Efficacy and safety of a patch vaccine containing heat-labile toxin from *Escherichia coli* against travellers’ diarrhoea: a phase 3, randomised, double-blind, placebo-controlled field trial in travellers from Europe to Mexico and Guatemala


**Summary**

**Background** Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of travellers’ diarrhoea. We investigated the efficacy and safety of a skin-patch vaccine containing the pathogen’s heat-labile toxin (LT) in a population of travellers to Mexico and Guatemala.

**Methods** In this phase 3, randomised, double-blind, placebo-controlled field trial, healthy adults (aged 18–64 years) travelling from Germany or the UK to Mexico or Guatemala were assigned in a 1:1 ratio by a dynamic electronic randomisation system to receive transcutaneous immunisation with a patch containing 37.5 µg of ETEC LT or a placebo patch. Participants, site staff, and the investigators who did the analyses were masked to group assignment. Participants were vaccinated before travel, with two patches given 14 days apart. In the destination country, participants tracked stool output in a diary and provided stool samples for pathogen identification if diarrhoea occurred. The primary endpoint was the proportion of participants with at least one episode of moderate-to-severe diarrhoea (defined as four or more unformed stools in a 24 h period) in which either or both ETEC enterotoxins (LT and heat-stable toxin [ST]) were detected. The study is registered at ClinicalTrials.gov, number NCT00993681.

**Findings** 2036 participants were recruited and randomly assigned between Oct 14, 2009, and Aug 13, 2010, with 1016 allocated to receive the LT patch and 1020 the placebo patch. 821 participants in the LT-patch group and 823 in the placebo group received both vaccinations and were analysed in the per-protocol population. 30 (3.7%, 95% CI 2.5–5.2) of 849 participants who received both vaccinations and returned for final assessment in the LT-patch group, compared with none of the 842 participants in the placebo group. The vaccine was immunogenic, with a post-vaccination geometric mean titre of LT-specific serum immunoglobulin G of 3400.29, compared with 315.41 in the placebo group.

**Interpretation** Although the LT antigen was delivered effectively by the skin patch, the vaccine did not protect travellers against diarrhoea caused by ETEC or other organisms. Future vaccines against travellers’ diarrhoea might need to include several antigens against various diarrhoeal pathogens, and might need to be able to generate mucosal and higher systemic immunity.

**Funding** Intercell USA.

**Introduction** Enterotoxigenic *Escherichia coli* (ETEC) causes roughly 300 000–500 000 deaths annually, mostly among children, and is also the leading cause of travellers’ diarrhoea.1 Some ETEC bacteria colonise the small intestine and secrete either or both of the enterotoxins associated with diarrhoea, the heat-labile (LT) and heat-stable (ST) enterotoxins.

Protection against ETEC diarrhoea is mediated by an anti-LT immune response,2 and as such LT has been a key virulence factor for vaccine development.3 If delivered orally, nasally, or parenterally, it remains a potent toxin, but it can be delivered safely via transcutaneous immunisation with a skin patch.4 Obvious advantages of a patch vaccine are the ease of application and the non-requirement of a cold chain. A skin-patch LT-antigen delivery system was

[1](#) Protection against ETEC diarrhoea is mediated by an anti-LT immune response, and as such LT has been a key virulence factor for vaccine development. If delivered orally, nasally, or parenterally, it remains a potent toxin, but it can be delivered safely via transcutaneous immunisation with a skin patch. Obvious advantages of a patch vaccine are the ease of application and the non-requirement of a cold chain. A skin-patch LT-antigen delivery system was
shown in a phase 2 study in travellers from the USA to Mexico and Guatemala to elicit immunity against ETEC and other forms of bacterial diarrhoea. The vaccine seemed to be immunogenic and effective against all-cause moderate-to-severe travellers’ diarrhoea, although protection against ETEC diarrhoea specifically was non-significant (on the basis of very few cases). We investigated the efficacy and safety of the same LT patch against ETEC and all-cause diarrhoea in a phase 3 study in travellers from Europe to destinations in Mexico and Guatemala.

