Bacterial, viral and parasitic pathogens analysed by qPCR: findings from a prospective study of travellers' diarrhoea

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Abbreviations:

Ct-value Cycle threshold value

DEC diarrhoeagenic *Escherichia coli*

EAEC enteroaggregative *Escherichia coli*

EHEC enterohaemorrhagic *Escherichia coli*

EIEC enteroinvasive *Escherichia coli*

EPEC enteropathogenic *Escherichia coli*

ETEC enterotoxigenic *Escherichia coli*

EV enterovirus

NoV norovirus

RoV rotavirus

qPCR quantitative PCR

SaV sapovirus

TD travellers' diarrhoea

Keywords: norovirus; antibiotic; Ct-value; diarrhoeagenic *Escherichia coli; EAEC; ETEC*

Running title: Bacteria, viruses and parasites in TD

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Authors' contributions: Study concept and design JA, JK, AK; acquisition of data JA, SM, JK, MR, AK; analysis and interpretation of results TL, JA, SM, SHP, JK, AK; drafting of manuscript TL, AK; statistical analysis TL; literature search TL; final approval of version published TL, JA, SM, SHP, JK, MR, AK

Potential conflicts of interest: AK has received an investigator-initiated grant (Valneva) and consulted an advisory board (Valneva). None of the interests listed above are relevant to the current manuscript. JK is employee of Mobidiag developing diagnostics test for infectious diseases. No commercial tests, however, are used in the study. All other authors report no potential conflicts of interest.

Highlights

- Prospectively collected stool specimens were tested by qPCR for bacteria, viruses and parasites
- Viruses were associated with severe TD

- Viral TD was associated with particularly high rate of antibiotic use

ABSTRACT

BACKGROUND:

The diagnostics of travellers' diarrhoea (TD) has been revolutionised by multiplex qPCR assays. While mostly of bacterial aetiology, viruses and parasites account for the disease among 10–20% of travellers. Despite this, prospective studies applying qPCR assays remain scarce that cover not only bacteria, such as the various diarrhoeagenic *Escherichia coli* (DEC), but also viral and parasitic pathogens.

METHOD:

We analysed by qPCR pre- and post-travel stool samples of 146 Finnish travellers for bacterial, viral and parasitic pathogens: enteropathogenic (EPEC), enteroaggregative (EAEC), enterotoxigenic (ETEC), enterohaemorrhagic (EHEC), and enteroinvasive (EIEC) *E. coli; Shigella*, *Campylobacter*, *Salmonella*, *Yersinia* and *Vibrio cholerae*; norovirus G1 and G2, rotavirus, enteroviruses, and sapovirus; and *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium*. Symptoms and medication data during travel were collected by questionnaires.

RESULTS: We detected bacterial pathogens in 102/146 samples (69.9%; EAEC, EPEC, ETEC most common), viral ones in 13 (8.9%; norovirus most common), and parasitic ones in one (0.7%; *Giardia*). Noroviruses were associated with severe symptoms (23.5% versus non-severe 4.9%). In the TD group, 41.7% (5/12) of those with viral pathogens (vs. 13.3%; 11/83 without) took antibiotics.

CONCLUSION: Viral pathogens, particularly noroviruses, prevail in severe TD. The symptoms of viral disease are often severe and lead to unwarranted use of antibiotics.

1. INTRODUCTION

Over the past decade, qPCR methods have revolutionised the diagnostics of travellers' diarrhoea (TD). While in studies conducted by conventional methods up to half of the stool samples remained negative [1], newer research using qPCR has revealed pathogens among almost all travellers with TD; a substantial proportion (up to 88%) had multiple findings [2–9]. The most common pathogens have comprised, besides enterotoxigenic *Escherichia coli* (ETEC; 7%–59%) and *Campylobacter* (0–31%), both enteropathogenic (EPEC; 0%–63%) and enteroaggregative (EAEC; 1%–59%) *E. coli*, whereas *Salmonella*, *Shigella*, enteroinvasive (EIEC), and enterohaemorrhagic (EHEC) *E. coli* have been found less frequently [1–17]. Even in more recent reports, the various diarrhoeagenic *E. coli* (DEC), particularly EPEC, have not been comprehensively covered [2,11,14,16,18–20]. While the disease is mostly of bacterial origin [1,18], viral pathogens, particularly noroviruses, have been detected among up to 24% [2,5,8,12–14,21–25], and parasitic pathogens among 18% [5,8,11] of travellers. Despite this, even many fresh studies have not encompassed viruses or parasites [3,10,17,26,27]. Most investigations describe travellers seeking care for diarrhoeal symptoms while abroad or after return home [2,4–13,15,16,21–27]. Studies using qPCR and conducted among volunteers recruited prospectively before travel have remained scarce [14,15,17].

