# Use of a patch containing heat-labile toxin from *Escherichia coli* against travellers' diarrhoea: a phase II, randomised, double-blind, placebo-controlled field trial

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## Summary

**Background** Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of travellers' diarrhoea. We investigated the rate of diarrhoea attacks, safety, and feasibility of a vaccine containing heat-labile enterotoxin (LT) from ETEC delivered to the skin by patch in travellers to Mexico and Guatemala.

**Methods** In this phase II study, healthy adults (aged 18–64 years) who planned to travel to Mexico or Guatemala and had access to a US regional vaccination centre were eligible. A centralised randomisation code was used for allocation, which was masked to participants and site staff. Primary endpoints were to investigate the field rate of ETEC diarrhoea, and to assess the safety of heat-labile toxins from *E coli* (LT) delivered via patch. Secondary endpoints included vaccine efficacy against travellers' diarrhoea and ETEC. Participants were vaccinated before travel, with two patches given 2–3 weeks apart. Patches contained either 37·5 µg of LT or placebo. Participants tracked stool output on diary cards in country and provided samples for pathogen identification if diarrhoea occurred. Diarrhoea was graded by the number of loose stools in 24 h: mild (three), moderate (four or five), and severe (at least six). Analysis was per protocol. The trial is registered with ClinicalTrials.gov, number NCT00516659.

**Findings** Recruitment closed after 201 participants were assigned patches. 178 individuals received two vaccinations and travelled and 170 were analysed. 24 (22%) of 111 placebo recipients had diarrhoea, of whom 11 (10%) had ETEC diarrhoea. The vaccine was safe and immunogenic. The 59 LT-patch recipients were protected against moderate-to-severe diarrhoea (protective efficacy [PE] 75%, p=0·0070) and severe diarrhoea (PE 84%, p=0·0332). LT-patch recipients who became ill had shorter episodes of diarrhoea (0·5 days *vs* 2·1 days, p=0·0006) with fewer loose stools (3·7 *vs* 10·5, p<0·0001) than placebo.

**Interpretation** Travellers' diarrhoea is a common ailment, with ETEC diarrhoea illness occurring in 10% of cases. The vaccine patch is safe and feasible, with benefits to the rate and severity of travellers' diarrhoea.

**Funding** IOMAI Corporation.

## Introduction

Enterotoxigenic *Escherichia coli* (ETEC) is the leading cause of diarrhoea in travellers to endemic areas and in young children living in developing countries.[1] Every year, acute diarrhoea afflicts about 27 million travellers and 210 million children, causing 380 000 paediatric deaths.[2] Travellers' diarrhoea generally lasts 4–5 days with frequent loose stools (18 per episode),[3] usually associated with nausea, vomiting, abdominal cramps, prostration, and dehydration.[3] We have recently described the development of a novel antigen delivery system using a patch to target the skin immune system to elicit the robust immunity that may be required to protect travellers against ETEC and other forms of bacterial diarrhoea.[4–6]

ETEC organisms are transmitted via contaminated food and beverages. They colonise the small intestine and secrete heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST)—or a combination—which cause secretory diarrhoea.[7] Globally, LT is found in about two-thirds of cases of ETEC diarrhoea.[8–10] Substantial data have shown that naturally acquired anti-LT immunity provides protection against ETEC disease in the field.[11–13] Several studies[10,14–17] of oral vaccines containing cholera toxin B subunit, which is both homologous in structure and immunologically cross-reactive with LT, have shown short-term protective effects against diarrhoea from LT-containing ETEC. LT is an ideal antigen but is too toxic to be delivered by oral, nasal, or parenteral routes, although it can safely be delivered via transcutaneous immunisation.[5,6] We postulated that a vaccine containing LT delivered to the skin would generate anti-toxin immunity that might prevent diarrhoea caused by the enteric pathogen, ETEC.