**Methods**

**Study design and participants**

In this phase 3, randomised, double-blind, placebo-controlled field trial, investigations took place across 20 sites—four in Germany and seven in the UK (referred to as countries of origin), and five in Mexico and four in Guatemala (referred to as destination countries). Participants were healthy adults (aged 18–64 years) of either sex who had either already planned to, or who agreed to, travel to Mexico or Guatemala. Recruitment and enrolment were timed so that most trips took place during the destination countries’ wet season (April to October) when diarrhoeal incidence is highest.

The study protocol was approved by local competent authorities and ethics committees, and the study followed Good Clinical Practice standards and adhered to the principles outlined in the Declaration of Helsinki. All participants provided written informed consent.

**Randomisation and masking**

We used a dynamic electronic randomisation system to assign participants in a 1:1 ratio to receive either the LT vaccine patch or the placebo patch. The stratification criteria were age (18–50 years vs >50–64 years) and destination city. Participants, site staff, and the investigators who did the analyses were masked to group assignment.

**Procedures**

Participants were given two transcutaneous immunisations with dermal patches containing 37.5 μg LT or placebo (ie, same patch and application without the LT protein) 14 days apart. Patches were applied as per the procedure described by Frech and colleagues. Briefly, each patch application was worn for 6 h on the deltoid region, with the procedure repeated with a second patch on the other arm 14 days later. Participants travelled to Mexico or Guatemala 7–30 days after receiving the second dose. They were required to stay in the destination country for at least 7 days, and were assessed on arrival and on day 7. A further surveillance visit took place 17 days after arrival in the destination country. A final study visit in the country of origin took place 180 days after random assignment to assess adverse events (only data for hyperpigmentation are reported) and irritable bowel syndrome (data not reported).

All participants who received at least one vaccination were included in the safety population. Adverse events were documented by each participant in a diary and assessed by a clinician via telephone 2–5 days after vaccination and at all clinic visits. Vaccination-site adverse events were assessed and photographed at clinic visits. After arrival in the destination country, participants kept a diary of daily stool passage by date, time, and stool consistency. Diaries were maintained for 17 days from arrival (the defined surveillance phase for efficacy and adverse events). When diarrhoea occurred, participants recorded detailed symptoms and their effect on daily activities.

Efficacy and immunogenicity analyses were done in the per-protocol population, which was defined as all participants who correctly received both assigned vaccinations, arrived in their destination country, successfully attended the destination country check-in and surveillance visits, and completed the surveillance-phase diary information for all days from arrival to the surveillance visit on day 17. Efficacy assessments were based on the diarrhoeal events reported in participants’ diaries. Passage of four or five unformed stools in a 24 h period was defined as moderate diarrhoea, with passage of six or more unformed stools defined as severe. Diarrhoeal episodes were deemed separate if they occurred at least 48 h apart.

If a participant had two unformed stools in a 24 h period, they provided a sample from the subsequent stool passed, which was delivered to a local designated laboratory as soon as possible (the target time was 8 h). Treatment was offered after the passage of four or more stools in 24 h. Stool samples were tested for a range of pathogens, including parasites (Entamoeba histolytica, Cryptosporidium spp, Cyclospora spp, Giardia spp, Microsporida spp) and bacteria (Salmonella spp, Shigella spp, Campylobacter jejuni, Vibrio spp, Aeromonas spp, and Plesiomonas shigelloides). Presence of ETEC toxins was investigated by two validated methods, PCR or DNA hybridisation.

The primary endpoint was the proportion of participants with at least one episode of moderate-to-severe diarrhoea during the surveillance period in which the ETEC toxins LT, ST, or LT plus ST were detected (in the absence of other detected pathogens). Secondary efficacy endpoints were the proportion of participants with moderate-to-severe all-cause diarrhoea, the proportion with all-cause diarrhoea of any severity, the frequency of unformed stools per episode of all-cause diarrhoea (mild, moderate, or severe), and the duration of all-cause diarrhoeal episodes during the surveillance phase. We also assessed the proportion of participants with ETEC diarrhoea of any severity (with or without a copathogen), and subdivided this result into LT-positive, ST-positive, and LT/ST-positive ETEC (non-prespecified analysis).

