Is It Useful To Treat Blastocystis sp.? A double-blind placebo-controlled randomised trial

Ludovico Cobuccio (ludovico@cobuccio.me)
University of Lausanne
Marie Laurent
University of Lausanne
Celine Gardiol
Centre for Primary Care and Public Health
Rahel Wampfler
Swiss Tropical and Public Health Institute
Sven Poppert
Swiss Tropical and Public Health Institute
Nicolas Senn
Centre for Primary Care and Public Health
Gilles Eperon
University Hospital of Geneva
Blaise Genton
Centre for Primary Care and Public Health
Serge de Vallière
University Hospital of Lausanne

Research Article

Keywords: gastrointestinal symptoms, irrespective of subtype, PCR, Entamoeba dispar

Posted Date: December 9th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-115389/v1

Abstract

Background

Blastocystis sp. is a protist with a worldwide distribution and able to colonise the gut of humans and of a great variety of animals. It is unclear whether it is just a commensal of a healthy gut microbiota or an infectious parasite that needs to be eradicated. Currently no treatment has proven its usefulness for patients complaining of gastro-intestinal symptoms and found to have *Blastocystis* sp.

The primary objective of this study was to evaluate the usefulness of metronidazole in patients with gastrointestinal symptoms harbouring only *Blastocystis* sp. In addition, we explored whether *Blastocystis* subtype or concomitant parasitic infection detected by polymerase chain reaction (PCR) may influence treatment outcome.

Methods

Adults with persistent gastrointestinal symptoms (> 14 days) visiting a primary care physician and in whom stool microscopy revealed only *Blastocystis* sp. were included. Eligible patients were randomised to receive ten days of metronidazole or placebo, followed by a crossover if still symptomatic. Stool samples were tested for 11 other protozoa with an in-house PCR and *Blastocystis* subtypes were determined by PCR and sequencing.

Results

We screened 474 outpatients for inclusion; 50 met the eligibility criteria. In the metronidazole group, 48% (12/25) reported an improvement of symptoms compared to 44% (11/25) in the placebo group (p = 0.78). After the crossover, again no differences in improvement of symptoms were seen between groups (placebo: 53% (8/15); metronidazole: 50% (8/16)). The in-house PCR was positive for other protozoa in 25% (10/40) of the patients. The protozoa identified were *Dientamoeba fragilis* (5), *Entamoeba dispar* (3) and Cyclospora *cayetanensis* (2). The most frequent *Blastocystis* subtypes were ST4 (11/36) and ST2 (10/36). Stratified analysis according to subtype or the presence of other protozoa showed no significant difference in treatment outcome with metronidazole or placebo.

Conclusion

Among patients infected with *Blastocystis* sp., metronidazole did not improve gastrointestinal symptoms, irrespective of subtype or microscopically undetected coinfection with other protozoa.

Trial registration

ClinicalTrials.gov: NTC01521403, 06-Nov-2012.

Background

Blastocystis sp. is a protistan living in the intestinal tract of humans and a wide range of animals, such as birds, reptiles, amphibians, fish, insects. It has high genetic diversity, with 22 different subtypes[1]. Of the ten subtypes infecting humans, subtype 3 (ST-3) is the single most frequently found.[2] *Blastocystis* sp. has a worldwide distribution with higher prevalence in low- and middle-income countries, which is probably due to poor hygienic conditions and exposure to animals, as well as consumption of contaminated food and water.[3] Travellers to tropical countries seem at increased risk of acquiring *Blastocystis* sp. [4][5] Asymptomatic individuals from temperate, high-income countries can also carry it, prevalence ranges from 14% in Spain [6] to 56% in Ireland [7] *Blastocystis* sp. pathogenicity has long been a matter of debate and it has been postulated that subtypes may differ in pathogenicity and sensitivity to drugs.[8]

The usefulness of treating *Blastocystis* sp. infection is unclear. Several observational studies have shown no association between the presence of *Blastocystis* sp. in the digestive tract and symptoms.[9–12] Similarly, in a case series of 100 patients with stool microscopy positive for *Blastocystis* sp. in Taiwan, only 10 had gastrointestinal symptoms and all improved without receiving any specific therapy.[13] A randomised, double-blind, placebo-controlled trial by Heyland et al. on a paediatric population compared the effect of cotrimoxazole on recurrent abdominal pain and no difference in pain reduction was seen between the 2 groups.[14] Lastly, various *in vitro* studies report evidence of *Blastocystis* drug resistance. [15–17]

Some studies suggest that treating *Blastocystis* sp. may improve non-specific gastrointestinal symptoms, such as nausea, anorexia, abdominal pain, bloating, flatulence, as well as acute and chronic diarrhoea. [18–21] Rossignol et al. conducted two double-blind, placebo-controlled trials evaluating the effect of nitazoxanide for 3 days on symptoms and microbiological eradication in patients without other identified enteric pathogens than *Blastocystis* sp. [22] One trial was conducted in 50 children aged 4–11 years and the other on 50 subjects aged 12 years or older. The clinical response rate was the same for both studies: 86% (18/21) in the nitazoxanide group compared with 38% (5/21) for the placebo group. Dinleyici et al. conducted a randomised, single-blind clinical trial assessing clinical cure rate and parasitological clearance in 48 children receiving the probiotic *Saccharomyces boulardii*, metronidazole or no treatment. [23] Clinical cure rates 15 and 30 days after the end of treatment were 77.7% and 94.4% with the probiotic, 66.6% and 73.3% with metronidazole; in the untreated group, 40% were cured at day 15, a course of metronidazole was given to those still symptomatic.

