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Travellers' diarrhoea: impact of TD definition and control group design on study results

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Abbreviations:

DEC	diarrhoeagenic *Escherichia coli*
EAEC	enteroaggregative *Escherichia coli*
EHEC	enterohaemorrhagic *Escherichia coli*

EIEC	enteroinvasive *Escherichia coli*
EPEC	enteropathogenic *Escherichia coli*
ETEC	enterotoxigenic *Escherichia coli*
qPCR	quantitative PCR
TD	travellers' diarrhoea

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Background (219/200 words)

Travellers' diarrhoea (TD) is a common health problem among visitors to the (sub)tropics. Much research deals with aetiology, prevention, and post-infection sequalae, yet the data may not allow comparisons due to incompatible definitions of TD and *No TD* control groups.

Method

The impact of defining *TD* and *No TD* control groups was explored by revisiting our recent data. We set up two TD groups: classical TD i.e. ≥3 loose or liquid stools/day and WHO TD (diarrhoea as defined by the WHO) i.e. any diarrhoea, and four *No TD* groups by TD definition and timing (no classical/WHO TD during travel, no ongoing classical/WHO TD).

Results

TD was recorded for 37% versus 65% of subjects when using classical versus WHO definitions, respectively; the proportions of the various pathogens proved similar. The strictest criterion for the *No TD* control group (no WHO TD during travel) yielded pathogens among 61% and the least strict (no ongoing classical TD) among 73% of the travellers; the differences were greatest for enteroaggregative *Escherichia coli* and *Campylobacter*.

Conclusions

Definition of TD and control group design substantially impact on TD study results. The WHO definition yields more cases, but the pathogen selection is similar by both definitions. Design of the *No TD* control group was found critical: only those remaining asymptomatic throughout the journey should be included.

1. INTRODUCTION

Travellers' diarrhoea (TD) is contracted by 10–40% of travellers to middle- or low-income countries [1]. A great deal of research has been conducted on its aetiology [1-19], prevention, risk factors [20-22] and associated consequences, such as acquisition of multiresistant *Enterobacteriaceae* [23-29] and development of post-infectious irritable bowel syndrome [30-35]. The results of the various studies may, however, not be comparable due to variation in defining TD and determining control groups; some aetiological studies even lack control groups. [6,7,9,11,15,16]

New molecular methods offer better coverage of pathogens [12,15,36,37] thus decreasing the proportion of TD cases with unknown aetiology in various studies from almost half of the travellers [1,8,10,13] to as low as 5–24% [11,12,14,18,19].

Many studies have applied the definition of classical *TD*, i.e. the passage of three or more watery or loose stools per day with or without one or more of the accompanying symptoms (nausea, abdominal pain, vomiting) (below referred to as classical TD, Figure 1) [5]. The WHO, however, defines diarrhoea as the passage of three or more loose or liquid stools per day or, alternatively, more frequently than is normal for the individual [38] (below referred to as the WHO TD, Figure 1). While the definitions overlap with respect to moderate and most severe cases, the WHO definition covers a large group of cases (24–39% of all) not included in the classical definition at all: those with a mild clinical picture [5,14,18,39,40]. It should be noted that bacterial findings have generally been found similar between travellers with mild and moderate/severe symptoms [7,16,18].

Studies applying PCR- and culture-based methods have revealed diarrhoeal pathogens in 9–45% of the travellers without TD [3,4,10,12-14,17-19,41]. Pathogen findings in asymptomatic individuals have been suggested to reflect the high sensitivity of new methods to detect low numbers of bacteria, continuing excretion of pathogens in travellers with resolved symptoms, weaker pathogenicity of the strains and/or host immunity [42]. Conversely, in some studies, the definition of the *No TD* control group has failed to exclude travellers with mild TD [2,10,12,17,19,41] or resolved symptoms, [10,12,13,17,41]; even individuals with no travel history have been used as controls [17]. Hence, investigations which suggest new

pathogens to be associated with TD but provide no data on control groups should be confirmed by further research [9].