We assessed immunogenicity as a tertiary study endpoint on the basis of ELISA measurement of serum anti-LT immunoglobulin G (IgG) and IgA, and
toxin-neutralising antibody titres, with measurements taken at prevaccination baseline and on entry into destination country. We calculated geometric mean titres, relative increases in geometric means, and proportions of seroconversions for each serological measurement. For anti-LT IgG and the toxin-neutralising assay, seroconversion was defined as a two-times or greater increase in titre; for anti-LT immunoglobulin A, seroconversion was defined as a four-times or greater increase in titre. Other tertiary endpoints assessed the proportion of participants with moderate-to-severe ETEC diarrhoea with or without a copathogen; the proportion with moderate-to-severe ETEC diarrhoea with a copathogen; disruption of daily activities due to all-cause diarrhoea; loss of days (ie, the cumulative sum of hours confined to room or toilet, or seeking medical help) due to all-cause diarrhoea; use of antibiotic treatment; and unscheduled clinic visits for medical treatment.

**Statistical analysis**

We calculated the needed sample size on the basis of a 90% power, a two-sided α of 0.05, an assumption that 12.5% of participants in the placebo group would be affected by ETEC diarrhoea of any severity (on the basis of the previous phase 2 patch study and the relevant scientific literature), and a vaccine efficacy of 67.5%. The success criterion required the lower limit of the two-sided 95% CI for vaccine efficacy to exceed 40%. With a participants assigned in a 1:1 ratio between the LT patch and placebo, a total per-protocol population sample size of 1542 was required. Assuming that 17% of participants would be lost from the analysis because of non-completion of vaccination or travel, we would need to recruit 1800 participants. We increased the sample size during the study when we realised that the proportion of participants with diarrhoea was lower than expected and the study could be underpowered. All statistical analyses were done with SAS version 9.2 and StatXact version 6.

The study is registered at ClinicalTrials.gov, number NCT00993681.

**Role of the funding source**

The sponsor funded and coordinated the study, and planned the study together with external consultants. Monitoring and data management were done by an independent contract research organisation (Kendle INC Research, Durham, NC, USA) and statistical analyses by EMMES Corporation (Rockville, MD, USA). The sponsor did not have any role in data collection.

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**Figure: Trial profile**

LT=heat-labile toxin. *Sum of reasons for exclusion does not match total because some participants were excluded for more than one reason.
Table 1: Vaccine efficacy against ETEC and all-cause diarrhoea (per-protocol population)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>LT-patch group (n=821)</th>
<th>Placebo group (n=823)</th>
<th>Vaccine efficacy (95% CI)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate-to-severe ETEC diarrhoea (primary endpoint)†</td>
<td>30 (3.7%, 2.5–5.2)</td>
<td>46 (5.6%, 4.1–7.4)</td>
<td>34.6% (–2.2 to 58.9)</td>
<td>0.0621</td>
</tr>
<tr>
<td>Moderate-to-severe all-cause diarrhoea</td>
<td>102 (12.4%, 10.2–14.9)</td>
<td>101 (12.3%, 10.1–14.7)</td>
<td>–1.24% (–31.0 to 21.8)</td>
<td>0.9404</td>
</tr>
<tr>
<td>Any ETEC diarrhoea (with or without copathogen)</td>
<td>57 (6.9%, 5.3–8.9)</td>
<td>68 (8.3%, 6.5–10.4)</td>
<td>16.0% (–17.9 to 40.1)</td>
<td>0.3522</td>
</tr>
<tr>
<td>LT-positive</td>
<td>7 (0.9%, 0.3–1.7)</td>
<td>18 (2.2%, 1.3–3.4)</td>
<td>61.0% (7.2 to 83.6)</td>
<td>0.0417</td>
</tr>
<tr>
<td>ST-positive</td>
<td>25 (3.0%, 2.0–4.5)</td>
<td>22 (2.7%, 1.7–4.0)</td>
<td>–3.9% (–30.4 to 32.5)</td>
<td>0.6606</td>
</tr>
<tr>
<td>LT/ST-positive</td>
<td>25 (3.0%, 2.0–4.5)</td>
<td>28 (3.4%, 2.3–4.9)</td>
<td>10.5% (–5.2 to 47.3)</td>
<td>0.7804</td>
</tr>
<tr>
<td>Any all-cause diarrhoea</td>
<td>140 (17.1%, 14.5–19.8)</td>
<td>125 (15.2%, 12.8–17.8)</td>
<td>–12.3% (–40.0 to 10.0)</td>
<td>0.3149</td>
</tr>
</tbody>
</table>