Despite the short (resolution in 2–3 days) and mostly mild or moderate natural course of TD – only 4–13% are severe – up to 45% of travellers take antibiotics to alleviate the symptoms [28–39]. The high prevalence of the disease (20–60% of travellers to middle- and low-income countries in the (sub)tropics [1]) implies a vast number of antibiotic courses, most of them presumably unnecessary [39]. In the light of recent studies demonstrating that taking antibiotics increases the risk of contracting multidrug-resistant bacteria [33, 34, 36, 37], their prophylactic use is not warranted, and even resorting to stand-by-antibiotics should not be encouraged [39].

To augment the scant literature on prospective TD studies covering concomitantly various bacterial pathogens, viruses and parasites by qPCR, we present data of 146 Finnish travellers analysed for all these applying that study design.

2. MATERIALS AND METHODS

2.2.Recruitment of travellers

The volunteers were recruited during pre-travel consultation at the Travel Clinic of the Medical Centre Aava, Helsinki, Finland, between March and August 2009. Travellers planning a journey outside the Nordic countries for a minimum of four days and a maximum of six months were eligible. Recruitment was carried on till February 2010, but only the 146 first volunteers were included in the present study. The subsequent investigations focused on bacterial pathogens [3,40–42], travellers' health [43], and multiresistant *Enterobacteriaceae* [33,44], all published earlier.

The study protocol was approved by the Ethics Committee of the Helsinki University Hospital. Written informed consent was obtained from all participants.

2.3.Questionnaires, definitions of TD and severity of symptoms, geographic regions

The pre-travel questionnaire Q1 gathered demographic and background information. The post-travel questionnaire Q2 covered diarrhoeal and other symptoms, countries visited, and medications taken during the journey.

Following the WHO criteria for diarrhoea, TD was defined as three or more loose or liquid stools per day or more frequently than normal for the individual [3,45]. Participants were considered asymptomatic if they remained free of any diarrhoeal symptoms throughout the journey [3]. The term mild TD was used to refer to 1–2 and moderate TD as 3–5 loose or liquid stools not accompanied by high fever, grossly bloody stools or need for hospitalisation. Severe TD was defined as six or more loose or liquid stools, or any number of diarrhoeal stools accompanied by high fever, gross blood in stools or need for hospitalisation. Mild and moderate TD were recategorised as non-severe TD.

The countries visited were grouped into seven regions, as described earlier [3,33]: (1) South Asia, (2) Southeast Asia, (3) East Asia, (4) North Africa and the Middle East, (5) Sub-Saharan Africa, (6) South

and Central America and the Caribbean, and (7) Europe, Australia, and North America. Travellers having visited more than one region were categorised by longest stay.

2.4.Analysis of bacterial, viral and parasitic pathogens

Faecal samples were collected as stool swabs (one pre-travel, two post-travel) and delivered to the laboratory in 1–3 days, where the total nucleic acids were extracted directly from the stools as described previously [46].

The pre- and post-travel stool samples were analysed for bacterial pathogens by a multiplex real-time qPCR assay [47,48] for DEC –[47,48] including EPEC, ETEC, EAEC, EHEC, and EIEC or *Shigella* – as well as *Salmonella*, *Yersinia spp.*, *Vibrio cholerae*, and *Campylobacter spp.* The analytical cut-off (lower limit of detection) cycle threshold (Ct) value was >40 [47].

The amount of nucleic acids obtained from pre-travel faecal samples (one swab) proved insufficient for analyses of viral pathogens which were, therefore, only analysed from post-travel stools (two swabs). The qPCR for viruses covered norovirus G1 and G2, and rota-, sapo-, adeno-, astro-, and enterovirus, as described previously [49]. Due to the insufficient amount of stools and, accordingly, low yield of nucleic acids, rotavirus could only be analysed from 136 post-travel stool samples, astrovirus and adenovirus from none.

Parasitic pathogens were analysed from pre-and post-travel stools by a multiplex qPCR assay[50] [50] covering *Cryptosporidium* spp, *Giardia lamblia* and *Entamoeba histolytica*.

2.5.Statistical analyses

Pearson's chi-square test, Fisher's exact test or binary logistic regression analysis were used to compare categorical variables, when applicable. Statistical significance was defined as $p < 0.05$ or 95% CIs ranging

only either above or below 1. The statistical analyses were carried out using SPSS 22 software (IBM Corp, Armonk, NY).

