Exploring the role of the microbiota: in defence against Clostridioides difficile and multidrug resistant Gram-negatives

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Chapter 7

Human transmission of *Blastocystis* by Fecal Microbiota Transplantation

without development of gastrointestinal symptoms in recipients

Clinical Infectious Diseases, 2019

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Clin Infect Dis. 2019;doi:10.1093/cid/ciz1122

Chapter 7. Human transmission of *Blastocystis* by Fecal Microbiota Transplantation without development of gastrointestinal symptoms in recipients

Abstract

Background: **Patients with multiple recurrent *Clostridioides difficile* infections (rCDI) are treated with fecal microbiota transplantation (FMT) 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 two donors with prior negative microscopy but positive PCR. Potential transmission of *Blastocystis sp.* to patients was assessed on 16 fecal patient samples, pre- and post-FMT, by PCR and subtype (ST) analysis. In addition, clinical outcome for treatment of rCDI (n=31), as well as development of gastrointestinal symptoms was assessed.**

Results: **There was one donor carried *Blastocystis* ST1, 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 a 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 difference in bowel complaints in the first week, after 3 weeks and the months following FMT.**

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

Key points

Transmission of *Blastocystis* by Fecal Microbiota Transplantation from colonized donors occurred in 50 % of treated patients. Transfer did not result in development of gastrointestinal symptoms or affect the outcome of the FMT treatment in patients with recurrent *Clostridioides difficile* infections.

Introduction

Blastocystis is a genus of common unicellular intestinal parasite in humans and animals which belongs to the stramenopiles, one of the eight major phylogenetic groups of eukaryotes. It is a diverse genus comprising of 17 characterized lineages, the so-called subtypes (ST1 – ST17), of which nine 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 (IBS) was suggested [10,11], but could not be confirmed in two large cohort studies [4,12]. Interestingly, *Blastocystis* sp. is found to be less prevalent in patients with inflammatory bowel disease (IBD), 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* infection (rCDI) 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 NDFB [21-26], resulting in considerable exclusion of donors (30-50 %). It is questionable whether this is justified. Therefore,

knowledge about potential side effects and treatment success of co-transplantation of *Blastocystis* sp. with FMT is warranted. This study reports the co-transmission of *Blastocystis* sp. from donor to patient, and the influence on the outcome and health of rCDI patients receiving FMT.

Methods

Donors and donor fecal suspensions for FMT

The Netherlands Donor Feces Bank (NDFB) is located within the Department of Medical Microbiology at the Leiden University Medical Center (LUMC) and started with treatment of patients with multiple rCDI with FMTs in 2016 [21]. All donors of the NDFB are healthy individuals between the age of 18 and 50, with normal weight (BMI 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 collection, preparation and storage of donor fecal suspensions as described earlier [21]. In short, donors deliver stool at the NDFB within two hours after defecation. Sixty grams of donor feces is used to prepare one fecal suspension. The feces was homogenized with sterile saline with use of mortar and pestle, sieved, centrifuged until an end volume of 200ml (containing 10 % glycerol). Two cc of the final fecal suspension, and two grams of original donor stool are separately aliquoted and stored as quality control. The fecal suspensions are stored within six 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 (125-250mg QID) for a minimum of four days,

followed by two liters of macrogol solution (bowel lavage) one day prior to FMT. The thawed fecal suspension is slowly infused through a duodenal tube, or via colonoscopy in selected patients.

Follow-up

Routine follow-up of patients consists of a standardized questionnaire three weeks post-FMT filled out by their local, treating physician and a telephonic interview performed by a member of the NDFB working group two months post-FMT. For this study an additional telephonic interview was performed between in January 2019, five 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 resolution of CDI symptoms without relapse of CDI within two months. A relapse of CDI was defined as the development of diarrhea for at least two consecutive days within two months following FMT, either in combination with positive free feces toxin test or PCR (proven relapse), or clinical suspicion for CDI (probable). A CDI episode occurring at a later timepoint than two 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 gastro-intestinal and other adverse events was also assessed, including nausea, vomiting, burping, abdominal pain, diarrhea not caused by rCDI, obstipation, hospital admittance, 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 three weeks after FMT. Stool samples were preserved until use at -80°C. Patients provided informed consent for 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 *Blastocystis sp.* presence by direct microscopy of the feces and Ridley-Allen sedimentation method [28]. This

screening was performed on fresh donor stool (<2 hours after defecation). With the introduction of a specific *Blastocystis* PCR at our department in 2018, two donors were identified with negative microscopy but positive PCR 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 sequence analysis as described previously **[29]**. Furthermore, 16 available pre- and post-FMT fecal samples of the patients treated by these two respective donors were tested with *Blastocystis* sp. PCR and when positive subsequently subtyped. *Blastocystis* sp. PCR positive patients and donors were regarded as *Blastocystis* sp. colonized

