Blastocystis sp.: Epidemiology and evidences of its pathogenic role

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Resumen

Blastocystis spp. es un parasito protozoario común en humanos. En los últimos años, su rol como patógeno se ha convertido en una tema común de discusión en la práctica clínica. Esta revisión describe avances recientes en la patogénesis, manifestaciones clínicas, diagnóstico y tratamiento de esta infección en humanos. Los subtipos de Blastocystis spp. y el estado inmune del hospedero podrían estar asociados con el desarrollo de la enfermedad. Muchos casos de infección por Blastocystis se han resuelto después de recibir un tratamiento adecuado. Ciertas poblaciones vulnerables desde el punto de vista inmune (VIH/SIDA, transplantados) pueden estar en mayor riesgo de infección por Blastocystis. En los últimos años, la evidencia científica soporta el rol patogénico de Blastocystis en humanos, pero estos mecanismo a nivel celular son aún poco conocidos por lo que más investigaciones son necesarias.

Palabras clave: Blastocystis | Blastocystis/inmunología | Blastocystis/genética | Blastocystis/patogenicidad | Urticaria.

Abstract

Blastocystis spp. is a common protozoan intestinal parasite in humans. In the last years, its role as pathogen has become a common topic of discussion in clinical practice. This review describes recent advances in pathogenesis, clinical manifestations, diagnosis and treatment of this infection in humans. Blastocystis spp. subtypes and host immune status may be associated with the development of disease. Resolution of symptoms after therapy have been reported in several cases of Blastocystis infection. Vulnerable populations such as immunocompromised (HIV/AIDS, transplanted) could be at a greater risk for Blastocystis infection. In recent years, scientific evidence supports the pathogenic role of Blastocystis in humans, but these mechanisms at a cellular level are still unknown and further research is warranted.

Key words: Blastocystis | Blastocystis/immunology | Blastocystis/genetics | Blastocystis/pathogenicity | Urticaria.
Introduction

Nine subtypes of Blastocystis spp. have been described to have the potential to infect humans. Each subtype is determined by analyzing the small-subunit ribosomal RNA gene. The old term Blastocystis hominis has been suggested to no longer be adequate because humans are not the most common host. The reservoirs for most subtypes are nonhuman primates, mammals, and birds. Blastocystis has been associated to a variety of gastrointestinal disorders such as irritable bowel syndrome, terminal ileitis, ulcerative colitis or associated with immunosuppression (AIDS, transplant population), but a definitive role as pathogen has not been established yet. In clinical practice, the challenge is to treat or not to treat a symptomatic patient who has Blastocystis in the stools. This is a common finding in patients from developing countries where clinicians are commonly encountered with a positive test result for Blastocystis, whereas in developed countries it is uncommon. Because of the controversial pathogenic role of this protozoan in humans, we reviewed the most recent advances in pathogenesis, clinical manifestations, diagnosis and treatment of Blastocystis spp. in humans.

Epidemiology

Blastocystis is an obligated anaerobic protozoan that resides in the colon of humans. It was previously known as a nonpathogenic yeast. Phylogenetic studies based on small subunit rRNA gene sequences have reallocated Blastocystis sp. into Sub-Kingdom Chromobiota, Infra-Kingdom Stramenopiles, Sub-Phylum Opalinata, Class Blastocystea and Genus Blastocystis. Microscopically, the size of Blastocystis can range from 5 to 40 µm, this variable size is explained due to the lack of cell wall.

In terms of transmission, the most accepted route is fecal-oral through contaminated food or water. Despite the fact that a zoonotic cycle of transmission seems plausible, risk factors associated with certain animal contact have not been yet established. Nevertheless, contaminated water seems to be the most important factor associated with Blastocystis infection. In developing countries, the consumption of unboiled water has 2.5 times higher the odds to have Blastocystis in stools.

The most vulnerable populations are travelers, children, immigrants, HIV individuals, transplant or chemotherapy patients. A higher rate of diarrhea associated with Blastocystis has been found in HIV positive patients when compared to non-HIV patients. Blastocystis was found in 14% of liver/kidney transplanted individuals with diarrhea in Brazil. There have been reports that about 56% of immigrants from developing to developed countries have Blastocystis in stools. Food-handlers infected by Blastocystis can transmit it to other people through contaminated food.