3. RESULTS

3.1. Traveller characteristics

In total, 58.2% (85/146) of our participants were women. The average age was 39.0 years (SD 17.1) (Table 1). Sub-Saharan Africa proved the most popular destination (n=61; 41.9%), followed by Southeast Asia (30; 20.5%), Latin America (18;12.3%) and South Asia (14; 9.6%) (Table 1). In total, 67.1% (n=98) of the participants reported TD during travel, and 57.1% had ongoing symptoms at the time of post-travel sampling (Table 1). Of those with TD, mild disease was reported by 31.3% (46/98), moderate disease by 23.8% (35/98), and severe by 17.3% (17/98); 32.9% (48/146) had remained asymptomatic. Eighteen travellers (12.6% of the 143 who provided information on medication use) reported having taken antibiotics during travel, 67.7% (12/18) for TD (12.2% of the 98 with TD).

3.2.Pathogen findings in stool samples

Of pre-travel stool samples, three (2.1%) proved positive for EAEC and one (0.7%) for EPEC; no other bacterial nor parasitic pathogens were identified. Viral pathogens were not analysed (see above).

From among post-travel samples, bacterial pathogen(s) were found in 69.9% (102/146), viral pathogen(s) in 8.9% (13/146), and parasitic pathogen(s) in 0.7% (n=1). None were detected in 28.1% (41/146) (Table 2.).

3.3.Pathogen findings in relation to TD symptoms

Table 2 shows how pathogen findings in post-travel stool samples related to TD symptoms.

Among the asymptomatic, 43.7% (21/48) had no pathogens, while for 56.3% (27/48) the most frequent findings were DEC with EPEC (n=16; 33.3%), EAEC (11; 22.9%), EHEC (5; 10.4%), and ETEC (4; 18.3%); one participant tested positive for enteroviruses; no noroviruses or *Campylobacter* were found.

Among the participants with TD (resolved or ongoing), a pathogen was found in 79.6% (78/98); DEC were detected in 56.3% (27/48) with EPEC (n=16; 33.3%), EAEC (11; 22.9%), EHEC (5; 10.4%), and ETEC (4; 18.3%) as the most frequent findings. A viral pathogen was identified in twelve (12.2%) specimens; noroviruses (NoV G2; 6; 6.1% and NoV G1: 4; 4.1%) and enteroviruses (4; 4.1%) were the most common findings.

Multiple pathogens were found in the samples of 43.9% (43/98) of participants with TD; of those with a bacterial pathogen, viral co-pathogens were identified in 13.3% (10/75) and, respectively, 76.9% (10/13) of those with viral pathogens had bacterial co-pathogens.

When compared with the asymptomatic, those with TD during travel showed a greater frequency of findings of any pathogen, multiple pathogens, any bacterial pathogen, DEC, ETEC, and EAEC (Table 3); with other pathogens the differences did not reach statistical significance.

3.4.Travellers with bacterial pathogens

Among the 146 participants, 102 (69.9%) had one or more bacterial pathogens; of these 75 (73.5%) encountered TD. Of travellers with bacterial pathogens, 10.0% (10/102) had taken antibiotics; pathogen findings did not differ between users and non-users (Table 3). Among those with any bacterial pathogen, 9.8% (10/102) had severe and 65.7% (65/102) non-severe diarrhoea (Table 3). EAEC was less common among those with severe (11.8%) than non-severe TD (53.1%; p=0.002; OR 0.1; 95% CI: 0.03–0.5); other pathogen differences did not reach statistical significance.

3.4.1. Ct-values of EAEC, EPEC, ETEC, and Campylobacter in relation to TD symptoms

The Ct-values for EAEC, EPEC, ETEC, and *Campylobacter* proved similar among the asymptomatic and those with TD (Table 4).

3.5.Participants with viral pathogens

Characteristics of the 13 (8.9%) with viral pathogens are detailed in Table 5. Of these participants, 12 (92.3%) reported TD during travel, two (1.4% of all 146) had NoV G1, six (4.1%) NoV G2, five (3.4%) enteroviruses, and one (0.7%) rotavirus. One had both NoV G1 and enterovirus (and EPEC). No sapoviruses were found. Five had visited Southeast Asia (16.7% of those travelling there), six (9.8%) Sub-Saharan Africa, one (7.1%) South Asia, and one (33.3%) East Asia.

Among the 98 with TD, eight (8.2%) had noroviruses, while none were detected among those asymptomatic. The respective figures for any viral pathogens were 12.2% (n=12) and 2.0% (n=1). Two of those with virus findings had visited Thailand together; both had NoV G2. Three others, one with rotavirus, two with NoVG2, reported that their travel companions had contracted TD, yet no viruses were found in the samples of these companions.

Half (50.0%; n=4) of the eight participants with norovirus (NoV G1 or G2) and 9.4% (13/139) of those with no noroviruses had experienced severe TD. Findings of any viral pathogen (p=0.032; OR: 4.4; 95% CI 1.2–16.2) and norovirus (p=0.029; OR: 5.9; 95% CI:1.3–26.7) were associated with severe clinical picture.