LT elicits robust immune responses when delivered to the skin in a patch.[5,6] In this study, we examined the safety, immunogenicity, and efficacy of LT delivered by transcutaneous immunisation as a travellers' diarrhoea vaccine patch given to individuals from the USA travelling to Mexico or Guatemala.

*Lancet* 2008; 371: 2019–25

Published Online June 12, 2008 DOI:10.1016/S0140-6736(08)60839-9

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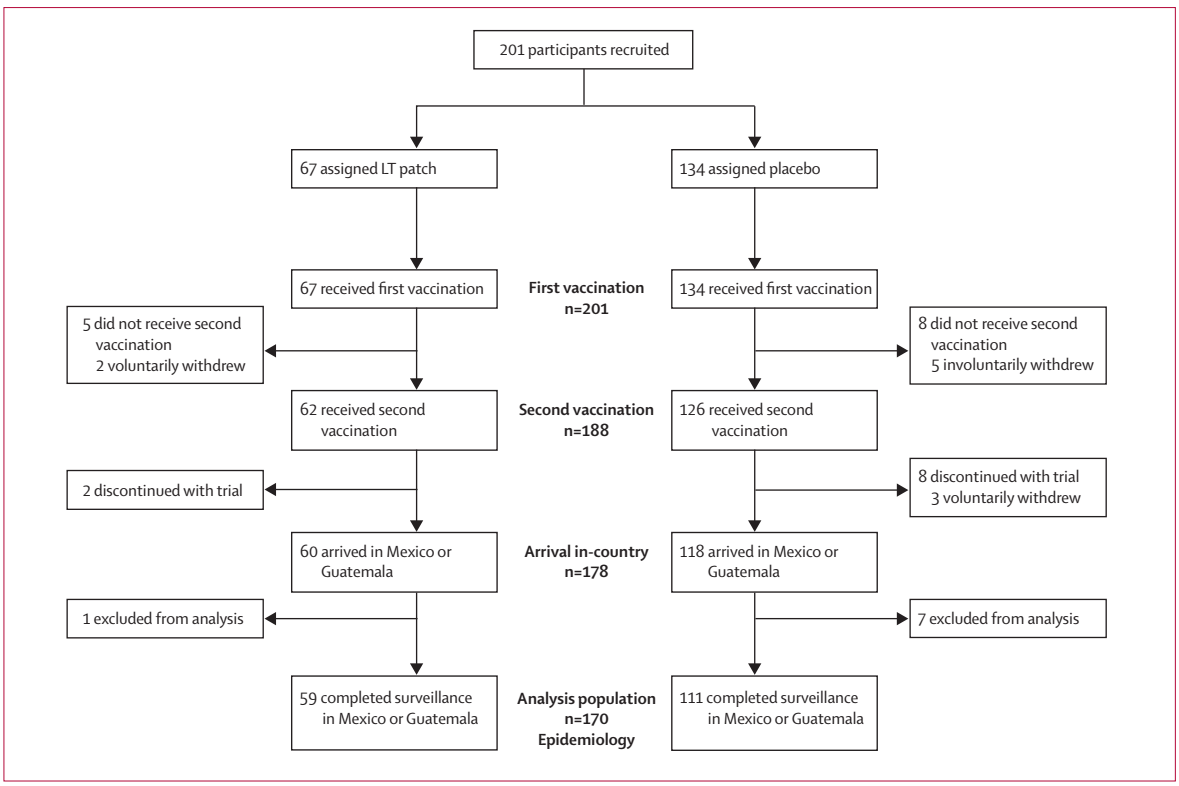


*Figure 1:* **Trial profile**

## Methods

### Participants

We enrolled healthy adults (aged 18–64 years) who planned to travel to Cuernavaca, Guadalajara, San Miguel, or Cancun (Mexico), or Antigua (Guatemala), and who had access to one of the 14 US regional vaccination centres. Exclusion criteria included history of travellers' diarrhoea travel to an endemic country in the previous 12 months; previous use of a cholera, LT, or ETEC vaccine; significant illness; immunosuppression; or, if female, pregnant, nursing, or unwilling to use an effective form of birth control.

