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Human Transmission of *Blastocystis* by Fecal Microbiota Transplantation Without Development of Gastrointestinal Symptoms in Recipients

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Background. Patients with multiple recurrent *Clostridioides difficile* infections (rCDI) are treated with fecal microbiota transplantation (FMT), using feces provided by healthy donors. *Blastocystis* colonization of donors is considered an exclusion criterion, whereas its pathogenicity is still under debate.

Methods. The introduction of molecular screening for *Blastocystis* sp. at our stool bank identified 2 donors with prior negative microscopies but positive polymerase chain reactions (PCRs). Potential transmission of *Blastocystis* sp. to patients was assessed on 16 fecal patient samples, pre- and post-FMT, by PCR and subtype (ST) analyses. In addition, clinical outcomes for the treatment of rCDI (n = 31), as well as the development of gastrointestinal symptoms, were assessed.

Results. There was 1 donor who carried *Blastocystis* ST1, and the other contained ST3. All patients tested negative for *Blastocystis* prior to FMT. With a median diagnosis at 20.5 days after FMT, 8 of 16 (50%) patients developed intestinal colonization with *Blastocystis*, with identical ST sequences as their respective donors. *Blastocystis*-containing fecal suspensions were used to treat 31 rCDI patients, with an FMT success rate of 84%. This success rate was not statistically different from patients transferred with *Blastocystis* sp.–negative donor feces (93%, 76/82). Patients transferred with *Blastocystis* sp.–positive donor feces did not report any significant differences in bowel complaints in the first week, after 3 weeks, or in the months following FMT.

Conclusions. We demonstrated the first transmission of *Blastocystis* ST1 and ST3 from donors to patients by FMT. This did not result in gastrointestinal symptomatology or have any significant effect on rCDI treatment outcomes.

Keywords. fecal microbiota transplantation; *Blastocystis* sp.; *Clostridioides difficile*; CDI; donor screening.

Blastocystis is a genus of a common unicellular intestinal parasite in humans and animals that belongs to the stramenopiles, 1 of the 8 major phylogenetic groups of eukaryotes. It is a diverse genus comprising 17 characterized lineages: the so-called subtypes (ST1 – ST17), of which 9 have been reported to occur in the human gastrointestinal tract [1, 2]. *Blastocystis* sp. carriage is very common but varies globally, from 0.5% in Japan to 100% in Senegal and 30–50% in Europe [3–6].

The pathogenicity of *Blastocystis* sp. is uncertain and, in general, it is considered an innocent parasite [7]. The presumed

entero-pathogenicity is based on anecdotal case reports and retrospective reviews and is mainly tested in animal models [8, 9]. The symptoms attributed to this organism include nausea, anorexia, abdominal pain, flatulence, and acute or chronic diarrhea [8]. However, outbreaks have never been reported and a human challenge model has not been applied. An association of *Blastocystis* sp. with irritable bowel syndrome was suggested [10, 11], but could not be confirmed in 2 large cohort studies [4, 12]. Interestingly, *Blastocystis* sp. is found to be less prevalent in patients with inflammatory bowel disease, a disorder which is associated with a reduced diversity of the gut microbiota [4, 13, 14], and asymptomatic *Blastocystis* sp. carriers tend to have a more diverse microbiota [4, 15–20]. These observations could indicate that the presence of *Blastocystis* sp. may reflect a more healthy and diverse state of the gut microbiota.

Patients with multiple recurrent *Clostridioides difficile* infections (rCDIs) are treated with fecal microbiota transplantation (FMT), prepared with feces of healthy donors. Carriership of *Blastocystis* sp. by healthy donors is considered an exclusion criterion for donation by several stool banks, including the Netherlands Donor Feces Bank (NDFB) [21–26], resulting in

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considerable exclusion of donors (30-50%). It is questionable whether this is justified. Therefore, knowledge about the potential side effects and treatment success of cotransplantation of *Blastocystis* sp. with FMT is warranted. This study reports the cotransmission of *Blastocystis* sp. from donor to patient, and its influence on the outcomes and health of rCDI patients receiving FMT.

