Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea (Review)

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TABLE OF CONTENTS

[Intervention Review]

Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

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ABSTRACT

Background

Infection with enterotoxigenic *Escherichia coli* (ETEC) bacteria is a common cause of diarrhoea in adults and children in developing countries and is a major cause of 'travellers' diarrhoea' in people visiting or returning from endemic regions. A killed whole cell vaccine (Dukoral®), primarily designed and licensed to prevent cholera, has been recommended by some groups to prevent travellers' diarrhoea in people visiting endemic regions. This vaccine contains a recombinant B subunit of the cholera toxin that is antigenically similar to the heat labile toxin of ETEC. This review aims to evaluate the clinical efficacy of this vaccine and other vaccines designed specifically to protect people against diarrhoea caused by ETEC infection.

Objectives

To evaluate the efficacy, safety, and immunogenicity of vaccines for preventing ETEC diarrhoea.

Search methods

We searched the Cochrane Infectious Disease Group Specialized Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, LILACS, and http://clinicaltrials.gov up to December 2012.

Selection criteria

Randomized controlled trials (RCTs) and quasi-RCTs comparing use of vaccines to prevent ETEC with use of no intervention, a control vaccine (either an inert vaccine or a vaccine normally given to prevent an unrelated infection), an alternative ETEC vaccine, or a different dose or schedule of the same ETEC vaccine in healthy adults and children living in endemic regions, intending to travel to endemic regions, or volunteering to receive an artificial challenge of ETEC bacteria.

Data collection and analysis

Two authors independently assessed each trial for eligibility and risk of bias. Two independent reviewers extracted data from the included studies and analyzed the data using Review Manager (RevMan) software. We reported outcomes as risk ratios (RR) with 95% confidence intervals (CI). We assessed the quality of the evidence using the GRADE approach.

Main results

Twenty-four RCTs, including 53,247 participants, met the inclusion criteria. Four studies assessed the protective efficacy of oral cholera vaccines when used to prevent diarrhoea due to ETEC and seven studies assessed the protective efficacy of ETEC-specific vaccines. Of these 11 studies, seven studies presented efficacy data from field trials and four studies presented efficacy data from artificial challenge studies. An additional 13 trials contributed safety and immunological data only.

Cholera vaccines

The currently available, oral cholera killed whole cell vaccine (Dukoral®) was evaluated for protection of people against 'travellers' diarrhoea' in a single RCT in people arriving in Mexico from the USA. We did not identify any statistically significant effects on ETEC diarrhoea or all-cause diarrhoea (one trial, 502 participants, *low quality evidence*).

Two earlier trials, one undertaken in an endemic population in Bangladesh and one undertaken in people travelling from Finland to Morocco, evaluated a precursor of this vaccine containing purified cholera toxin B subunit rather than the recombinant subunit in Dukoral®. Short term protective efficacy against ETEC diarrhoea was demonstrated, lasting for around three months (RR 0.43, 95% CI 0.26 to 0.71; two trials, 50,227 participants). This vaccine is no longer available.

ETEC vaccines

An ETEC-specific, killed whole cell vaccine, which also contains the recombinant cholera toxin B-subunit, was evaluated in people travelling from the USA to Mexico or Guatemala, and from Austria to Latin America, Africa, or Asia. We did not identify any statistically significant differences in ETEC-specific diarrhoea or all-cause diarrhoea (two trials, 799 participants), and the vaccine was associated with increased vomiting (RR 2.0, 95% CI 1.16 to 3.45; nine trials, 1528 participants). The other ETEC-specific vaccines in development have not yet demonstrated clinically important benefits.

Authors' conclusions

There is currently insufficient evidence from RCTs to support the use of the oral cholera vaccine Dukoral® for protecting travellers against ETEC diarrhoea. Further research is needed to develop safe and effective vaccines to provide both short and long-term protection against ETEC diarrhoea.