We sought to understand the impact of TD definitions and accurate control groups on the results of the TD studies. To this end, we investigated the TD and *No TD* definitions by reanalysing the data of our previous study of 382 Finnish travellers with no antimicrobial use during travel. We chose to focus on findings with enteroaggregative (EAEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC) *Escherichia coli*, and *Campylobacter jejuni/coli*, as these pathogens were associated with TD symptoms in our previous report [18].

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2. METHODS

2.1. Study population

We reanalysed our recent data [18] on pathogen findings of 382 travellers who had not used antibiotics during their journey. They had provided pre- and post-travel stool samples and completed questionnaires before and after travel. Recruitment of volunteers, handling of stool specimens, and identification of bacterial pathogens were detailed in our previous reports [18,36]. The study protocol was approved by the Ethics Committee of the Helsinki University Hospital. All participants had given written informed consent. For the same volunteers, we previously reported the findings of resistant *Enterobacteriacae* [24,28], travel-related health problems [43], stool pathogen findings in various geographical regions [44] as well as of those 382 travellers who used no antimicrobials [18].

2.2. Definitions of TD and *No TD*

For the presence/absence of TD symptoms, the travellers were classified in three categories: Asymptomatic (no diarrhoea during travel), resolved TD (no TD at the time of sampling but TD during the journey), and ongoing TD (ongoing TD at the time of sampling) (Figure 1).

The severity of TD was classified as mild if it comprised one or two loose or liquid stools per day without high fever or blood in stools, and moderate/severe with three or more diarrhoeal stools. The classical TD definition covered those with moderate/severe TD, but not those with mild TD; the WHO TD definition covered all cases with diarrhoea (Figure 1).

The possible impact on the pathogen findings resulting from the use of various TD and *No TD* definitions was approached by forming one group for each TD definition (classical versus WHO TD), and four groups for the *No TD* definitions (no ongoing classical TD, no classical TD during travel, no ongoing WHO TD, and no WHO TD during travel, Figures 1, 2, and 3). Assignment to group depended on whether travellers with resolved symptoms were included (no ongoing versus no TD during travel) and whether mild symptoms were included (no classical versus WHO TD).

2.4. Statistical analysis

For categorical variables, statistical analyses were carried out with Chi-square tests, Fisher's exact test, or binary logistic regression analysis when applicable. The binominal regression model was used in order to obtain profile likelihood confidence intervals. Statistical significance was defined as $p<0.05$ or when confidence intervals did not overlap. The statistical analysis was conducted using SPSS 22 software (IBM Corp, Armonk, NY).

3. RESULTS

3.1. Traveller demographics, itineraries, and pathogen findings

This study comprised 382 volunteers who had not taken antimicrobials during travel outside Nordic countries. Demographic and travel data have been described in detail in our previous article [18]. In brief, 233 (61%) travellers were women and 149 (39%) men. The median age was 36 years (IQR 27), and the median duration of travel was 16 days (IQR 10). The most popular destination was Sub-Saharan Africa (171 travellers; 45%), followed by South East Asia (91; 25%), South Asia (52; 14%), and Latin America (36; 9%).

The results of the PCR analyses for pathogens have been reported earlier [18]. In brief, a bacterial pathogen was detected in 75% of post-travel samples: EPEC (46%) and EAEC (45%) were the most common findings, followed by ETEC (20%), and *Campylobacter* (7%). Multiple pathogens were found in 40% of post-travel samples.

3.2. Proportions of travellers with TD by classical or WHO criteria

The difference between the two TD definitions concerns those with mild symptoms: they are defined as TD cases only when the WHO definition is used (Table 1). In the present data, 107/242 (44%) travellers in our study population had mild TD (ongoing or resolved). Diarrhoeal symptoms experienced during travel or immediately after return were classified as TD for 140 (37%) cases if the classical TD criteria were used, and for 247 (65%) if applying the WHO criteria. At the time of post-travel stool sampling, 73 (19%) had ongoing TD by classical and 115 (30%) by WHO criteria, and among 67 (18%) and 132 (35%) the symptoms had already resolved, respectively.