Signed, informed consent was obtained from all participants. Recruitment occurred from May to December, 2006, with vaccinations given in the USA from July, 2006, to January, 2007, and surveillance done in Mexico and Guatemala from August, 2006, to February, 2007. This study was given ethics approval by Schulman Institutional Review Board (Cincinnati, OH, USA); University of Texas Health Science Center at Houston (Houston, TX, USA); Western Institutional Review Board (Olympia, WA, USA), Universidad Del Valle De Guatemala EC (Guatemala City, Guatemala), and Inovamed Hospital Institutional Review Board (Cuernavaca, Mexico).

### Procedures

The study used a web-based, audit-trail enabled, centralised randomisation code and allocation system (Amarex LLC, Germantown, MD, USA). Participants were stratified on the basis of destination city and sex, after which they were randomly assigned in block sizes of six before first vaccination. Vaccination sites accessed a web page, entered participants into the system, and received unique patch numbers for every study participant. Dose information was masked at allocation, as well as on primary and secondary product packaging. Participants and site staff, including those assessing study outcomes, remained masked until database lock.

At pre-travel vaccination sites, consented and enrolled participants received a brief physical examination, and blood was taken for measurement of baseline LT-antibody titres. Vaccinations with either an LT patch or placebo patch were given to alternate upper arms a minimum of 3 weeks (first vaccination) and 1 week (second vaccination) before departure. On the day of vaccination, participants had the site area marked with an ink pen and prepared by use of two mild strokes with a skin preparation device containing a mild abrasive affixed to a pressure-controlled device. The device was a single-use, disposable system and was discarded immediately after use. Following skin preparation, the patch was applied within the marked area and worn for 6 h at each vaccination (allowable range was 5–8 h), then removed and discarded by the participant.[18] LT patches contained 37·5 μg of LT and placebo patches contained all the components of the LT patch, but with no LT included in

the formulation. Assessment for adverse events was done by phone 3 days after every vaccination, and participants recorded adverse events on a diary card from first vaccination until arrival in Mexico or Guatemala (range 21–42 days).

Within 24 h of arrival in Mexico or Guatemala, participants reported to the clinic for blood to be taken and for review of the diary card; and, they returned weekly for diary review and other applicable procedures such as blood draw and stool sample submission up to the final in-country visit. Participants were trained to designate a stool as loose if it took the form or shape of the toilet bowl. Diarrhoea was defined as three loose stools in 24 h. Three loose stools in 24 h constituted mild diarrhoea, four to five was moderate diarrhoea, and at least six was severe diarrhoea. Symptom reports were not used to grade diarrhoea events. Diarrhoea episodes were deemed as separate if there was a 48-h interval between loose stools. If participants had at least three loose stools in a 24-h period, they were asked to collect the third and fourth stools and submit them to the clinic. Ciprofloxacin (500 mg twice daily for 3 days) was given if individuals had moderate or severe diarrhoea, or mild diarrhoea accompanied by moderate or severe symptoms (eg, vomiting, fever ≥37·8°C, blood or mucus in stool, abdominal pain, faecal urgency, or tenesmus), or if the investigator deemed that treatment was needed. Participants were contacted 6 months after returning to the USA to solicit any changes in health.

The primary objectives of this study were to assess the rate of ETEC illness in placebo recipients in a field setting and the safety of LT delivered by trancutaneous immunisation compared with placebo. Secondary objectives included measurement of stool frequency per episode of ETEC illness in placebo recipients, immunogenicity of LT delivered by transcutaneous immunisation, and frequency of LT-containing ETEC in placebo and LT-patch recipients. The occurrence and severity of all diarrhoea, ETEC-associated diarrhoea episode duration, and concomitant medication use were also prospectively analysed and compared between treatment groups.