PLAIN LANGUAGE SUMMARY

Vaccines for preventing diarrhoea caused by enterotoxigenic *Escherichia coli* bacteria

Enterotoxigenic *E. coli* (ETEC) is a type of bacteria that can infect both children and adults, causing diarrhoea. In particular, it affects people in developing countries. However, it is also a major cause of 'travellers' diarrhoea' in people visiting or returning from regions where this infection is common. It is transmitted from person to person by eating or drinking unclean food or water. Typically it causes watery diarrhoea, with abdominal pains and vomiting, that can last for several days. Vaccines are being considered as a way to prevent diarrhoea caused by ETEC bacteria. ETEC bacteria share some similarities with the bacteria that cause cholera. In this review, we examined the effectiveness of either vaccines designed to prevent cholera or vaccines designed specifically to prevent ETEC infection for preventing ETEC diarrhoea. We compared these vaccines against the use of a control vaccine (either an inert vaccine or a vaccine normally given to prevent an unrelated infection), no intervention, an alternative ETEC vaccine, or a different dose or schedule of the same ETEC vaccine.

We examined the research published up to 07 December 2012. We included 24 randomized controlled trials and 53,247 participants in this review. Four studies assessed the use of oral cholera vaccines to prevent diarrhoea caused by ETEC and eight trials assessed the use of ETEC-specific vaccines to prevent diarrhoea. Seven studies presented data from field trials and four studies presented data from studies where people were artificially infected with ETEC bacteria. Also, 13 trials gave safety and immunological data only.

There is currently insufficient evidence to support the use of the oral cholera vaccine Dukoral® to protect travellers against ETEC diarrhoea. Based on a single trial in people travelling from the USA to Mexico, the oral cholera vaccine Dukoral® may have little or no effect in preventing ETEC diarrhoea (one trial, 502 participants, *low quality evidence*). Two earlier trials, one undertaken in an endemic population in Bangladesh and one undertaken in people travelling from Finland to Morocco, evaluated a precursor of the oral cholera vaccine Dukoral®. Short term protection against ETEC diarrhoea was demonstrated, lasting for around three months (RR 0.43, 95% CI 0.26 to 0.71; two trials, 50,227 participants). However, this vaccine is no longer available.

An ETEC-specific, killed whole cell vaccine, which also contains the recombinant cholera toxin B-subunit, was evaluated in people travelling from the USA to Mexico or Guatemala, and from Austria to Latin America, Africa, or Asia. There were no statistically significant differences in ETEC-specific diarrhoea or all-cause diarrhoea (two trials, 799 participants) found and the vaccine was associated with increased vomiting (RR 2.0, 95% CI 1.16 to 3.45; nine trials, 1528 participants). The other ETEC-specific vaccines in development have not yet demonstrated clinically important benefits. Further research is needed to develop safe and effective vaccines to provide both short and long-term protection against ETEC diarrhoea.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

Cholera killed whole cells plus recombinant B-subunit vaccine for enterotoxigenic *E. coli* (ETEC) diarrhoea

Patient or population: People travelling from non-endemic settings
Settings: Endemic settings
Intervention: Cholera killed whole cells plus recombinant B-subunit vaccine (WC-rCTB Cholera)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Vaccine				
ETEC diarrhoea	**99 per 1000**	**120 per 1000** (72 to 198)	**RR 0.93** (0.61 to 1.41)	502 (1 study)	⊕⊕◯◯ **low**[1,2,3,4]	
Severe ETEC diarrhoea	-	-	-	(0 studies)	-	
All-cause diarrhoea	**492 per 1000**	**512 per 1000** (428 to 610)	**RR 1.04** (0.87 to 1.24)	502 (1 study)	⊕⊕◯◯ **low**[1,4,5]	
Adverse events	-	-	-	502 (1 study)	[6]	

*The basis for the **assumed risk** (eg the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] This single study was conducted in adults travelling from the USA to Mexico (Scerpella 1995). Although the paper does not clearly describe the methods used to prevent selection bias, we have not downgraded the evidence as selection bias is probably unlikely in a trial where everyone is healthy at enrolment.