3.3. Comparison of pathogen findings when using classical and WHO TD definitions

For ongoing TD, the proportions of pathogens proved similar regardless of the TD definition used, classical or WHO (table 1). Applying the classical TD criteria yielded one or more pathogens in 61

(84%) stool samples, EPEC in 41 (56%), EAEC in 39 (53%), ETEC in 31 (42%), and *Campylobacter* in 6 (8%). The respective figures with the WHO criteria gave one or more bacterial pathogens in 96 (83%) stool samples, EPEC in 63 (55%), EAEC in 58 (50%), ETEC in 42 (37%), and *Campylobacter* in 9 (8%).

Likewise, for those with resolved symptoms, the findings were similar with both TD definitions (classical and WHO) (Table 1). In contrast, when compared to those with ongoing TD, the proportions of EPEC and ETEC were lower among travellers with resolved, compared to ongoing symptoms with both definitions, whereas for EAEC and *Campylobacter*, the difference was not significant.

3.4. Proportions of travellers in *No TD* control groups

When *No TD* was defined as no ongoing TD symptoms at the time of post-travel stool sampling (but possibly during travel), 309 (81%) and 267 (70%) travellers were categorised into the control group according to the classical and WHO criteria, respectively.

When travellers with resolved symptoms were excluded from the *No TD* control groups, the classical criteria yielded 242 TD cases (63%; no classical TD during travel) and, if also excluding those with mild symptoms, i.e. using the WHO criteria (no WHO TD during travel), gave 135 (35%) cases as *No TD*.

3.5. Comparison of bacterial findings with different definitions for *No TD* control group

If the *No TD* control group was described as no ongoing TD at the time of sampling, a pathogen was detected in 73% (95% CI 68-78%) and 72% (66-77%) of cases by the classical and WHO TD criteria; *Campylobacter* was found in 7% (4-10%) and 6% (4-10%), and EAEC in 43% (37-48%) and 42% (36-48%) of cases, respectively.

If the *No TD* control group was defined as no TD during travel, the proportion of travellers with positive pathogen findings was 70% (64-77%) versus 61% (52-69%) when using the classical versus WHO definitions, respectively; *Campylobacter* was found in 4% (2-7%) and 1% (95% CI 0-3%), and EAEC in 37% (31-43%) and 28% (21-36%) of cases, respectively.

3.6. Impact of *No TD* definitions on the interpretation of causative agents for TD

The *No TD* definition used had an impact on the interpretation of the role of each pathogen as causative agent of TD (Table 1): when no classical TD during travel was chosen as the *No TD* control group, travellers with ongoing symptoms did not differ from controls with respect to EPEC and *Campylobacter* findings. When travellers with resolved symptoms were included in the control groups (no ongoing classical or WHO TD), no significant differences were found for EAEC and *Campylobacter*.

When the *No TD* control group comprised only travellers without any diarrhoeal symptoms during the journey (no WHO TD during travel), EPEC, EAEC, ETEC, and *Campylobacter* were all significantly more prevalent among those with ongoing TD than in the *No TD* control group.

4. DISCUSSION

Diarrhoea remains the most common reason for travellers to contact health care both when on a journey and after their return [43,45-47]. The aetiology and consequences of TD have been widely studied, but the comparability and even reliability of various studies may have been jeopardized by incompatible definitions used for TD and *No TD* control groups. We scrutinised these differences by revisiting the findings of our aetiological study and comparing the results obtained when applying the differing criteria.

4.1. Definition of TD: classical versus WHO

The major difference between the two definitions (classical and WHO) concerns cases with mild diarrhoea: these are included in the WHO definition, while the classical criteria only denote cases with three or more unformed stools with or without additional symptoms. The population with mild symptoms was substantial, 44% of all subjects. This indicates a significant effect on the number of TD cases: they were recorded by 37% versus 65% when evaluating by the classical versus WHO criteria, respectively. Indeed, the definition of TD is evidently reflected in the number of cases recorded. Comparing TD risk between various regions is valid only when using the same TD definition. For this reason, we suggest that when analysing TD rates, the results should be reported according to both (classical and WHO) definitions.

4.2. Pathogen findings among travellers with ongoing TD

Travellers with milder symptoms are in many studies excluded from subject groups [15,48] or included in the *No TD* group [10,13]. Findings among such subjects with mild symptoms are only described in a few papers [5,7]. Our previous report on the same travellers [18], however, did not show significant differences between those with mild symptoms and those with moderate or severe symptoms in the pathogens detected, a finding consistent with the studies by Jiang et al [7] and Frickmann et al [16]. With respect to pathogen findings of EPEC, EAEC, ETEC, and *Campylobacter*, both definitions (classical and WHO) for TD are applicable.