Data are n (%), unless otherwise indicated. ETEC=enterotoxigenic Escherichia coli. LT=heat-labile toxin. ST=heat-stable toxin. *Exact unconditional test of treatment equality. †Diarrhoeal cases with four or more unformed stools in any 24 h period with LT, ST, or both LT and ST detected by PCR or DNA hybridisation from diarrhoeal stool samples taken during the first diarrhoeal episode, which were otherwise pathogen-free.

Table 2: Severity and duration of all-cause diarrhoea (per-protocol population)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>LT-patch group (n=140)</th>
<th>Placebo group (n=125)</th>
<th>Difference (95% CI)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of unformed stools per all-cause diarrhoeal episode</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>9.8 (9.0)</td>
<td>12.0 (9.9)</td>
<td>2.2 (0.1 to 4.5)</td>
<td>0.0371</td>
</tr>
<tr>
<td>Median (range)</td>
<td>7.0 (3.0–7.9)</td>
<td>9.0 (3.0–48.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of all-cause diarrhoeal episode (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>2.2 (2.3)</td>
<td>3.0 (2.8)</td>
<td>0.8 (0.2 to 1.4)</td>
<td>0.0080</td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.6 (&lt;1 to 14.0)</td>
<td>2.2 (&lt;1 to 16.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LT=heat-labile toxin. *Wilcoxon test for difference between treatments.

Table 3: Immunogenicity (per-protocol population)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>LT-patch group</th>
<th>Placebo group</th>
<th>Geometric mean titre</th>
<th>Relative increase</th>
<th>Seroconversion, n (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-LT IgG*</td>
<td>386 (11)</td>
<td>397 (86)</td>
<td>817</td>
<td>0.79</td>
<td>106 (13%)</td>
</tr>
<tr>
<td>Prevaccination†</td>
<td>3400 (29)</td>
<td>315 (41)</td>
<td>111</td>
<td>0.72</td>
<td>95 (12%)</td>
</tr>
<tr>
<td>Post-vaccination†</td>
<td>88 (77)</td>
<td>79 (10)</td>
<td>14</td>
<td>0.74</td>
<td>106 (13%)</td>
</tr>
<tr>
<td>Anti-LT IgA*</td>
<td>44 (40)</td>
<td>44 (46)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevaccination†</td>
<td>127 (22)</td>
<td>36 (21)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-vaccination†</td>
<td>286 (82)</td>
<td>82 (82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxin-neutralising assay*</td>
<td>330 (41%)</td>
<td>15 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevaccination†</td>
<td>20 (9)</td>
<td>20 (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-vaccination†</td>
<td>45 (24)</td>
<td>20 (17)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LT=heat-labile toxin. IgG=immunoglobulin. *For anti-LT IgG and anti-LT IgA, n=809 for LT-patch group and n=814 for placebo group. †Post-vaccination assessment took place on arrival in destination country. ‡For anti-LT IgG and the toxin-neutralising assay, seroconversion was defined as a two-times or greater increase in titre. §For anti-LT IgA, seroconversion was defined as a four-times or greater increase in titre. ¶For the toxin-neutralising assay, n=788 for the LT-patch group and n=804 for the placebo group.