[2] Two older trials evaluated a prototype of this vaccine which contained purified cholera B-subunit rather than the recombinant subunit contained in this vaccine. Although both trials found some evidence of benefit, the evidence may no longer be applicable due to changes in both composition and dosing of the vaccine.

[3] Downgraded by one for indirectness: in this study the vaccine was provided in two doses 10 days apart after the travellers had arrived in Mexico. Most cases of ETEC diarrhoea occurred between doses or within seven days of administration of the second dose. The authors conducted a subgroup analysis of only those participants who had diarrhoea > 7 days after the second dose, which excluded 75% of cases. We did not find a statistically significant difference in our analysis of this data.

[4] Downgraded by one for imprecision: this trial was small and underpowered to reliably prove or exclude a clinically important effect with the vaccine.

[5] Downgraded by one for indirectness: in this study the vaccine was provided in two doses 10 days apart after the travellers had arrived in Mexico. Further studies are required which assess administration prior to travel to a variety of destinations.

[6] Scerpella 1995 reported no differences in the frequency of gastrointestinal symptoms, headaches, or febrile illnesses between vaccinees, or placebo recipients but data were not presented.

BACKGROUND

Description of the condition

Enterotoxigenic *Escherichia coli* (ETEC) is the most common bacterial cause of diarrhoea in adults and children in developing countries (Qadri 2005; Walker 2007). The annual incidence of this disease is highest in young children and susceptibility to the disease declines with age. Children born in endemic regions are likely to experience two to three episodes of ETEC diarrhoea before their fifth birthday (Wennerås 2004). The practical difficulties associated with making an accurate diagnosis of ETEC in low-resource settings mean that its significance has often been underestimated (Wennerås 2004; Qadri 2005). However, a review of microbiological studies conducted in endemic regions between 1992 and 2000 found that ETEC was the causative organism in approximately 25% of all diarrhoeal episodes in children aged between one and four years (Wennerås 2004). Many more children were shown to carry the organism asymptomatically in their gut (Walker 2007). A global burden of approximately 280 million clinical episodes and 380,000 deaths annually are estimated (WHO 2009).

Person-to-person transmission of ETEC occurs via ingestion of faecally-contaminated food or water. In developed countries where sanitation standards are usually higher, ETEC infection is rare. However, it remains a major cause of 'travellers' diarrhoea' which occurs in people visiting or returning from ETEC-endemic regions (Qadri 2005; DuPont 2008; Widermann 2009). Epidemics of ETEC diarrhoea have also occurred during natural disasters, such as floods, where there has been an acute deterioration in the quality of drinking water and sanitation (Schwartz 2006; Harris 2008).

The clinical illness is characterized by a profuse watery diarrhoea that lasts for several days and may be associated with abdominal cramp, malaise, vomiting, and a low grade fever. Without adequate treatment this can lead to dehydration. If people have a prolonged infection or are infected again, this can lead to malnutrition or growth inhibition in young children (Black 1984; Qadri 2005; Qadri 2007).

Following ingestion, ETEC bacteria adhere to the lining of the gut and secrete either one or both types of enterotoxins: the heat labile toxin (LT) and the heat stable toxin (ST). These toxins induce the hypersecretion of fluids and electrolytes, which cause the typical watery diarrhoea (Gill 1980). Different strains of ETEC can be further characterized on the basis of the antigens expressed on the cell surface: the colonization factor (CF), and the 'O' and 'H' antigens (Wolf 1997). Some of these antigens have been shown to be important in inducing natural immunity and therefore represent key targets for vaccine development (Rao 2005; Svennerholm 2008). Over 100 different "O" antigens can be present on ETEC and therefore have not been considered important for vaccine development. Since both antitoxic and antibacterial antibodies are important for protection, most vaccine formulations have been based on the enterotoxins and CFs of ETEC (Svennerholm 1984; Ahren 1998). Important antigens considered until now for vaccine development include the LT and CFs. Over 25 CFs that have been characterized and most common CFs present on clinical isolates include CFA/I, CS1, CS2, CS3, CS5, and CS6. These CFs have been included as vaccine antigens on ETEC vaccines to date (Harro 2011; Tobias 2011; Tobias 2012).

