# Travel-related acquisition of diarrhoeagenic bacteria, enteral viruses and parasites in a prospective cohort of 98 Dutch travellers

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## ABSTRACT

### Background

Limited prospective data are available on the acquisition of viral, bacterial and parasitic diarrhoeagenic agents by healthy individuals during travel.

### Methods

To determine the frequency of travel associated acquisition of 19 pathogens in 98 intercontinental travellers, qPCR was used to detect 8 viral pathogens, 6 bacterial enteric pathogens and 5 parasite species in faecal samples collected immediately before and after travel.

### Results

We found high pre-travel carriage rates of *Blastocystis* spp. and *Dientamoeba fragilis* of 32% and 19% respectively. Pre-travel prevalences of all other tested pathogens were below 3%. *Blastocystis* spp. (10%), *Plesiomonas shigelloides* (7%), *D. fragilis* (6%) and *Shigella* spp. (5%) were the most frequently acquired pathogens and acquisition of enteral viruses and hepatitis E virus in this relatively small group of travellers was rare or non-existent.

### Conclusions

Our findings suggest that the role of viruses as the cause of persisting traveller's diarrhoea is limited and bacterial pathogens are more likely as a cause of traveller's diarrhoea. The substantial proportion of travellers carrying *Blastocystis* spp. and *D. fragilis* before travel warrants cautious interpretation of positive samples in returning ravellers with gastrointestinal complaints.

## INTRODUCTION

Although diarrhoea ranges in the top of the most frequently occurring travel associated conditions [1], limited data are available on the acquisition of viral, bacterial and parasitic diarrhoeagenic agents by previously healthy individuals during travel. In addition to the epidemiological relevance, such data may help to understand the clinical relevance of detected pathogens in the era of extremely sensitive diagnostic testing with real-time PCR (qPCR). This study was initiated to determine the frequency of travel associated acquisition of 19 (entero)pathogens by qPCR.

## MATERIALS & METHODS

### Study population

The COMBAT-study is a multicenter longitudinal cohort study primarily focussing on the acquisition of ESBL- and carbapenemase-producing *Enterobacteriaceae* [2,3]. A total of 2001 Dutch adults travelling intercontinentally for 1–12 weeks were included from November 2012 until November 2013. All subjects received a faeces collection swab (Fecal Swab; Copan, Brescia, Italy) with transport medium and a questionnaire before and after travel. The questionnaires comprised information about previous travel, health, travel destination and behaviour during travel. Stool samples were stored at −80 °C for future analysis. For this study, 100 travellers were randomly selected from the complete study population of 2001 travellers.

### Extraction and PCR

Automated nucleic acid extraction was performed using the MagNA Pure 96 instrument (Roche Applied Science, Roche Diagnostics B.V., the Netherlands) with the inclusion of internal controls for generic RNA and DNA. Reverse-transcriptase PCR was applied immediately after extraction to form cDNA. qPCR was performed using the LightCycler480 (Roche Applied Science, Roche Diagnostics B.V., the Netherlands) to detect 8 viral pathogens, 6 bacterial enteric pathogens and 5 parasite species in faecal samples collected immediately before and after travel (see Table 1 for targeted micro-organisms and Supplementary Table for primer and probe sequences). A positive (Phocine Herpesvirus, PhoHV) and negative extraction control (STAR-buffer) were included in every qPCR run.

### Descriptive and statistical analysis

Acquisition was defined as a negative pre-travel and a positive post-travel test result for the examined micro-organism, irrespective of signs and symptoms of illness. Acquisition rates were calculated for travellers with a negative pre-travel test: the population at risk.

Traveller's diarrhoea (TD) was defined as three or more unformed stools within a 24-h period during travel.

**Table 1.** Micro-organisms studied by qPCR in the cohort of travellers.

| Viruses | Bacteria | Parasites |
|---|---|---|
| Adenovirus (40, 41, 52) | *Campylobacter* spp. | *Blastocystis* spp. |
| Astrovirus | *Clostridium difficile* | *Cryptosporidium* spp. |
| Enterovirus | *Plesiomonas shigelloides* | *Dientamoeba fragilis* |
| Hepatitis E virus | *Salmonella* spp. | *Entamoeba histolytica* |
| Norovirus | *Shigella* spp. | *Giardia lamblia* |
| Parechovirus | *Yersinia enterocolitica* | |
| Rotavirus (A, C) | | |
| Sapovirus | | |