The impact of Blastocystis in public health is unknown. Human cases of Blastocystis seem to have been undoubtedly disregarded in the past and there have not been estimates of infected people around the world or by country. Nonetheless, the prevalence of the disease may be high since Blastocystis has a worldwide distribution, occurs in both children and adults and is commonly seen in clinical practice. A university-based hospital in Peru reported that almost one third (35%; n=2,056) of the patients examined for intestinal parasites had Blastocystis in their stools. In a European retrospective study, Blastocystis was the most common protozoan found (7%) from 5,351 patients (hospital-based population). Furthermore, Blastocystis has also been found in endemic indigenous communities from South America whose prevalence rates were 46% in children from Venezuela and from 9.9% to 46% in Peru.
These findings stress the importance of studying some unresolved issues in the epidemiology of Blastocystis including the burden of disease, role of asymptomatic carriage, genetic predisposition of some native populations, risk factors to develop subsequent gastrointestinal symptoms and determination of Blastocystis spp. subtypes in the affected populations.

Pathogenesis

In vivo and in vitro studies suggest that some subtypes of Blastocystis are more likely to cause disease in humans. In other words, humans may harbor several subtypes but not all of them cause disease. The host specificity of Blastocystis is low, numerous zoonotic isolates can be found in several animals or humans. These subtypes are indistinguishable under the routine microscopic examination. DNA-based tests are needed to identify a subtype. By phylogenetic analysis, it has been reported about 12 different species of Blastocystis within the genus.

Among them, the most common is subtype 3 whose geographic distribution seems to be cosmopolitan, followed by subtypes 1, 2, and 4. However, the distribution of these genetic subtypes may vary according to the population. In Amazonic indigenous communities, the most common subtype found was 1 followed by 2 and 3, whereas the subtype 4 is more restricted geographically to Europe and North America. Furthermore, another study suggested that the vast genetic variation of subtype 3, in contrast to subtype 4 (almost a clonal subtype), could explain the relatively recent colonization of humans by subtype 4 because of its restricted geographic distribution to Europe and North America.

In regards to the virulent factors, some subtypes have been associated to have certain antigens that could play a key role on the pathogenesis. For instance, cystein proteases seem to play an important role on the pathogenesis. The pathogenic role of subtype 3 can be directly associated to the production of cystein proteases. They can also increase directly the IL-8 production in human colonic epithelial cells through a NF-kB dependent mechanism. In the subtype 4, the cystein proteases cause local host responses including mild goblet cell hyperplasia and upregulation of the expression of interferon-γ, interleukin (IL)-12, tumor necrosis factor alpha and mild goblet cell hyperplasia in the cecal mucosa of rats. Further research is needed to continue clarifying the role of these and other cystein proteases.

In the past, the controversy between Blastocystis and disease was based on the fact that this parasite may not invade the colonic mucosa. However, recent basic research studies have demonstrated the opposite. Urinary levels of hyaluronidase, IL-6 and IL-8 were significantly increased in infected experimental rats by Blastocystis when compared to controls, which indicate that tissue destruction and inflammation occurred in the infected rat. By humoral immune response, IgA was the most common immunoglobulin found in intestinal secretions from infected mice whereas IgM was found in the serum, which demonstrates a clear immunogenic reaction to the parasite. Finally, the evasion of the immune response by the parasite has also been described. Nitric oxide (NO) induces apoptosis-like cell death in Blastocystis, but this parasite can evade the host defense by depressing the host NO production, similar to other parasites such as Giardia or Entamoeba.

