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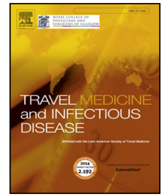
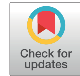


Carriage of *Blastocystis* spp. in travellers - A prospective longitudinal study



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ABSTRACT

Introduction: A lack of prospective and longitudinal data on pre- and post-travel carriage of *Blastocystis* spp. complicates interpretation of a positive test post-travel. Therefore we studied dynamics of *Blastocystis* carriage in a cohort of Dutch travellers.
Methods: From the prospective, multicentre COMBAT study among 2001 Dutch travellers, a subset of 491 travellers was selected based on travel destination to 7 subregions (70 or 71 travellers each). Faecal samples taken directly before and after travel were screened for *Blastocystis* with qPCR, followed, when positive, by sequence analysis to determine subtypes.
Results: After exclusion of 12 samples with missing samples or inhibited qPCR-reactions, stool samples of 479 travellers were analysed. Before travel, 174 of them (36.3%) carried *Blastocystis* and in most of these, the same subtype was persistently carried. However, in 48/174 of those travellers (27.6%; CI95 20.8–36.6%) no *Blastocystis* or a different subtype was detected in the post-travel sample, indicating loss of *Blastocystis* during travel. Only 26 (5.4%; CI95 3.7%–8.0%) of all travellers acquired *Blastocystis*, including two individuals that were already positive for *Blastocystis* before travel but acquired a different subtype during travel.
Discussion: This study shows that *Blastocystis* carriage in travellers is highly dynamic. The observed acquisition and loss of *Blastocystis* could either be travel-related or reflect the natural course of *Blastocystis* carriage. We demonstrate that the majority of *Blastocystis* detected in post-travel samples were already carried before travel.

1. Introduction

Blastocystis spp. are among the most commonly observed intestinal protozoan parasites in humans [1]. Thus far, 17 different *Blastocystis* spp. (or simply *Blastocystis*) subtypes (STs) have been distinguished genetically; with ST1, ST2, ST3 and ST4 being the most frequently reported subtypes [2–5]. Of these, ST4 is possibly associated with increased pathogenicity in humans [6]. Simultaneous colonisation with different subtypes is not uncommon. Transmission most often occurs by the faecal-oral route or through ingestion of contaminated water [6,7]. Since *Blastocystis* is found in many animal species, zoonotic transmission may also occur [8–10].

Since asymptomatic carriage is common in humans and results from previous studies are unclear on whether elimination of the parasite is associated with clinical improvement. Therefore the pathogenic potential of *Blastocystis* for humans had been debated for decades [11–14]. It has been demonstrated that the prevalence of *Blastocystis* in stool samples submitted for routine parasitological examination of patients

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who returned from tropical countries is significantly higher than among patients without such a travel history [15]. These findings suggest that travel is associated with a risk of acquiring *Blastocystis*. This is particularly relevant in travel medicine where many travellers return with abdominal complaints and/or diarrhoea and are tested positive for *Blastocystis*. In those cases, clinicians are faced with the dilemma whether treatment should be offered or not, especially if no other potential pathogen is detected [16].

A wide range of bacteria, viruses and parasites that can be acquired during travel are associated with traveller's diarrhoea (TD) [17]. Non-prospective studies in travellers found *Blastocystis* to be more prevalent in subjects with TD than in those without TD [18,19] whilst others did not find this difference [12]. However, prospective and longitudinal data on acquisition rates of *Blastocystis* during travel in symptomatic and asymptomatic travellers are needed to better understand the clinical relevance of *Blastocystis* detection post travel, but such data are largely lacking. Therefore we studied pre- and post-travel carriage and subtype distribution of *Blastocystis* in a cohort of Dutch international travellers.

Although hard to realize, sequential and frequent sampling before, during and after travel would be ideal in understanding colonisation dynamics of travel related pathogens. In absence of such frequent samples, mathematical modelling maximizes insight in colonisation dynamics of *Blastocystis* and these pathogens in general.

2. Methods

2.1. Study population

The COMBAT-study is a multicentre longitudinal cohort study of 2001 healthy Dutch adults who travelled abroad for a time period of 1–12 weeks. Details of this cohort study have been reported previously [20,21]. In short, subjects were included from November 2012 until November 2013. All received a faeces collection swab (Fecal Swab; Copan, Brescia, Italy) with transport medium and a questionnaire immediately before and after travel. The questionnaires comprised detailed information about previous travel, health, medication use and details about destination and behaviour during travel. All stool samples were stored at −80 °C for future analysis.