**Table 2.** Baseline characteristics of current study group and the full study population.

| | current study, n/N (n = 98[a]) | full study population, n/N (n = 2001[a]) |
|---|---|---|
| Female | 51/97 (53%) | 1079/1996 (54%) |
| Median age, years (range) | 48 (19–76) | 51 (18–82) |
| Median travel duration, days (range) | 20 (7–72) | 20 (3–105) |
| Chronic illness | 17/97 (18%) | 450/1977 (23%) |
| Antibiotic use last 3 months | 11/98 (11%) | 197/1988 (10%) |
| Diarrhoea pre-travel | 10/97 (10%) | 242/1993 (12%) |
| Travel reason: vacation | 84/97 (87%) | 1655/1964 (84%) |
| Travellers' diarrhoea | 34/97 (35%) | 734/1962 (37%) |
| Travel destination: | | |
| South-Eastern Asia | 30/97 (31%) | 578/1965 (29%) |
| South America | 13/97 (13%) | 192/1965 (10%) |
| Eastern Africa | 10/97 (10%) | 206/1965 (10%) |
| Northern Africa | 7/97 (7%) | 83/1965 (4%) |
| Multiple sub-regions in Asia | 6/97 (6%) | 76/1965 (4%) |
| Western Africa | 4/97 (4%) | 114/1965 (4%) |
| Other sub-regions | 27/97 (28%) | 716/1965 (36%) |

a. Numbers do not always add up to 98 and 2001 because of missing data.

## RESULTS

For one of the 100 randomly selected travellers a sample was missing, and for another one, DNA extraction failed. Therefore, 98 travellers were analysed. Distributions of the population characteristics were similar to the distributions in the full study population

(Table 2). The median age was 48 years (range 19–75) and 53% was female. Median travel duration was 20 (7–72) days. Most frequently visited sub-regions were South-Eastern Asia (n = 30), South America (n = 13) and Eastern Africa (n = 10). Thirty-five percent of travellers (34/97) reported TD.

Pre-travel carriage rates for *Blastocystis* spp. and *Dientamoeba fragilis* were 31/98 (32%) and 19/98 (19%), respectively of which seven participants carried both species (Table 3). Pre-travel prevalences of the other tested species were much lower and ranged from 0 to 3%. A total of six travellers carried one or more pathogenic bacteria before travel. None of these six travellers had diarrhoeal complaints before travel.

**Table 3.** Pre-travel carriage and acquisition rates of diarrhoeagenic bacteria, parasites and viruses.

| Pathogen | Pre travel (n-98) | | Acquisition[a] | | | | |
|---|---|---|---|---|---|---|---|
| | | | TD[b] | % | No TD[b] | % | Total |
| **Bacteria** | | | | | | | |
| *Campylobacter* spp. | 0 | 0% | 3/34 | 9% | 1/63 | 2% | 4/98 (4%) |
| *Clostridium difficile* | 0 | 0% | 1/34 | 3% | 0/63 | 0% | 1/98 (1%) |
| *Plesiomonas shigelloides* | 1 | 1% | 1/34 | 3% | 6/62 | 10% | 7/97 (7%) |
| *Salmonella* spp. | 0 | 0% | 2/34 | 6% | 1/63 | 2% | 3/98 (3%) |
| *Shigella* spp. | 2 | 2% | 4/34 | 12% | 1/61 | 2% | 5/96 (5%) |
| *Yersinia enterocolitica* | 3 | 3% | 2/33 | 6% | 0/61 | 0% | 2/95 (2%) |
| **Parasites** | | | | | | | |
| *Blastocystis* spp. | 31 | 32% | 3/25 | 12% | 4/41 | 10% | 7/67 (10%) |
| *Cryptosporidium* spp. | 0 | 0% | | | | | 0 |
| *Dientamoeba fragilis* | 19 | 19% | 1/24 | 4% | 4/54 | 7% | 5/79 (6%) |
| *Entamoeba histolytica* | 0 | 0% | | | | | 0 |
| *Giardia lamblia* | 0 | 0% | 0/34 | 0% | 1/63 | 2% | 1/98 (1%) |
| **Viruses** | | | | | | | |
| Adenovirus (40, 41, 52) | 0 | 0% | | | | | 0 |
| Astrovirus | 0 | 0% | | | | | 0 |
| Enterovirus | 0 | 0% | 1/34 | 3% | 0/63 | 0% | 1/98 (1%) |
| Hepatitis E virus | 2 | 2% | | | | | 0 |
| Norovirus | 1 | 1% | 2/34 | 6% | 0/62 | 0% | 2/97 (2%) |
| Parechovirus | 0 | 0% | 0/34 | 0% | 2/63 | 3% | 2/98 (2%) |
| Rotavirus (A, C) | 0 | 0% | | | | | 0 |
| Sapovirus | 0 | 0% | | | | | 0 |

TD: traveller's diarrhoea.
Numbers add up to 97 instead of 98 because of one missing post travel questionnaire.
a. Acquisition rates were calculated for travellers with a negative pre-travel test: the 'population at risk'.
b. Denominator is the number travellers with and without TD.