Data analysis was by an independent body commissioned by the sponsor. Data interpretation was by the authors, among whom the sponsor was by represented SD, who also contributed to the writing of the report. RHB, JPC, HLD, and SD had full access to all the data in the study. The corresponding author had final responsibility for the decision to submit for publication.

Results

The study recruited 2036 participants between Oct 14, 2009, and Aug 13, 2010. 2034 participants received at least one vaccination and were therefore analysed in the safety population. Because of various protocol violations (procedural errors), the total per-protocol population was 1644 participants, with 821 participants in the LT-patch group and 823 in the placebo group (figure). The mean age of participants in the per-protocol population was 28–8 years (median 26 years; range 18–64 years), and 888 (54%) were women. 880 (54%) participants were recruited in the UK and 764 (46%) in Germany. 1130 (69%) participants visited Mexico and 514 (31%) visited Guatemala. The mean time between the second vaccination and arrival in the destination country was 20 · 3 days in both groups.

The proportion of participants with moderate-to-severe ETEC diarrhoea was very low in both groups, with a non-significantly lower proportion affected in the LT-patch group than in the placebo group (table 1). The results for the modified intention-to-treat population (ie, all travellers) were similar (vaccine efficacy 27·8%, 95% CI −10·5 to 53·3; p=0·13). The study therefore did not meet the primary endpoint. We also noted no difference in the proportion of participants affected by moderate-to-severe all-cause diarrhoea (table 1).

In a subgroup analysis by ETEC enterotoxin type, the vaccine seemed to be somewhat protective against LT-positive ETEC, but not against ST-positive or LT/ST-positive ETEC (table 1). With respect to the secondary endpoints, we noted significant reductions in duration of all-cause diarrhoeal episodes and frequency of unformed stools per all-cause diarrhoeal episode in the LT-patch group (table 2). We noted no significant differences in any of the tertiary efficacy endpoints (appendix p 1).

Table 3 shows the prevaccination and post-vaccination immunogenicity results. We did not note any association between immunogenicity outcomes and frequency of primary endpoint events (table 4). 12 (3%) of 417 LT-vaccinated participants who had toxin-neutralising antibody seroconversions had primary endpoint events, compared with 46 (6%) of the 804 participants assessed the placebo group (only four of whom had...
toxin-neutralising antibody seroconversions; p=0.045). The small number of LT-only primary endpoint events in the LT-patch group (n=5) limited the usefulness of doing an association analysis in this subgroup.

Throughout the study 9333 local adverse events were reported in 943 (93%) of 1015 participants in the LT-patch group, compared with 1444 local adverse events in 574 (56%) of 1019 participants in the placebo group (p<0.0001). No difference was seen in the frequency of systemic adverse events incidence between the groups (appendix p 1). Serious adverse events occurred in 25 participants (14 in the LT-patch group and 11 in the placebo group), including two deaths from a road traffic accident in the LT-patch group; all serious adverse events were regarded as either unrelated or possibly related to treatment.

Table 5 lists the solicited (ie, from a predefined list rather than spontaneously described) local adverse events after the first and second vaccinations. All solicited local adverse events (erythema, rash, pruritus, hyperpigmentation, pain, hypopigmentation, and oedema) occurred in a significantly higher proportion of participants in the LT-patch group than in the placebo group. Most of these events were mild or moderate in severity, as self-assessed by the participants; however, some severe and grade 4 events were reported in both groups (appendix pp 2–3). Mild-to-moderate hyperpigmentation persisted for at least 180 days after vaccination in 150 (18%) of 849 participants who received both vaccinations and returned for final assessment in the LT-patch group, compared with none of 842 participants in the placebo group.