Improvements in public health and sanitation conditions represent the ideal solution to preventing transmission of ETEC and other faecally-transmitted organisms. However, this can be difficult to achieve given the financial and logistical constraints in low-resource regions. Thus prophylactic measures, including vaccines, are being considered as alternative short-term strategies (Walker 2007).

Description of the intervention

Only one vaccine (Dukoral® produced by SBL Sweden) is currently available for the prevention of ETEC diarrhoea. This vaccine has been recommended to prevent 'travellers' diarrhoea' in people visiting endemic regions from developed countries (Steffen 2005). This vaccine is primarily designed and licensed to prevent diarrhoea due to *Vibrio cholerae* (cholera), but it contains a recombinant B subunit of the cholera toxin that is antigenically very similar to the LT of ETEC (Walker 2007). In an early clinical trial, using a prototype of this vaccine which contained purified cholera B subunit rather than the recombinant form, significant cross protection against ETEC diarrhoea was demonstrated (Clemens 1988).

Many alternative vaccine candidates designed specifically to protect people against ETEC diarrhoea are now at various stages of clinical development (Table 1). The vaccine candidates can be broadly categorized in to two groups: inactivated vaccines containing killed whole cells, purified CF antigens, or inactivated LT; and live attenuated vaccines containing genetically modified, non-pathogenic strains of ETEC, or alternative carrier bacteria expressing the important ETEC antigens (Svennerholm 2008). Given the number of antigenically different strains of ETEC, it is likely that a vaccine formulation capable of providing broad protection would need to contain a combination of the most commonly expressed antigens (Walker 2007; Svennerholm 2008).

How the intervention might work

Epidemiological and experimental data suggest that natural immunity to ETEC does occur following natural infection and antibodies against CF antigens and the B subunit of LT have been detected (Qadri 2005; Rao 2005). Vaccine candidates aim to induce similar immunity (without the associated clinical illness) and to provide lasting protection against a broad range of the pathogenic ETEC strains (Svennerholm 2008). Attempts have been made to find immunological markers of protection, including toxin- or

CF-specific immune responses, or both. CF-specific antibodies have been used to determine 'take rates' (the proportion of vaccinations that induce high antibody levels to vaccine) for ETEC vaccines containing CFs as components (Wenneras 1999; Qadri 2003; Rao 2005). However, adequate and lasting protection cannot be assumed without demonstrating reduced incidence of the clinical illness in large, well conducted clinical trials.

The route of administration of a vaccine may influence both its immunogenicity and acceptability. Oral vaccines have the potential to stimulate local immunity within the mucosa of the gut, preventing the colonization and multiplication of the bacteria. ETEC is transmitted through the faecal-oral route and vaccines designed to be given orally have been developed (Holmgren 2005). Such vaccines are easy to administer in all settings and have a reduced risk of transmitting blood-borne infections.

Why it is important to do this review

Assessment of the level of mortality and morbidity associated with ETEC diarrhoea and the extent of the global disease burden has resulted in several initiatives to develop effective vaccines (Walker 2007). ETEC vaccine development is at an earlier stage than some other vaccines (eg cholera vaccine) but data from phase II and phase III trials in endemic areas and non-primed participants are available and these need to be reviewed.

This review aims to evaluate the efficacy, safety, and immunogenicity of current vaccine candidates tested in randomized controlled trials (RCTs), including the oral cholera vaccine Dukoral®, when used to protect against ETEC diarrhoea.

OBJECTIVES

To evaluate the efficacy, safety, and immunogenicity of vaccines for preventing enterotoxigenic ETEC diarrhoea.

METHODS

Criteria for considering studies for this review

Types of studies

RCTs and quasi-RCTs, for which the unit of randomization is the individual participant or a cluster of participants.

Types of participants

Healthy adults and children living in endemic regions, intending to travel to endemic regions, or receiving an artificial challenge.

Types of interventions

Intervention

Any vaccine being used to prevent ETEC diarrhoea. Studies evaluating vaccines which have not yet been evaluated for clinical outcomes will be excluded.