In light of the above evidence, it has been suggested that some subtypes of Blastocystis can produce inflammation and invasion in the host. However, the underlying basic mechanisms into the pathogenesis at a cellular level by each subtype are still poorly understood but promising studies are expected in the near future. For sure, a variable inflammatory response seems to occur in the infected host.
Clinical cases

Blastocystis is the term that refers Blastocystis infection in humans. The most common symptoms are bloating, vomiting, diarrhea, increased abdominal sounds and abdominal pain. Immunocompromised individuals are more susceptible to develop symptoms associated with Blastocystis, whereas immunocompetent subjects may have a self-limited disease.\(^\text{29}\) Since the relationship between Blastocystis and clinical infection is controversial and part of the objective of this review is to update the reader with clinical information, a search of case reports was performed in the literature (Database: Medline) between 2001 and 2011 using the keywords "Blastocystis" AND "Disease" OR "case reports". Inclusion criteria consisted of cases with a definitive microscopic diagnosis of Blastocystis in stools (not serology) and clinical response to treatment. Reports in the following languages were accepted; Spanish, English and Portuguese. Cases reported in other languages or those inaccessible were not included in this study.

Results are presented in table 1.\(^\text{38-52}\) Nineteen cases were published between 2001 and 2011. The median age was 45 year-old (range: 11-67) and 37% were male. About 58% presented with gastrointestinal symptoms such as diarrhea and abdominal pain mainly. The most common extra-intestinal symptom was urticaria in about 37% of the patients. This non-gastrointestinal symptom can be a clue for the clinician to suspect Blastocystis infection. Furthermore, it has been postulated that urticaria may be associated to particular subtypes of Blastocystis.\(^\text{53,54}\)

<table>
<thead>
<tr>
<th>Age / Gender</th>
<th>Risk factor</th>
<th>Clinical Manifestations</th>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>47 / M</td>
<td>Traveler</td>
<td>Diarrhea, abdominal bloating, rectal bleeding</td>
<td>Colonic ulcer biopsy</td>
<td>Metronidazole for 10 days</td>
<td>38</td>
</tr>
<tr>
<td>46 / F</td>
<td>Colon cancer</td>
<td>Peritonitis, intestinal perforation</td>
<td>Peritoneal fluid cytology</td>
<td>N/A</td>
<td>39</td>
</tr>
<tr>
<td>21 / F</td>
<td>N/A</td>
<td>Chronic angioedema and urticaria</td>
<td>Stool examination</td>
<td>Paromomycin for 7 days</td>
<td>40</td>
</tr>
<tr>
<td>67 / M</td>
<td>HCV</td>
<td>Chronic diarrhea, rectal bleeding (ulcers)</td>
<td>Stool examination</td>
<td>Metronidazole for 10 days</td>
<td>41</td>
</tr>
<tr>
<td>65 / F</td>
<td>Dialysis</td>
<td>Abdominal pain, diarrhea, eosinophilia</td>
<td>Stool examination</td>
<td>Metronidazole for 7 days</td>
<td>42</td>
</tr>
<tr>
<td>12 / M</td>
<td>N/A</td>
<td>RLQ abdominal pain, nausea, vomiting</td>
<td>Stool examination</td>
<td>Metronidazole and cotrimoxazole</td>
<td>43</td>
</tr>
<tr>
<td>11 / M</td>
<td>Rural</td>
<td>Watery diarrhea, nausea, vomiting</td>
<td>Stool examination</td>
<td>Metronidazole and cotrimoxazole</td>
<td>43</td>
</tr>
<tr>
<td>24 / F</td>
<td>Traveler</td>
<td>Mild chronic diarrhea, urticaria</td>
<td>Stool examination</td>
<td>Metronidazole for 10 days</td>
<td>44</td>
</tr>
<tr>
<td>32 / F</td>
<td>N/A</td>
<td>Chronic urticaria</td>
<td>Stool examination</td>
<td>Metronidazole for 10 days</td>
<td>45</td>
</tr>
<tr>
<td>45 / F</td>
<td>N/A</td>
<td>Chronic urticaria</td>
<td>Stool examination</td>
<td>N/A</td>
<td>46</td>
</tr>
<tr>
<td>62 / F</td>
<td>N/A</td>
<td>Itching, pruritus</td>
<td>Stool examination</td>
<td>Paromomycin for 10 days</td>
<td>47</td>
</tr>
<tr>
<td>34 / F</td>
<td>N/A</td>
<td>Urticaria, pruritus</td>
<td>Stool examination</td>
<td>Metronidazole and paromomycin (failed),</td>
<td>47</td>
</tr>
<tr>
<td>69 / F</td>
<td>N/A</td>
<td>Urticaria, pruritus</td>
<td>Stool examination</td>
<td>Metronidazole for 10 days</td>
<td>47</td>
</tr>
<tr>
<td>45 / F</td>
<td>N/A</td>
<td>Diarrhea, anasarca, hypoalbuminemia</td>
<td>Stool examination</td>
<td>Metronidazole for 10 days</td>
<td>48</td>
</tr>
<tr>
<td>44 / M</td>
<td>Renal transplant</td>
<td>Pain, vomiting, diarrhea, abdominal pain</td>
<td>Stool examination</td>
<td>Metronidazole for 7 days</td>
<td>49</td>
</tr>
<tr>
<td>64 / M</td>
<td>Renal transplant</td>
<td>Loose diarrhea, low grade fever</td>
<td>Stool examination</td>
<td>Metronidazole for 7 days</td>
<td>49</td>
</tr>
<tr>
<td>39 / M</td>
<td>AIDS</td>
<td>Diarrhea</td>
<td>Stool examination</td>
<td>Metronidazole, nitazoxanide</td>
<td>50</td>
</tr>
<tr>
<td>60 / F</td>
<td>N/A</td>
<td>Chronic urticaria</td>
<td>Stool examination</td>
<td>Paromomycin for 10 days</td>
<td>51</td>
</tr>
<tr>
<td>35 / F</td>
<td>N/A</td>
<td>Chronic urticaria and pruritus</td>
<td>Stool examination</td>
<td>Paromomycin for 7 days</td>
<td>52</td>
</tr>
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</table>
In a case-control study matched by age, gender and socioeconomic group, 61% of the cases with urticaria were positive for Blastocystis in stools in comparison to only 8% in the control group or people without urticaria (p<0.001).\(^{(55)}\)