*Blastocystis* spp. were acquired most frequently, followed by *Plesiomonas shigelloides*, Dientamoeba fragilis and *Shigella* spp. (Table 3). Pathogenic bacteria were acquired by 19/98 (19%) of travellers, of whom 7 acquired *P. shigelloides*.

*Entamoeba histolytica*, *Cryptosporidium* spp., Rotavirus (A, C), Adenovirus (40, 41, 52), Astrovirus and Sapovirus were neither detected in any of the pre-travel samples nor in the post-travel samples. Hepatitis E virus (HEV) was detected in 2 pre-travel samples only. Norovirus and Parechovirus were both acquired by two travellers and Enterovirus by one traveller, respectively. Of note, the traveller who acquired an Enterovirus travelled to China only and did not visit a polio-endemic area.

4/5 travellers that acquired *Shigella* spp. and 3/4 that acquired *Campylobacter* spp. reported TD (OR 8.0; p = 0.054 by Fisher's exact test and OR 6.0; p = 0.12 respectively). Of the 7 travellers that acquired *P. shigelloides* only one reported TD (OR 0.3; p = 0.42) (Table 3).

## DISCUSSION

This study shows that *Dientamoeba fragilis* and *Blastocystis* spp. were highly prevalent before travel. *Blastocystis* spp., *Plesiomonas shigelloides*, Dientamoeba fragilis and *Shigella* spp. were the most frequently acquired pathogens and acquisition of enteral viruses and HEV in this relatively small group of travellers was rare or non-existent.

To our knowledge, travel associated acquisition of a wide spectrum of nineteen viral, bacterial and parasite species has not been reported before. The PCR's used for this study are validated and used for routine diagnostics and are considered very sensitive. However, as with all molecular tests, the detection of DNA does not necessarily mean that detected pathogens are viable. Also, the possibility exists that pathogens acquired during travel were already cleared by time the traveller returned and did the post travel sampling, and some brief intermittent acquisitions may therefore have been missed in this analysis. However, post-travel samples were provided within a mean of 3 days (range 0–19 days; median 2 days) and the mean duration between sample collection and processing in the laboratory was 2 days (range 1–23 days) so we do not regard this possibility as a significant source of bias. A major limitation of this study is the sample size that is probably too small to detect rarely acquired enteropathogens. Therefore, no firm conclusions on the acquisition of HEV and other rare pathogens can be drawn from this study. Also, an association between the acquisition of studied microorganisms and TD was not found, but because a limited number of travellers were tested, the statistical power to study this association was low. Unfortunately we were not able to study *Cyclospora cayetanensis*, which is considered an important parasitic cause of traveller's diarrhoea in some regions [4].

Lääveri and co-workers performed a prospective study on bacterial TD pathogens in 382 Finnish travellers [5] and found acquisition of *Salmonella* spp. and *Shigella*/EIEC to be slightly lower than in the present study; namely 2.4% and 0.8% respectively; and *Campylobacter* was acquired by slightly more (6.8%) travellers. However, they did not test for Clostridium, *Yersinia*, *Plesiomonas*, parasites and viruses.

A non-prospective case-control study in 114 returning travellers with diarrhoea and 56 travellers without diarrhoea [6] found 19/170 (11.2%) to be positive by microscopy for *Blastocystis* spp. after travel and only one positive for *Dientamoeba fragilis*. Probably, these lower post-travel prevalences are the result of lower diagnostic sensitivity of microscopy compared to PCR. Interestingly, they found 10 cases of *Giardia lamblia* and 3 cases of *Cryptosporidium* spp., whereas in the present study only one acquisition of *Giardia lamblia* and no *Cryptosporidium* spp. was found. In their study, PCR for Norovirus was positive in 10.5% of the cases and 3.6% of the controls, whereas we found only 2.1% acquisition in the total studied population. The observed differences might be explained by a selection towards a more symptomatic population in the study by Paschke [6], because all of their participants presented as patients to the Department of Infectious Diseases of which two-third with diarrhoea [6].

Travel associated acquisition of HEV is a potential threat to blood safety [7], but no acquisitions were found in the present study. Accordingly, in a Dutch study, no seroconversions to anti-HEV were found in 1206 travellers to (sub)tropical countries [8]. An Australian study estimated the risk of acquiring HEV to be 0.01 to 18 per 10,000 travellers [7] indicating that our limited sample size likely is too small to reliably determine travel-associated acquisition of HEV. Furthermore, the incubation period of symptomatic HEV infections is approximately 40 days (range 2–10 weeks) [9], hence infections may be missed by analysis of samples that were collected shortly after return.