Control

No intervention, a control vaccine (either an inert vaccine or a vaccine normally given to prevent an unrelated infection), an alternative ETEC vaccine, or a different dose or schedule of the same ETEC vaccine.

Types of outcome measures

Primary outcomes

Protective efficacy as measured against:

- Episodes of ETEC diarrhoea (any severity)
- Severe episodes of ETEC diarrhoea

Secondary outcomes

Protective efficacy as measured against:

- Episodes of all-cause diarrhoea
- Severe episodes of all-cause diarrhoea

Safety measured as:

- The number of adverse events, including systemic and local reactions.

Immunological outcomes:

- Any immunological measure of response to vaccination, eg an increase in CF, or toxin-specific, immune responses in serum/plasma, or both, or an increase in antibody-secreting cell responses in lymphocytes.

Search methods for identification of studies

We attempted to identify all relevant studies regardless of language or publication status (published, unpublished, in press, or in progress).

Electronic searches

Published studies

We searched the Cochrane Infectious Disease Group Specialized Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, LILACS and http://clinicaltrials.gov/, using the search terms detailed in Table 2.

Ongoing studies

We searched the metaRegister of Controlled Trials (mRCT) and the WHO International Clinical Trials Registry Platform (ICTRP) for ongoing trials using 'Enterotoxigenic' and 'vaccin*' as search terms.

Searching other resources

Reference lists

We searched the reference lists of all included studies for additional references relevant to this review.

Data collection and analysis

Selection of studies

Tanvir Ahmed (TA) and Taufiqur Bhuiyan (TB) independently screened all citations and abstracts identified by the search strategy to identify potentially eligible studies. We obtained full-text articles of potentially eligible studies. TA and TB independently assessed these articles for inclusion in the review using a pre-designed eligibility form based on the inclusion criteria.

In the event that it was unclear whether a trial was eligible for the review, we resolved any differences in opinion through discussion with Firdausi Qadri (FQ). We excluded any studies that did not meet the inclusion criteria and we documented the reasons for exclusion.

Data extraction and management

For each included trial, TA and TB independently extracted information on the characteristics of the trial (study design, study dates and duration, study location, setting, and source of funding), the participants recruited (the inclusion and exclusion criteria), and the intervention (the type of vaccine, type of placebo, dose, and immunization schedule), and listed the outcomes presented in the papers using a pre-tested data extraction form. For all outcomes, we extracted the number of patients randomized to each treatment group and the number of patients for whom an outcome was available. For dichotomous outcomes, we extracted the number of participants that experienced the event and the number of patients in each treatment group. We extracted adverse event data for each individual type of event wherever possible. Where adverse events were reported for more than one dose, the number of people reporting each side-effect after each dose was recorded. Where trials reported the occurrence of adverse events over time following a single dose, we recorded the proportion of people affected during each time period. If the denominator or total number of people affected for each time period was not clear, then we only recorded the events that occurred in the first time period (typically 72 hours) after each dose. Where data were missing or incomplete, we contacted the authors for clarification. In cases of disagreement, we double-checked the data extraction and we resolved any disagreements through discussion.

Assessment of risk of bias in included studies

Two authors (TA and TB) independently assessed the risk of bias of each trial using 'The Cochrane Collaboration's tool for assessing the risk of bias' (Higgins 2008). We followed the guidance to assess whether steps were taken to reduce the risk of bias across six domains: sequence generation; allocation concealment; blinding (of participants, personnel, and outcome assessors); incomplete outcome data; selective outcome reporting; and other sources of bias.

For sequence generation and allocation concealment, we reported the methods used. For blinding, we described who was blinded and the blinding method. For incomplete outcome data, we reported the percentage and proportion lost to follow-up in each group. For selective outcome reporting, we stated any discrepancies between the methods used and the results, in terms of the outcomes measured or the outcomes reported. For other biases, we described any other trial features that we thought could have affected the trial result (eg if the trial was stopped early).