METHODS

Donors and Donor Fecal Suspensions for Fecal Microbiota Transplantation

The NDFB is located within the Department of Medical Microbiology at the Leiden University Medical Center, and started with the treatment of patients with multiple rCDI with FMTs in 2016 [21]. All donors of the NDFB are healthy individuals between the ages of 18 and 50, with normal weight (body mass index, 18.5–25) and no relevant medical history or medication use. All donors are extensively screened and rescreened for disorders associated with a perturbed microbiota and potential transmissible infectious diseases [21].

The NDFB uses standardized procedures for the collection, preparation, and storage of donor fecal suspensions, as described previously [21]. In short, donors deliver stool at the NDFB within 2 hours after defecation. It takes 60 grams of donor feces to prepare 1 fecal suspension. The feces are homogenized with sterile saline with the use of a mortar and pestle, sieved, and centrifuged until an end volume of 200 ml (containing 10% glycerol). Then, 2 cc of the final fecal suspension and 2 grams of the original donor stool are separately aliquoted and stored as quality controls. The fecal suspensions are stored within 6 hours following defecation. Storage is accommodated by a certified, centralized biobanking facility in a dedicated -80°C freezer with connected alarm notification and biobanking information and management system (BIMS SampleNavigator).

Patient Selection and Treatment

Requests for FMT in rCDI patients are carefully evaluated by the working group of the NDFB. Upon approval, the NDFB facilitates FMT by providing ready-to-use fecal suspensions for treatment at the local hospital, as previously described [21]. Patients are preferably pretreated with vancomycin (125–250 mg means 4 times each day) for a minimum of 4 days, followed by 2 liters of macrogol solution (bowel lavage) 1 day prior to FMT. The thawed fecal suspension is slowly infused through a duodenal tube, or via a colonoscopy in selected patients.

Follow-Up

The routine follow-up of patients consists of a standardized questionnaire filled out 3 weeks post-FMT by their local, treating physician and a telephonic interview performed by a member of the NDFB working group at 2 months post-FMT. For this study, an additional telephonic interview was performed in January 2019, between 5 to 33 months post-FMT. In addition, treating

physicians were asked to contact the NDFB in case of any adverse events or treatment failures. Success of FMT was defined as the resolution of CDI symptoms without a relapse of CDI within 2 months. A relapse of CDI was defined as the development of diarrhea for at least 2 consecutive days within 2 months following FMT, either in combination with a positive free-feces toxin test or polymerase chain reaction (PCR; proven relapse), or clinical suspicion for CDI (probable). A CDI episode occurring at a later time point than 2 months post-FMT was regarded as a new CDI episode, as proposed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) *C. difficile* treatment guideline [27]. The development of gastrointestinal and other adverse events was also assessed, including nausea, vomiting, burping, abdominal pain, diarrhea not caused by rCDI, obstipation, hospital admittance, and antibiotic use, and we included an open field for other complaints. In addition, participants were asked to evaluate their defecation pattern post-FMT, compared to pre-FMT (improved, similar, or deteriorated).

Stool samples of patients were collected before and approximately 3 weeks after FMT. Stool samples were preserved until use at -80°C . Patients provided informed consent for the collection of stool samples and outcome data of FMT for research purposes, which was approved by the Medical Ethics Committee at the Leiden University Medical Center (P15.145).

Blastocystis sp. Diagnostics and Typing

Stool samples of the donors were routinely screened for the presence of *Blastocystis* sp. by direct microscopy of the feces and the Ridley-Allen sedimentation method [28]. These screenings were performed on fresh donor stool (<2 hours after defecation). With the introduction of a specific *Blastocystis* PCR at our department in 2018, 2 donors were identified with negative microscopies but positive PCRs for *Blastocystis* sp. In retrospect, all donated fecal samples used to treat patients were tested for the presence of *Blastocystis* sp. with a specific PCR targeting approximately 360 bp of the small subunit ribosomal RNA gene (see [Supplementary Material](#)). Positive samples were subtyped using a sequence analysis, as described previously [29]. Furthermore, 16 available pre- and post-FMT fecal samples of the patients treated by these 2 respective donors were tested with *Blastocystis* sp. PCR and, when positive, were subsequently subtyped. Patients and donors that were PCR positive for *Blastocystis* sp. were regarded as *Blastocystis* sp. colonized.