Antibiotic use (Table 3.) was associated with findings of any viral pathogen (p=0.012; OR 5.6; 95% CI 1.6–19.7) or norovirus (p=0.063; OR 4.8; 95% CI:1.0–22.0). Among participants with TD, those with viral pathogens had taken antibiotics most frequently (41.7%; 5/12 vs. 8.1% (7/86) of those with TD but no viral pathogens). Viruses were detected from 31.3% (5/16) of the users and 8.9% (7/79) of the non-users (p=0.028; OR 4.7; 95% CI 1.3–17.4).

3.6.Traveller(s) with parasitic pathogens

All 146 faecal samples tested negative for *Cryptosporidium spp.* and *E. histolytica*. One participant (0.7%) with ongoing diarrhoea at the time of sampling had *Giardia lamblia* but no other pathogens after visiting Namibia for 15 days. However, four others reported having been given nitroimidazoles for TD; two had a viral pathogen (enterovirus and NoV G2).

4. DISCUSSION

Ours is one of the few prospective studies employing qPCR assays to cover all major bacterial, viral and parasitic TD pathogens within one single research design. The data confirmed the aetiology of TD to be principally bacterial. A major finding was the greater frequency of viral pathogens among those with severe than non-severe TD. Another result worthy of note is that travellers with viral pathogens were particularly prone to take antibiotics for TD.

4.1 Travellers with bacterial pathogens

Our results accord with previous research reporting DEC as the most frequent findings in TD [1–9,11–13,15,18]. Even recent studies have shown considerable variation in the rates for EPEC (0%–63%), EAEC (1%–59%), and ETEC (7%–59%) [2–13,15,17], however, and many have not covered all DEC [1,2,11,18–20]. Besides traveller population or geographic region, the differences in detection rates can be ascribed to the diagnostic method applied (culture/PCR, primers or cut-off Ct-values used for qPCR). In our data, the most common findings for TD were EPEC (46.9%) and EAEC (45.9%) followed by ETEC (22.4%). *Campylobacter* has been shown to be the primary pathogen in Southeast Asia especially among US military personnel [18,51,52], yet in more recent studies among civilian travellers, equal or lower rates have been reported for *Campylobacter* than for DEC [7,9,16,40,53,54]. Our rates for *Shigella*/EIEC (2.0%) and *Salmonella* (2.0%) proved low compared with some other reports [7,11,12,20,26,54]. This may be explained by study design: *Campylobacter* and *Shigella* rates appear higher when looking at travellers who seek care for acute TD symptoms [7,9,11,12,20,26,54] than in prospective studies [15,19,55].

4.2.Travellers with viral pathogens

Our results (12.2% viruses) agree with a great deal of recent research into TD showing significant rates for viral pathogens, particularly noroviruses [2,5,8,9,12–14,16,21–25]. The rates of norovirus

infections appear to vary greatly between studies (0–24%) [2,5,8,9,11–14,16,21–25]. These differences may be of methodological nature, or due to possible outbreaks or seasonal variations with highest prevalence in winter. Consistent with our findings, rotavirus rates appear low in most previous studies [2,5, 9,11–14,16,21–25]. Interestingly, a study among French child travellers admitted to an emergency ward detected rotaviruses in 15% [8], the high rate presumably ascribable to lack of pre-existing immunity because of low rotavirus vaccine coverage in the country [56]. Astroviruses, enteroviruses, adenoviruses, and sapoviruses have been less common findings [4,6–9,11,12,14,15].

4.3.Low frequency of parasitic pathogens

Of our 146 participants, the sample of only one was found positive for a parasite, *G. lamblia.* The low rate (0.7%) accords with an earlier prospective study [14] which reported one case of (1%) *G. lamblia* but no *E. histolytica* or *Cryptosporidium* among 98 Dutch travellers with TD. However, one of our travellers had been diagnosed with giardiasis and another with amoebiasis while abroad, both treated locally. With these two cases included, our parasite rate totals two cases of giardiasis (2.0% of those with TD) and one amoebiasis (1.0%). Similar frequencies were reported in a study conducted in Bangkok, Thailand among short-term civilian travellers [7]. However, higher *G. lamblia* rates have been reported among travellers seeking care for TD after return (5–13%)[2,6,8,57] and among military personnel commissioned abroad (13%) [5] or long-term travellers/expats (4–11%) [9,25,54]. Consistent with our data, the rates of *Cryptosporidium* and *E. histolytica* have mostly proved low in these studies, yet one conducted in France found *Cryptosporidium* in 18% of child travellers treated for severe TD [8]. Indeed, high parasite rates appear common in investigations focusing mostly on patients given medical care for prolonged or severe TD symptoms, while lower rates prevail in prospective studies with only a minor proportion having symptoms severe enough to contact health care.

