Travel, infection and immunity

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Analysis of efficacy of CVD 103-HgR live oral cholera vaccine against all-cause travellers' diarrhoea in a randomised, double-blind, placebo-controlled study

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Abstract

Enterotoxigenic *Escherichia coli* (ETEC), which produces heat labile toxin (LT) and/or heat stable toxin (ST), is considered to be the most common known cause of travellers' diarrhoea (TD). Owing to the antigenic similarity between cholera toxin and LT, immunization with inactivated oral B-subunit/whole-cell cholera vaccine (BS–WC) offers short term (3 months) but significant (>67%) protection against TD caused by LT-related ETEC. Since it expresses the cholera toxin B (CTB) subunit, the live attenuated oral cholera vaccine strain CVD 103-HgR, may induce similar protection. A trial was performed to determine if CVD 103-HgR live oral cholera vaccine would provide a protective efficacy of at least 50% against TD. In addition, the protective efficacy of the vaccine against TD specifically due to LT–ETEC and LT/ST–ETEC was determined. Volunteers ($n = 134$) travelling to Indonesia, India, Thailand or West-Africa were randomised to receive either a placebo ($n = 65$) or the vaccine ($n = 69$). In the placebo group, 46% reported an episode of diarrhoea, compared to 52% in the vaccine group. No significant group differences were found with regard to incidence, duration or severity of all caused TD or ETEC-associated TD. However, ETEC-associated TD occurred earlier in the placebo group (median 5 days), compared to the vaccine group (median 15 days).

In conclusion, CVD 103-HgR live oral cholera vaccine failed to provide a 50% protection against TD. This study does not exclude that the vaccine may offer a short-lived protection against ETEC-associated TD. However, the power of the study was limited by the unexpected low incidence of LT–ETEC-associated diarrhoea (9% of all TD) compared to ST-associated TD (24% of all TD).

Keywords: Travel; Diarrhoea; Cholera vaccine

1. Introduction

Travellers from industrialized countries visiting (sub)tropical regions often develop diarrhoea. Large-scale studies among European and North American travellers to high-risk destinations, report an incidence rate of diarrhoea of 20–50% per 2 weeks' stay [1,2]. Though a self-limiting illness, travellers' diarrhoea (TD) can ruin holidays and cause substantial financial and emotional damage, creating a need for prophylactic and therapeutic agents. In both respects antibiotic drug therapy has proven effective. However, the use of antibiotics carries disadvantages when administered to a large number of people [3]. Therefore, consensus opts against the prophylactic use of antibiotics, and the need for a preventive agent that is both effective and safe, persists.

Although the prevalence of etiologic agents that cause TD differs from area to area, enterotoxigenic *Escherichia*

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coli (ETEC) is deemed to be the most common cause [4]. Based on a meta-analysis, the median isolation rate of ETEC associated TD is 42% in Latin America, 36% in Africa and 16% in Asia [3,5]. Despite the use of modern methods, in approximately 50% of cases, no pathogen is detected [3,4,6]. A significant proportion of this pathogen-undetected TD is probably caused by ETEC [7].

ETEC expresses a heat-labile toxin (LT), a heat-stable toxin (ST) or both (LT/ST). Reports on the proportion of ETEC strains producing a certain type of toxin vary. Approximately 25–30% of strains express LT, 35–45% ST and 30–35% LT/ST [8,9]. LT is very similar to cholera toxin in both structure and mode of action. It is composed of an enzymatically active (CTA) subunit surrounded by 5 identical binding (CTB) subunits. It binds to the same ganglioside receptors via its CTB moiety that are recognized by the cholera toxin, and its enzymatic activity is identical to that of cholera toxin. This explains why immunization with oral B-subunit/whole-cell cholera vaccine (BS–WC) can induce an increase in intestinal IgA directed against LT antigens [10]. Most likely these antibodies account for the effect found by previous studies, that showed that BS–WC offered significant (>67%) short-term (3 months) protection against diarrhoea caused by LT–ETEC and LT/ST–ETEC [11,12]. CVD 103-HgR, a live oral cholera vaccine, may induce similar protection. It contains a genetically modified strain of *Vibrio cholerae* O1, attenuated via deletion of about 95% of the *ctxA* locus encoding the toxic CTA subunit, and elicits seroconversion with high titres of vibriocidal antibody. Since the strain expresses normal quantities of immunogenic CTB, it is also known to induce a significant antitoxin immune response in intestinal fluid [13–16]. Owing to antigenic similarity between cholera toxin and LT, one may expect CVD 103-HgR to induce antibodies directed against LT, offering protection against diarrhoea caused by LT–ETEC and LT/ST–ETEC.