The best evidence for treating adult symptomatic patients infected by *Blastocystis* sp. with metronidazole derives from a single-blind placebo-controlled trial in which Nigro et al. screened 616 subjects and enrolled 76 patients complaining of diarrhoea with *Blastocystis* sp. as the only identified microorganism and without other identifiable causes. Among subjects receiving metronidazole, 88% reported cessation of symptoms one month after therapy compared to 14% in those receiving placebo. [24]

Our study main objective was to evaluate if, in a primary care setting, treatment with metronidazole would improve persistent gastrointestinal symptoms of patients without significant comorbidities found to harbour *Blastocystis* sp. by stool microscopy. The secondary objective was to describe evolution of gastro-intestinal symptoms with treatment. A post-hoc analysis was performed to determine *Blastocystis* subtypes and to test stools with an in-house real-time PCR for protozoa other than *Blastocystis* sp.

Methods

In this double-blind, randomised crossover trial, all physicians of the general primary care outpatient clinic of the University Hospital of Lausanne, practitioners of the Tropical and Humanitarian division of the Geneva University Hospitals and ten private general practitioners of the neighbouring region acted as recruiters. They evaluated their patients according to their usual practice. When a parasitological exam was deemed necessary, they sent three stool samples fixed in sodium acetate-acetic acid formalin solution (SAF) for parasitology microscopy and for detection of stool antigen of *Giardia lamblia.* Investigators received a notification for specimens positive for *Blastocystis* sp. and contacted the patients for inclusion in the study.

The inclusion criteria were age ≥ 18 years, gastrointestinal symptoms for more than 14 days, a collection of 3 separate stool specimens, identification of *Blastocystis* sp. as the only pathogen on microscopy and negative *Giardia lamblia* stool antigen. Exclusion criteria were as follows: significant alteration of general condition, axillary temperature > 37,5 °C, visible blood in the stool, known chronic digestive symptoms, immunosuppression (due to drugs or disease), oncological disease, pregnancy or lactation, weight loss > 10% from the usual weight, antimicrobial treatment within the last three months. Patients being treated with anti-vitamin K or disulfiram were also excluded as metronidazole is known to increase their activity.

After signing the informed consent, patients had to complete a questionnaire evaluating the following symptoms: number of stools per day during the three previous days, presence of soft or unformed stool, as well as the severity of abdominal pain, bloating and flatulence on a scale from 0 to 10. After that, patients were randomised with a 1:1 ratio in a double-blind fashion to a regimen of metronidazole 500 mg or placebo three times a day for ten days.

The University Hospital Pharmacy oversaw the capsules production and drug packages preparation. Metronidazole and placebo capsules were visually indistinguishable. Drug packages were prepared with four, visually indistinguishable containers labelled A to D, and filled with either metronidazole or placebo. The Pharmacy generated a randomisation list with the containers' content to ensure that each patient received sequentially the two drugs. The randomisation list was kept in a secure place within the Pharmacy and was not available to the physicians and the investigators.

Treating physicians gave a drug package to the included patients according to the administration sequence list. Ten to fourteen days after the end of treatment, we assessed the symptoms again with the questionnaire. In addition, patients had to self-evaluate the impact of the treatment on their general

wellbeing according to an ordinal scale consisting of four categories: no improvement – slight improvement – significant improvement – cured.

The second drug was given to patients in both groups who considered themselves as not cured. Symptoms were again assessed 10 to 14 days after the end of the 2nd-course treatment using the questionnaire as mentioned above.

The primary study endpoint was the proportion of participants reporting any improvement (defined as either “slight improvement”, “significant improvement” or “cured”) of their general wellbeing. Secondary endpoints were the proportion of participants reporting unformed or soft stool, the mean number of stools per day, maximal abdominal pain, bloating and flatulence during the three previous days.

After the end of the trial, we determined the *Blastocystis* subtype by sequencing the 18SrRNA gene and tested 40 frozen stool samples for 11 protozoa (*Entamoeba dispar, Entamoeba histolytica, Cryptosporidium hominis, Cryptosporidium parvum, Encephalitozoon* spp. *Entamoeba moshkovskii, Entamoeba polecki, Dientamoba fragilis, Giardia lamblia, Cyclospora cayetanensis, Cystoisospora belli*) by an in-house TaqMan probe-based real-time PCR developed at the Swiss Tropical and Public Health Institute (SwissTPH) (See Appendix A). Samples positive for *Blastocystis* 18SrRNA real-time PCR were subsequently amplified by PCR to obtain a 600 bp long fragment suitable for sequencing and determination of the *Blastocystis* subtype according to Scicluna *et al*.[25]. All details of the molecular methods are provided in the supplementary material. (Molecular Microbiology Procedures)

We calculated the sample size based on the proportion of patients still reporting unformed or soft stools after treatment in the study from Nigro et al.[24] With a two-sided 5% significance level, a power of 90% and anticipating a dropout rate of 20% we estimated that we would need 200 patients to show a reduction of the proportion of patients reporting unformed or soft stool from an initial 50–25%. Statistical analysis included a chi-square analysis for dichotomous outcomes and a Wilcoxon rank-sum test for ordinal outcomes. We chose an alpha level of .05 for all statistical tests. Statistical analyses were performed with STATA 15. All methods were carried out in accordance with relevant guidelines and regulations. The study was registered on ClinicalTrials.gov under the number NTC01521403 on the 6th of November 2012.