Primary outcome measures of diarrhoea occurrence, stool frequency, and severity were derived from study participant diary data. We determined the severity of diarrhoea illness by recording the highest number of loose stools produced in any 24-h period during an episode and graded it as mild (three loose stools), moderate (four to five), or severe (at least six). ETEC illness was defined as a positive result for LT, LT/ST, or ST by DNA hybridisation (University of Texas Medical School at Houston, Houston, TX, USA) or toxin-specific PCR assays (ViroMed Laboratory, Minnetonka, MN, USA) on diarrhoea stools. Colony picks from stool culture (five per assay) were used in both PCR and DNA hybridisation. Whole stool was also investigated in the PCR assay, but not done for DNA hybridisation. We measured the sensitivity of the PCR by spiking stool samples with serial dilutions of known positives (LT, ST, and LT/ST strains). The results of this study yielded a limit of detection of two bacteria per reaction that corresponded to an analytical sensitivity of 1000 bacteria per mL of stool sample (or one bacterium per µL). Both the analytical specificity and accuracy were 100% during assay validation (all strains were characterised correctly, and negative samples tested negative).

Local and regional field laboratories also tested for the following pathogens using standardised laboratory procedures: *Salmonella* spp, *Shigella* spp, *Campylobacter* spp, *Vibrio* spp, *Aeromonas* spp, *Plesiomonas* spp, *Giardia* spp, *Cryptosporidium*, *Entamoeba histolytica*, *Cyclospora* spp, *Microsporidia* spp, entero-

| | All treatments | LT patch | Placebo | p* |
|---|---|---|---|---|
| **Application 1** | | | | |
| Total | 201 | 67 | 134 | |
| Local | | | | |
| Pruritus | 50 (25%) | 45 (67%) | 5 (4%) | <0·0001 |
| Rash | 43 (21%) | 41 (61%) | 2 (1%) | <0·0001 |
| Pigmentation changes | 5 (2%) | 5 (7%) | 0 | 0·0014 |
| Systemic | | | | |
| Fever | 2 (1%) | 1 (1%) | 1 (1%) | 0·6153 |
| Malaise | 14 (7%) | 5 (8%) | 9 (7%) | 0·8447 |
| Headache | 23 (1%) | 9 (13%) | 14 (10%) | 0·5308 |
| Diarrhoea | 21 (10%) | 9 (13%) | 12 (9%) | 0·3279 |
| **Application 2** | | | | |
| Total | 188 | 62 | 126 | |
| Local | | | | |
| Pruritus | 28 (15%) | 27 (44%) | 1 (1%) | <0·0001 |
| Rash | 34 (18%) | 33 (53%) | 1 (1%) | <0·0001 |
| Pigmentation changes | 5 (3%) | 4 (6%) | 1 (1%) | 0·0227 |
| Systemic | | | | |
| Fever | .. | .. | .. | .. |
| Malaise | .. | .. | .. | .. |
| Headache | 7 (4%) | 3 (5%) | 4 (3%) | 0·5637 |
| Diarrhoea | 5 (3%) | .. | 5 (4%) | 0·1133 |

Data are n (%) of patients having one or more treatment-emergent episodes of specified adverse event, unless otherwise stated. *$\chi^2$ test.

***Table 1:*** **Selected local and systemic adverse events reported after vaccination**

| Anti-LT | Timepoint | LT patch (n=60) | | | Placebo (n=110) | | |
|---|---|---|---|---|---|---|---|
| | | GMT | GMFR | Seroconversion | GMT | GMFR | Seroconversion |
| IgG | Baseline | 665 | n/a | n/a | 573 | n/a | n/a |
| IgG | Arrival | 8914 | 13·4 | 92% | 526 | 0·9 | 3% |
| IgG | Exit | 11060 | 16·6 | 92% | 640 | 1·1 | 6% |
| IgA | Baseline | 39 | n/a | n/a | 49 | n/a | n/a |
| IgA | Arrival | 398 | 10·1 | 78% | 49 | 1·0 | <1% |
| IgA | Exit | 442 | 11·2 | 82% | 64 | 1·3 | 2% |

GMT=geometric mean titre. GMFR=geometric mean fold rise. Arrival=arrival in country. Exit=exit from country.