Statistics

Statistical analysis was performed using SPSS 23.0 statistical software. To test for differences between the prevalence rate of relapses and gastrointestinal symptoms of *Blastocystis* sp. positive versus negative donors and patients a Chi-square test or Fischer exact in cases of n < 5, was performed. An odds ratio (OR) was calculated using logistic regression and presented with a 95% confidence interval **[95% CI]**. For ordinal data a linear-by-linear association test was used. In addition, a Kaplan-Meier curve and log-rank test to compare CDI free survival between patients receiving *Blastocystis* sp. positive or negative donor feces was performed. For statistical comparisons, a p-value below 0.05 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 two out of 10 donors, *Blastocystis* sp. testing revealed a negative stool microcopy but in retrospect a positive PCR, with Cycle quantification (Cq) value's ranging from 18.95 to 25.13 (Table 1). Subtype analysis revealed one donor with *Blastocystis* subtype (ST) 1, and the other donor with ST3. The *Blastocystis* ST1 donor carried the *Blastocystis* for at least three donating months, and the second donor carried the *Blastocystis* ST3 for at least nine donating months.

Table 1. Details of donor to patient transfer of *Blastocystis* ST1 and ST3 by FMT

Donors				Recipients pre-FMT		Recipients post-FMT				
Donor ID	Subtype of *Blastocystis*	*Blastocystis* Cq-value	Feces collection (days pre-FMT)	Patient ID	*Blastocystis* status pre-FMT	Feces collection (days post-FMT)	*Blastocystis* status post-FMT	*Blastocystis* Cq-value	Subtype of *Blastocysti*	Colonization with *Blastocystis* 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/aa	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/ab	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: patient identification, n/a: not available or not applicable, Neg: negative, Pos: positive, ST: subtypes.

a Transplanted donor feces not available, samples 6 days prior and 2 days post-FMT positive with Blastocystis ST3.

b Transplanted donor feces not available, samples 30 days prior and 3 days post-FMT positive with Blastocystis ST3.

Patients treated with *Blastocystis* sp. containing FMT suspensions

Donor feces suspensions of *Blastocystis* sp. positive donors were used for rCDI treatment of 31 patients; four patients were treated with donor feces containing *Blastocystis* ST1, 27 with *Blastocystis* ST3. From 16 of 31 patients, stool samples pre-FMT and post-FMT were available. All fecal samples of the patients prior to FMT tested *Blastocystis* sp. negative *(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 rRNA region were 100 % identical to the sequences of their respective donors.

Patient follow-up rCDI treatment

Of the 113 FMT's performed in 110 patients to cure rCDI, 31 FMTs were performed with feces from the *Blastocystis* sp. positive donors, 82 with *Blastocystis* sp. negative donor feces. Patients treated with *Blastocystis* sp. positive donor feces had a 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 rate was not significant *(Table 2, Figure 1)*. Moreover, no significant difference in the number of confirmed (three versus three) and probable CDI relapses (two versus three) was found (OR 1.5, 95 % CI [0.14, 16.54], p-value 1). Of a total of 11 relapses of CDI, three were challenged by antibiotic treatment, whereas eight (five in *Blastocystis* positive and three in *Blastocystis* negative treated patients) developed a relapse without antibiotics as predisposing factor. The ST1 and ST3 *Blastocystis* sp. positive donor fecal suspensions were used for treatment of four, and respectively 27 rCDI patients. 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 [0.04, 6.36], p-value = 0.525). In addition, no difference was found in relapse rate between patients with (12.5 %, 1/8) or without (0 %, 0/8) *Blastocystis* sp. colonization following FMT with a *Blastocystis* sp. containing donor suspension (OR 1.143, 95 % CI [0.88, 1.49], p-value 1).

Table 2. Follow-up of rCDI FMT treatment success of patients transferred with *Blastocystis* sp. positive versus negative donor feces.