Table 5

<table>
<thead>
<tr>
<th>Frequency of primary endpoint events*</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log-transformed immunoglobulin G titre at entry to destination country</td>
<td>0.4256</td>
</tr>
<tr>
<td>First tertile (≤0.352; n=268)</td>
<td>11 (4.1%, 2.0–7.1)</td>
</tr>
<tr>
<td>Second tertile (0.352–3.821; n=272)</td>
<td>12 (4.4%, 2.3–7.5)</td>
</tr>
<tr>
<td>Third tertile (&gt;3.821; n=259)</td>
<td>7 (2.7%, 1.1–5.4)</td>
</tr>
<tr>
<td>Log-transformed immunoglobulin A titre at entry to destination country</td>
<td>1.0000</td>
</tr>
<tr>
<td>First tertile (≤1.839; n=264)</td>
<td>9 (3.4%, 1.6–6.3)</td>
</tr>
<tr>
<td>Second tertile (1.839–3.382; n=279)</td>
<td>12 (4.3%, 2.3–7.5)</td>
</tr>
<tr>
<td>Third tertile (&gt;3.382; n=264)</td>
<td>9 (3.4%, 1.5–6.3)</td>
</tr>
</tbody>
</table>

Data are n (%), unless otherwise indicated. A primary endpoint event was an episode of moderate-to-severe ETEC diarrhoea. *Percentages are based on the number of participants in each tertile. †Cochran-Armitage exact test for trend.

Table 4: Frequency of primary endpoint events in the LT-patch group, by immunogenic response tertile (per-protocol population)

Table 5: Solicited local adverse events after first and second vaccinations (safety population)

Discussion

Although the LT antigen was delivered effectively by the skin patch, the trial did not reach its primary endpoint of prevention of moderate-to-severe ETEC diarrhoea. The vaccine also did not protect travellers against all-cause diarrhoea (panel). Our results did show a small amount of protection against LT-positive ETEC diarrhoea, and the vaccine seemed to be effective at slightly reducing the severity and duration of all-cause diarrhoea. Our results are similar to those of a parallel run, phase 2 study in travellers to India, which showed similarly low efficacy against all-cause and ETEC diarrhoea in 299 LT-patch recipients.

Our findings contrast with those of the phase 2 study by Frech and colleagues, in which the same vaccine system in participants travelling to the same destination countries protected 75% of recipients against all-cause moderate-to-severe diarrhoea, although protection against ETEC diarrhoea specifically was non-significant. Surprisingly, in Frech and colleagues’ study no apparent protection was seen against diarrhoea associated with LT-producing ETEC, leading the investigators to speculate that the vaccine had a broader effect beyond LT-producing ETEC. However, Frech and colleagues’ study was smaller than ours, with efficacy results based on 59 participants who received an LT patch, and was not designed to assess vaccine efficacy. The proportion of participants affected by all-cause, all-severity diarrhoea in the placebo group of Frech and colleagues’ study was higher (22%) than in the present study (15%). ETEC isolation from the placebo group was similarly higher in Frech and colleagues’ study than in our own (10% vs 8%). That the proportion of participants affected by moderate-to-severe ETEC diarrhoea in the placebo group of our study was lower (7%) than expected might have reduced the precision of our findings. The...
Articles

Panel: Research in context

Systematic review
We searched PubMed and Embase for reports published in English up to Nov 5, 2013, using the MeSH terms “Escherichia coli vaccines”, “randomized controlled trial”, “administration, cutaneous”, and “human”. We identified three studies that assessed a vaccine patch containing enterotoxigenic Escherichia coli (ETEC) heat-labile enterotoxin (LT). Glenn and colleagues described the safe delivery of the LT antigen and the induction of increased anti-LT antibodies. The delivery method and antigen were then examined for safety and efficacy in a double-blind, placebo-controlled challenge study, in which participants were given 50 μg of LT protein via the skin patch. The results suggested that the vaccine generated some reduction in the severity of ETEC diarrhea in volunteers challenged orally with ETEC pathogens. Finally, a phase 2 randomised, double-blind, placebo-controlled field trial was done in volunteers visiting Mexico or Guatemala. The vaccine seemed to be immunogenic and effective against all-cause moderate-to-severe travellers’ diarrhoea, although protection against ETEC diarrhoea specifically was non-significant. The results also suggested that the patch vaccine could reduce the severity and duration of diarrhoeal episodes.