***Table 2:*** **Immune responses to LT patch and placebo**

| | LT patch (n=59) | Placebo (n=111) | p* | Protective efficacy (95% CI) |
|---|---|---|---|---|
| Individuals with diarrhoea of any cause | 9 (15%) | 24 (22%) | 0·32 | 29% (−13 to 72) |
| Moderate to severe | 3 (5%) | 23 (21%) | 0·007 | 75% (48 to 103) |
| Severe | 1 (2%) | 12 (11%) | 0·033 | 84% (54 to 115) |
| Individuals with ETEC diarrhoea | 3 (5%) | 11 (10%) | 0·28 | 49% (−8 to 105) |
| Moderate to severe | 2 (3%) | 11 (10%) | 0·13 | 66% (19 to 112) |
| Severe | 1 (2%) | 5 (5%) | 0·34 | 62% (−11 to 136) |

Data are n (%) unless stated otherwise. *$\chi^2$ or Fisher's exact test.

***Table 3:*** **Diarrhoea incidence and severity**

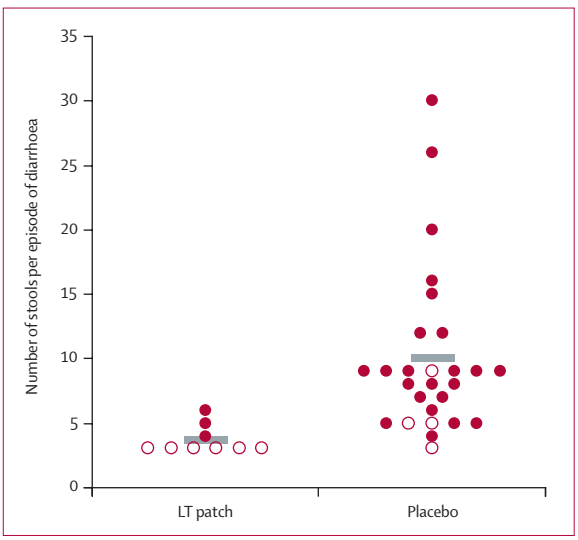


***Figure 2:*** **Severity of diarrhoeal episodes and number of stools by treatment group**
Cumulative stools from individual episodes in LT-patch recipients versus placebo recipients, including analysis population as well as two individuals who had more than one episode (two and three episodes, respectively). Solid spot=moderate to severe episode. White spot=mild episode. Solid bar=mean number of diarrhoea stools per group. Note that placebo-to-vaccine rate is 1 to 1·88.

aggregative *E coli*, and Norwalk-like virus.[19,20] Safety of the LT patch was compared with placebo by review of diary data and clinician assessments. Serum samples taken at baseline, arrival in country, and 1–2 weeks after arrival were simultaneously tested for anti-LT IgG and anti-LT IgA by ELISA and reported in ELISA units.[5]

### Statistical analysis

We designed this phase II, double-blind, randomised, placebo-controlled study to investigate the epidemiology of natural infection with ETEC in placebo recipients with a planned enrolment of 300 individuals, at a placebo-to-LT patch ratio of 2:1. The rate of infection in controls was estimated to range from 5% to 15% (SD 1·5 to 2·5, respectively). With a placebo group of 200 people, the sample size was intended to establish an estimated rate of natural infection with ETEC to aid in the determination of sample size for future studies, and was not anticipated to provide power to detect significant differences between placebo and LT recipients.

The study was halted when enrolment reached 201, because the planned interval for conduct had been exceeded, and it was thought that a placebo group greater than 100, although less powerful than the original 200, would be sufficient to assess the ETEC attack rate in placebo recipients.