The use of CVD 103-HgR has some advantages over BS–WC. Firstly, it induces a strong immune response after only a single dose [17,18], as opposed to the multiple doses required for immunisation with BS–WC. Secondly, CVD 103-HgR possibly elicits higher vibriocidal titres than BS–WC [13]. However, the antitoxin response is probably similar to that induced by BS–WC.

We performed a randomised, double-blind, placebo controlled trial to determine if CVD 103-HgR live oral cholera vaccine would provide a protective efficacy of at least 50% against (severe) travellers' diarrhoea. In addition, the protective efficacy of the vaccine against travellers' diarrhoea specifically due to LT–ETEC and LT/ST–ETEC, was determined.

2. Materials and methods

2.1. Study design

The study was performed at the Leiden University Medical Center (LUMC). The primary outcome was the attack rate of TD in the placebo group compared to the vaccine group. The difference between the two groups, regarding attack rate of TD caused by LT–ETEC and LT/ST–ETEC was chosen as secondary outcome. The protocol (KV 9506) was approved by the ethical committee of the LUMC, The Netherlands.

2.2. Inclusion of subjects

Dutch volunteers were enrolled between May 1995 and February 1996. Travellers were recruited from the travel clinics of the LUMC ($n = 131$), the Municipal Health Centre at Leiden ($n = 5$) and the Harbour Hospital at Rotterdam ($n = 9$). All adults who made an appointment at the travel clinic between May 1995 and February 1996 and who were intending to travel to Indonesia, Thailand, the Indian subcontinent or West Africa (Gambia or Senegal) for a period of 1–4 weeks were invited to take part in the trial and were subsequently sent an informative letter concerning the study.

2.3. Exclusion criteria

The following subjects were excluded from the study. People suffering an acute or chronic inflammatory disease of the intestinal tract; prior recipients of WC–BS cholera vaccine or CVD 103-HgR vaccine; subjects receiving immunosuppressive drugs; persons known to be immunodeficient; anyone having received an experimental drug within the last 3 months; subjects participating in other clinical trials and women who were either pregnant or breast-feeding. Information on the concomitant use of medication, treatment or vaccination was obtained by way of a standardised questionnaire.

2.4. Randomisation

After having obtained written informed consent, subjects were stratified according to region, and were subsequently randomised (1:1) to two groups. For randomisation a computer-generated randomisation list, was used, which had been produced at the Berna Biotech AG (formerly Swiss Serum and Vaccine Institute), Bern, Switzerland. Sachets and suspensions of vaccine ($n = 100$) and placebo ($n = 100$), that were identical in appearance, were labelled by a coded number from 1 to 200. Within each stratum, for each permutation of 20, the weighing of randomisation was adjusted to 1:1 (vaccine to placebo). Participants were subsequently enrolled in the trial. At least 2 weeks prior to departure they consumed the appointed sachet. The key to the coded sachets was stored at the hospital pharmacy in a sealed envelope. The envelope was only to be opened by the investigator in case of an emergency that required knowledge of the identity of the trial medication in order to manage the participant's condition. At the end of the trial the coded envelope was returned to the Berna Biotech AG and checked to ensure that the seal had remained unbroken.

2.5. Vaccine and placebo

The vaccine consisted of a single dose of 5×10^8 colony forming units (CFU) of lyophilised CVD 103-HgR live oral cholera vaccine (CVD 103-HgR). CVD 103-HgR is an attenuated strain of *Vibrio cholerae* O1 derived from the wild-type classic Inaba strain 569B by deleting the genes that encode for the A subunit of cholera toxin and by inserting a marker gene encoding for resistance to Hg^{2+} into the *hlyA* locus of the bacterial chromosome. Genes encoding for the synthesis of the immunogenic, non-pathogenic, B-subunit remain intact. A placebo dose consisted of 5×10^8 heat killed *Escherichia coli* K-12. Both vaccine and placebo were administered in a glass of water together with a buffer containing 2.65 g $NaHCO_3$, 1.65 g ascorbic acid and 0.2 g lactose. A nurse supervised administration. Volunteers were urged not to eat or drink anything 1 h before and after vaccination.

