Treatment failure in patients with chronic *Blastocystis* infection

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This article reports long-term infection and treatment failure in 18 symptomatic individuals infected with *Blastocystis* spp. Patients were initially treated with either metronidazole, iodoquinol or triple combination therapy consisting of nitazoxanide, furazolidone and secnidazole. Following treatment, resolution of clinical symptoms did not occur and follow-up testing revealed ongoing infection with the same subtype. Patients then underwent secondary treatment with a variety of antimicrobial agents but remained symptomatic with *Blastocystis* spp. still present in faeces.

Sequencing of the SSU rDNA was completed on all isolates and four subtypes were identified in this group: ST1, ST3, ST4 and ST5. This study highlights the lack of efficacy of several commonly used antimicrobial regimens in the treatment of *Blastocystis* and the chronic nature of some infections. It also demonstrates the need for further research into treatment options for *Blastocystis* infection.

**INTRODUCTION**

*Blastocystis* is the most common enteric parasite present in humans (Tan et al., 2002). There have been up to 14 subtypes described in humans and animals, with subtype (ST) 3 being the predominant subtype identified in most epidemiological studies (Parkar et al., 2010; Meloni et al., 2011; Forsell et al., 2012; Roberts et al., 2013). Transmission has been attributed to the faecal-oral route as well as consumption of contaminated food and water (Leelayoova et al., 2004). Although there is still some debate about the pathogenicity of *Blastocystis*, symptoms attributed to *Blastocystis* infection include diarrhoea, abdominal pain, bloating and vomiting (Boorom et al., 2008; Stensvold et al., 2009). Due to the lack of knowledge of *Blastocystis* pathogenicity, treatment may not be offered, resulting in ongoing symptoms and possible transmission to family and household members. Metronidazole is the most commonly prescribed drug for the treatment of *Blastocystis*, with a large variation in efficacy ranging from 0 to 100 % (Nigro et al., 2003; Moghaddam et al., 2005; Stensvold et al., 2008). Other antimicrobial agents which have been used to treat *Blastocystis* infection include paromomyycin, nitazoxanide, iodoquinol and trimethoprim/sulfamethoxazole, with varying results (Cimerman et al., 2003; Pasqui et al., 2004; Rossignol et al., 2005; Andiran et al., 2006).

**METHODS**

Stool samples were collected from 18 individuals complaining of intestinal symptoms including diarrhoea, abdominal cramps and bloating. All samples were submitted to microscopy of a permanent modified iron haematoxylin stain according to the manufacturer’s instructions (Fotedar). DNA was extracted using the Bioline Isolate faecal DNA kit as per manufacturer’s instructions, and underwent PCR for the detection of *Blastocystis* spp. using a previously described method (Stensvold et al., 2007). DNA sequence analysis was performed on all PCR products generated. PCR products were purified using the QIAquick PCR purification kit (Qiagen) as per the manufacturer’s instructions and sent to the Australian Genome Research Facility (Westmead Millennium Institute, Sydney) for sequencing in both directions, and reads were assembled into a consensus. The SSU rDNA sequences were then compared with those available in the GenBank database using the BLASTN program run on the National Centre for Biotechnology Information server (http://www.ncbi.nlm.nih.gov/BLAST). Samples also underwent PCR for the detection of *Dientamoeba fragilis* using a previously published method (Stark et al., 2010). PCR was performed on all samples positive for *Entamoeba histolytica* complex by microscopy for species identification (Fotedar et al., 2007). All samples were also screened for bacterial pathogens including *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Campylobacter* sp., *Clostridium difficile* and *Aeromonas* sp.

**Abbreviation:** ST, subtype.
RESULTS

Eighteen patients were identified as infected with Blastocystis spp. by microscopy, and this was confirmed by PCR. All patients were symptomatic. A total of four different subtypes (ST) were identified by sequencing: ST1 (n=1), ST3 (n=14), ST4 (n=2) and ST5 (n=1). No bacterial pathogens were isolated. Owing to the chronic nature of infection no viral testing was performed. The cohort consisted of eight females and ten males with a mean age of 37 years (6–62 years). Results are summarized in Table 1.