Data analyses were done with SAS statistical software (version 8.2) and StatXact software (version 5). Occurrence of diarrhoea illness, ETEC diarrhoea, diarrhoea severity, and irritable bowel syndrome were presented as binary variables. $\chi^2$ test or Fisher's exact test were used to compare the occurrence rates between groups for these variables. The formula used for protective efficacy was: ([occurrence in placebo–occurrence in LT patch]/occurrence in placebo)×100%. The stool frequency and duration per diarrhoea episode were calculated for every participant, and the mean differences between groups were analysed by Kruskal-Wallis test. Safety data were analysed for all participants. Adverse events were tabulated by MedDRA code according to severity, and compared between groups by Cochran-Mantel-Haenszel tests implemented in StatXact software.

All serological analyses were done in the $\log_{10}$ scale so that the distribution of titres and fold ratios were closer to Gaussian. We compared geometric mean titres and fold ratios with a two-sample *t* test as well as a generalised linear model adjusted for age, sex, and baseline titre. Seroconversion (defined as at least a two-fold increase in ELISA units for IgG and at least a four-fold increase in ELISA units for IgA) was compared by use of Fisher's exact test and logistic regression with the same covariates. Analysis was per protocol. The trial is registered with ClinicalTrials.gov, number NCT00516659.

### Role of the funding source

The sponsor of the study made the full data available to investigators at the Johns Hopkins Bloomberg School of Public Health, principal investigators at the University of Texas, Houston School of Public Health, and in-country collaborators. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

## Results

201 participants received a first vaccination (intention-to-treat population). 188 received a second vaccination as per protocol. Of the 178 participants who travelled to Mexico or Guatemala and checked in at the clinical study site, five did not provide diary cards and three did not attend the final in-country visit, leaving 170 participants in the per-protocol population (figure 1).

Study participants were aged 18–64 years (median 29 years) and were mainly tourists, language students, and missionaries. 104 (61%) travelled to Mexico, and 66 (39%) to Guatemala. Mean duration of stay was

12·4 days (11·8 days, LT-patch group; 12·8 days, placebo group; p=0·32). Safety was assessed in all participants who received a first and second vaccination. Overall, the LT patch was safe and well tolerated, with most adverse events reported as mild (table 1). No vaccine-related serious adverse events were recorded and, before arrival in Mexico or Guatemala, no significant differences were seen between the LT-patch and placebo groups in either occurrence or severity of systemic adverse events (eg, fever, malaise, headache, and diarrhoea). Transient, application site reactions included mild (and occasionally moderate) pruritus, rash, and hyperpigmentation.

170 participants received two vaccinations of LT and had blood drawn at all timepoints (figure 1, table 2). Baseline anti-LT IgG and IgA titres did not differ between the groups before vaccination, but were significantly higher in the LT-patch group than placebo at the time of arrival in country (p<0·0001 for IgG and IgA; table 2) and exit (both p<0·0001; table 2). At time of arrival in country, seroconversion for LT-IgG and LT-IgA occurred in many more individuals in the LT-patch group than in the placebo group (both p<0·0001), and anti-LT IgG and IgA increased at least ten times in the LT-patch group compared with no increase in the placebo group.

Nine (15%) participants in the LT-patch group versus 24 (22%) in the placebo group developed diarrhoea (p=0·3117; table 3). The rate of moderate-to-severe diarrhoea of any cause was also higher in placebo recipients than in LT-patch recipients (21% *vs* 5%, protective efficacy [PE] 75%, p=0·0070; figure 2, table 3). Similarly, the number of severe cases of diarrhoea was also significantly increased in placebo (11% *vs* 2%, PE 84%, p=0·0332; table 3).