We categorized our judgements as either low, high, or unclear risk of bias. We used this information to guide our interpretation of the data. Where our judgement was unclear risk of bias, we attempted to contact the trial authors for clarification and we resolved any differences of opinion through discussion.

Measures of treatment effect

We expressed dichotomous outcomes using risk ratios (RR), and presented with 95% confidence intervals (CI).

Unit of analysis issues

We ensured that the same patients were not included in the same meta-analysis more than once, by grouping or splitting the data in multi-arm trials as appropriate.

Dealing with missing data

If data from the trial reports were insufficient, unclear, or missing, we attempted to contact the trial authors for additional information. We aimed to carry out an intention-to-treat analysis. However if the duration of follow-up of all patients was not known, we carried out a complete case analysis, in which we only included patients for whom an outcome was available.

Assessment of heterogeneity

We assessed heterogeneity between the trials by examining the forest plot to check for overlapping CIs, by using the Chi^2 test for heterogeneity with a 10% level of significance, and by using the I^2 statistic with a value of 50% to represent moderate levels of heterogeneity.

Assessment of reporting biases

We did not assess publication bias using funnel plots because the number of trials per comparison were insufficient.

Data synthesis

We analyzed the data using Review Manager (RevMan).
We used the Mantel-Haenszel method to combine dichotomous data. If there was no heterogeneity present, we used a fixed-effect model. If there was moderate heterogeneity and it was still appropriate to combine studies, we used a random-effects model. When it was deemed inappropriate to combine studies due to methodological or statistical heterogeneity, we presented the data in tables. We stratified the primary analysis by vaccine type.

Subgroup analysis and investigation of heterogeneity

We planned to conduct subgroup analyses to investigate causes of heterogeneity but the data were too limited.

Sensitivity analysis

As the number of trials per comparison were insufficient, we did not conduct the pre-planned sensitivity analysis to assess the robustness of the results against risk of bias judgements.

Assessment of the quality of the evidence

We assessed the quality of evidence using the GRADE approach (Guyatt 2008). The GRADE system considers 'quality' to be a judgment of the extent to which we can be confident that the estimates of effect are correct. The level of 'quality' is judged on a four-point scale. Evidence from RCTs is initially graded as high and downgraded by either one, two, or three levels after full consideration of: any limitations in the design of the studies, the directness (or applicability) of the evidence, the consistency and precision of the results, and the possibility of publication bias.