We recommend that studies of the aetiology of TD use the WHO definition to ensure that the *No TD* group is fully asymptomatic. On the other hand, as antibiotics should only be considered for severe diarrhoea, the classical definition appears reasonable for studies comparing various antibiotics. This also applies to research exploring preventive strategies: the definition should be made according to purpose (which degree of severity prevention is aimed at). Also in such studies, recording milder symptoms would enable subgroup analyses of the various cases.

4.3. **Pathogen findings among travellers with resolved symptoms**

We scrutinized separately travellers with resolved TD because in some studies they have been categorised into TD and in others into no ongoing TD groups. Our results suggest that if travellers with resolved TD are included in the TD group, the proportions of EPEC and ETEC will be underestimated. By contrast, the results of the comparison between those with resolved symptoms with the controls (no TD during travel) depended of by TD criteria used: when we applied the classical criteria, ETEC and *Campylobacter* proved more prevalent among travellers with resolved symptoms than in the control group; when we applied the WHO criteria the difference was significant for EAEC and *Campylobacter*. It thus appears that certain pathogens are found in the stools after the symptoms have resolved, a finding consistent with extended excretion of nontyphoidal *Salmonella* [49] and *Campylobacter jejuni* [50] for weeks after recovery from clinical illness. Diarrhoeagenic *Escherichia coli* have also been found in faecal samples after the resolution of symptoms [18,41]: in the research by Adachi et al [41], the proportion of travellers with EAEC increased over the four study weeks. Indeed, the findings of travellers with diarrhoea during any time of travel should be analysed separately from those asymptomatic throughout the journey, irrespective of time elapsed between resolution of symptoms and stool sampling.

4.4. Pathogen findings among four different *No TD* control groups

The main point where the definitions of *No TD* groups differ concerns inclusion of travellers with resolved and/or mild symptoms: when defined most strictly, i.e. absence of any, even mild, diarrhoeal

symptoms throughout the journey (no WHO TD during travel), a pathogen was detected in the stool samples of 61% of the travellers. By contrast, if *No TD* was defined by the least strict definition, i.e. not having ongoing moderate/severe diarrhoea (no ongoing classical TD) at the time of sampling, 73% of the travellers had one or more pathogens; the respective figures were 28% and 43% for EAEC, and 1% and 7% for *Campylobacter*. As the pathogen findings between the *No TD* groups differ substantially by definition, we recommend that the composition of the control groups should be described in greater detail in future studies.

4.5. Possible impact of *No TD* definitions on results of aetiological studies of TD

The definition of TD and control group design were also reflected in the evaluation of the role of the pathogens causing the symptoms. Had the *TD* group in our study been defined as 'ongoing classical TD' and the *No TD* group as 'no classical TD during travel' (i.e. those with ongoing and resolved mild symptoms included in control group) (Table 1), no difference would have been found in the EPEC and *Campylobacter* rates. If, on the other hand, travellers with resolved symptoms (either classical or WHO) had been included in the *No TD* control group, EAEC and *Campylobacter* would not have been observed as significant pathogens. In contrast, when the *No TD* control group comprised only travellers without any diarrhoeal symptoms (not even mild ones) during the journey, all four pathogens appeared significant. These examples may partly explain the differing results in studies analysing the role of some pathogens, for example EAEC [3,51] and EPEC [3,10] in causing TD. Hence, the role of various pathogens should only be evaluated in study settings with a *No TD* control group comprising those fully asymptomatic (not showing even mild symptoms) during the journey.

4.6. Limitations

The stool samples were collected only after return, thus allowing new bacteria to possibly colonize the intestine in cases with resolved TD and, likewise, some pathogens to disappear; ETEC, for example, is known to vanish rather quickly [18,41,52]. As for the limitations of the PCR method per se, they have been discussed in our previous article [18].