Statistics

The statistical analysis was performed using SPSS 23.0 statistical software. To test for differences between the prevalence rates of relapses and gastrointestinal symptoms of *Blastocystis* sp.–positive versus –negative donors and patients, a Chi-square test or Fischer exact was performed in cases of $n < 5$. An odds ratio (OR) was calculated using logistic regression and presented with a 95% confidence interval (CI). For ordinal data, a

linear-by-linear association test was used. In addition, Kaplan-Meier curve and log-rank tests to compare CDI-free survival rates between patients receiving *Blastocystis* sp.–positive or –negative donor feces were performed. For statistical comparisons, a *P* value below .05 was considered statically significant.

RESULTS

Blastocystis sp.–Positive Donors

In the period between May 2016 and December 2018, 110 patients were treated with 113 FMTs, using fecal suspensions of 10 donors. In 2 out of 10 donors, *Blastocystis* sp. testing revealed a negative stool microcopy but, in retrospect, a positive PCR, with cycle quantification values ranging from 18.95 to 25.13 (Table 1). A subtype analysis revealed that 1 donor had *Blastocystis* ST1 and the other donor had ST3. The *Blastocystis* ST1 donor carried the *Blastocystis* for at least 3 donating months, and the second donor carried the *Blastocystis* ST3 for at least 9 donating months.

Patients Treated With *Blastocystis* sp. Containing Fecal Microbiota Transplantation Suspensions

Donor feces suspensions of *Blastocystis* sp.–positive donors were used for rCDI treatment of 31 patients; 4 patients were treated with donor feces containing *Blastocystis* ST1 and 27 with *Blastocystis* ST2. From 16 of 31 patients, stool samples pre-FMT and post-FMT were available. All fecal samples of the patients tested *Blastocystis* sp.–negative prior to FMT (Table 1). With a median of 20.5 days (5–53 days) post-FMT, 8 of 16 (50%) patients developed intestinal colonization with

Blastocystis: 7 of 14 with ST3 and 1 of 2 with ST1 (Table 1). Patient DNA sequences of part of the *Blastocystis* small subunit ribosomal RNA region were 100% identical to the sequences of their respective donors.

Patient Follow-Up for Recurrent *Clostridioides difficile* Infections Treatment

Of the 113 FMTs performed in 110 patients to cure rCDI, 31 FMTs were performed with feces from the *Blastocystis* sp.–positive donors and 82 with *Blastocystis* sp.–negative donor feces. Patients treated with *Blastocystis* sp.–positive donor feces had an FMT success rate (cure without relapse <2 months) of 84% (26/31), whereas treatment with *Blastocystis* sp.–negative donor feces had a success rate of 93% (76/82). This difference in success rates was not significant (Table 2; Figure 1). Moreover, no significant difference in the numbers of confirmed (3 versus 3) and probable CDI relapses (2 versus 3) was found (OR, 1.5; 95% CI, .14–16.54; *P* value = 1). Of a total of 11 relapses of CDI, 3 were challenged by antibiotic treatment, whereas 8 (5 in *Blastocystis*-positive and 3 in *Blastocystis*-negative treated patients) developed a relapse without antibiotics as a predisposing factor. The ST1 and ST3 *Blastocystis* sp.–positive donor fecal suspensions were used for the treatment of 4 and 27 rCDI patients, respectively. Treatment with feces of the *Blastocystis* sp. ST1 donor resulted in a treatment success of 75% (1/4), whereas the ST3 donor had a success rate of 85% (4/27; OR, 0.522; 95% CI, .04–6.36; *P* value = .525). In addition, no difference was found in the relapse rates between patients with (12.5%, 1/8) or without (0%, 0/8) *Blastocystis* sp. colonization following FMT

Table 1. Details of Donor to Patient Transfer of *Blastocystis* Subtypes 1 and 3 by Fecal Microbiota Transplantation