We identified an enteropathogen in 12 placebo and three LT-patch participants (table 4). ETEC diarrhoea was detected in 11 placebo recipients with an overall attack rate of 10%, accounting for 46% of all placebo diarrhoea cases (three LT, three LT/ST, and five ST). Furthermore, enteroaggregative *E coli* was detected in two placebo recipients (one with ST co-infection). 11 (10%) participants in the placebo group and three (5%) in the LT-patch group contracted ETEC diarrhoea (table 5), with a higher rate of moderate or severe disease in the placebo group (10% [11/111] *vs* 3% [2/59]; PE 66%, p=0·1278, table 3). Five (5%) placebo recipients and one (2%) LT-patch recipient contracted severe ETEC diarrhoea (PE 62%, p=0·3446). Although the rates of diarrhoea with LT-containing ETEC did not differ between groups (5%), individuals given the LT patch had fewer stools per episode (4·3 *vs* 8·2, p=0·0381) and diarrhoea of shorter duration (0·4 *vs* 2·2 days, p=0·0201; table 5) than placebo recipients. The number of diarrhoea stools per episode in participants who became ill was also significantly lower in LT-patch recipients than in placebo recipients for diarrhoea of any cause (3·7 *vs* 10·5, p<0·0001) and for all ETEC diarrhoea (4·3 *vs* 10·5, p=0·0150; table 5). Diarrhoea episode duration of any cause did not exceed 24 h for any participant in the LT-patch group compared with a mean of more than 2 days in the placebo group (p<0·0001; table 5). Results for all ETEC cases and cases with LT-containing ETEC only were identical (p=0·0201 for both; table 5). Placebo recipients used antibiotics more frequently than LT-patch recipients (14% [16/111] *vs* 5% [3/59], p=0·0661).

| | LT patch (n=59) | Placebo (n=111) |
|---|---|---|
| Diarrhoea cases | 9 | 24 |
| Cases with any enteropathogen | 3 | 12 |
| ETEC | 3 | 11 |
| -LT | 1 | 3 |
| -LT/ST | 2 | 3 |
| -ST (total) | 0 | 5 |
| -ST only | 0 | 4 |
| -ST with enteroaggregative *E coli* | 0 | 1 |
| Enteroaggregative *E coli* only | 0 | 1 |

Data are number of cases.

***Table 4:*** **Pathogens detected in study participants**

| | LT patch | Placebo | p* |
|---|---|---|---|
| Total | 59 | 111 | |
| Mean stool frequency (range) | 3·7 (3–6) | 10·5 (4–30) | <0·0001 |
| Duration (days [range]) | 0·5 (0·1–1·0) | 2·1 (0·2–5·8) | 0·0006 |
| ETEC diarrhoea (n) | 3 | 11 | |
| Mean stool frequency (range) | 4·3 (3–6) | 10·5 (5–30) | 0·015 |
| Duration (days [range]) | 0·4 (0·3–0·5) | 2·2 (0·4–4·3) | 0·024 |
| LT or LT/ST ETEC diarrhoea (n) | 3 | 6 | |
| Mean stool frequency (range) | 4·3 (3–6) | 8·2 (5–12) | 0·038 |
| Duration (days [range]) | 0·4 (0·3–0·5) | 2·2 (0·9–3·8) | 0·020 |

*Kruskal-Wallis test.

***Table 5:*** **Diarrhoea episode duration and mean number of loose stools per episode**

## Discussion

The present study was designed to assess the logistics of conducting a future larger field trial in Mexico and Guatemala, and assess the ETEC attack rate in placebo recipients. The study, therefore, specified a 1:2 vaccine-to-placebo ratio to increase the ability to estimate diarrhoea illness in untreated participants, and was not powered to show efficacy. Despite this limitation, reductions in occurrence and severity were seen in LT-patch recipients. In this multinational trial of adult travellers from the USA to Mexico and Guatemala, the rate of travellers' diarrhoea was 22%, with a 10% rate of ETEC diarrhoea. The LT patch vaccine conferred some protective efficacy against moderate-to-severe travellers' diarrhoea of any cause, severe diarrhoea, and moderate-to-severe ETEC diarrhoea. Furthermore, recipients of the LT patch who became ill had a milder

course of illness, with reduced stool output and duration of diarrhoea illness compared with placebo. Taken together, the LT patch conferred a meaningful benefit to the recipients.