**Diagnosis**

Blastocystis is a polymorphic organism, and four major morphologic forms—vacuolar, cyst, granular, and amoeboid—have been reported in stools or cultures. Stool examinations are the preferred method for diagnosis. Stools can be stained with trichrome to facilitate identification.\(^{(56)}\) DNA-based techniques can detect Blastocystis and subtypes, which may be an important molecular biological tool for future clinical studies. The following parasitological techniques have been used to detect Blastocystis in stools: spontaneous sedimentation technique in tube (SSTT),\(^{(57)}\) FLOTAC-400 dual technique and formalin-ether concentration technique (EFCT).\(^{(58)}\) 

SSTT was superior significantly when compared to direct smear test and EFCT (p<0.05).\(^{(57)}\) FLOTAC-400 was not superior than EFCT for detecting Blastocystis (p =0.4).\(^{(58)}\) Besides the technique used, multiple stool samples are required to increase the likelihood of detecting Blastocystis cysts. Diagnostic yield increases significantly from 68% to 100% with 1 and 3 stool samples, respectively.\(^{(59)}\) In vitro cultures have also been described in the literature. A short-term in vitro culture on Boeck and Derbholav's medium can have better results (35.5%) than Trichrome-stained smear (27.5%) and saline-sedimentation concentrated smear (21%).\(^{(60)}\)

**Treatment**

In the management of this infection, some factors such as immunity, travel, age, length and severity of symptoms in each individual should be taken into consideration. An acute infection that resolves by itself may not need any therapy.

### Table 2. Therapeutic options for Blastocystosis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Duration (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>Adults 1.5 grams daily; 750 mg Q8h</td>
<td>7 to 10</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Children 15 mg/kg Q12h</td>
<td>7 to 10</td>
<td></td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>Children 6 mg/kg TMP, 30 mg/kg SMX</td>
<td>7 to 10</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Adults 320 mg TMP, 1600 mg SMX</td>
<td>7 to 10</td>
<td></td>
</tr>
<tr>
<td>Furazolidone</td>
<td>100 mg orally Q6h</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Nitazoxanide</td>
<td>500 mg twice a day</td>
<td>3</td>
<td>66</td>
</tr>
<tr>
<td>Paromomycin</td>
<td>25 mg/kg orally divided every 8 h</td>
<td>7 to 10</td>
<td></td>
</tr>
<tr>
<td>Secnidazole</td>
<td>4 grams daily</td>
<td>3</td>
<td>64</td>
</tr>
<tr>
<td>Saccharomyces</td>
<td>250 mg twice a day</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>boulardii</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In general, those with persistent abdominal bloating, diarrhea, abdominal pain or urticaria may require treatment and follow up (see table 2).