Patients 1–3 were family members living at the same residence. Symptoms of diarrhoea were described after the ingestion of water from a contaminated water tank from a property in rural New South Wales, Australia. Blastocystis spp. and D. fragilis were detected by microscopy in all three patients. The patients were prescribed a dose of metronidazole 400 mg three times daily but remained symptomatic following treatment. Follow-up testing 1 month post-therapy revealed that all patients were still infected with Blastocystis and patient 2 was still positive for D. fragilis. Subtyping of the isolates demonstrated the same subtypes were present in both the pre- and post-treatment samples (ST5 in patient 1 and ST3 in patients 2 and 3). All three patients were then treated with paromomycin (25 mg kg⁻¹ three times daily). Follow-up clinical consultation and subsequent stool samples revealed clearance of Blastocystis (and D. fragilis in patient 2) with resolution of symptoms.

Patient 4 was positive for Blastocystis ST1. This patient also had E. histolytica complex by microscopy, which was subsequently confirmed as the non-pathogenic Entamoeba dispar by PCR. The patient complained of diarrhoea, nausea and abdominal pain. Initial treatment with metronidazole 400 mg three times daily for 7 days resulted in the clearance of E. dispar; however, Blastocystis was still present and symptoms persisted. The patient was then prescribed a single dose of tinidazole but remained symptomatic and failed to clear Blastocystis. Finally, norfloxacin was administered for 4 weeks. The patient reported a slight reduction in gastrointestinal symptoms and follow-up samples 6 months later showed that, while the patient was no longer symptomatic, Blastocystis was still present in the stool but in very low numbers. The patient’s household contacts were tested for Blastocystis and both the housemate and pet dog tested negative for Blastocystis by microscopy and PCR.

Patient 5 first presented with intestinal symptoms of diarrhoea and abdominal cramps after travelling to Borneo. A laboratory diagnosis of Blastocystis infection was made, but the physician did not prescribe anti-parasitic treatment. The patient’s gastrointestinal symptoms continued for 12 months. The patient was subsequently diagnosed with Blastocystis (ST4) in the absence of any other pathogens. Treatment was commenced immediately with metronidazole 400 mg three times daily for 10 days. Three months following treatment the patient was still symptomatic and follow-up samples confirmed that Blastocystis (ST4) was still present. The patient was then treated with ciprofloxacin. However, 1 year later gastrointestinal symptoms remained and the faeces were confirmed positive for Blastocystis ST4 by microscopy and PCR.

Patient 6 had a history of abdominal cramps, vomiting, diarrhoea and bloating for 3 years. This patient saw several gastroenterologists and was diagnosed with irritable bowel syndrome without having any stool samples collected to exclude infective pathogens. After another year a stool sample was finally obtained and Blastocystis ST4 was identified. The patient was prescribed metronidazole 400 mg three times daily. Symptoms partially subsided after treatment but 2 years later the patient had ongoing gastrointestinal symptoms and Blastocystis ST4 was still present. The patient had several animals which were all tested for Blastocystis. One chicken was positive for Blastocystis ST2 and two guinea fowls were positive for ST7.

Patients 7–18 were all diagnosed with Blastocystis ST3 infection after seeing a gastroenterologist, complaining of diarrhoea and abdominal pain. Five patients were initially treated with metronidazole (400 mg three times daily) for 10 days, two with iodoquinol (630 mg three times daily) and doxycycline (50 mg twice daily) for 20 days, and five with a triple therapy of nitazoxanide, furazolidone and seceidazole. After treatment all patients continued to describe ongoing gastrointestinal symptoms. None of these patients owned pets or had other household members complaining of gastrointestinal symptoms.

DISCUSSION

There is increasing debate over the pathogenicity of Blastocystis, with some studies stating that it is not pathogenic while others argue the validity of Blastocystis being considered a pathogen (Stark et al., 2007; Tan, 2008; Roberts et al., 2013). One viewpoint is that some subtypes of Blastocystis may be pathogenic. This study’s results are consistent with that viewpoint, as we demonstrated by the presence of chronic symptoms in the absence of any other infectious agents (Hussein et al., 2008; Ozyurt et al., 2008; Roberts et al., 2013). This study reports treatment failure for 18 individuals identified with chronic Blastocystis infection. All patients complained of intestinal symptoms including diarrhoea, abdominal cramps and nausea. All patients were infected with Blastocystis and four different subtypes were identified from this group: ST1 (n=1), ST3 (n=14), ST4 (n=2) and ST5 (n=1).