Results

From December 2012 to April 2017, we screened 474 patients of which 424 refused or did not met inclusion criteria (Fig. 1)

Thus, fifty patients met the eligibility requirements and were enrolled. Twenty-five patients were randomised to each group with comparable baseline characteristics. (Table 1)

Table 1
Baseline patient characteristics by treatment group

	Placebo (n = 25)	Metronidazole (n = 25)	All (n = 50)
Age, mean (SD), years	42.92 (11.49)	40.92 (14.06)	41.92 (12.75)
Male/Female	14/11	11/14	25/25
Weight, kg, median (interquartile range)	72.0 (65–81) Missing data = 2	66.5 (63–75) Missing data = 2	69 (63–80) Missing data = 4
Travel history (%)	19/25 (76.0)	22/25 (88.0)	41/50 (82.0)
By destination (%)			
Sub-Saharan Africa	4/19 (21.0)	3/22 (13.6)	7/41 (17.1)
Caribbean–Central America	1/19 (5.3)	1/22 (4.5)	2/41 (4.9)
South America	3/19 (15.8)	4/22 (18.2)	7/41 (17.1)
Indian subcontinent–Southeast Asia	10/19 (52.6)	6/22 (27.3)	16/41 (39.0)
Europe	2/19 (10.5)	8/22 (36.4)	10/41 (24.4)
Asia	1/19 (5.3)	2/22 (9.1)	3/41 (7.3)
Northern Africa	1/19 (5.3)	3/22 (13.6)	4/41 (9.7)
Abnormal stool consistency (%)	20/25 (80.0)	19/25 (76.0)	39/50 (78.0)
Bowel movements, median (interquartile range)	2 (1–3)	2 (2–2)	2 (1–3)
Abdominal pain, median (interquartile range)	4 (2–6)	3 (0–6) Missing data = 1	4 (0–6) Missing data = 1
Bloating, median (interquartile range)	6 (3–7)	3.5 (0–6) Missing data = 1	5 (1–7) Missing data = 1
Flatulence, median (interquartile range)	7 (4–8)	4.5 (2.5-7) Missing data = 1	6 (3–8) Missing data = 1

The mean age was 41.9 years, 82% reported travel abroad in the previous 12 months. The most frequent destinations were the Indian subcontinent and Southeast Asia, 78% reported loose stools. Median (interquartile range) number of bowel movements, maximum abdominal pain, bloating and flatulence

severity were 2 (1–3), 4 (0–6), 5 (1–7), 6 (3–8) respectively (Table 1). Before the start of the study, eight patients withdrew their consent or were lost to follow-up (Specific reasons mentioned in the study flow, Fig. 2).

Patients in both treatment groups experienced an improvement in symptoms 10 to 14 days after treatment completion. In the intention-to-treat analysis, 48% of patients in the metronidazole group reported an improvement of their general wellbeing compared to 44% in the placebo group. This difference was not significant ($\chi^2(1, n = 50) = 0.08$, $p = .78$, OR = 1.17, 95% CI 0.062–2.29) and the effect size was very small (Cohen's $h = 0.08$). The proportion of patients reporting abnormal stool consistency decreased from 80–44% in the placebo group and from 76–36% in the metronidazole group. Median abdominal pain (interquartile range) decreased from 4 (2–6) to 1 (0–5) in the placebo group and from 3 (0–6) to 1.5 (0-3.5) in the metronidazole group (Table 2).

Table 2
Baseline patient characteristics by treatment group

	After the 1st course of treatment			**After crossover**	
Variables	Placebo (n = 25)	Metronidazole (n = 25)	p value	Placebo (n = 15)	Metronidazole (n = 16)
Abnormal stool consistency (proportion, %)	11/25 (44%)	9/25 (36%)	0.56	7/15 (47%)	10/16 (63%)
Number of bowel movements per day in the last 3 days (median, interquartile range)	2 (1–2) Missing data = 1	2 (1–2) Missing data = 3	0.89	2 (1–2) Missing data = 1	1 (1–2) Missing data = 1
Abdominal pain on a scale from 0 to 10 (median, interquartile range)	1 (0–5)	1.5 (0-3.5)	0.79	2 (0–4)	1.5 (0-4.5)
Bloating on a scale from 0 to 10 (median, interquartile range)	4.5 (0–6)	2.5 (0–3)	0.23	3 (0–6)	3.5 (0.5-6)
Flatulence on a scale from 0 to 10 (median, interquartile range)	5 (3–6)	3 (2-5.5)	0.17	4 (2–7)	4.5 (2.5–6.5)
Positive impact on general wellbeing (proportion, %) *(Per protocol)*	11/22 (50%)	12/20 (60%)	0.52	8/15 (53%)	8/16 (50%)
Positive impact on general wellbeing (proportion, %) *(Intention to treat)*	11/25 (44%)	12/25 (48%)	0.78	N/A	N/A

In the 2nd treatment phase, 64% (16/25) from the placebo group received metronidazole, and 60% (15/25) from the metronidazole group received a placebo. Again, no differences in improvement of

symptoms were seen between treatment groups (53% (8/15) in the placebo group versus 50% (8/16) in the metronidazole group) (Table 3). No serious adverse events or unintended events were registered.