Patients outcome	*Blastocystis* sp. positive donor feces	*Blastocystis* sp. negative donor feces	Significance (OR [95% CI], p-value)	
FMT success rate	83.9 % (26/31)	92.7 % (76/82)	OR 0.411 [0.12, 1.46]	p-value 0.159
Relapses of CDI	16.1 % (5/31)	7.3 % (6/82)	OR 2.436 [0.69, 8.65]	p-value 0.159
New CDI episode (> 2 months after FMT)	9.7 % (3/31)	7.3 % (6/82)	OR 1.357 [0.32, 5.80]	p-value 0.704
CDI event (relapse or new episode	25.8 % (8/31)	14.6 % (12/82)	OR 2.029 [0.74, 5.88]	p-value 0.165

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

Percentages and final odds ratio with 95 % confidence intervals of the FMT treatment outcome between patients treated with Blastocystis sp. positive versus negative donor feces. A chi-square test or Fischer exact test in cases of n < 5, was performed.

Nine (8.0 %, 9/113) patients experienced a new episode of CDI later than two months after FMT, with a median of four months (range 63 – 402 days) post-FMT. All new episodes could be attributed to initiation of antibiotic treatment shortly before 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 (7.3 %, 6/82), Table 2, *Figure 1*. Moreover, no statistically significant difference in development of a new initial CDI episode was found between ST1 (0 %, 0/4) and ST3 (11.1 % 3/27) transferred patients (OR 0.889, 95 % CI [0.78, 1.02], p-value 1), or between patients that were demonstrable *Blastocystis* colonized post-FMT using *Blastocystis* containing donor feces (12.5 %, 1/8) versus demonstrable *Blastocystis* negative post-FMT (0 % 0/8) (OR 1.143, 95 % CI [0.88, 1.49], p-value 1).

Table 3. Potential side-effects due to newly acquired *Blastocystis* sp. infections after FMT

Side-effect	FMT with *Blastocystis* sp. negative donor (n=82)						FMT with *Blastocystis* sp. positive donor (n=31)						Blastocystis sp. colonized post-FMT (n=8) [a]					
	Week 1		Week 2+3		LTFU		Week 1		Week 2+3		LTFU		Week 1		Week 2+3		LTFU	
Nausea (% yes) [a]	11.0%	(9/69)	12.2%	(0/70)	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 [b] (% yes)	22.0%	(18/70)	18.3%	(15/71)	35.0%	(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)

[a] Subgroup of patients receiving Blastocystis sp. positive fecal suspensions with proven intestinal colonization of Blastocystis sp. post-FMT.

[b] Prevalence of nausea, abdominal pain or diarrhea were not significantly different between the groups as tested with chi-square or Fischer exact in cases of n < 5.

[c] Statistically significant difference in the self-evaluated defecation pattern at long-term follow-up between patients that received Blastocystis sp. positive versus Blastocystis sp. negative donor feces as tested by Chi-square linear-by-linear test, p = 0.043.

Abbreviation: FMT: fecal microbiota transplantation, LTFU: long-term follow-up (median 35 weeks, range 10-143 weeks), n/a: not applicable

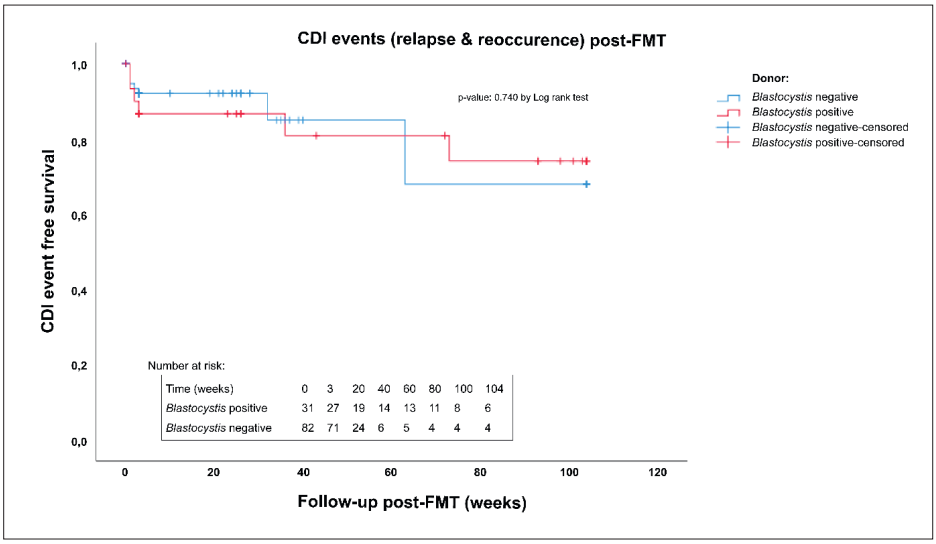

Figure 1. Kaplan Meier curve of *C. difficile* infection (CDI) event free survival in patients post-FMT treated with *Blastocystis* sp. positive versus *Blastocystis* sp. negative fecal suspensions.