ST3 is the most common subtype found in most epidemiological studies and there has been a low association between subtype and symptoms. There have been several previous
### Table 1. *Blastocystis* subtype results, treatment and household contacts for patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Subtype</th>
<th>Symptoms</th>
<th>Initial treatment</th>
<th>Clearance of symptoms</th>
<th>2nd line treatment</th>
<th>Clearance of symptoms</th>
<th>Time elapsed between initial and latest positive sample</th>
<th>Household contacts and subtype</th>
<th>Travel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>5</td>
<td>Diarrhoea</td>
<td>Metronidazole</td>
<td>No</td>
<td>Paromomycin</td>
<td>Yes</td>
<td>7 months</td>
<td>2 ST3</td>
<td>Rural N.S.W.</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>3</td>
<td>Diarrhoea</td>
<td>Metronidazole</td>
<td>No</td>
<td>Paromomycin</td>
<td>Yes</td>
<td>7 months</td>
<td>1 ST5, 1 ST3</td>
<td>Rural N.S.W.</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>3</td>
<td>Diarrhoea</td>
<td>Metronidazole</td>
<td>No</td>
<td>Paromomycin</td>
<td>Yes</td>
<td>7 months</td>
<td>1 ST5, 1 ST3</td>
<td>Rural N.S.W.</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>1</td>
<td>Diarrhoea, nausea, abdominal pains</td>
<td>Metronidazole</td>
<td>No</td>
<td>Tinidazole, norfloxacin</td>
<td>No</td>
<td>4 years</td>
<td>Household and dog both PCR negative</td>
<td>Southeast Asia</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>4</td>
<td>Diarrhoea, abdominal pains</td>
<td>Metronidazole</td>
<td>No</td>
<td>Ciprofloxacin</td>
<td>No</td>
<td>3 years</td>
<td></td>
<td>Borneo</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>4</td>
<td>Diarrhoea, nausea, bloating, abdominal pains</td>
<td>Metronidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>4 years</td>
<td>Chicken ST2, guinea fowl ST7</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Metronidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Metronidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Iodoquinol, doxycycline</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>48</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Iodoquinol, doxycycline</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>49</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Metronidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>26</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Metronidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>38</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Metronidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>54</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Triple therapy: nitazoxanide, furazolidone, secnidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>29</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Triple therapy: nitazoxanide, furazolidone, secnidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>39</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Triple therapy: nitazoxanide, furazolidone, secnidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>17</td>
<td>51</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Triple therapy: nitazoxanide, furazolidone, secnidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>62</td>
<td>3</td>
<td>Diarrhoea, abdominal pains</td>
<td>Triple therapy: nitazoxanide, furazolidone, secnidazole</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>1 year</td>
<td>No</td>
<td>–</td>
</tr>
</tbody>
</table>
studies that have shown approximately 40–60% of patients with ST3 have some sort of gastrointestinal symptom (Jones et al., 2009; Janert-mort et al., 2013; Roberts et al., 2013). ST3 was the most common subtype isolated in this group and all patients had symptoms suggesting that this subtype is pathogenic. Although four patients had other parasites present on initial testing, after the first treatment these parasites were cleared while intestinal symptoms still persisted. No other pathogens were identified, which suggests that Blastocystis was the probable cause of these symptoms.

The three family members that presented with Blastocystis stated that symptoms started after the consumption of water from a water tank whilst on holiday in rural Australia. There were four members of the family that were infected with Blastocystis, but after the initial treatment, one patient had resolution of symptoms and diagnostic methods confirmed that infection had been cleared. There is the possibility that re-infection rather than treatment failure occurred with the other three members of the family, but as two different subtypes were isolated within this group and as the fourth member did not have any further symptoms, this appears unlikely. It has been previously suggested that treatment failure could be mistaken for re-infection. This should be considered in all cases where symptoms persist (Stensvold et al., 2010).

