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

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**A R T I C L E   I N F O**

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* Carriage*  
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* Loss*  
* Dynamics*

**A B S T R A C T**

**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.

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1. Introduction

*Blastocystis* spp. are among the most commonly observed intestinal protozoan parasites in humans \cite{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 \cite{2–5}. Of these, ST4 is possibly associated with increased pathogenicity in humans \cite{6}. Simultaneous colonisation with different subtypes is not uncommon. Transmission most often occurs by the faecal-oral route or through ingestion of contaminated water \cite{6,7}.

Since *Blastocystis* is found in many animal species, zoonotic transmission may also occur \cite{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 \cite{11–14}. It has been demonstrated that the prevalence of *Blastocystis* in stool samples submitted for routine parasitological examination of patients...
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). |
|-----------------|-----------------|
| Sex | n | % |
| Female | 263 | 54.9% |
| Male | 216 | 45.1% |
| Age (median, range in years) | 52 | 19-81 |
| Chronic illness | Yes | 105 | 21.9% |
| Joint disorder | > 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% |
| 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 ICs 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 state 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 nitazoxanide 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 0.0014 per day during travel (95%CI: 0.001–0.0068) 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
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 carriage 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 carriage due to a higher prevalence, dissemination in the food chain and/or lower hygiene standards at the travel destination.

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.

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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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