4.4.Severity of TD in relation to pathogen findings

Noroviruses, *Campylobacter*, or *Shigella* have been connected with severe TD [7,21,25,58] more often than DEC or other bacterial pathogens. Indeed, up to 90% of those with noroviruses have been bedridden [25]. One of our central findings was higher detection rates for norovirus among travellers with severe (23.5%; 4/17) than non-severe TD (4.9%; 4/81; OR: 5.9; 95% CI:1.3–26.7), according with other studies [7,21,25]. Moreover, travellers with viral pathogen findings were more likely to have had fever than those with a pathogen finding but no viruses detected (4/11; 36.4% vs. 4/53; 7.5%; p=0.024; OR 7.0, 95% CI 1.4–34.5); none of our travellers had gross blood in stools. Although our earlier report describing bacterial agents among travellers with no antibiotic use suggested that EAEC, EPEC, and ETEC cause similar clinical symptoms [3], the present data show EAEC less frequently in severe than non-severe TD (11.8% vs. 53.1%; OR 0.1; 95% CI: 0.03–0.5), a finding agreeing with some earlier studies [4,59]. These findings highlight the importance of broad pathogen coverage in investigations evaluating the clinical significance of each pathogen.

4.5.Antibiotic use was associated with findings of norovirus / viral pathogens

Looking at those with TD, viral pathogens proved more common among antibiotic users than non-users (31.3% vs. 8.9%; p=0.028; OR 4.7; 95% CI 1.3–17.4). This was not an unexpected finding, as noroviruses and other viral pathogens tended to cause severe TD and antibiotic treatment was mostly started empirically. We are aware of only one other study scrutinizing the relation between viral pathogens and antibiotic use: among travellers in Nepal, rotavirus was found more frequent among antibiotic users (17%) than non-users (5–8%; p=0.006); for norovirus, no association was observed (15% vs. 16–19%; p=0.635) [9]. As evident, when given for TD caused by viruses, the benefits of antibiotics against TD are lost but the adverse effects remain, and, as an additional harm, the risk of colonization by multidrug-resistant enteropathogens will increase [33,34,36,37]. Many other studies have also shown viruses to cause more severe symptoms (including research designs where severity of TD is judged by degree of incapacitation) [7,21,27]: this further contests the justification of

antibiotic use – and instead, encourages use of anti-diarrhoeals as self-treatment. For those with high fever or gross blood in stools or those in poor clinical condition a medical evaluation is needed. At hospitals, to ensure an early start of antibiotics for patients with bacteraemia, it is unavoidable that some unnecessary antibiotics should be given.

4.6.Ct-values similar among the asymptomatic and those with TD

In our data, for EAEC, EPEC, ETEC, or *Campylobacter*, the qPCR Ct-values proved similar regardless of presence or absence of TD symptoms. These results agree with the findings of an earlier report [12] which did not describe differences in Ct-values between those with or without diarrhoea. By contrast, a study carried out in Thailand [7] connected lower Ct-values of *Campylobacter* (i.e. higher pathogen loads) and, at the highest pathogen loads, also LT-ETEC with diarrhoeal symptoms. This might not serve as a good example, however, because of the rarity of *Campylobacter* findings among asymptomatic travellers [3,7,19,27,28,60,61]. For the other pathogens, they found no association. Moreover, they did not take prior antibiotic use (32%) into account, and in addition, since the classical definition of TD was used, the control group may have included travellers with mild or resolved TD. Likewise, our data had limitations which may distort the results (low numbers of cases, 35.6% with multiple pathogens, time point of sampling etc.). Indeed, Ct-values should be explored in a study setting where travellers are sampled while still abroad (not merely after travel), a sufficient number of single pathogen infections are analysed, and prior antibiotic users excluded.

4.7.Limitations

Our qPCR assay for parasites did not cover *Cyclospora*, *Blastocystis*, and *Dientamoeba fragilis*. However, *Cyclospora* is rarely found from travellers (table 1). *Blastocystis*, on the other hand, is generally considered apathogenic [55,62] and *D. fragilis* is typically identified in patients with

prolonged symptoms [63]. A prospective study among 98 Dutch travellers found *Dientamoeba* in 19% of pre- and 24% of post-travel stools; the authors recommended caution in interpreting *Dientamoeba* as a cause of acute diarrhoeal symptoms solely on the basis of findings in post-travel stools [55]. Of viral pathogens, we did not cover adenovirus or astrovirus, the detection rates of which have been reported low [4,6–8,11,12,14,15]. Neither could we analyse viral pathogens from pre-travel samples, yet the rates should be low: in the Dutch study [55], one (1%) pre-travel sample out of 98 was positive for norovirus; pre-travel rates also proved low in a Finnish study among travellers to Benin, West Africa (A. Kantele, personal communication).