Donors			Recipients Pre-FMT			Recipients Post-FMT				
Donor ID	Subtype of <i>Blastocystis</i>	<i>Blastocystis</i> Cq Value	Feces Collection, Days Pre-FMT	Patient ID	<i>Blastocystis</i> Status Pre-FMT	Feces Collection, Days Post-FMT	<i>Blastocystis</i> Status Post-FMT	<i>Blastocystis</i> Cq Value	Subtype of <i>Blastocystis</i>	Colonization With <i>Blastocystis</i> Due to FMT
A	ST1	25.13	119	1	Neg	21	Neg	n/a	n/a	No
A	ST1	23.57	199	2	Neg	21	Pos	25.05	ST1	Yes
B	ST3	24.19	43	3	Neg	20	Pos	22.28	ST3	Yes
B	ST3	20.16	34	4	Neg	5	Neg	n/a	n/a	No
B	n/a ^a	n/a	66	5	Neg	18	Pos	22.57	ST3	Yes
B	ST3	19.51	64	6	Neg	53	Pos	27.64	ST3	Yes
B	ST3	18.95	119	7	Neg	15	Pos	27.77	ST3	Yes
B	ST3	20.94	124	8	Neg	20	Neg	n/a	n/a	No
B	ST3	19.81	140	9	Neg	48	Pos	25.78	ST3	Yes
B	ST3	23.21	152	10	Neg	20	Neg	n/a	n/a	No
B	ST3	21.11	255	11	Neg	31	Neg	n/a	n/a	No
B	ST3	21.68	360	12	Neg	29	Neg	n/a	n/a	No
B	ST3	21.68	376	13	Neg	23	Neg	n/a	n/a	No
B	ST3	19.96	385	14	Neg	20	Pos	23.86	ST3	Yes
B	n/a ^b	n/a	509	15	Neg	20	Neg	n/a	n/a	No
B	ST3	20.29	521	16	Neg	27	Pos	19.56	ST3	Yes

Abbreviations: Cq, cycle quantification; FMT, fecal microbiota transplantation; ID, identification; n/a, not available or not applicable; Neg, negative; Pos, positive; ST, subtypes.

^aTransplanted donor feces were not available; samples 6 days prior and 2 days post-FMT were positive with *Blastocystis* ST3.

^bTransplanted donor feces were not available; samples 30 days prior and 3 days post-FMT were positive with *Blastocystis* ST3.

Table 2. Follow-Up of Recurrent *Clostridioides difficile* Infection Fecal Microbiota Transplantation Treatment Success of Patients Transferred With *Blastocystis* sp.–Positive Versus –Negative Donor Feces

Patients Outcome	<i>Blastocystis</i> sp.– Positive Donor Feces	<i>Blastocystis</i> sp.– Negative Donor Feces	Significance, OR [95% CI], <i>P</i> value
FMT success rate	83.9% (26/31)	92.7% (76/82)	0.411 [0.12, 1.46], <i>P</i> value = .159
Relapses of CDI	16.1% (5/31)	7.3% (6/82)	2.436 [0.69, 8.65], <i>P</i> value = .159
New CDI episode, >2 months after FMT	9.7% (3/31)	7.3% (6/82)	1.357 [0.32, 5.80], <i>P</i> value = .704
CDI event: relapse or new episode	25.8% (8/31)	14.6% (12/82)	2.029 [0.74, 5.88], <i>P</i> value = .165

Percentages and final ORs with 95% CIs of the FMT treatment outcome between patients treated with *Blastocystis* sp.–positive versus –negative donor feces. A χ^2 test or Fischer exact test was performed in cases of $n < 5$.

Abbreviations: CDI, *Clostridioides difficile* infection; CI, confidence interval; FMT, fecal microbiota transplantation; OR, odds ratio.

with a donor suspension containing *Blastocystis* sp. (OR, 1.143; 95% CI, .88–1.49; *P* value = 1).

There were 9 (8.0%, 9/113) patients who experienced a new episode of CDI later than 2 months after FMT, at a median of 4 months (range 63–402 days) post-FMT. All new episodes could be attributed to the initiation of antibiotic treatment shortly before the development of CDI symptoms. The frequency of development of a new initial episode of CDI was not statistically different in patients transferred with *Blastocystis* sp.–positive feces (9.7%, 3/31), versus *Blastocystis* sp.–negative feces (7.3%, 6/82; Table 2; Figure 1). Moreover, no statistically significant difference in the development of a new initial

CDI episode was found between patients transferred with ST1 (0%, 0/4) and ST3 (11.1% 3/27; OR, 0.889; 95% CI, .78–1.02; *P* value = 1), or between patients that were demonstrably colonized with *Blastocystis* post-FMT using *Blastocystis*-containing donor feces (12.5%, 1/8), versus those demonstrably *Blastocystis* negative post-FMT (0%, 0/8; OR, 1.143; 95% CI, .88–1.49; *P* value = 1).