Although there have been several publications that have reported the eradication of Blastocystis with metronidazole, there have been few studies that have examined treatment failure in relation to subtype. Metronidazole is considered first-line treatment but reported success rates vary between 0 and 100% (Stensvold et al., 2010). Metronidazole treatment failure has been reported in one patient with ST2-related urticaria and gastrointestinal disease (Vogelberg et al., 2010), in another patient with severe intestinal symptoms associated with ST8 (Stensvold et al., 2008), and for six ST3 and one ST1 infections in patients who presented with both urticarial and gastrointestinal symptoms (Jones et al., 2009). A study of 11 symptomatic patients (five with ST1, four ST3, four ST4 and one ST6 with three mixed infections) treated with either metronidazole or trimethoprim/sulfamethoxazole reported that no infection was cleared by treatment (Nagel et al., 2012). The 11 patients in our study who were initially treated with metronidazole were shown to have treatment failure along with the other seven patients treated with combination therapy. It has previously been suggested that some subtypes might be more resistant or are more likely to fail treatment than others. However, our study, where four different subtypes were identified, suggests that any subtype could result in treatment failure. These results also highlight the fact that Blastocystis should be considered a pathogen as all patients noted severe symptoms in the absence of any other pathogen.

This study also highlights the need for other treatment options for Blastocystis infection. One study has demonstrated the efficacy of Saccharomyces boulardii (250 mg twice a day, Reflot) (Dinleyici et al., 2011) while others have reported treatment with trimethoprim/sulfamethoxazole with varying results: 22% eradication (Moghaddam et al., 2005), 95% clearance (Ok et al., 1999) and 100% efficacy (Stensvold et al., 2008). Other studies suggest paromomycin is the most effective agent for clearing Blastocystis, with up to 100% efficacy, and in our study paromomycin also appeared to be effective (Armentia et al., 1993; Kick et al., 2002; Pasqui et al., 2004; Valsecchi et al., 2004; van Hellemont et al., 2013). A number of other studies have highlighted the variable efficacy of a number of antimicrobial agents, including nitazoxanide, iodoquinol, tinidazole, emetine, pentamidine, iodochlorohydroxyquine and furazolidone (Markell & Udkov 1986; Romero-Caballo et al., 1997; Moghaddam et al., 2005; Rossignol et al., 2005; Mirza et al., 2011).

Both the Centre for Disease Control (CDC) and the Australian Therapeutic Guidelines refer to the clinical significance of Blastocystis as controversial. The CDC recommends treatment with metronidazole, trimethoprim/sulfamethoxazole or nitazoxanide, while the Therapeutic Guidelines recommend Blastocystis is treated with tinidazole, metronidazole or nitazoxanide. There is a minor comment that says that pregnant woman should be treated with paromomycin. Unfortunately paromomycin is not readily available in Australia and is accessed on a case by case basis via the Special Access Scheme. From this and other studies it would appear that the recommended treatments should be revised and that further studies are required to determine the most effective treatment options for Blastocystis infection. Blastocystis should be considered a potential pathogen when in the presence of symptoms and the absence of any other infectious agents. Metronidazole should no longer be considered the first-line treatment prescribed.

Host factors such as age and ethnicity may play a role in the severity and length of Blastocystis infection. The mean age for patients in this group was 37, with only one child in the group. A previous study showed that clearance rates for Blastocystis infection increase as age increases and this could be a factor in this group with almost all of the patients above the age of 27 (Pipatsatitpong et al., 2012). It has previously been shown that IL-8 and IL-10 single nucleotide polymorphisms (SNPs) play a role in Blastocystis infection (Olivo-Diaz et al., 2012). SNPs at IL-10 have been shown to vary between populations and this may play a role in this Australian Caucasian study group in terms of disease and clearance (Meenagh et al., 2002).

CONCLUSION

This study reports the failure of treatment to clear Blastocystis infections in 18 patients treated with a number of different antimicrobial agents, in particular the recommended treatment agent, metronidazole. This study identified four different subtypes – ST1, ST3, ST4 and ST5 – and demonstrated that there is not one particular
subtype that has a higher rate of treatment failure. This study also highlights the pathogenic role of Blastocystis and reasserts that it should be considered a pathogen when found in conjunction with symptoms and no other infectious agents. Treatment failures highlight the need for further antimicrobial testing to be performed to expand therapeutic options for the management of Blastocystis when treatment failure does occur.

REFERENCES