We only collected stool samples before and after travel. Because some pathogens, such as ETEC, are considered to disappear quite quickly, looking merely at post-travel samples is likely to result in underestimating their proportions. On the other hand, noroviruses in particular are known to be shed several weeks after resolution of symptoms [64,65]. As stool pathogens are also found from asymptomatic travellers and molecular methods fail to distinguish between viable and non-viable pathogens, we only included in our control group those having remained asymptomatic throughout the journey (mild and resolved symptoms excluded). To our knowledge, there are no prospective studies that have employed qPCR and collected faecal specimens soon after onset of symptoms while abroad.

As pathogens are also detected from asymptomatic travellers [2,7,12,14,16,17,28,54,55,61], the significance of these findings should always be interpreted with caution. Moreover, attention should be paid to the design of the control group, i.e. whether those with mild or resolved disease have been excluded or not [42]. Indeed, we recently demonstrated that the results of aetiological studies depend less on the definition of TD than that of the control group [42].

After this research of ours with data from 2009–10, the epidemiological situation may have changed in various regions. This appears not to be a major issue, however: two more recent studies by van

Hattem et al. [14,17] and one by Sow et al. [15] report pathogen findings according with ours. Furthermore, a TD vaccine trial we recently completed in Benin, West Africa, yielded pathogen profiles consistent with the present results (Kantele A, unpublished data).

The last point to be mentioned here concerns recruitment which accounts for visitors to Africa being overrepresented in our study population. As the travellers most likely to seek pre-travel advice (vaccinations, antimalarials), their proportion agrees with that in many other prospective European studies conducted at travel clinics [14,17,28,34,36,37,55].

4.8.Conclusions

This was one of the few prospective traveller studies to date screening a broad coverage of bacterial, viral and parasitic pathogens employing qPCR assays. As expected, bacterial pathogens, particularly DEC, were found in most post-travel samples, while parasitic ones proved rare. Viral pathogens, noroviruses in particular, were common in severe TD, and, worthy of particular note, associated with antibiotic use during travel. The possibility of noroviruses in severe TD has been paid surprisingly scant attention in treatment guidelines recommending stand-by antibiotics for TD [66]. Our data imply that even in severe diarrhoea the aetiological agent may be a virus, and the overall results further demonstrate the need of caution in using stand-by antibiotics to treat TD.

Acknowledgements: This work was supported by a Finnish government subsidy for health science research (grant numbers: TYH2012141, TYH 2013218 and TYH 2014216), the SSAC Foundation (grant number SLS-504141) and the Paulo Foundation. We express our gratitude to the late Dr Jukka Riutta and the nurses at the Travel Clinic of Aava Medical Centre for help in recruiting the volunteers.

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Table 1. Demographics of study population (146 Finnish travellers)

	Travellers in study	Percent
Total	146	100.0
Travellers' diarrhoea (TD)		
Asymptomatic	48	32.9
TD ongoing	56	38.4
TD resolved	42	28.9
Antibiotic (AB) use		
AB (–)	125	87.7
AB (+)	18	12.3
Age		
mean ± SD	39.0 ± 16.5	
median; IQR	34; 28–54	
Duration of travel		
mean ± SD	22.0 ± 17.1	
median; IQR	16; 11–27	
Gender		
Male	61	41.8
Female	85	58.2
Travel destination		
South Asia	14	9.6
Southeast Asia	30	20.5
Latin America	18	12.3
Europe, Australia and North America	13	8.9
East Asia	3	2.1
North Africa and Middle East	7	4.8
Sub-Saharan Africa	61	41.9

Abbreviations used: TD – travellers' diarrhea; AB – antibiotic; SD – standard deviation; IQR – interquartile range

Table 2. Pathogen findings among 146 Finnish travellers; data presented in relation to TD symptoms