Interpretation
Although in our trial the LT antigen was delivered effectively by the skin patch, leading to an LT-specific immunogenic response, the vaccine did not protect travellers against diarrhoea. The vaccine did seem to provide a small amount of protection against LT-positive ETEC, but not against ST-positive or ST-positive ETEC, nor against diarrhoea caused by other pathogens. Future vaccines against travellers’ diarrhoea might need to include several antigens against various diarrhoeal pathogens, and might need to be able to generate mucosal and higher systemic immunity.

The LT component in the patch probably caused this vigorous dermal immune response in nearly all vaccine recipients, resulting in both local discomfort and skin changes during the vaccination and surveillance phase. Dermatitis associated with the skin-patch delivery of protein antigens needs to be better understood and the system refined to improve future tolerability of transdermal patch delivery with other candidate vaccines.

Immunogenicity results confirmed delivery of the LT antigen, with vaccine recipients having higher anti-LT IgG titres than placebo recipients. Functional antibodies against LT toxin, measured by toxin-neutralising antibody assay, were much higher in the LT-patch group than in the placebo group, with seroconversion apparent in more than half of the LT-patch recipients. However, serum IgG and IgA titres were not associated with vaccine efficacy, suggesting that such titres cannot be used as a correlate of protection. This finding is in agreement with the results of McKenzie and colleagues’ study, in which participants given transdermal vaccination with 50 μg LT antigen were challenged orally with LT/ST-positive ETEC. The investigators noted significantly raised titres of IgG and toxin-neutralising antibody, with much larger increases in geometric mean titres of IgG, IgA, and toxin-neutralising antibody than in the present study. However, this immune response did not translate into protection against clinical diseases, although it did reduce disease severity.

The inability of the patch vaccine to achieve protection might be related to the low proportion (40%) of IgA seroconversions, although higher IgA seroconversion in the Indian study (60%) did not improve the outcome. The proportion of participants who required antibiotic rescue treatment did not differ between the LT-patch and placebo groups in our study, despite the differences in severity and duration of diarrhoea. We could not have predicted the low efficacy of the LT-patch vaccine on the basis of the apparent benefits seen in a previous volunteer challenge study and in the phase 2 trial. The anti-LT IgG response was several times lower than in the phase 2 study and earlier challenge studies. The skin preparation system and patch used in this study were the same as those described by Frech and colleagues and used by Frech and colleagues in the phase 2 study. It is therefore unlikely that the reduced immunogenicity in this phase 3 study can be attributed to the vaccine delivery system. The low efficacy might be partly related to the suboptimum antibody and mucosal immune response to neutralise the toxin. McKenzie and colleagues’ challenge study assessed protection against ingestion of one very large ETEC challenge, whereas in nature exposure seems to occur recurrently with lower doses of pathogen. Our findings might have been more precise (ie, with smaller CIs) if the incidence of travellers’ diarrhoea in the placebo group been higher. An important contribution to the low efficacy with respect to all-cause diarrhoea was the high prevalence of non-ETEC diarrhoea that we
identified; in this trial a 100% effective ETEC vaccine would still have had only about 50% vaccine efficacy for moderate-to-severe all-cause diarrhoea.

The incidence of travellers’ diarrhoea is affected by several factors related to the host and the environment. Robust epidemiological data for the true incidence of ETEC diarrhoea in travellers is not available, but the proportion of participants affected by ETEC diarrhoea in our study was lower than expected. One review reported ETEC infections in 11–16% of travellers to endemic areas, although incidence varies substantially with destination and exposure. ETEC diarrhoea has been reported in 24% of travellers to Goa, India, 35% of travellers to Mombasa, Kenya, and 37.5% of travellers to Latin America and the Caribbean.1,14 The proportion of participants with all-cause, all-serotype travellers’ diarrhoea in the placebo group of the present study (15.2%) was also lower than figures reported previously for these countries—eg, 16% for Mexico and 39% for Guatemala.1 In the parallel study in travellers to India,1 of 304 participants in the placebo group had all-cause travellers’ diarrhoea.