Table 3
Endpoints evaluated 10 days after the end of the 2nd course of treatment

Variables	Placebo (n = 15)	Metronidazole (n = 16)
Abnormal stool consistency (proportion, %)	7/15 (47%)	10/16 (63%)
Number of bowel movements per day in the last 3 days (median, interquartile range)	2 (1–2) Missing data = 1	1 (1–2) Missing data = 1
Abdominal pain on a scale from 0 to 10 (median, interquartile range)	2 (0–4)	1.5 (0-4.5)
Bloating on a scale from 0 to 10 (median, interquartile range)	3 (0–6)	3.5 (0.5-6)
Flatulence on a scale from 0 to 10 (median, interquartile range)	4 (2–7)	4.5 (2.5–6.5)
Positive impact on general wellbeing (proportion, %)	8/15 (53%)	8/16 (50%)

For the post-hoc study aimed at determining the presence of protozoa by PCR and *Blastocystis* sp. subtyping, enough samples were available for 80% (40/50) of patients. Twenty-five per cent (10/40) had a positive PCR for other protozoa undetected by microscopy (Fig. 3), distribution did not differ between the 2 groups.

In the placebo group the following protozoa were identified by PCR: *Dientamoeba fragilis* = 3, *Entamoeba dispar* = 2, *Cyclospora cayetanensis* = 1. In the metronidazole group the PCR showed the presence of the following additional protozoa: *Dientamoeba fragilis* = 2, *Entamoeba dispar* = 1, *Cyclospora cayetanensis* = 1. *Blastocystis* PCR was negative in 4/40 stool specimens positive for *Blastocystis* sp. by microscopy. *Blastocystis* subtyping was successful in 31/36 patients. The most frequent subtypes were ST4 (11) and ST2 (10), followed by ST3 (8) and ST1 (2). Analyses stratified by subtype or the simultaneous presence of other protozoa did not show any difference in the benefit of treatment with metronidazole.

Discussion

In this double-blind placebo-controlled randomised study, there was no difference in improvement in general wellbeing and specific digestive symptoms in patients infected with *Blastocystis* sp. and treated with metronidazole or placebo.

A strength of our study resided in the stringent inclusion and exclusion criteria, which allowed to test the usefulness of metronidazole on a very homogenous population. Most previous studies included more heterogeneous populations, such as patients with comorbidities. However, at the same time, our

approach limited patient recruitment which ultimately prevented us from reaching the planned sample size of 200 patients. Indeed, we decided to stop the study after 52 months due to slow recruitment.

It can be argued that viral or bacterial agents causing diarrhoea have not been investigated prior to inclusion, but such infectious agents do usually not cause abdominal symptoms lasting more than 14 days. Patients who had received antibiotics in the previous three months were excluded to avoid possible delayed effect by a previous anti-infectious treatment. We also decided purposefully not to perform stool tests after the two treatment periods, because in general practice only clinical cure is relevant. To show a microbiological effect of metronidazole was not the aim of our pragmatic study.

Three out of the 4 randomised trials which investigated the efficacy of antiparasitic drugs for symptomatic *Blastocystis* sp. infections were either focusing on a different drug than metronidazole or on paediatric patients. It is therefore difficult to compare our results to those studies[14, 22–24].

The recommendation for metronidazole use in adult patients with gastrointestinal symptoms and *Blastocystis* sp. infection is mainly based on a study from Italy.[24] Several methodological reasons may explain the divergent results between the Italian study and ours. First, in the Italian study physicians who collected the data were also aware of patient group allocation. Second, the authors assessed abdominal pain, fatigue and stomach gurgling without clearly defining measurement criteria. Finally, the mean duration of diarrhoea was 10 ± 3 days for the metronidazole group and 11 ± 2 days in the placebo group. Therefore, one could not reasonably exclude the presence of further undetected pathogen sensitive to metronidazole. In contrast, our study was double-blinded, we assessed symptoms with a linear analogue scale and, although we did not measure the duration of symptoms, we included only patients complaining of persistent symptoms, i.e. of at least 14 days. We may point out that including only patients with persistent symptoms may have led to the selection of patients with travel related post-infectious irritable bowel syndrome for which anti-parasitic drugs are ineffective.

An in-house PCR panel for intestinal protozoa found a concomitant pathogenic protozoa in 25% of our stool specimens. Our results therefore support to some extent the widely believed theory that the use of metronidazole has sometimes a positive effect on patients with *Blastocystis* infection, because the drug is active against a concomitant infection with another protozoa.[26] In 5 subjects we identified *Dientamoeba fragilis*, a protozoon commonly treated by metronidazole. However, this was not the case, but the numbers were too small to draw any firm conclusion. It is useful to remember that the diagnosis of *Dientamoeba fragilis* and *Cyclospora cayetanensis* by microscopy requires special stains while other protozoa such as *Giardia lamblia* are reliably detected.

The relative distribution of *Blastocystis* subtypes was different from previous studies.[27] We found ST4 (31%) and ST2 (28%) being the most frequent subtypes, and ST3 was present only in 22% of samples. These results suggest that a large proportion of our subjects acquired their *Blastocystis* sp. infection locally, as ST4 is rarely reported outside of Europe.[28] This finding is in line with other studies supporting the hypothesis that ST4 may have higher virulence. [29–31]

Conclusions

Our results suggest that metronidazole effectiveness does not depend on the genetic subtypes of *Blastocystis* sp. The rates of improvements of symptoms were indeed identical with all subtypes, although the small numbers do not permit firm conclusions.