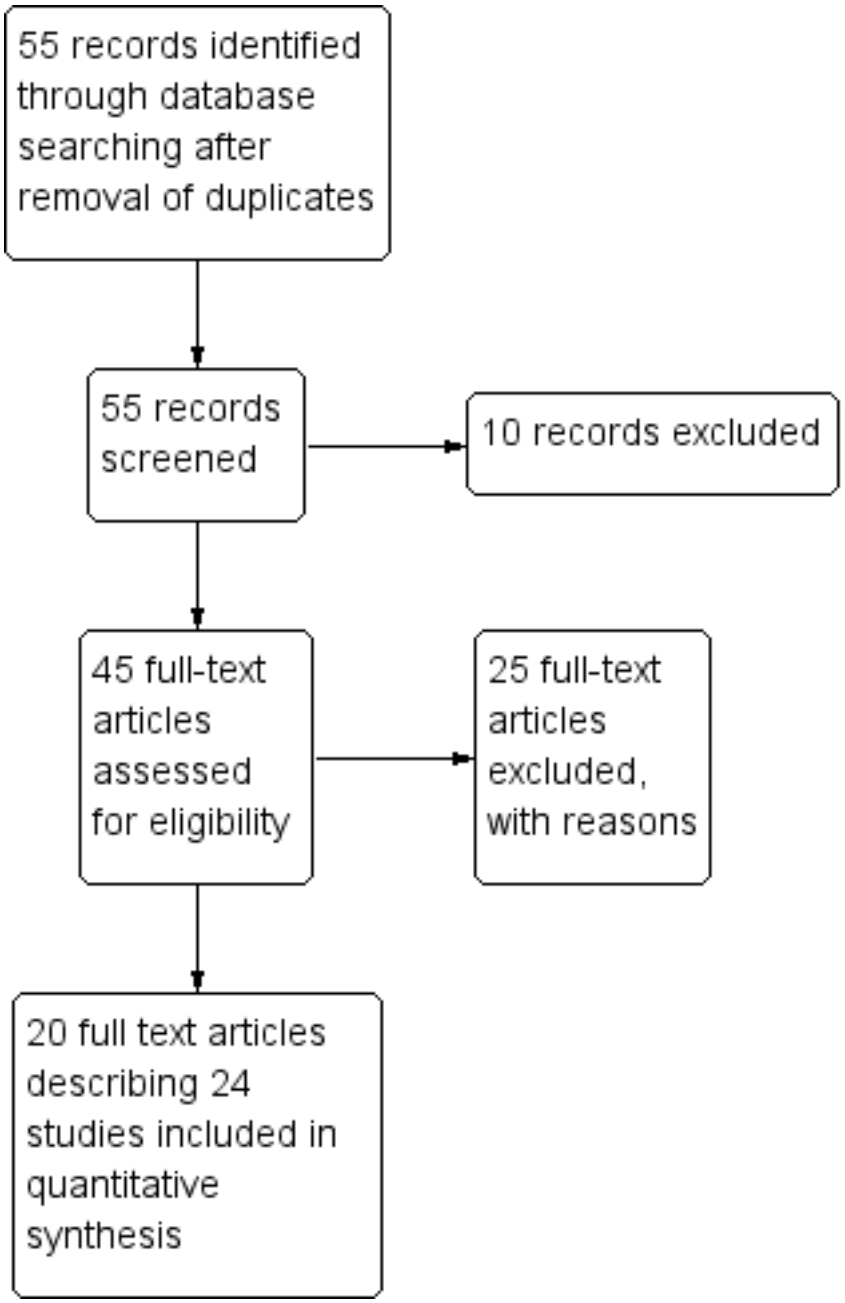
RESULTS

Description of studies

Results of the search

We identified 55 potentially relevant articles for inclusion. We assessed these articles using the pre-stated inclusion criteria (see Figure 1).

Figure 1. Study flow diagram.



Included studies

We included 20 individual papers describing 24 trials which evaluated eight different vaccines. Of these, seven trials presented efficacy data from field trials (Clemens 1988; Peltola 1991; Scerpella 1995; Wiedermann 2000; Sack 2007; Leyten 2005; Frech 2008), and four trials presented efficacy data from artificial challenge studies (Freedman 1998; Tacket 1999; McKenzie 2007; McKenzie 2008). A summary of the main characteristics of these trials is given in Table 3. For further details see the Characteristics of included studies tables. An additional 13 trials only contributed safety and immunogenicity data.

Interventions

Three different killed whole cell vaccines were evaluated in efficacy trials: the oral cholera vaccine with purified B-subunit (Cholera WC-BS: Clemens 1988; Peltola 1991), the oral cholera vaccine with recombinant B-subunit (Cholera WC-rCTB: Scerpella 1995), and an ETEC vaccine with recombinant cholera B-subunit (ETEC WC-rCTB: Wiedermann 2000; Sack 2007). Two live attenuated vaccines have undergone evaluation of clinical efficacy: one oral cholera vaccine (CVD 103-HgR: Leyten 2005) and one ETEC-specific vaccine (PTL-003: McKenzie 2008). Two additional studies evaluated an LT subunit vaccine delivered by transcutaneous patch (McKenzie 2007; Frech 2008) and two evaluated passive immunization using hyperimmune anti-*E. coli* CFA (Freedman 1998; Tacket 1999).

Populations

Only one vaccine was evaluated for use among an endemic population in a low income country and this vaccine is no longer available (Cholera WC-BS: Clemens 1988). Five vaccines were evaluated among travellers to endemic settings: Cholera WC-BS (Peltola 1991). Cholera WC-rCTB (Scerpella 1995), ETEC WC-rCTB (Wiedermann 2000; Sack 2007), CVD 103-HgR (Leyten 2005), and the LT transcutaneous patch (Frech 2008). The remaining three vaccines were evaluated among volunteers in artifical challenge studies.