2.6. Definition of travellers' diarrhoea

TD was defined as any episode of three or more unformed stools per 24 h, or two such bowel movements accompanied by vomiting, abdominal cramps or subjective fever, with an onset during travel until 3 days after returning home. Diarrhoeal episodes were registered from the time of getting on the plane. Diarrhoea was recorded as episodes, which were considered separate when the symptom-free interval was 5 days or more.

2.7. Recording incidence of diarrhoea and collecting stool specimens

All participants kept a diary of their defecation pattern during their stay abroad. On return they filled out a questionnaire, concerning defecation pattern, use of medication and information regarding travel, accommodation, and dietary hygiene. Each participant submitted a stool specimen. Subjects who had experienced an episode of diarrhoea during travel collected a sample during the first diarrhoeal episode, prior to having taken any medication. The remaining travellers collected and submitted a sample within 3 days after returning home. Written instructions were given on how to collect the stool specimen. The sample was preserved in a plastic vial on a specific transport medium, chosen because of its capacity to preserve ETEC for a minimum of 4 weeks (Para-Pak Enteric Plus system, Meridian diagnostics Inc., Cincinnati, OH, USA) [18–20]. After returning home, the vials were collected and sent to the laboratory for microbiology at the Academic Medical Center, Amsterdam where specimens were analysed for presence of enterotoxin producing *E. coli*.

2.8. Laboratory evaluation of stool samples

All samples, submitted by subjects who had experienced an episode of diarrhoea were examined for enterotoxigenic *E. coli*. In addition the first 28 samples, taken on return home, by people who had not suffered an episode of diarrhoea were subjected to the same examination. Stool samples were inoculated onto Cystine Lactose Electrolyte Deficient (CLED) agar plates. After 18 h of incubation at 37 °C, a sweep of the complete bacterial growth on the agar was collected using a sterile cotton swab, and stored in glycerol–pepton at −70 °C, as described previously [21]. This frozen material was inoculated on a CLED agar plate, from which a new sweep was taken. This material was diluted in PBS and subjected to PCR for detection of ETEC–LT, STIa and STIb genes, as described previously [21]. All PCR-positive samples were submitted to repeated PCR detection from the sweeps stored at −70 °C. The detection limit for ETEC is 10^2 CFU/g of feces [21].

2.9. Statistical analysis

The aim of this trial was to estimate the difference (δ) in chance of acquiring travellers' diarrhoea after having taken the placebo (p_p) compared to the vaccine (p_v). The attack rate of TD per group reflects these chances. The null hypothesis (H_0) implies that placebo and vaccine are equally effective in preventing TD ($\delta = p_p - p_v = 0$). The alternative hypothesis (H_1) states that $\delta \neq 0$. The number of subjects required for this trial was 100 per group (vaccine/placebo). This was calculated on the basis of a one-sided test with a power of the study of at least 0.9, a type I error of less than 0.025 and an expected incidence rate for travellers' diarrhoea of 35% with an expected protection rate of the vaccine of at least 50%. Proportions were compared using univariate analysis for numerical data and the χ^2-test for categorical data. Numerical data that were not normally distributed were analysed with Mann–Whitney *U*-test. The study was terminated after an ad hoc[1] interim analysis. During the interim analysis the key to the randomisation code remained blinded from the principal investigators.

3. Results

In total, 343 volunteers, meeting the inclusion criteria were approached, of which 198 either refused to take part or matched one or more of the prior mentioned exclusion criteria. At the moment of interim analysis, 145 volunteers had been stratified according to region and subsequently randomised to receive either placebo or vaccine. Since three individuals cancelled their journey, and eight did not fill out the questionnaire, 134 participants were evaluable. A total of 65 subjects received a placebo and 69 received CVD 103-HgR (Fig. 1). Except for the category 'duration of stay' and for

[1] Due to changes in the law regarding the use of genetically modified products, the study was temporarily put on halt, pending the outcome of an investigation of the vaccine and the study design. Though the trial was allowed to continue, the study was terminated based on the results of the interim analysis.