In conclusion, our study although limited by a low recruitment suggests that treating *Blastocystis* sp. with metronidazole is not useful.

Declarations

Ethics approval and consent to participate

The ethics Committee of Canton Vaud, Canton Genève and Swissmedic, the national authorisation and supervisory authority for drugs and medical products in Switzerland, approved the study. The study was registered on ClinicalTrials.gov under the number NTC01521403. The Clinical Research Unit of the University Hospital of Lausanne monitored the study. All patients provided informed consent to participate.

Availability of data and materials

Access to data: data set and statistical analysis is fully available on Zenodo at the following link : https://doi.org/10.5281/zenodo.3905068. SdV and LGC have full access to the data, SdV is the guarantor for the data. Full protocol (in French) is available on demand from the corresponding author.

Competing interests

The authors declare no competing interests.

Funding

The study was funded by Loterie Romande which had no active role in the study.

Authors' contributions

Conceptualisation: SDV; Investigation: ML, CG, GE, RW, SP; Methodology: SDV, ML, BG, NS, GE, LGC; Formal Analysis: LGC; Visualisation: LGC; Project Administration: SDV; Funding Acquisition: SDV; Writing – Original Draft: LGC; Writing – Review & Editing: LGC, SDV, BG, SP, GE, CG, NS ;

Acknowledgements

Preliminary results from this study have been presented as a poster at the biennial Conference of the International Society of Travel Medicine, June 2019, Washington, USA. The authors would like to acknowledge Ksenija Kocur, SwissTPH, who performed the PCRs on the stool specimens, Christelle

Weibel, HUG, for her contribution to patient recruitment, the pharmacy for the manufacture of the study drugs and, last, but not least, all the patients for their essential contribution.

References

[1] Stensvold CR, Clark CG. Pre-empting Pandora's Box: Blastocystis Subtypes Revisited. Trends Parasitol 2020;36:229–32. https://doi.org/10.1016/j.pt.2019.12.009.

[2] Wong KHS, Ng GC, Lin RTP, Yoshikawa H, Taylor MB, Tan KSW. Predominance of subtype 3 among Blastocystis isolates from a major hospital in Singapore. Parasitol Res 2008;102:663–70. https://doi.org/10.1007/s00436-007-0808-0.

[3] Tan KSW. New Insights on Classification, Identification, and Clinical Relevance of Blastocystis spp. Clin Microbiol Rev 2008;21:639–65. https://doi.org/10.1128/CMR.00022-08.

[4] Batista L, Pérez Jove J, Rosinach M, Gonzalo V, Sainz E, Loras C, et al. Low efficacy of metronidazole in the eradication of Blastocystis hominis in symptomatic patients: Case series and systematic literature review. Gastroenterol Hepatol 2017;40:381–7. https://doi.org/10.1016/j.gastrohep.2016.11.003.

[5] El Safadi D, Cian A, Nourrisson C, Pereira B, Morelle C, Bastien P, et al. Prevalence, risk factors for infection and subtype distribution of the intestinal parasite Blastocystis sp. from a large-scale multi-center study in France. BMC Infect Dis 2016;16:451. https://doi.org/10.1186/s12879-016-1776-8.

[6] Reh L, Muadica AS, Köster PC, Balasegaram S, Verlander NQ, Chércoles ER, et al. Substantial prevalence of enteroparasites Cryptosporidium spp., Giardia duodenalis and Blastocystis sp. in asymptomatic schoolchildren in Madrid, Spain, November 2017 to June 2018. Eurosurveillance 2019;24:1900241. https://doi.org/10.2807/1560-7917.ES.2019.24.43.1900241.

[7] Scanlan PD, Stensvold CR, Rajilić-Stojanović M, Heilig HGHJ, De Vos WM, O'Toole PW, et al. The microbial eukaryote Blastocystis is a prevalent and diverse member of the healthy human gut microbiota. FEMS Microbiol Ecol 2014;90:326–30. https://doi.org/10.1111/1574-6941.12396.

[8] Zierdt CH. Pathogenicity of Blastocystis hominis. J Clin Microbiol 1991;29:662–3.

[9] Shlim DR, Hoge CW, Rajah R, Rabold JG, Echeverria P. Is Blastocystis hominis a cause of diarrhea in travelers? A prospective controlled study in Nepal. Clin Infect Dis Off Publ Infect Dis Soc Am 1995;21:97–101.

[10] Miller RA, Minshew BH. Blastocystis hominis: an organism in search of a disease. Rev Infect Dis 1988;10:930–8.

[11] Senay H, MacPherson D. Blastocystis hominis: epidemiology and natural history. J Infect Dis 1990;162:987–90. https://doi.org/10.1093/infdis/162.4.987.

[12] Kain KC, Noble MA, Freeman HJ, Barteluk RL. Epidemiology and clinical features associated with Blastocystis hominis infection. Diagn Microbiol Infect Dis 1987;8:235–44.

[13] Kuo H-Y, Chiang D-H, Wang C-C, Chen T-L, Fung C-P, Lin C-P, et al. Clinical significance of Blastocystis hominis: experience from a medical center in northern Taiwan. J Microbiol Immunol Infect Wei Mian Yu Gan Ran Za Zhi 2008;41:222–6.

[14] Heyland K, Friedt M, Buehr P, Braegger CP. No advantage for antibiotic treatment over placebo in Blastocystis hominis-positive children with recurrent abdominal pain. J Pediatr Gastroenterol Nutr 2012;54:677–9. https://doi.org/10.1097/MPG.0b013e31823a29a7.