Many different reasons could account for the low proportion of participants affected by travellers’ diarrhoea in the present study—eg, participants might have maintained good hygienic standards. Another factor that affects the number of people who will get travellers’ diarrhoea is the duration of stay. According to Cabada and colleagues,11 staying less than 7 days is a protective factor against travellers’ diarrhoea. To rule out the potential effect of length of stay, we did an additional analysis in the subpopulation of participants who stayed in the destination country for at least 2 weeks (n=286 for the LT-patch group, n=321 for the placebo group), but the proportion affected by ETEC travellers’ diarrhoea did not change (data not shown).

Our study follows on from previous attempts to develop a vaccine to prevent ETEC diarrhoea in non-immune travellers to endemic countries. Zhang and Sack1 have reviewed the effectiveness of ETEC vaccines and noted that little evidence exists for efficacy in paediatric populations in endemic countries, but some benefit has been seen in travellers. The cholera toxin B subunit is a very closely related structure to the ETEC LT toxin and seems to provide cross-reactive mucosal immunity to the LT toxin.15,16 Much ETEC vaccine research has focused on combination vaccines containing ETEC colonisation factor antigen and LT or recombinant cholera toxin B subunit antigen, with some effect seen when mucosal immunity has been induced.18 A Cochrane review19 has assessed the evidence for the protective efficacy of both oral cholera B subunit and ETEC-specific vaccines in the prevention of travellers’ diarrhoea. An oral cholera vaccine containing killed whole cells and recombinant cholera toxin B subunit did not have any significant effects on ETEC or all-cause diarrhoea (one trial, n=502). An earlier oral cholera vaccine containing killed whole cells and purified cholera toxin B subunit provided short-term protective efficacy against ETEC diarrhoea, lasting for roughly 3 months (risk ratio [RR] 0.43, 95% CI 0.26–0.71; two trials; n=50 227). An oral ETEC-specific combined killed whole-cell and recombinant cholera toxin B subunit vaccine did not have a significant effect on ETEC or all-cause diarrhoea (two trials; n=799), and was associated with increased vomiting (RR 2.0, 95% CI 1.16–3.45; nine trials; n=1528). The Cochrane investigators also noted that other ETEC-specific vaccines in development have not yet shown clinically important benefits.

Our findings have shown that a transdermal patch can effectively deliver an antigen from which an IgG and IgA antibody response can be generated. However, despite this immunogenic response, the vaccine did not prevent travellers’ diarrhoea. Using a single-antigen vaccine to prevent the syndrome of travellers’ diarrhoea, which can be caused by several different pathogens or other insults (such as drugs and toxins), might not be realistic. Future vaccines against travellers’ diarrhoea might need to include several antigens against various diarrhoeal pathogens, and might need to be able to generate mucosal immunity, as with the orally administered anti-cholera vaccine.20 Furthermore, any future studies to assess vaccines against ETEC in travellers must be adequately powered to be able to show efficacy, taking into account the possibility of low numbers of ETEC infections in the study population.

Contributors
RHB was a study investigator, interpreted the results, cowrote the first draft of the report, and edited all versions of the report. SD cowrote the first draft of the report and reviewed subsequent drafts. DMS developed the statistical plan and did the statistical analysis, interpreted the results, and reviewed the final report. JPC, TJ, HS, FvS, DW, TW, DJB, EA, HLEP, RM, and MP-P were study investigators and reviewed the final report. Z-DJ analysed the study samples and reviewed the final report. GMG contributed to the original product and study design, and reviewed and contributed to the final report. HLD contributed to the study design, interpreted the results, and reviewed and edited the final report.

Conflicts of interest
SD is employed by Intercell AG. All participating institutions received funding from Intercell USA for contract research in conjunction with this clinical trial, from which salaries of investigators have been partly covered. HLD has served as a consultant to, and received funding for research from, Salix Pharmaceuticals. RHB is on the advisory board of, and has received educational support from, Norgine Pharmaceuticals. JPC has been a clinical investigator and member of the data safety monitoring board of other Intercell-sponsored vaccine trials.

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