2.2. Selection of travellers

To be able to determine geographic distribution of acquired *Blastocystis* subtypes, seven United Nations' defined geographical subregions that were visited by more than 70 travellers were selected [Table 1]. After excluding subjects who had travelled to more than one subregion, 70 travellers were randomly selected from six subregions and 71 travellers from the remaining one. In total, a subset of 491 travellers was selected from the entire cohort. From these travellers, pre- and post-travel samples were analysed for the presence of *Blastocystis*. Subjects of whom one or both samples were missing were excluded from final analyses.

2.3. DNA extraction

Automated nucleic acid extraction was performed using the MagNA Pure 96 instrument (Roche Applied Science, the Netherlands) according to the manufacturer's protocol. DNA was eluted in 100 µl elution buffer (Roche Applied Science). Phocine Herpes Virus (PhoHV) DNA was added to all samples as an internal control (IC) for extraction and amplification efficiency.

2.4. Testing for Blastocystis

Presence of *Blastocystis* infection was assessed by real-time PCR targeting the small subunit ribosomal RNA gene (SSU-rDNA) [22].

Table 1
Characteristics of included travellers (n = 479).

		n	%
Sex	Female	263	54.9%
	Male	216	45.1%
Age (median, range in years)		52	19–81
Chronic illness	Yes	105	21.9%
	Joint disorder	20	4.2%
	> Cardiovascular disease	19	4.0%
	Lung disease	18	3.8%
	Bowel disease	10	2.1%
	Multiple	8	1.7%
	Diabetes	7	1.5%
	Cancer	6	1.3%
	Other	17	3.5%
	No	374	78.1%
Antibiotic use within 3 months before travel	Yes	45	9.4%
	No	434	90.6%
Median duration of travel in days (IQR)		18	(14–23)
Purpose of travel	Holiday	395	82.5%
	Work or internship	38	7.9%
	Visiting friends or relatives	19	4.0%
	Other	27	5.6%
Subregion visited during travel	South Eastern Asia	69	14.4%
	Eastern Africa	68	14.2%
	Northern Africa	68	14.2%
	Southern Africa	70	14.6%
	Western Africa	67	14.0%
	South America	67	14.0%
	Southern Asia	70	14.6%
Accommodation during travel	Hotel or apartment	121	25.3%
	Luxury	109	22.8%
	Low budget	53	11.1%
	Family or local people	20	4.2%
	Tent	12	2.5%
	Several	146	30.5%
	Other	18	3.7%
Traveller's diarrhoea	Yes	183	38.2%
	No	296	61.8%
Antibiotic use during travel	Yes	28	5.8%
	No	451	94.2%
Medical care during travel	Visited doctor or hospital	16	3.3%
	No medical care	463	96.7%

IQR, interquartile range.

Positive controls consisting of a plasmid containing the target sequence were included in every run, as well as negative extraction controls and negative PCR controls. Subjects were excluded from further analyses if IC's tested negative in one or more samples.

For subtyping of *Blastocystis* and confirmation of the presence of *Blastocystis* DNA in samples, PCR-amplicons were sequenced using separate primers. Both DNA strands of amplicons were sequenced with BigDye™ Terminator chemistry (Applied Biosystems) and analysed on an ABI 3900 sequencer (Applied Biosystems). Obtained sequences were aligned to Genbank® reference sequences using CodonCode Aligner program (CodonCode Corporation) and MEGA 6.0 [23]. Subtypes were identified according to the nomenclature proposed by Stensvold et al. [24]. For sequences that contained mixed signals of multiple subtypes, the samples were considered to carry multiple *Blastocystis* subtypes. All primers are listed in the Supplementary Material.

2.5. Definitions

Travel-related acquisition of *Blastocystis* was defined as a combination of a negative PCR for *Blastocystis* in the pre-travel sample and a positive PCR in the post-travel sample, or a positive PCR in both samples but for different *Blastocystis* subtypes. Persistent carriers were defined as those with positive PCRs for the same subtype in both samples and non-carriers as those with negative PCRs in both samples. Loss of carriage was defined as a positive pre-travel PCR and a negative PCR for

the same subtype in the post-travel sample. Because travellers who were positive before travel could acquire *Blastocystis* of a different subtype, all travellers were considered 'at risk' for acquisition. All travellers that carried *Blastocystis* before travel were considered at risk for losing *Blastocystis*.

Pre-travel diarrhoea and TD were defined as 3 or more unformed stools within 24 h, with or without accompanying symptoms.