Potential Side Effects Due to Newly Acquired *Blastocystis* sp. Colonization Following Fecal Microbiota Transplantation

Compared to patients treated with *Blastocystis* sp.–negative donor feces, patients treated with *Blastocystis* sp.–positive donor feces did not report significantly more bowel complaints (nausea, abdominal pain, or diarrhea) after 1 week, after 3 weeks, or at the long-term follow-up (median, 35 weeks; range, 10–143 weeks; Table 3). Moreover, no difference in side effects was observed in the subgroup of patients with demonstrable *Blastocystis* sp. colonization after FMT. Interestingly, a significant difference towards an improvement of the self-evaluated defecation pattern was observed at long-term follow-up in patients receiving *Blastocystis* sp.–positive donor feces (Table 3).

DISCUSSION

Healthy stool donors colonized with *Blastocystis* sp. are usually excluded from FMT donorship [21–26], though the enteropathogenicity of *Blastocystis* sp. remains debatable [7]. Through a combination of PCR and subtyping techniques of donors and of patient pre-FMT and post-FMT fecal samples, the first human-to-human transmission by FMT of *Blastocystis* sp. ST1 and ST3 was described. This transmission did not

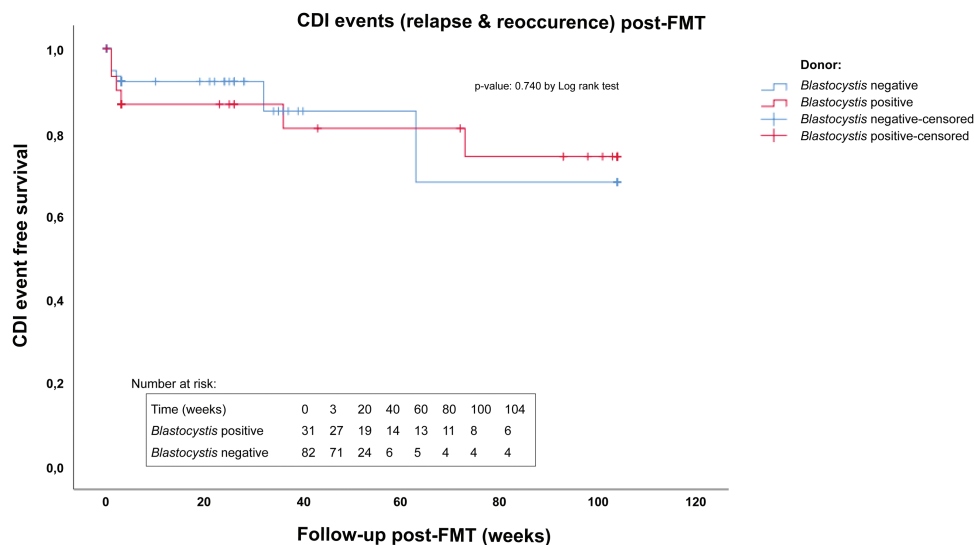


Figure 1. Kaplan-Meier curve of CDI event-free survival in patients post-FMT who were treated with *Blastocystis* sp.–positive versus *Blastocystis* sp.–negative fecal suspensions. CDI-free survival is defined as survival without a relapse (<2 months post-FMT) or new CDI infection (>2 months post-FMT) within 2 years (104 weeks) after FMT. Follow-up data exceeding 2 years were censored at 104 weeks. Patients suffering from a new CDI event after 104 weeks were counted as having no CDI event. Abbreviations: CDI, *Clostridioides difficile* infection; FMT, fecal microbiota transplantation.

Table 3. Potential Side Effects Due to Newly Acquired *Blastocystis* sp. Infections After Fecal Microbiota Transplantation

