection with ST2 and ST3 was detected only in the stools. The SSU rDNA gene sequences

1These authors contributed equally to this article.
The child completely recovered after an appendectomy, removal of a stercolith from the appendix lumen, and treatment with tinidazole, 20 mg/kg/d, and ceftriaxone, 50 mg/kg/d for 10 days, together with gentamicin, 5 mg/kg/d for 5 days. Although tinidazole is not the first line medication for treatment of Blastocystis infection, the child recovered completely and showed total clearance of parasites at day 73: using microscopy and real-time PCR on fecal samples, we found negative results for Blastocystis. Data obtained from the child’s mother revealed simultaneous cases of gastroenteritis in 26 family members: 13 adults, 34–98 years of age, and 13 children, 18 months–15 years of age, who lived in the same building at the residential “Mohammadi” area of Casablanca. Adults had mild or moderate diarrhea but symptoms were more severe in children, who all had abundant diarrhea, vomiting, and weight loss. Repatriation in France of an 18-month-old baby was obtained in this study have been deposited in GenBank under accession nos. KJ605630–KJ605649.

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Here, we report dissemination of Blastocystis into the lumen, the mucosa, and the recto-uterine pouch exudate, associated with appendicular acute inflammation, and no other infectious agent was detected. These observations, together with the well-documented acute or chronic inflammation occurring in humans or animals with Blastocystis infections (3,9), likely support the contribution of this infection to the inflammatory process. Infection with ST3 further reinforced this hypothesis. Indeed, the presence of pathogenic strains among ST3 has been confirmed through experimental infections in rats (9). Additionally, a substantial inflammatory reaction and an increased propagation of human colorectal cancer cells exposed to Blastocystis ST3 antigens has been demonstrated in vitro (10). For humans, the pathogenicity of different STs is unclear and remains a debatable issue. ST1 isolates were found to be more prevalent among symptomatic patients in Lebanon (5), but ST3 was found to be the only ST that showed pathogenic potential in Malaysian patients when compared with ST1 and ST2 (11). ST3 was also found to be significantly associated with diarrhea in Libya (p = 0.008) (12). For this case, the fact that only ST3 was detected in all analyzed samples, whereas a mixed infection with ST2 and ST3 was found in the child’s stools, further supports the high invasive potential of ST3. ST3 is the most common ST in Europe, but, in African countries, its frequency varies from 17.8% in Libya (12) to 61.9% in Egypt (13). In Morocco, a 28.7% prevalence of blastocystosis has been

### Table. Microbiological examination of samples of feces, appendix, recto-uterine pouch, and peritoneal fluid from child who had peritonitis, Casablanca, Morocco®

<table>
<thead>
<tr>
<th>Variable</th>
<th>Aug 30</th>
<th>Sept 1</th>
<th>Nov 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure/result</td>
<td>Feces</td>
<td>Appendix</td>
<td>Recto-uterine pouch</td>
</tr>
<tr>
<td>Microscopic examination</td>
<td></td>
<td>Presence of rare Blastocystis†</td>
<td>ND</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td></td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Blastocystis spp.</td>
<td></td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequencing Blastocystis spp.</td>
<td></td>
<td>ST2, ST3</td>
<td>ST3</td>
</tr>
<tr>
<td>Bacterial culture</td>
<td></td>
<td>Negative for Salmonella, Shigella, Campylobacter, Yersinia enterocolitica</td>
<td>ND</td>
</tr>
<tr>
<td>Viral antigen detection</td>
<td></td>
<td>Negative for aenovirus and rotavirus</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND, not done; ST, subtype.*
†Figure, panels B.C.
reported, but data concerning the ST distribution of the parasite are not available (14). Furthermore, although \textit{Blastocystis} infection could not be confirmed among the child’s relatives, the simultaneous occurrence of gastroenteritis cases in the same family and the absence of other infectious agents in the child’s stools suggest a potential outbreak of \textit{Blastocystis} infection. \textit{Blastocystis} parasites could have spread within the child’s family, as previously reported in Italy, where 2 adopted children originating from India and the Côte d’Ivoire transmitted \textit{Blastocystis} parasites to their adoptive parents and grandmother (15). Possible acquisition of this parasite from a common source such as contaminated water could also explain family transmission in this report. Altogether, these data highlight 1) the need for both systematic parasitologic examinations of stools in patients with invasive infections who are traveling from countries with high \textit{Blastocystis} prevalence and 2) the need for routine provision of imidazoles for empiric treatment of peritonitis.

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Dr. Fréalle is a hospital practitioner and researcher in Lille University Hospital Center and Pasteur Institute of Lille. Her primary research interests are respiratory fungal and intestinal parasitic infections, with special emphasis on biodiversity and pathogenesis.

**References**


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