Pathogen	Total	Asymptomatic	TD during travel	TD during travel vs. asymptomatic	
	n (%)	n (%)	n (%)	p-value	OR (95% CI)
	146	48 (32.9)	98 (67.1)		
Any pathogen	105 (71.9)	27 (56.3)	78 (79.6)	0.003	3.1 (1.5–6.6)
Multiple pathogens	52 (35.6)	9 (18.8)	43 (43.9)	0.003	3.4 (1.5–7.7)
Any bacterial pathogen	102 (69.9)	27 (56.3)	75 (76.5)	0.012	2.5 (1.2–5.2)
DEC	101 (69.2)	27 (56.3)	74 (75.5)	0.018	2.4 (1.2–5.0)
EPEC	62 (42.2)	16 (33,3)	46 (46.9)	0.118	1.8 (0.9–3.6)
EAEC	56 (38.1)	11 (22.9)	45 (45.9)	0.007	2.9 (1.3–6.2)
ETEC	26 (17.8)	4 (8.3)	22 (22.4)	0.036	3.2 (1.0–9.8)
Campylobacter	6 (4.1)	0	6 (6.1)	0.178	n/a
EHEC	12 (8.2)	5 (10.4)	7 (7.1)	0.530	0.7 (0.2–2.2)
Salmonella	3 (2.1)	1 (2.0)	2 (2.0)	1.000	n/a
EIEC/*Shigella*	2 (1.4)	0	2 (2.0)	1.000	n/a
Vibrio cholerae	0	0	0	n/a	n/a
Yersinia spp.	0	0	0	n/a	n/a
Any viral pathogen	13 (8.9%)	1 (2.1)	12 (12.2)	0.061	6.6 (0.8–52.0)
SaV	0	0	0	n/a	n/a
EV	5 (3.4)	1 (2.1)	4 (4.1)	1.000	2.0 (0.2–18.4)
Norovirus (G1+G2)	8 (5.5)	0	8 (8.2)	0.053	n/a
NoV G1	2 (1.4)	0	2 (2.0)	1.000	n/a
NoV G2	6 (4.1)	0	6 (6.1)	0.178	n/a
RoV	1 (0.7)	0	1 (1.1)	1.000	n/a
Any parasitic pathogen	1 (0.7)	0	1 (1)	n/a	n/a
Giardia lamblia	1 (0.7)	0	1 (1.1)	1.000	n/a
Cryptosporidium	0	0	0	n/a	n/a
Entamoeba histolytica	0	0	0	n/a	n/a

TD – travellers' diarrhoea; DEC – diarrhoeagenic *Escherichia coli*; EPEC – enteropathogenic *E. coli*; EAEC – enteroaggregative *E. coli*; ETEC – enterotoxigenic *E. coli*; EHEC – enterohaemorrhagic *E. coli;* EIEC – enteroinvasive *E.coli*. NoV – norovirus; RoV – rotavirus; EV – enterovirus; SaV– sapovirus

Table 3. Severity of TD and proportion of travellers with antibiotic use in relation to pathogen findings: any pathogen, multiple pathogens, any bacterial pathogen, DEC, EPEC, EAEC, ETEC, and *Campylobacter*; any viral pathogen, NoV, EV

	Total	Asymptomatic	Non-severe TD	Severe TD	Severe TD vs. Non-severe TD		AB* users	AB* non-users	AB users vs. AB non-users	
	n (%)	n (%)	n (%)	n (%)	p-value	OR (95% CI)	n (%)	n (%)	p-value	OR (95% CI)
Total	146	48 (32.9)	81 (55.5)	17 (11.6)			18 (12.6)	125 (87.4)		
Any pathogen	105 (71.9)	27 (56.3)	68 (84.0)	11 (64.7)	0.091	0.4 (0.1–1.1)	11 (61.1)	92 (73.6)	0.270	0.6 (0.2–1.6)
Multiple pathogens	52 (35.6)	9 (18.8)	36 (44.4)	7 (41.2)	0.805	0.9 (0.3–2.5)	5 (27.8)	45 (36.0)	0.494	0.7 (0.2–2.0)
Bacterial pathogens										
Any bacterial pathogen	102 (69.9)	27 (56.3)	65 (80.2)	10 (58.8)	0.111	0.4 (0.1–1.1)	10 (55.6)	89 (71.2)	0.179	0.5 (0.2–1.4)
DEC	101 (69.2)	27 (56.3)	65 (80.2)	9 (52.9)	0.028	0.3 (0.1–0.8)	9 (50.0)	89 (71.2)	0.070	0.4 (0.1–1.1)
EPEC	62 (42.2)	16 (33,3)	39 (48.1)	7 (41.2)	0.601	0.8 (0.3–2.2)	4 (22.2)	56 (44.8)	0.070	0.4 (0.1–1.1)
EAEC	56 (38.1)	11 (22.9)	43 (53.1)	2 (11.8)	0.002	0.1 (0.03–0.5)	4 (22.2)	51 (40.8)	0.130	0.4 (0.1–1.3)
ETEC	26 (17.84)	4 (8.3)	19 (23.5)	3 (17.6)	0.756	0.7 (0.2–2.7)	1 (5.6)	24 (19.2)	0.199	0.2 (0.03–2.0)
Campylobacter	6 (4.1)	0	6 (7.4)	0	0.308	n/a	0 (0)	4 (3.2)	1.000	n/a
Viral pathogens										
Any viral pathogen	13 (8.9%)	1 (2.1)	7 (8.6)	5 (29.4)	0.032	4.4 (1.2–16.2)	5 (27.8)	8 (6.4)	0.012	5.6 (1.6–19.7)
NoV (G1+G2)	8 (5.5)	0	4 (4.9)	4 (23.5)	0.029	5.9 (1.3–26.7)	3 (16.7)	5 (4.0)	0.063	4.8 (1.0–22.1)
EV	5 (3.4)	1 (2.1)	4 (4.9)	0	1.000	n/a	2 (11.1)	3 (2.4)	0.118	5.1 (0.8–32.8)

*Data on AB use missing for three travellers.