Side Effect	FMT With <i>Blastocystis</i> sp.–Negative Donor, n = 82			FMT With <i>Blastocystis</i> sp.–Positive Donor, n = 31			<i>Blastocystis</i> sp. Colonized Post-FMT, n = 8 ^a		
	Week 1	Weeks 2 + 3	LTFU	Week 1	Weeks 2 + 3	LTFU	Week 1	Weeks 2 + 3	LTFU
Nausea, % yes ^a	11.0% (9/69)	12.2% (10/70)	35.0% (7/20)	13.0% (3/23)	3.2% (1/23)	12.5% (2/16)	0.0% (0/8)	0.0% (0/8)	0.0% (0/3)
Abdominal pain, % yes ^b	22.0% (18/70)	18.3% (15/71)	27.8% (5/18)	34.8% (8/23)	16.1% (5/23)	25.0% (3/12)	25.0% (2/8)	12.5% (1/8)	33.3% (1/3)
Diarrhea ^b	32.9% (23/70)	22.0% (18/70)	35.0% (7/20)	26.1% (6/23)	26.1% (6/23)	25.0% (4/16)	0.0% (0/8)	37.5% (3/8)	33.3% (1/3)
Defecation pattern									
Improved	n/a	16.1% (9/56)	17.6% ^c (3/17)	n/a	13.6% (3/22)	53.8% ^c (7/13)	n/a	12.5% (1/8)	33.3% (1/3)
Similar	n/a	67.9% (38/56)	58.8% ^c (10/17)	n/a	68.2% (15/22)	38.5% ^c (5/13)	n/a	62.5% (5/8)	66.7% (2/3)
Worsened	n/a	16.1% (9/56)	23.5% ^c (4/17)	n/a	18.2% (4/22)	7.7% ^c (1/13)	n/a	25.0% (2/8)	0.0% (0/3)

The LTFU median duration was 35 weeks, and the range was 10–143 weeks.

Abbreviation: FMT, fecal microbiota transplantation; LTFU, long-term follow-up.

^aA subgroup of patients receiving *Blastocystis* sp.–positive fecal suspensions with proven intestinal colonization of *Blastocystis* sp. post-FMT.

^bPrevalences of nausea, abdominal pain, or diarrhea were not significantly different between the groups, as tested with either a χ^2 or Fischer exact test in cases of $n < 5$.

^cA statistically significant difference in the self-evaluated defecation pattern at LTFU between patients that received *Blastocystis* sp.–positive versus *Blastocystis* sp.–negative donor feces, as tested by a χ^2 linear-by-linear test ($P = .043$).

influence the success rate of the FMT to treat rCDI. More importantly, it did not result in gastrointestinal symptomatology of the recipients.

Symptoms attributed to *Blastocystis* sp. infection that have been described in anecdotal case reports, series, and retrospective cohorts include nausea, anorexia, abdominal pain, flatulence, and acute or chronic diarrhea [8]. The high prevalence of *Blastocystis* sp. colonization in healthy individuals suggests that *Blastocystis* sp. does not harm most hosts. As *Blastocystis* consists of 17 subtypes, initially the idea was raised that the subtype correlated with pathogenicity [30]. Numerous, globally performed studies comparing the subtypes of *Blastocystis* could not confirm a consistent correlation and could not explain the pathogenicity in some patients [30]. Currently, it is mostly acknowledged that *Blastocystis* sp. may colonize many hosts, but the infection's potential depends on the interplay between the virulence of the parasite, the number of infecting parasites present, the duration of infection (acute versus chronic), and host factors like genetics, immune competence, or gut microbiota composition [3, 4, 20, 30, 31]. The 2 identified subtypes in this study, ST1 and ST3, are the most commonly found subtypes in Europe and the Netherlands [3]. In a Dutch study in which the stool samples of 442 patients were evaluated by routine parasitological examination, 107 (24%) stool samples contained *Blastocystis* sp., of which 40% had *Blastocystis* ST3 and 21% had *Blastocystis* ST1 [3]. The sustained colonization with *Blastocystis* ST1 and ST3 observed in 50% (median, 20.5 days) of *Blastocystis*-transferred patients in this study did not result in gastrointestinal symptomatology, as determined by patient follow-up questionnaires. In contrast, these *Blastocystis* sp.–transferred patients evaluated their defecation pattern as being significantly better post-FMT, compared to patients receiving *Blastocystis* sp.–negative donor feces.