4.7. Conclusion

Our data imply that specifying *No TD* is at least equally important as defining TD. This applies not only to studies of the aetiology of TD but most likely also to those presenting risk factor analyses or evaluations of post-infection sequelae, such as irritable bowel syndrome or colonization with multiresistant *Enterobacteriaceae*. The classical and WHO definition of TD yielded identical selections of pathogens, a finding suggesting that the criteria for TD can be chosen according to focus of study. However, further attention should be paid to *No TD* control group design and findings among travellers with resolved TD symptoms: *No TD* groups should only consist of travellers who have not shown any gastrointestinal symptoms throughout the journey.

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Figure 1. Definitions used in this paper:

Definition of TD	**Classical TD**	Three or more loose or liquid stools +/-accompanying symptoms
	WHO TD	Any number of diarrhoeal stools more frequently than normal for the individual
Severity of TD	**Mild TD**	1-2 diarrhoeal stools per day (i.e. diarrhoea not meeting the classical TD criteria)
	Moderate TD	3-5 diarrhoeal stools per day
	Severe TD	6 or more diarrhoeal stools per day or diarrhoea plus fever, grossly bloody stools or diarrhoea requiring hospitalisation
Timing of TD (Classical or WHO)	**Ongoing TD**	Diarrhoeal symptoms ongoing at the time of sampling
	Resolved TD	Diarrhoeal symptoms resolved at the time of sampling
No TD control group (Classical or WHO)	**TD during travel**	Ongoing or resolved diarrhoeal symptoms
	No ongoing TD	No ongoing diarrhoeal symptoms at the time of sampling (but possibly during travel)
	No TD during travel	No ongoing or resolved diarrhoeal symptoms at the time of sampling

Figure 2. Definitions of TD and *No TD* when applying classical criteria for TD

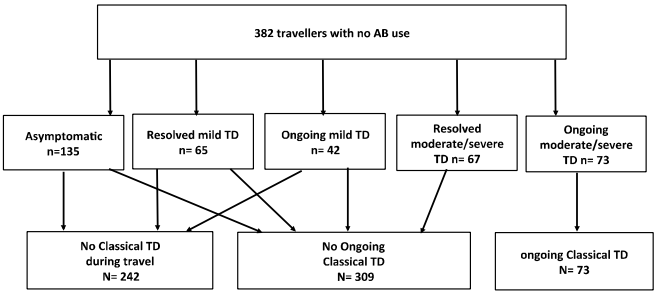

Figure 3. Definitions of TD and *No TD* when applying the WHO criteria for TD

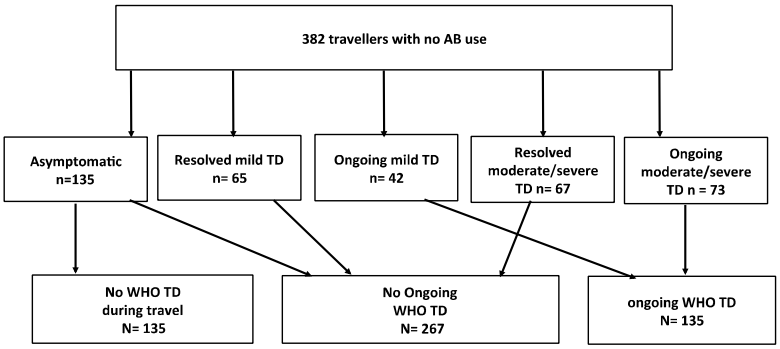

Table 1. Findings of EPEC, EAEC, ETEC, and *Campylobacter* in relation to TD symptoms among 382 travellers not having taken antibiotics during their journey. The findings are presented separately for TD defined by classical and WHO criteria, and whether TD was ongoing, resolved, or absent. Statistical comparisons are given for the various TD and *No TD* definitions, the data showing the significance of definitions and the apparent role of EPEC, EAEC, ETEC, and *Campylobacter* as causative agents for TD.