CDI free survival is defined as survival without relapse (<2 months post-FMT) or new CDI infection (>2 months post-FMT) within two years (104 weeks) after FMT. Follow-up data exceeding 2 years, was censored at 104 weeks. Patients suffering from a new CDI event after 104 weeks were counted as no CDI even.

Abbreviations: CDI: *Clostridioides difficile* infection, FMT: fecal microbiota transplantation

Potential side-effects due to newly acquired *Blastocystis* sp. colonization following FMT

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 one week, three weeks or at 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 donor, 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 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 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 *Blastocystis* sp. does not harm most hosts. As *Blastocystis* consists of 17 subtypes, initially the idea was raised that subtype correlated with pathogenicity [30]. Numerous, globally performed studies comparing the subtypes of *Blastocystis* could not confirm such 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, number of infecting parasites present, duration of infection (acute versus chronic) and host factors like genetics, immune competence or gut microbiota composition [3, 4, 20, 30, 31]. The two 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 % *Blastocystis* ST3 and 21% *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 gastro-intestinal symptomatology, as determined by patient follow-up questionnaires. In contrast, these *Blastocystis* sp. transferred patients evaluated their defecation pattern 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. transfer 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 IBD 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 is a result from an absence of *Blastocystis* sp., or from the inability of *Blastocystis* to colonize and sustain in a dysbiotic gut microbiota composition is an interesting question which merits further research.

In this study the importance of performing appropriate *Blastocystis* sp. diagnostics is shown. The NDFB used microscopy on unfixed material, and Ridley-Allen sedimentation to detect *Blastocystis* sp., in contrast to the more superior techniques using microscopy on two sodium acetate formalin (SAF) fixated 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 two weeks post-FMT. Together with the relative low Cq values (high load) found in these rCDI patients post-FMT suggests actual *Blastocystis* 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 setting, with co-transplantation with *Blastocystis* sp.

Our study is the first study that indicates that *Blastocystis* sp. transmission does not result in gastrointestinal symptoms of recipients. In the setting of rCDI, transmission

of *Blastocystis* ST1 and ST3 via FMT did not result in a significant decrease in efficacy of FMT, although there was a non-significant 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 as one could have extrapolated from recent metagenomic studies, in which *Blastocystis* sp. is correlated with a more diverse and healthy microbiota, a general prerequisite of a good donor **[4, 15-20]**. In a large cohort of 1106 healthy Flemish individuals, *Blastocystis* sp. carriership was associated with higher microbial diversity, richness and composition. Tito et al, found that the most common subtypes in Europe, ST1, ST2, ST3 and ST4, were all associated with a higher diversity, though ST1 and ST3 (which were identified in our study) had a lower diversity increase than ST2 and ST4 **[4]**. 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, eradication of multi-drug resistant organisms or hepatic encephalopathy **[4, 36, 37]**.

In this study only transfer of *Blastocystis* ST1 or ST3 was studied. To assess the contribution of *Blastocystis* sp. transfer 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 is described up to 6 – 10 years **[38]**. An important limitation of this study is voluntary reporting by the treating physicians of late CDI relapses (after three weeks) or new CDI episodes (after two 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 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 development of gastrointestinal symptoms. This study is an important step towards a possible exempt of *Blastocystis* sp. (ST1 and ST3) as donor exclusion criterion in FMT.

Funding

This work was supported by the Netherlands Organization for Health Research and Development, ZonMW (VIMP number 1708810011).

Acknowledgements

We would like to acknowledge and thank Patricia E. Broekhuizen – van Haaften for the excellent technical support.

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Potential conflicts of interest

E.M. Terveer, J.J. Keller and E.J. Kuijper report grants from the Netherlands Organization for Health Research and Development, ZonMW, during the conduct of this study and an unrestricted research grant from Vedanta, outside the submitted work.

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