AB – antibiotic; TD – travellers' diarrhoea; DEC – diarrhoeagenic *Escherichia coli*; EPEC – enteropathogenic *E. coli*; EAEC – Enteroaggregative *E. coli*; ETEC – enterotoxigenic *E. coli*; EHEC – enterohaemorrhagic *E. coli;* EIEC – enteroinvasive *E.coli.* NoV– norovirus; RoV – rotavirus; EV – enterovirus; SaV– sapovirus

Table 4. Ct-values for EPEC, EAEC, ETEC, and *Campylobacter* in relation to experienced symptoms

	Total		Asymptomatic		Ongoing TD		Resolved TD	
	n	Ct-value, mean ± SD (95% CI)	n (%)	Ct-value, mean ± SD (95% CI)	n (%)	Ct-value, mean ±SD (95% CI)	n (%)	Ct-value, mean ±SD (95% CI)
EPEC	68	29.5 ± 6.8 (27.9–31.1)	19 (27.9)	28.1 ± 8.0 (24.2–31.9)	29 (42.6)	29.0 ± 7.4 (26.2–31.8)	20 (29.4)	31.7 ± 4.1 (29.8–33.6)
EAEC	53	27.6 ± 5.4 (26.2–29.1)	12 (22.6)	28.9 ± 4.7 (25.9–31.9)	21 (39.6)	28.4 ± 6.0 (25.7–31.1)	20 (37.7)	26.1 ± 4.9 (23.8–28.4)
ETEC	26	27.0 ± 5.2 (24.9–29.1)	5 (19.2)	27.9 ± 5.7 (20.8–35.0)	16 (61.5)	26.5 ± 5.0 (23.8–29.2)	5 (19.2)	27.7 ± 6.2 (20.0–35.3)
Campylobacter	4	29.5 ± 2.6 (25.4–33.6)	0	n/a	1 (25.0)	n/a	3 (75.0)	29.8 ± 3.0 (22.2–37.4)

TD – travellers' diarrhoea; EPEC – enteropathogenic *E. coli*; EAEC – enteroaggregative *E. coli*; ETEC – enterotoxigenic *E. coli*

Table 5. Characteristics of 13 travellers with viral pathogens detected in post-travel stool samples

Gender	Age (years)	Travel destination(s)	Duration of travel (days)	Viruses	Bacteria	Antibiotic use	TD symptoms	Severity of TD
male	31	Indonesia, Thailand	56	RoV	0	No	resolved	severe
female	31	Thailand, Laos, Cambodia	31	NoV G2	EAEC	ciprofloxacin	resolved	moderate
male	32	Indonesia (Bali, Gili), Singapore	32	NoV G2	*Salmonella*	ciprofloxacin	resolved	severe
female	24	Nigeria	18	EV	0	ciprofloxacin+ nitroimidazole	ongoing	moderate
female	31	India	26	EV	EPEC, EAEC, *Campylobacter*	No	resolved	mild
male*	45	Thailand	24	NoV G2	EPEC	No	resolved	mild
female*	50	Thailand	24	NoV G2	EPEC	No	resolved	severe
male	54	Ghana, Togo, Benin	13	NoV G2	EPEC	No	ongoing	severe
female	46	Benin	91	NoV G2	EHEC	amoxycillin+ metronidazole	ongoing	severe
male	34	China (Shanghai, Changsha,Huaihua)	8	NoV G1	0	No	ongoing	moderate
male	33	West Africa	45	EV, NoV G1	EPEC	No	ongoing	mild
male	36	Côte d'Ivoire, Benin, Togo, Ghana	10	EV	EPEC, EAEC	No	asymptomatic	asymptomatic
female	29	Senegal	17	EV	EPEC, ETEC	cotrimoxazole	ongoing	moderate

*visited Thailand together

EPEC – enteropathogenic *E. coli*; EAEC – enteroaggregative *E. coli*; ETEC – enterotoxigenic *E. coli*; NoV – norovirus; RoV – rotavirus; EV – enterovirus; SaV– sapovirus