[15] Rajamanikam A, Hooi HS, Kudva M, Samudi C, Kumar S. Resistance towards metronidazole in Blastocystis sp.: A pathogenic consequence. PLoS ONE 2019;14. https://doi.org/10.1371/journal.pone.0212542.

[16] Zaman V, Zaki M. Resistance of Blastocystis hominis cysts to metronidazole. Trop Med Int Health TM IH 1996;1:677–8.

[17] Wu Z, Mirza H, Tan KSW. Intra-Subtype Variation in Enteroadhesion Accounts for Differences in Epithelial Barrier Disruption and Is Associated with Metronidazole Resistance in Blastocystis Subtype-7. PLoS Negl Trop Dis 2014;8. https://doi.org/10.1371/journal.pntd.0002885.

[18] Taylor DN, Houston R, Shlim DR, Bhaibulaya M, Ungar BL, Echeverria P. Etiology of diarrhea among travelers and foreign residents in Nepal. JAMA 1988;260:1245–8.

[19] Sheehan DJ, Raucher BG, McKitrick JC. Association of Blastocystis hominis with signs and symptoms of human disease. J Clin Microbiol 1986;24:548–50.

[20] Qadri SM, al-Okaili GA, al-Dayel F. Clinical significance of Blastocystis hominis. J Clin Microbiol 1989;27:2407–9.

[21] Doyle PW, Helgason MM, Mathias RG, Proctor EM. Epidemiology and pathogenicity of Blastocystis hominis. J Clin Microbiol 1990;28:116–21.

[22] Rossignol J-F, Kabil SM, Said M, Samir H, Younis AM. Effect of nitazoxanide in persistent diarrhea and enteritis associated with Blastocystis hominis. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc 2005;3:987–91.

[23] Dinleyici EC, Eren M, Dogan N, Reyhanioglu S, Yargic ZA, Vandenplas Y. Clinical efficacy of Saccharomyces boulardii or metronidazole in symptomatic children with Blastocystis hominis infection. Parasitol Res 2011;108:541–5. https://doi.org/10.1007/s00436-010-2095-4.

[24] Nigro L, Larocca L, Massarelli L, Patamia I, Minniti S, Palermo F, et al. A Placebo-Controlled Treatment Trial of Blastocystis hominis Infection with Metronidazole. J Travel Med 2003;10:128–130.

[25] Scicluna SM, Tawari B, Clark CG. DNA Barcoding of Blastocystis. Protist 2006;157:77–85. https://doi.org/10.1016/j.protis.2005.12.001.

[26] Markell EK, Udkow MP. Blastocystis hominis: pathogen or fellow traveler? Am J Trop Med Hyg 1986;35:1023–6. https://doi.org/10.4269/ajtmh.1986.35.1023.

[27] Alfellani MA, Stensvold CR, Vidal-Lapiedra A, Onuoha ESU, Fagbenro-Beyioku AF, Clark CG. Variable geographic distribution of Blastocystis subtypes and its potential implications. Acta Trop 2013;126:11–8. https://doi.org/10.1016/j.actatropica.2012.12.011.

[28] Stensvold CR, Ahmed UN, Andersen LO, Nielsen HV. Development and Evaluation of a Genus-Specific, Probe-Based, Internal-Process-Controlled Real-Time PCR Assay for Sensitive and Specific Detection of Blastocystis spp. J Clin Microbiol 2012;50:1847–51. https://doi.org/10.1128/JCM.00007-12.

[29] Domínguez-Márquez MV, Guna R, Muñoz C, Gómez-Muñoz MT, Borrás R. High prevalence of subtype 4 among isolates of Blastocystis hominis from symptomatic patients of a health district of Valencia (Spain). Parasitol Res 2009;105:949–55. https://doi.org/10.1007/s00436-009-1485-y.

[30] Stensvold CR, Christiansen DB, Olsen KEP, Nielsen HV. Blastocystis sp. subtype 4 is common in Danish Blastocystis-positive patients presenting with acute diarrhea. Am J Trop Med Hyg 2011;84:883–5. https://doi.org/10.4269/ajtmh.2011.11-0005.

[31] Mattiucci S, Crisafi B, Gabrielli S, Paoletti M, Cancrini G. Molecular epidemiology and genetic diversity of Blastocystis infection in humans in Italy. Epidemiol Infect 2016;144:635–46. https://doi.org/10.1017/S0950268815001697.