The low acquisition rates of enteric viruses during travel could indicate that the role of viruses as the cause of persisting TD is limited or that viral RNA or DNA is rapidly cleared before returning. For Norovirus however, virus is shed for a median of 28 days after inoculation in previously healthy persons [10] and for Rotavirus, median duration of shedding is approximately 3 weeks [11]. Since bacterial pathogens were acquired more often, they are likely to be more important as a cause of traveller's diarrhoea. For *Blastocystis* and *Dientamoeba*, pathogenicity of these parasites is under debate. To that end, a positive test in a symptomatic returning traveller must be interpreted with caution, keeping in mind that a substantial proportion probably would already have been carrier before travel.

Since *Blastocystis* spp were acquired relatively often, it would be interesting to study if acquisition could be assigned to specific travel destinations.

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**Appendix A.** Supplementary data

| | Species | Gene targeted | Primer-F | Primer-R | Probe |
|---|---|---|---|---|---|
| Viruses | Adenovirus (40, 41) | | TTC CAG CAT AAT AAC TCW GGC TTT G | AAT TTT TCT GWG TCA GGC TTG G | CCW TA+CCC+C+CTT ATT+GG |
| | Adenovirus (52) | | AAC AGA TAC CGC AAC CAC CC | CCT GCC ACT TTA TCA TTA GTG CCT A | TAT CAA+CCT+GAA+C+CA+CAA G |
| | Astrovirus | | GAC TGC WAA GCA GCT TCG T | GCC ATC ACA CTT CTT TGG T | TCA CAG AAG AGC AAC TCC ATC GCA TTT G |
| | Enterovirus | | GGC CCT GAA TGC GGC TAA T | GGG ATT GTC ACC ATA AGC AGC C | GCG GAA CCG ACT ACT TTG GGT |
| | Hepatitis E virus | | CGG TGG TTT CTG GGG TGA | GCR AAG GGR TTG GTT GG | ATT CTC AGC CCT TCG C |
| | Norovirus G1 | | ATG TTC CGC TGG ATG CG | CGT CCT TAG ACG CCA TCA TC | TGG ACA GGA GAT CGC |
| | Norovirus G2 | | CAA GAI CCI ATG TTY AGI TGG ATG AG | TCG ACG CCA TCT TCA TTC AC | TGG GAG GGC GAT CG |
| | Parechovirus | | CTG GGG CCA AAA GCC A | GGT ACC TTC TGG GCA TCC TTC | AAA CAC TAG TTG TAW GGC CC |
| | Rotavirus A | | ACC ATC TWC ACR TRA CCC TC | GGT CAC ATA ACG CCC C | ATG AGC ACA ATA GTT AAA AGC TAA CAC TG+T+CAA |
| | Rotavirus C | | CTA CAA GTA ATG GAA TCG GAT G | TGG GTG TCA TTT GAT ACA ACT TCA | ACC AGC TAG TA+C+A+G+A+AAC |
| | Sapovirus | | F1: GAC CAG GCT CTC GCY ACC TAC<br>F2: TTG GCC CTC GCC ACC TAC | CCC TCC ATY TCA AAC ACT AWT TTG | TGG TT+C ATA+G+GT+G+GT AC |
| Bacteria | *Campylobacter* spp.* | 16S | | | |
| | *Clostridium difficile* | toxB | CAAAYGAGTATTCAAARGAKATAGATGAA | TCTTTCTACYAACTCTTGTTCATATAADTTGAAT | AATAGTGGRAATGATGTTAGAAA |
| | *Plesiomonas shigelloides** | gyrB | | | |
| | *Salmonella* spp.* | ttr | | | |
| | *Shigella* spp.* | ipaH | | | |
| | *Yersinia enterocolitica** | gyrB | | | |
| Parasites | *Blastocystis* spp. | 18S rRNA | CGTTGTTGCAGTTAAAAAGCTCGT | GATTAATGAAAACATCCTTGGTAAATGC | CAGTTGGGGGTATTCATATTC |
| | *Cryptosporidium* spp. | DNAJ-like protein | CGCTTCTCTAGCCTTTCATGA | CTTCACGTGTGTTTGCCAAT | CCAATCACAGAATCATCAGAATCGACTGGTATC |
| | *Dientamoeba fragilis* | 18S rRNA | CAA CGG ATG TCT TGG CTC TTT A | TGC ATT CAA AGA TCG AAC TTA TCA C | CAA TTC TAG CCG CTT AT |
| | *Entamoeba histolytica* | 18S rRNA | ATTgTCgTggCATCCTAACTCA | gCggACggCTCATTATAACA | UCAUUGAAUGAAUUGGCCAUUU |
| | *Giardia lamblia* | 18S rRNA | GAC GGC TCA GGA CAA CGG TT | TTG CCA GCG GTG TCC G | CCC GCG GCG GTC CCT GCT AG |

* Detection of these bacteria was performed using The LightMix® Modular Assay (Roche Applied Science, Roche Diagnostics B.V., the Netherlands) for which no primer and probe sequences are available.