Unfortunately, a human challenge model to study the presumed enteropathogenicity of *Blastocystis* sp. has not been

described [7]. In our study, the transfer of *Blastocystis* sp. was accompanied by a healthy donor microbiota. This may not reflect the effects of *Blastocystis* sp. transfers from individuals with intestinal complaints or a disturbed microbiota to individuals with a healthy microbiota. Interestingly, *Blastocystis* sp. may not be able to maintain itself in a dysbiotic rCDI microbiota, since we found that none of the rCDI patients carried *Blastocystis* sp. pre-FMT. Low *Blastocystis* sp. colonization rates in diseased individuals were previously also reported in patients with active inflammatory bowel disease or hepatic encephalopathy [4, 13, 14, 32]. These diseased individuals and rCDI patients have a perturbed gut microbiota in common. Whether the association between a perturbed microbiota and low *Blastocystis* sp. colonization results from an absence of *Blastocystis* sp. or from the inability of *Blastocystis* to colonize and sustain itself in a dysbiotic gut microbiota composition is an interesting question that merits further research.

In this study, the importance of performing appropriate *Blastocystis* sp. diagnostics is shown. The NDFB used microscopy on unfixed material and used Ridley-Allen sedimentation to detect *Blastocystis* sp., in contrast to the more superior techniques, which use microscopy on 2 sodium acetate formalin-fixed stool samples or molecular detection of a single stool sample [3]. *Blastocystis* sp. colonization of the donors or patients was, therefore, defined by positive PCR, irrespective of microscopic findings. Post-FMT stool samples with a positive *Blastocystis* sp. PCR were taken more than 2 weeks post-FMT. Together with the relatively low cycle quantification values (high load) found in these rCDI patients post-FMT, this suggests actual *Blastocystis* sp. colonization instead of *Blastocystis* passage after FMT.

There is no consensus among FMT centers and stool banks about *Blastocystis* sp. screening of donors, though published guidelines still recommend screening, especially for immunocompromised patients [24]. Many centers do not screen for *Blastocystis* sp. and, according to a recent systemic review, only

14.5% of 168 studies reported specific *Blastocystis* sp. screening [33]. In addition, the method of screening for ova and parasites was often not stated [21–26]. Consequently, we assume that a substantial number of patients has received FMT treatment for rCDI or other diseases in experimental settings, with cotransplantation with *Blastocystis* sp.

Our study is the first study that indicates that *Blastocystis* sp. transmission does not result in gastrointestinal symptoms in recipients. In the setting of rCDI, the transmission of *Blastocystis* ST1 and ST3 via FMT did not result in a significant decrease in the efficacy of FMT, although there was a nonsignificant trend towards an increased rate of CDI events (both relapses and new episodes) in patients treated with *Blastocystis* sp.–positive donors (8/31) versus *Blastocystis* sp.–negative donors (12/82). Interestingly, this contrasts with expected outcomes that could have extrapolated from recent metagenomic studies, in which *Blastocystis* sp. is correlated with a more diverse and healthier microbiota, a general prerequisite of a good donor [4, 15–20]. In a large cohort of 1106 healthy Flemish individuals, *Blastocystis* sp. carriage was associated with higher microbial diversity, richness, and composition. Tito et al [4] found that the most common subtypes in Europe—ST1, ST2, ST3, and ST4—were all associated with higher diversity, though ST1 and ST3 (which were identified in our study) had lower diversity increases than ST2 and ST4. For FMT treatment of rCDI, super donors have not been detected [34, 35] and all donors display a high cure rate, of around 85% [21]. The role of super-donors could play a more significant role in possible future FMT indications other than rCDI, such as ulcerative colitis, metabolic syndrome, the eradication of multidrug resistant organisms, or hepatic encephalopathy [4, 36, 37].

In this study, only the transfer of *Blastocystis* ST1 or ST3 was studied. To assess the contribution of *Blastocystis* sp. transfers to FMT success, it is important to include microbiota data of donors and patients, other subtypes of *Blastocystis*, and longer-term follow-up, as colonization has been described for up to 6–10 years [38]. An important limitation of this study is voluntary reporting by the treating physicians of late CDI relapses (after 3 weeks) or new CDI episodes (after 2 months) to the NDFB. However, physicians had a low threshold to contact the NDFB, since an excellent relationship was developed during the entire process of the FMT request and treatment of the patient.

In conclusion, to the best of our knowledge we demonstrate the first transmission of *Blastocystis* ST1 and ST3 from donor to recipient via FMT without the development of gastrointestinal symptoms. This study is an important step towards a possible exemption of *Blastocystis* sp. (ST1 and ST3) as a donor exclusion criterion in FMT.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the

posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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