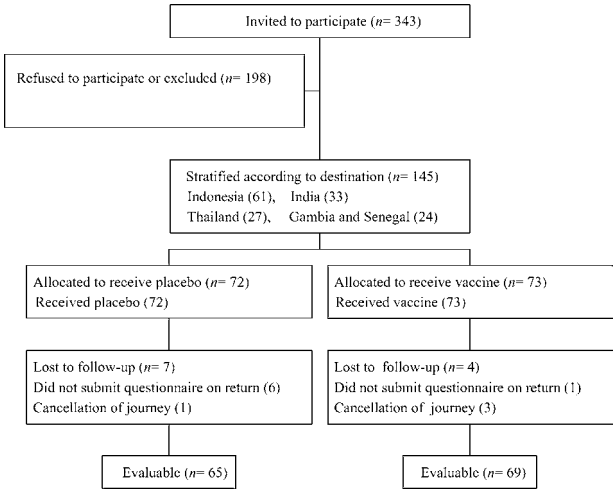

Fig. 1. Flow diagram of the progress through the phases of the randomised trial.

the subcategory 'accommodation with locals', demographic data and distribution of risk factors for diarrhoea did not differ significantly between the two groups (Table 1). Vaccine tolerability was excellent, with 10% of vaccines reporting mild abdominal discomfort compared to 17% in the placebo group.

3.1. Primary endpoint

Of the 134 participants, 66 (49%) reported at least one episode of diarrhoea. No significant difference was observed between the placebo and vaccination groups with respect to attack rate (Table 2). No significant differences existed

Table 1
Base-line characteristics of the study population consisting of 134 Dutch travellers

Parameter	Placebo ($n=65$)	Vaccine ($n=69$)	All ($n=134$)	p-value
Average age (years)[a]	38.7	40.3	39.5	NS
Sex (m/f)[a]	26/39	36/33	62/72	NS
Interval from vaccination to departure (days)[a]	18.0	17.1	17.6	NS
Average duration of stay (days)[a]	22.5	20.1	21.3	0.01
Travel destination (n)				
Indonesia[b]	30	28	58	NS
India[b]	12	17	29	NS
Thailand[b]	14	13	27	NS
Gambia + Senegal[b]	9	11	20	NS
Prior travel to (sub)tropics (n)[b]	45	48	93	NS
Antacid medication (n)	1	0		
Accommodation (n)				
Large hotel[b]	20	27	47	NS
Budget hotel[b]	18	13	31	NS
Guesthouse[b]	13	21	34	NS
Camping	0	2	2	
With locals[b]	14	6	20	0.04
Followed advise on diet and hygiene (n)[b]				NS
Always	27	34	61	
Sometimes	38	35	73	
Use of antibiotic prior to onset of TD	1	0	1	

Placebo: heat-killed *Escherichia coli*-K1; vaccine: a single dose of CVD 103-HgR live oral cholera vaccine; mean duration of stay abroad: 20 days, range (7–30) days; NS: not significant.

[a] Statistics: univariate analysis comparing placebo group to vaccine group.

[b] χ^2-test comparing placebo group to vaccine group; p-value significant at <0.05.

Table 2
Attack rate of travellers' diarrhoea (TD) among 134 Dutch travellers

Parameter	No. (%)				
	Placebo ($n=65$)	Vaccine ($n=69$)	Total ($n=134$)	p-value	ETEC associated diarrhoea ($n=17$)
Subjects with TD	30 (46)	36 (52)	66/134 (49)	NS	
Subjects with TD specified for					
Indonesia ($n=58$)	15	18	33/58 (50)	NS	11/33 (33)
India ($n=29$)	5	9	14/29 (48)	NS	4/14 (29)
Thailand ($n=27$)	6	3	9/27 (33)	NS	0/9 (0)
Gambia and Senegal ($n=20$)	4	6	10/20 (50)	NS	2/10 (20)

Placebo: heat-killed *Escherichia coli*-K12; vaccine: a single dose of CVD 103-HgR live oral cholera vaccine; mean duration of stay abroad: 20 days, range (7–30) days; statistics: χ^2-test comparing placebo group to vaccine group; p-value significant at <0.05; NS: not significant.