Several drugs have showed good activity against Blastocystis. In vitro, the most effective drugs against Blastocystis are the 5-nitroimidazoles (e.g. metronidazole, tinidazole, secnidazole), followed by emetine, furazolidone and trimethoprim.\(^{(60)}\)

Despite metronidazole being the drug of choice for blastocystosis, the rates for cure are variable. Furthermore, resistance has been described several years ago.\(^{(62)}\) An alternative to metronidazole could be furazolidone since it has been reported as being just as potent as metronidazole for Blastocystis.\(^{(61)}\) In a placebo-controlled trial, 88% of patients infected by Blastocystis spp. had clinical resolution after receiving metronidazole.\(^{(63)}\) In another study, 54 out of 81 people achieved cure for blastocystosis (66.6%) after metronidazole 500 mg orally three times a day for 10 days.\(^{(64)}\) The same authors recommended a higher dose of metronidazole at 750 mg three times a day for 10 days when needed. Although the mechanism of action of metronidazole against Blastocystis is unknown, it may work by inhibiting the cystein proteases of Blastocystis. For example, in vitro studies have showed that metronidazole can inhibit the cystein protease WR1 to produce IL-8.\(^{(61)}\)

Other alternative antimicrobials are paromomycin, nitazoxanide, Saccharomyces boulardii-based probiotics.\(^{(65,66)}\) A single-blinded clinical trial compared S. boulardii 250 mg orally twice a day for 10 days versus metronidazole 30 mg/kg daily for 10 days; at day 15 the clinical cure rate was 77.7% versus 66.6%, respectively; whereas at day 30 the clinical cure rate was 94% versus 73%, respectively. Those differences were not statistically significant.\(^{(65)}\) However, the major limitation of this study was the small sample size in each arm (about 15 participants). A randomized, double-blinded, placebo-controlled trial for nitazoxanide showed that the 86% of the group who received nitazoxanide had resolution of symptoms at day 4 versus 38% of cases in the placebo group.\(^{(66)}\)

Limitations of the study were the small sample size and short-term follow up. A clinical trial of trimethoprim-sulfamethoxazole (TMP-SMX) for Blastocystis showed an eradication rate of 94.7% in children and 93.3% in adults,\(^{(67)}\) but in clinical practice in other endemic areas this antibiotic has low cure rate (Terashima, personal communication). Other therapeutic options are secnidazole 4 g daily for 3 days (in children 30 mg/kg daily for 3 days) with a cure rate of about 90.9%.\(^{(64)}\)

If the symptoms persist despite antimicrobials, further assessment is recommended. Other infectious or non-infectious causes can be present concomitantly. For example, a randomized controlled clinical trial compared TMP/SMX versus placebo to eradicate Blastocystis from stools and to decrease recurrent abdominal pain in children, but there was not a statistically significant difference between both groups. Furthermore, it was not proven that Blastocystis was necessarily the cause of the recurrent abdominal pain in those children either,\(^{(68)}\) and further work up may be necessary. Interestingly, Blastocystis has been recently associated with irritable bowel syndrome.\(^{(69)}\) Further clinical trials should evaluate response to treatment according to Blastocystis subtypes, host immune status, age and other comorbidities.

**Conclusions**

Blastocystis spp. (subtypes 1-9) affects humans, but the underlying mechanism of action and pathogenesis is still poorly understood. Blastocystosis is commonly seen in clinical practice in developing countries; and in travelers, immigrants, children and immunocompromised individuals in developed countries. Its impact in public health is increasing and it will continue growing in the
overwhelming tendency that Blastocystis may be pathogenic in humans.

Acknowledgments. We thank Claudia Quiroz for manuscript review.

Conflict of Interest: None

References


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