In this study, we obtained data on stool patterns using a well-developed diary card system and standardised laboratory procedures to identify enteropathogens from submitted samples.[20,21] Pathogens were identified in about half of all cases of diarrhoea, with ETEC as the main isolate, consistent with previous field studies and a dry season ETEC attack rate (10% in placebo recipients).[15,16,21,22] The diagnosis of enteric pathogens from stool cultures in field trials lacks sensitivity and is likely to have missed some ETEC cases. However, an increase in the rate of diagnosed moderate or severe cases of LT-containing ETEC may not fully explain the level of efficacy seen against all-cause diarrhoea. The presence of five ST-only cases and two cases of enteroaggregative *E coli* in the placebo group suggests that the vaccine immunity could exert effects beyond LT-containing ETEC. Similar findings have been made in other field trials with orally administered vaccines based on cholera toxin B, which protected against LT/ST-containing ETEC,[14] against S*almonella* and *Salmonella*/LT ETEC mixed infections,[15] and against *Campylobacter*.[23] Preclinical studies have shown that exposure of the enterocytes to LT conditions the gut for initial colonisation of ETEC, a key step in the pathogenesis of ETEC illness,[24–26] and can enhance the virulence of non-ETEC pathogens.[27] We suggest that immunity to LT blocks the conditioning of the gut wall for enhanced enteric pathogenicity, decreasing the attack rate of LT-containing and non-LT-containing ETEC as well as other pathogens.

We analysed two main potential confounders that might have affected diarrhoea outcomes: surveillance time and antibiotic use. The mean duration of surveillance did not differ greatly between the two groups (about 12–13 days). With respect to antibiotic treatment for diarrhoea episodes, ciprofloxacin was used in three (33%) of nine participants in the LT-patch group, compared with 16 (66%) of 24 participants in the placebo group. This increased antibiotic use by participants in the placebo group could have reduced the severity and duration of diarrhoea, thus potentially biasing the results in favour of the placebo group. Despite the antibiotic use in the placebo group, a large number of placebo recipients with diarrhoea were quite ill, and as a group had an average of more than ten stools per episode, which is consistent with other studies indicating that traveller's diarrhoea is far more than an inconvenience to travellers even when antibiotic treatment is used.[12]

The combination of LT, a highly immunogenic antigen and a key pathogenic factor from ETEC, and the delivery of this antigen to the dendritic cells in the skin, might explain the robust immunity and protection seen in this trial. Additionally, both preclinical and clinical investigations have shown that transcutaneous immunisation results in mucosal immunity to LT, which may contribute to protection.[6] In addition to the biological rationale for delivering antigen to the skin, the transcutaneous patch lends itself to a simple and needle-free application, use of the vaccine outside the cold chain, and has been designed to be suitable for both travellers and use in the developing world. This study suggests that transcutaneous immunisation with LT in a patch could protect travellers against this common, debilitating ailment but the recorded efficacy will need confirmation in a phase III trial.

#### Contributors

All authors participated in the design, implementation, analysis, and interpretation of the study. HD was the principal investigator of the study and coordinated the study sites in Mexico along with PO as subinvestigator. AB coordinated the study sites in Guatemala in conjunction with RM and JH. SF led the IOMAI clinical team with GG, TV, and RK. JB, JF, NG, FMS, AH, and EA ran the field sites. Laboratory analyses of pathogens were done by OT and AJ, with support by BA, HD, AB, and RM. ELISAs were generated by DF and BA. Statistical and data analysis were undertaken by KK, SB, JC, GG, SF, BA, and DF.

#### Conflict of interest statement

SF, GG, TV, BA, DF, JC, and RK are shareholders in IOMAI Corporation.

#### Acknowledgments

IOMAI Corporation provided funding for all phases of the trial. We thank Wanda Hardy for her support in preparation of this manuscript.

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