Table 3
Detection of enterotoxigenic *Escherichia coli* in faeces of 134 Dutch travellers to (sub)tropical destinations

Parameter	No. (% of analysed samples)						
	Travellers' diarrhoea			No travellers' diarrhoea			All ($n=134$)
	Placebo ($n=30$)	Vaccine ($n=36$)	Total ($n=66$)	Placebo ($n=35$)	Vaccine ($n=33$)	Total ($n=68$)	
Stool samples analysed	28	31	59	14	14	28	87
Sample negative for ETEC	21 (75)	21 (68)	42 (71)	13 (93)	11 (79)	24 (86)	66 (76)
Sample positive for ETEC	7 (25)	10 (32)*	17 (29)	1 (7)	3 (21)	4 (14)	21 (24)
ETEC LT only	1	0	1	0	0	0	1
ETEC LT and ST	2	3	5	1	3	4	9
ETEC ST only	4	7	11	0	0	0	11

Placebo: heat-killed *Escherichia coli*-K12; vaccine: a single dose of CVD 103-HgR live oral cholera vaccine; Mean duration of stay abroad: 20 days, range (7–30) days; samples: diarrheic stool specimens taken during episodes of diarrhoea, non-diarrheic specimens taken a maximum of 3 days after return home; statistics: χ^2-test comparing placebo group to vaccine group; p-value significant at <0.05.
* $p > 0.05$.

regarding number of episodes, time to first onset, duration or severity of diarrhoea (Table 4). In the placebo group 30 of 65 subjects (46%) developed diarrhoea, compared to 36 of 69 (52%) in the group of vaccines (Table 2). Comparison of the two groups, stratified according to travel destination, did not yield significant differences either (Table 2). The study was ended prematurely, because the primary endpoint, a vaccine efficacy of at least 50%, would not be reached by continuing the study until 200 subjects were included.

Table 4
Severity, number of episodes and duration of travellers' diarrhoea (TD) in 134 Dutch travellers to (sub)tropical destinations

Parameter	No.						
	ETEC associated diarrhoea					All diarrhoea	
	Placebo ($n=7$)	Vaccine ($n=10$)	Toxin			Placebo ($n=30$)	Vaccine ($n=36$)
			LT	ST	LT and ST		
Severity of episode of TD[a]							
2 stools/day	1	1	0	1	1	10	11
3–6 stools/day	6	3	1	5	3	15	16
>6 stools/day	0	6	0	5	1	5	9
Number of episodes of TD[a]							
1 episode	6	9				23	20
2 episodes	0	1				5	12
3 episodes	1	0				2	4
Mean duration (days) [range][a]	2.7 [1,11]	3.7 [1,10]				2.5 [1,14]	4.1 [1,24]
Median interval to onset of TD (days) [range][b]	5 [4,17]	15 [5,23]*				9 [4,25]	9 [3,25]

Placebo: heat-killed *Escherichia coli*-K12; vaccine: a single dose of CVD 103-HgR live oral cholera vaccine; mean duration of stay abroad: 20 days, range (7–30) days; a separate episode of TD is defined as an episode occurring after five consecutive days without diarrhoea.
[a] Statistics: χ^2-test comparing placebo group to vaccine group.
[b] Mann–Whitney U-test comparing placebo group to vaccine group.
* $p = 0.043$.

3.2. Secondary endpoint

Analysis could be performed on 59 stool samples obtained from the 66 participants who had reported an episode of diarrhoea. In the placebo group ETEC was isolated from 7 of 28 samples (25%), compared to 10 of 31 samples (32%) in the vaccine group (Table 3). The majority of sweeps (65%) were only positive in the PCR detecting the ST-genes. In the placebo group 4 sweeps contained only ST-, 2 LT/ST- and 1 LT-genes. For the vaccine group this was 7, 3 and 0, respectively (Table 3). This means that per 100 travellers, only 4.6 experienced an episode of LT–ETEC or LT/ST–ETEC associated diarrhoea. For all travellers, the (detected) incidence rate of ETEC associated TD was 11% in the placebo group and 15% in the group of vaccines. Travellers to Indonesia experienced the highest incidence of ETEC associated diarrhoea (33%), followed by travellers to India (29%) and West-Africa (20%). No ETEC diarrhoea was found in people visiting Thailand (Table 2). The median time from departure to onset of ETEC-associated diarrhoea was shorter in the placebo group (5 days, range 4–17 days) compared to the vaccine group (15 days, range 5–23 days) ($p = 0.043$) (Table 4). Six of 7 subjects from the placebo group developed diarrhoea within 12 days compared to 4 of 10 vaccinated travellers. However, there was no difference in time from departure to onset of diarrhoea when all TD were taken into account.

Of the samples obtained from travellers who had not suffered an episode of diarrhoea, the first 28 were analysed for ETEC. In the placebo group 1 of 14 contained ETEC, compared to 3 of 14 in the vaccine group (Table 3).