Figures

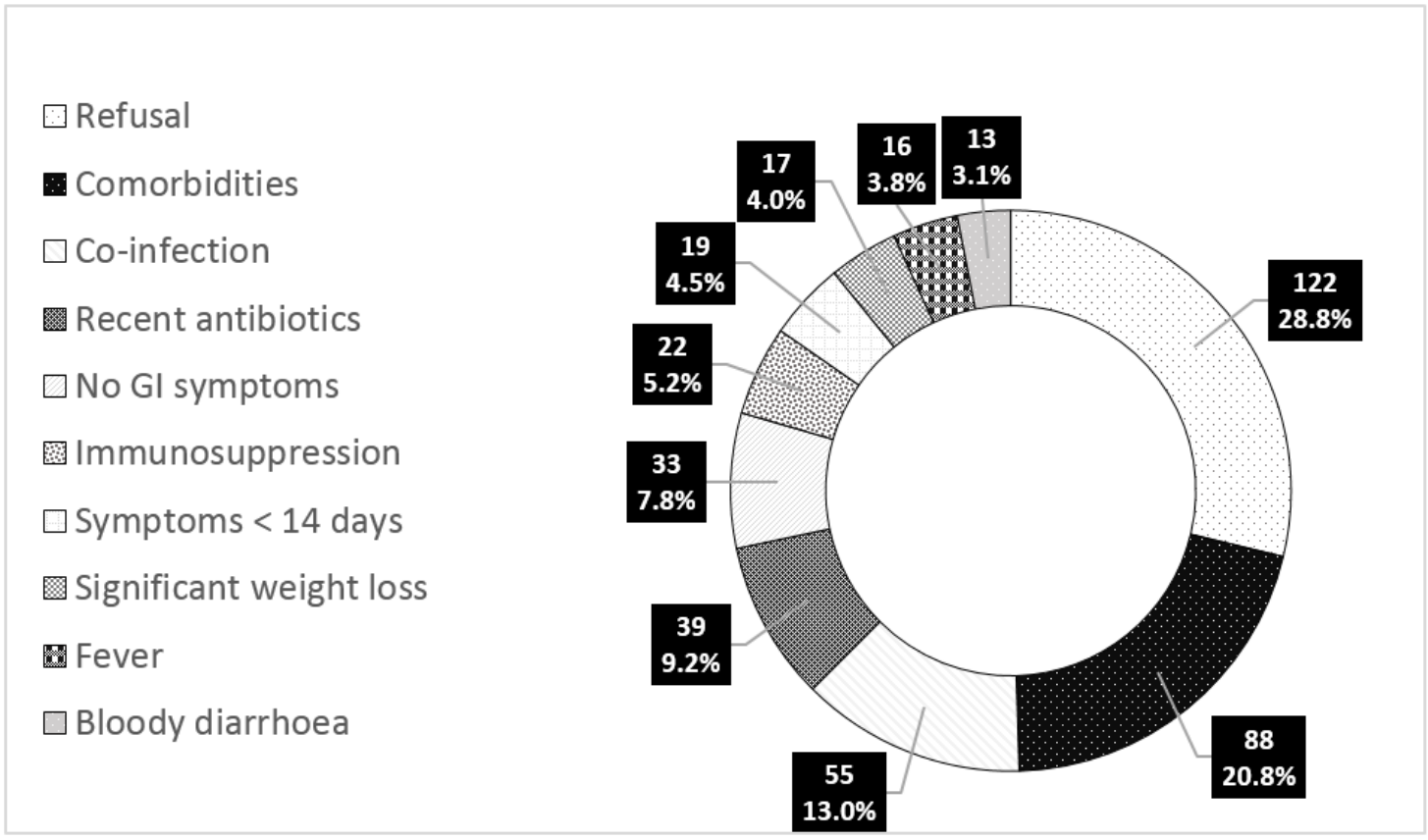

Figure 1

Reasons for subject exclusion. (n=424)