2.6. Statistical analysis

Statistical analyses were done using IBM SPSS Statistics (version 24.0). Incidence proportions (IP) and incidence per 100 person-days of travel (IR/100 pdt) and accompanying 95% CIs for acquisition and loss were calculated for each subregion. If 95% CIs between subregions did not overlap, the difference in acquisition and loss were considered significant. The association between carriage, acquisition and loss of *Blastocystis* and antibiotic usage and TD was calculated with the Chi-squared test ($\chi 2$) using MedCalc [25]. P-values below 0.05 were considered statistically significant.

2.7. Mathematical modelling

Data on travel duration, time between pre- and post-travel sampling and departure/return as well as the *Blastocystis* colonisation state before and after travel were used in a mathematical model to estimate median acquisition rates with 95% credibility intervals at home and during travel, as well as decolonisation rates and duration of carriage. A detailed description of this method has been included in the Supplementary Material.

In short, we assume that individuals can be either colonised or uncolonised. Colonised individuals may lose colonisation at a fixed rate, independent of whether the individual is travelling or at home. Uncolonised individuals can acquire colonisation. The acquisition rate depends on the location (during travel or at home) and the time an individual spends at this location. If we put our data on the colonisation status before and after travel, the time between sampling, the travel duration and the subregion that were visited into a mathematical Markov Chain Monte Carlo model, we are able to calculate loss of colonisation, acquisition during travel for each of the included subregions and acquisition at home.

3. Results

Of the 491 selected travellers, one or more samples were missing from 7 subjects and samples of 5 subjects had negative IC-PCR's. Therefore 479 travellers were included in further analyses.

Median travel duration was 18 days (IQR 14–23) and leisure (82.5%) was the main purpose of travel. Travellers to Northern Africa travelled shorter than those to other regions with median durations of 12 days (IQR 8–14) and 19 days (IQR 15–24), respectively. Travellers' diarrhoea was reported by 183 travellers (38.2%) [Table 1].

Of the 479 travellers, 281 (58.7%) were negative before and after travel. Before travel, 174 of the 479 travellers (36.3%) were already carrying *Blastocystis* and in most of these (126 of 174; 72.4%) the same subtype was persistently carried. In the remaining 48 travellers, no *Blastocystis* or *Blastocystis* belonging to a different subtype than before travel were detected in the post-travel sample, indicating loss of *Blastocystis* during travel (IP 27.6%; CI95 20.8–36.6% and IR 1.45/100 pdt; 1.07–1.92) [Fig. 1; Supplementary Table 1]. This included one traveller who had lost two subtypes (ST1 and ST3) and one traveller who had lost an ST1 and acquired an ST3 [Supplementary Table 2]. No statistically significant differences in the rates of loss between subregions [Fig. 1, Supplementary Table 1] or between different *Blastocystis* subtypes were found (data not shown); however, numbers per subregion and subtype were small [Supplementary Table 2].

Of 305 travellers who were negative before travel, 281 (92.1%) were still negative after travel and 24 (7.9%; CI95 5.3–11.7%) acquired *Blastocystis*. In addition, acquisition of another subtype was observed in two travellers who carried *Blastocystis* before travel, resulting in a total number of 26 acquisitions in the cohort of 479 travellers (5.4%; CI95 3.7%–8.0% and IR 0.28/100 pdt; 0.18–0.40). Acquisition rates were highest in Southern Africa (IP 8.6%; IR 0.43/100 pdt) and lowest in travellers to Eastern Africa (IP 2.9%; IR 0.15/100 pdt), but differences were not statistically significant [Fig. 1].

Before travel, ST1, ST2, ST3 and ST4 accounted for more than 90% of all *Blastocystis* subtypes. ST3 was the most frequently carried subtype, present in 56/174 (32.2%) *Blastocystis*-positive subjects [Table 2]. *Blastocystis* ST1 and ST3 were the most frequently acquired subtypes [Supplementary Table 2], but acquisition rates and rates of loss [Table 2] were not statistically significant between different *Blastocystis* subtypes. One traveller acquired a ST6/9 hybrid *Blastocystis* strain and one acquired a *Blastocystis* that could not be subtyped due to technical reasons.

Median travel duration and median time between pre- and post-travel samples were comparable between non-carriers, persistent carriers and travellers with loss and acquisition [Supplementary Table 3]. Antibiotic use during travel was not associated with acquisition and antibiotic use was slightly, but not significantly, higher in non-carriers than in travellers with acquisition (21/281 [7.5%] VS 1/26 [3.8%]; p = 0.4934). In addition, antibiotic use was not associated with loss: none of the 48 travellers that lost *Blastocystis* used antibiotics during travel compared to 4 of 126 that were persistent carriers. One traveller used nitrofurantoin and one used metronidazole during travel. Both were non-carriers. No usage of antiparasitic nitazoxanide or furazolidone was reported.