4. Discussion

Interim analysis of 134 travellers to different tropical destinations failed to demonstrate a 50% protective efficacy of CVD 103-HgR live oral cholera vaccine against all-cause travellers' diarrhoea. In addition, no significant differences between placebo and vaccine group were found with regard to time of first onset, duration, severity or recurrence of TD. A vaccine-induced protection against TD of at least 50% was chosen as primary endpoint because we assumed that a lower protection rate would not be relevant to clinical practice. The study was not continued until all 200 participants were included because a statistical significant difference would not have been reached with an attack rate of TD of 46% in the placebo group, even in the unlikely event that all additionally included vaccinated travellers were protected against diarrhoea.

Most travellers from the vaccine group developed ETEC-associated diarrhoea after two weeks. Therefore, this study does not exclude a short-lived protection of CVD 103-HgR against TD specifically caused by ETEC. The duration of protection was much shorter than observed for BS-WC (3 months) in a field trial in Bangladesh [11]. However, because of the high incidence of LT–ETEC and LT/ST–ETEC diarrhoea in this country, it is likely that BS–WC had boosted pre-existent immunity against ETEC resulting in longer protection. Further studies should confirm our observation and evaluate whether a second oral dose of CVD 103-HgR could boost the primary response and prolong protection.

Several potential limitations of this study require comment: (1) the detected incidence of LT-associated diarrhoea was much lower than expected. Only 4.6 subjects per 100 travellers experienced an episode of LT–ETEC or LT/ST–ETEC associated TD. The vaccine's protective efficacy is based on the putative production of cross reacting antibodies against LT. The low incidence of LT–ETEC associated diarrhoea may have limited the power of this study to demonstrate a protective effect of CVD 103-HgR on incidence, duration and severity of LT–ETEC or LT/ST–ETEC associated TD; (2) the preservation of the stool sample in a faecal transport medium may have adversely affected the recovery of ETEC. This would result in an underestimation of the true incidence of ETEC-associated diarrhoea. However, *E. coli* can be recovered from the faecal transport medium up to 49 days after inoculation in the laboratory [18–20]. The mean (±S.D.) interval between collection and microbiologic analysis in this study was 19 (±7.6) days (range 7–34 days). Furthermore, the attack rate of ETEC associated diarrhoea according to travel destination in the present study was in accordance with published literature [2,4,6]. Finally, PCR detection of LT- and ST-genes in sweeps of the complete bacterial growth is far more sensitive than the conventional DNA-probe hybridisation of *E. coli* like colonies [7,21]. Therefore, we do not think that the incidence of ETEC associated diarrhoea was underestimated; (3) seroconversion of the participants for anti-cholera toxin or anti-heat-labile enterotoxin was not documented in this study. No doubts exist concerning the placebo, as it has been proven not to elicit an antitoxin antibody response [22]. The biological activity of the vaccine was extensively tested in the laboratory prior to supervised administration. Dosage and method of delivery were similar to those known to induce an anti-cholera toxin antibody response in 72–83% of vaccinated healthy Swiss or American volunteers 21 days after vaccination [14]. The mean interval from vaccination to departure was 17.1 days allowing enough time to mount an immune response; and (4) other enteropathogens than ETEC have not been looked for. Several studies have found mixed infections with other pathogens along with ETEC in stools of travellers affected by TD [6,12,24]. CVD 103-HgR may not protect against TD caused by such mixed infections.

Remarkably 65% of all detected ETEC strains isolated from stool specimens of subjects with TD were sole producers of ST. Furthermore, none of the asymptomatic participants, whose specimens were analysed, carried ST–ETEC. This suggests that ST–ETEC is more pathogenic than LT–ETEC or LT/ST–ETEC.

In summary, a 50% protective efficacy against TD could not be demonstrated for CVD 103-HgR live oral cholera vaccine. This may be due to the low incidence of LT-producing

ETEC strains. The study does not exclude a short-lived protective effect against ETEC-associated TD. However, the small sample size, lack of antibody-response measurements and selective testing of faeces, limit the predictive power.

Future studies attempting to prevent TD through vaccination may focus on ETEC, as it remains the most common causative pathogen [5,6,7], but should target a broader range of strains, because ST–ETEC seems to have a higher incidence than suggested in earlier studies [8,9,23]. Recent studies have done just that by developing vaccines including colonization factor antigens expressed by ETEC [24]. Furthermore, it is recommended that future trials stating attack rate of TD as a primary outcome should include large numbers of travellers, or limit the investigation to countries for which detailed data concerning aetiology of TD is available.

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