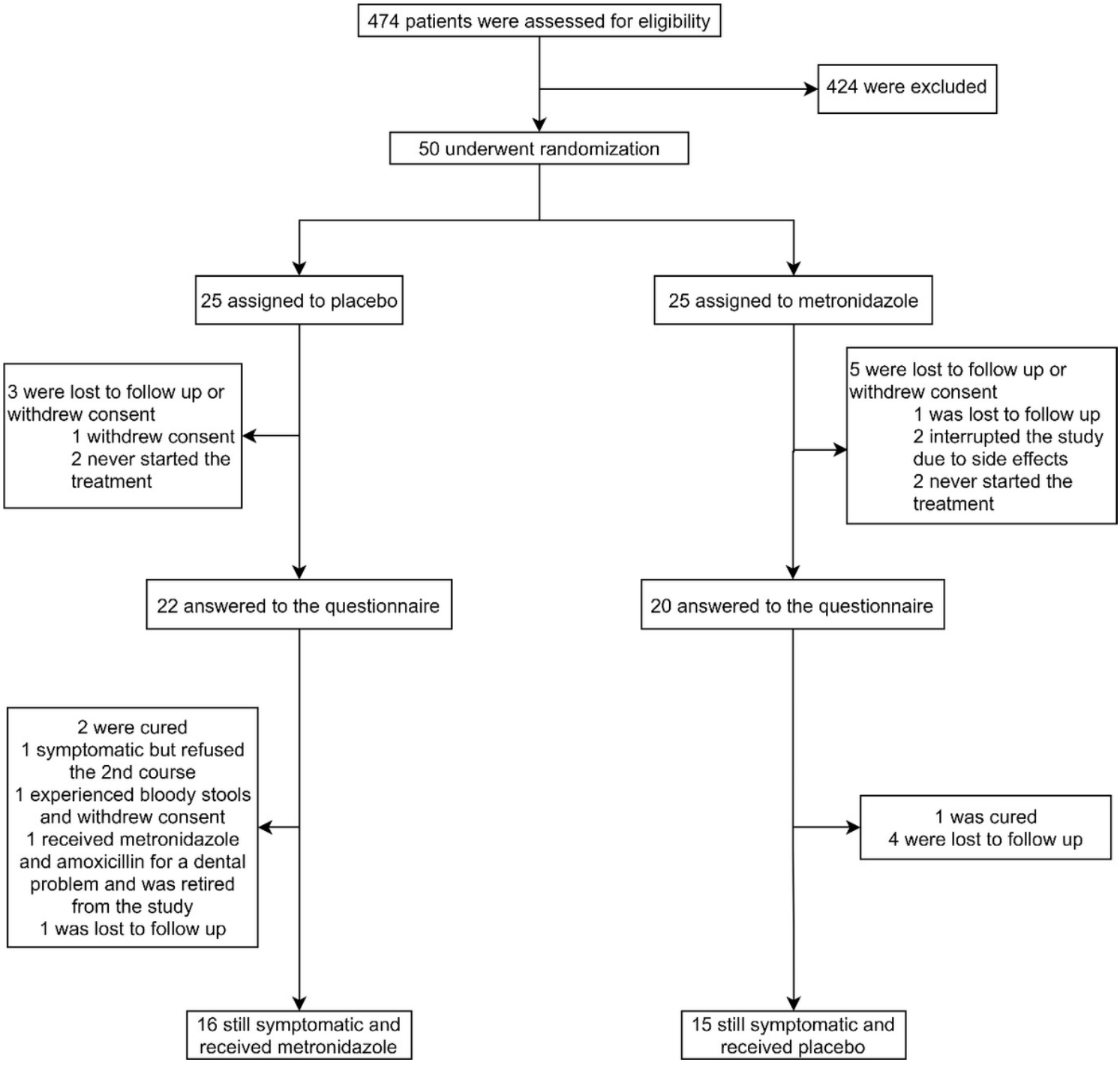

Figure 2

Study flow

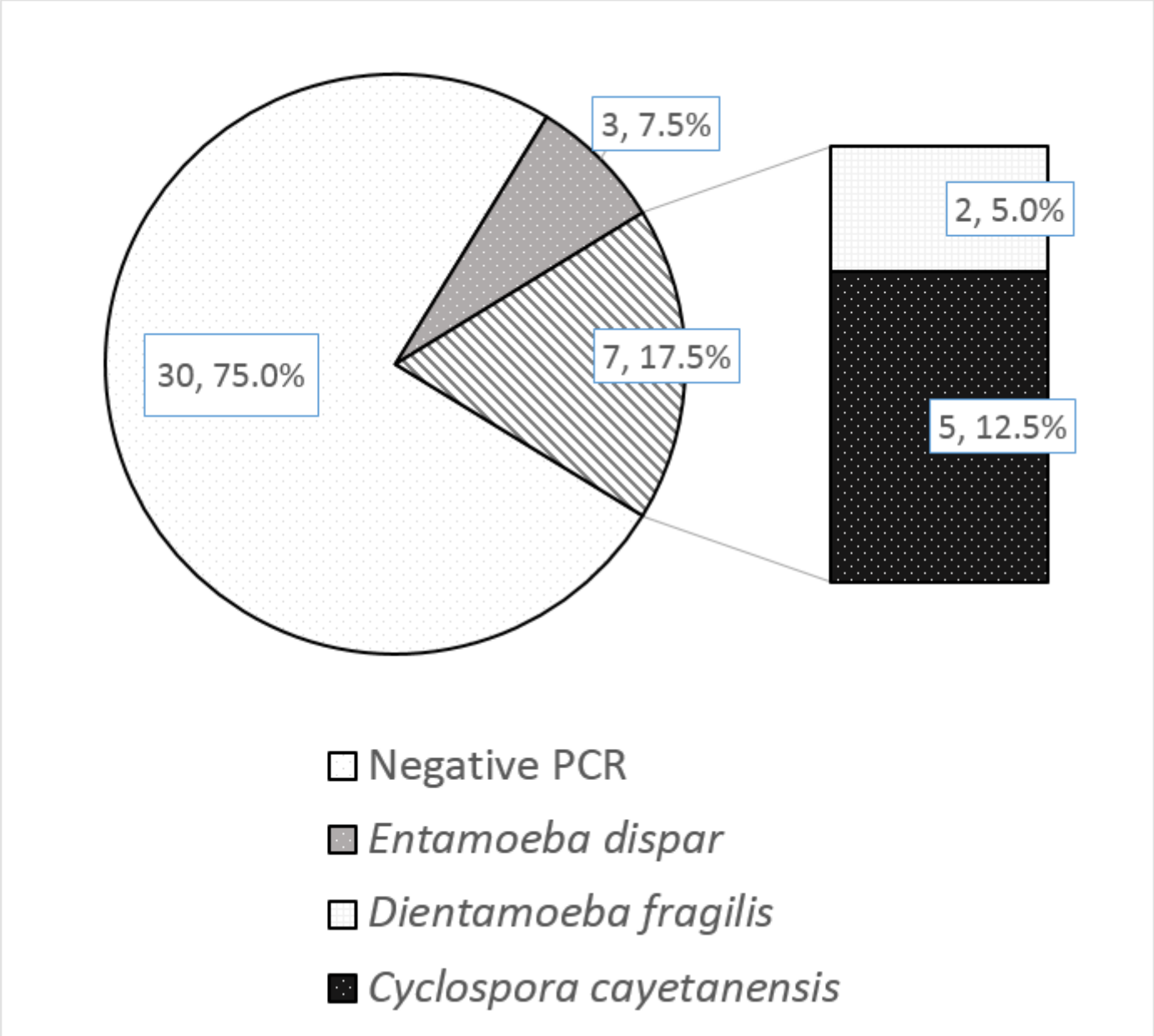

Figure 3

Concomitant protozoa detected by in-house multiplex PCR (n=40)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MolecualMicrobiologyMethodsv2.docx