	All travellers		Any bacterial pathogen		EPEC		EAEC		ETEC		*Campylobacter*	
	n (%)	95% CI*	n (%)	95% CI*	n (%)	95% CI*	n (%)	95% CI*	n (%)	95% CI*	n (%)	95% CI*
	382		287 (75)		174 (46)		171 (45)		76 (20)		26 (7)	
Ongoing TD												
Classical TD definition	73 (19)	15-23	61 (84)	74-91	41 (56)	45–67	39 (53)	42–65	31 (42)	32–54	6 (8)	3–16
WHO TD definition	115 (30)	26-35	96 (83)	76–90	63 (55)	46–64	58 (50)	41–60	42 (37)	28–46	9 (8)	4–14
No ongoing TD symptoms at the time of stool sampling												
-TD resolved												
Classical TD definition	67 (18)	14-22	56 (84)	74–91	25 (37)	26-49	42 (63)	51-74	12 (18)	10-28	10 (15)	8-25
WHO TD definition	132 (35)	30-39	109 (83)	76-88	54 (41)	33-49	75 (57)	48-65	22 (17)	11-24	16 (12)	7-18
-No ongoing TD control group												
Classical TD definition	309 (81)	77-85	226 (73)	68–78	133 (43)	38–49	132 (43)	37–48	45 (15)	11–19	20 (7)	4–10
WHO TD definition	267 (70)	65-74	191 (72)	66–77	111 (42)	36–47	113 (42)	36–48	34 (13)	9–17	17 (6)	4–10
-No TD during travel control group												
Classical TD definition	242 (63)	58-68	170 (70)	64–77	108 (45)	38–51	90 (37)	31–43	33 (14)	10–18	10 (4)	2–7
WHO TD definition	135 (35)	31-40	82 (61)	52–69	57 (42)	34–51	38 (28)	21–36	12 (9)	9–14	1 (1)	0–3
Univariate statistics for Classical TD definition												
Classical TD ongoing vs. No Classical TD ongoing	OR (95% CI)		1.9 (1.0-3.6)		1.7 (1.0-2.8)		1.5 (0.9-2.6)		4.3 (2.5-7.6)		1.3 (0.5-3.3)	
	P		0.064		0.043		0.098		<0.001		0.606	
Classical TD ongoing vs. No Classical TD during travel	OR (95% CI)		2.2 (1.1-4.2)		1.6 (0.9-2.7)		1.9 (1.1-3.3)		4.7 (2.6-5.4)		2.1 (0.7-5.9)	
	P		0,027		0,085		0,014		<0.001		0,171	
Classical TD resolved vs. No Classical TD during travel	OR (95% CI)		2.2 (1.1-4.4)		0.7 (0.4-1.3)		2.8 (1.6-5.0)		1.4 (0.7-2.9)		4.0 (1.6-10.2)	
	P		0,032		0,286		<0.001		0,382		0,003	
Classical TD resolved vs. Classical TD ongoing	OR (95% CI)		1.0 (0.4-2.5)		0.5 (0.2-0.9)		1.5 (0.7-2.9)		0.3 (0.1-0.6)		2.0 (0.7-5.7)	
	P		0.997		0.026		0.268		0.002		0.213	
Univariate statistics for WHO TD definition												
WHO TD ongoing vs no WHO TD during travel	OR (95% CI)		3.3 (1.8-6.0)		1.7 (1.0-2.7)		2.6 (1.5-4.4)		5.9 (2.9-11.9)		11.4 (1.4-91.2)	
	P		<0.001		0.048		<0.001		<0.001		0.022	
WHO TD ongoing vs no WHO TD ongoing	OR (95% CI)		2.0 (1.1-3.5)		1.7 (1.1-2.6)		1.4 (0.9-2.2)		3.9 (2.3-6.7)		1.2 (0.5-2.9)	
	P		0,013		0,017		0,144		<0,001		0,603	
WHO TD resolved vs. no WHO TD during travel	OR (95% CI)		3.1 (1.7-5.4)		0.9 (0.6-1.5)		3.4 (2.0-5.6)		2.1 (1.0-4.3)		18.5 (2.4-141.5)	
	P		<0,001		0,828		<0,001		0,060		0,005	

WHO TD resolved vs. WHO TD ongoing	OR (95% CI)	0.9 (0.5-1.8)	0.6 (0.3-0.9)	1.3 (0.8-2.1)	0.4 (0.2-0.6)	1.6 (0.7-3.8)
	P	0.851	0.030	0.316	<0.001	0.286

* 95% Confidence Intervals (CI) are profile likelihood intervals for %.

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