3.1. Association between Blastocystis carriage and TD

No association between *Blastocystis* acquisition and TD was found: TD was reported in 10 of 26 travellers (38.5%) who acquired Blastocystis and in 173 of 453 (38.2%) travellers who did not acquire Blastocystis (p = 0.9779). Additionally, no association was found between loss of *Blastocystis* and TD: 19 of 47 (40.4%) travellers who lost *Blastocystis* reported TD, compared to 44 of 125 (35.2%) persistent carriers (p = 0.5274). Finally, there was no significant difference in the proportion of post-travel carriage between travellers that did and did not report TD. Among those travellers that experienced TD, 54 of 183 (29.5%) carried *Blastocystis* post-travel and among travellers that did not report TD, 97 of 296 (32.8%) were post-travel carriers (p = 0.4558).

3.2. Mathematical modelling

Results of our mathematical model showed that the decolonisation rate per day was 0.0110 (0.0081–0.01453) with a mean duration of carriage of 91 days (95%CI 69–123). The chance of acquisition was calculated to be 0.0041 per day during travel (95%CI 0.0014–0.0068) and 0.0011 (0.0001–0.0043) per day when not travelling. Acquisition rates were not significantly different between subregions. [Supplementary table 4].

4. Discussion

This is the first prospective longitudinal study addressing the dynamics of *Blastocystis* carriage during travel. We found high pre-travel carriage rates and limited acquisition among travellers overall and for each of the studied subregions. An interesting finding of this study is the large proportion of travellers that appears to loose *Blastocystis* during travel.

The 'weighted selection' strategy and the large sample size made it possible to study acquisition in less visited sub regions. Ideally, multiple samples should be taken to determine exactly when and where the

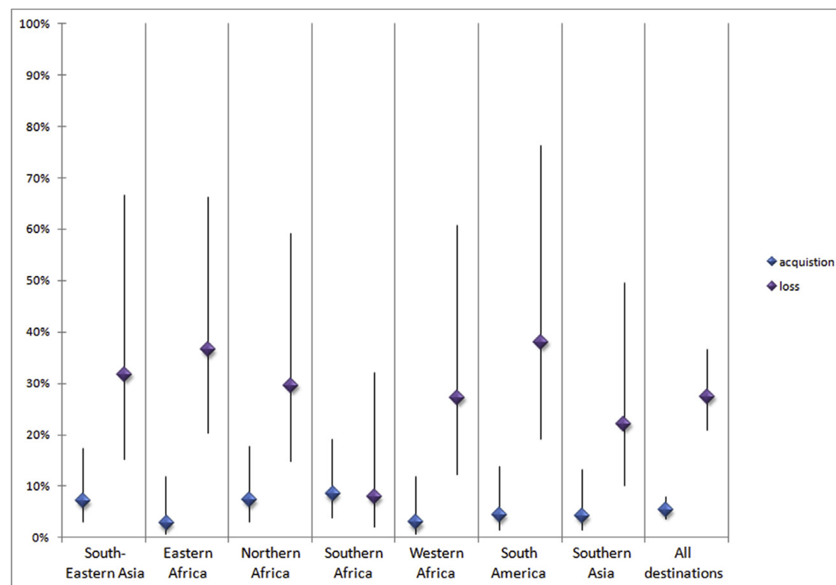

Fig. 1. Acquisition and loss of *Blastocystis* per subregion visited. Blue box: acquisition rate (incidence proportions). Purple triangle: rate of loss (incidence proportions). Black line: 95% confidence interval (95%CI). In case 95%CI's do not overlap, the difference in rates is considered significant.

Table 2
Pre and post travel carriage of *Blastocystis* subtypes.

	Before travel	After travel			
		Negative (loss)		Positive (persistent carrier)	
ST1	29	5	17.2%	24	82.8%
ST2	38	9	23.7%	29	76.3%
ST3	54	16	29.6%	38	70.4%
ST4	41	13	31.7%	28	68.3%
ST6	6	2	33.3%	4	66.7%
ST7	2	1	50.0%	1	50.0%
Mixed[a]	4	2	50.0%	2	50.0%
Total	**174**	**48**	**27.6%**	**126**	**72.4%**

Only subjects that carried *Blastocystis* before travel are shown. ST: subtype.

[a] Of these four travellers, one lost both an ST1 and ST3, one persistently carried an ST2 and ST3, one lost an ST1 and acquired an ST3 and one persistently carried an ST2 and additionally acquired an ST3 during travel.

Blastocystis were acquired and lost. However, multiple sampling during travel however is a great challenge in terms of compliance and preservation of the samples.

Theoretically, a small number of mixed infections, where one subtype is present in a much lower concentration than the other, could have been missed by using this PCR- and sequencing-based method. However, mixed infections up to a ratio of 1:10 can be detected with the approach used in this study [unpublished data] and indeed a number of subjects were found to have combined infections with 2 different *Blastocystis* subtypes.

The observed high rate of *Blastocystis* carriage of 36.3% before travel and 31.5% after travel is comparable to rates reported for healthy individuals in another Dutch study, but much higher than the prevalence among ulcerative colitis patients [26]. In a study in returning travellers presented at one of our units (Center of Tropical Medicine and Travel Medicine of the AMC, Amsterdam), a comparable proportion carried *Blastocystis* was found in post-travel samples, but the proportion was much lower in patients from other departments in the same hospital [15].

In a case control study, investigating post-travel stool samples by direct microscopy from 795 German tourists returning from tropical countries, only 11.3% were found to be positive for *Blastocystis* [19]. Another non-prospective German study, performed between August 2006 and November 2009, found 14.9% of the travellers with TD and 3.6% of travellers without TD to be positive for *Blastocystis* ($p = 0.03$) [18]. The lower post-travel prevalences compared to the present study might be explained by the higher sensitivity of the PCR-based method applied in our study as compared to microscopy.

The most frequently detected *Blastocystis* subtypes in our study were subtypes 1–4, reflecting the most prevalent subtypes in humans. ST4 accounted for a relatively large proportion of 21.2% (32/151) of post-travel carried subtypes. This is significantly more than the 11.7% (12/103) found in the before mentioned study in returning travellers in our hospital ($p = 0.0490$) [15]. One traveller acquired a *Blastocystis* ST7, which is possibly a zoonotic transmission from birds [27]. Subtype 6 and 9 are genetically similar and it is debated whether they should be seen as variants of a single subtype [3,4]. The detection of a *Blastocystis* subtype 6/9 hybrid in a traveller to Southeastern Asia may also indicate that these subtypes form a continuum rather than distinct entities [28–30]. As sample size was calculated to detect differences in acquisition between subregions, the study was insufficiently powered to detect differences in loss and acquisition per subtype.

Two mechanisms could potentially explain the observed acquisition and loss of *Blastocystis* in this study. First, both could be related to travel. Travel is potentially associated with an increased risk of acquisition due to a higher prevalence, dissemination in the food chain and/or lower hygiene standards at the travel destination. Theoretically, travel could also result in loss of *Blastocystis*, for example by alterations in intestinal microbiota that could be unfavourable for *Blastocystis* [31]. It is hypothesized that the presence of *Blastocystis* is a sign of a healthy gut [32], although in our study no association between *Blastocystis* carriage and TD was found.

Most likely, the observed acquisition and loss could merely reflect the natural course of *Blastocystis* carriage. A few studies investigated *Blastocystis* carriage in sequential samples. Vennila and colleagues, described fluctuation in the shedding of the parasite from day to day in one patient [33]. In a Turkish randomized clinical trial on the efficacy of *Saccharomyces boulardii* and metronidazole for treatment of assumed symptomatic *Blastocystis* infections, spontaneous clearance, as detected by microscopy, was observed within 14 days in 4/15 children (26.7%) receiving no treatment [34]. Of 17 Swiss placebo-treated children with recurrent abdominal pain, 5 (29.4%) became microscopically negative within 3 weeks [35]. These studies and our study found comparable proportions of loss of carriage within a similar time frame. In our study,

no statistically significant differences in travel duration and the time between samples were found between non-carriers, persistent carriers and travellers with acquisition and loss, suggesting that *Blastocystis* carriage can be relatively short-lived regardless of travel. In contrast, individuals that were consistently positive for the same *Blastocystis* strain (determined at allele level) over a time period ranging from 6 to 10 years have also been reported [36]. Future longitudinal studies investigating *Blastocystis* carriage in healthy, non-travelling individuals could give insight in the natural course of *Blastocystis* carriage.

Our observation that the vast majority of *Blastocystis* detected after travel were already carried before travel and only few travellers acquired *Blastocystis* during travel, indicates that clinicians should be reluctant to offer treatment for *Blastocystis* carriage in returning travellers.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tmaid.2018.06.005.

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