Outcomes

Ten trials reported episodes of ETEC diarrhoea, six reported on severe ETEC diarrhoea, and ten reported on all-cause diarrhoea. The definitions of these outcomes varied between trials and we have presented these in Table 4.

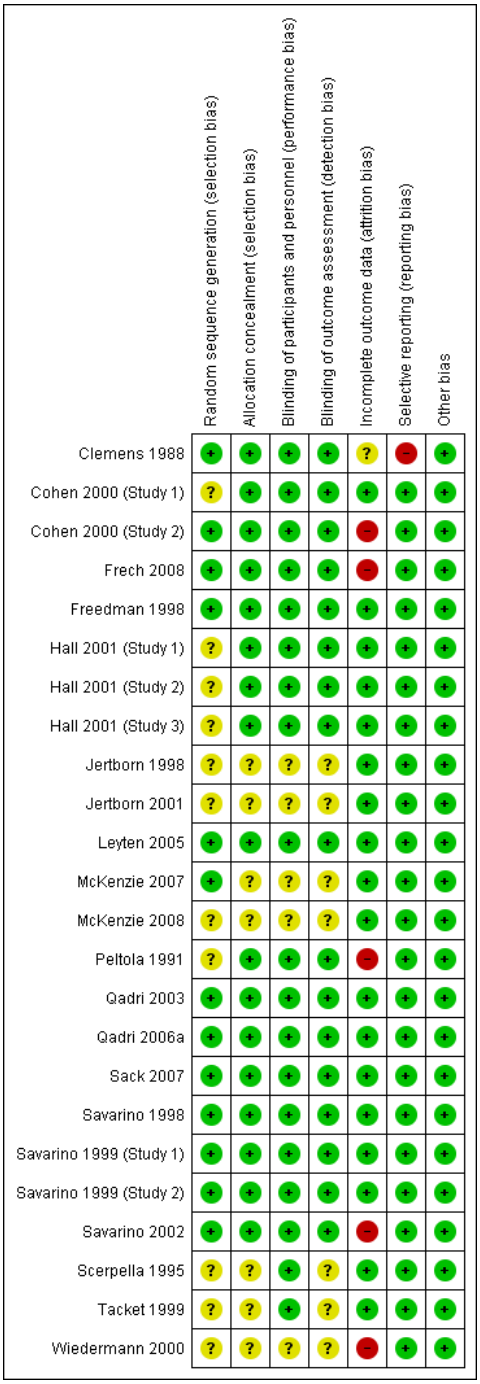
Excluded studies

We excluded 35 studies and we listed the reasons for exclusion in the Characteristics of excluded studies table.

Risk of bias in included studies

We summarized the risk of bias assessments in Figure 2.

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Efficacy studies

We judged five natural challenge studies to have adequately described allocation concealment and we considered them to be at low risk for selection bias (Clemens 1988; Peltola 1991; Sack 2007; Leyten 2005; Frech 2008). In two natural challenge studies we judged the risk of bias to be unclear (Scerpella 1995; Wiedermann 2000). Of the five artificial challenge studies, only one adequately described a method of allocation concealment (Freedman 1998).

Safety and immunogenicity only studies

We judged 11 of the 13 safety and immunogenicity studies at low risk of selection bias.

Blinding

Efficacy studies

We found that blinding of participants and study personnel was clearly described in eight out of 11 efficacy trials (Clemens 1988; Peltola 1991; Scerpella 1995; Freedman 1998; Tacket 1999; Leyten 2005; Sack 2007; Frech 2008), and was unclear in three. Outcome assessors were blinded to treatment allocation in six trials (Clemens 1988; Peltola 1991; Freedman 1998; Leyten 2005; Sack 2007; Frech 2008), and was unclear in five trials (Scerpella 1995; Tacket 1999; Wiedermann 2000; McKenzie 2007; McKenzie 2008).

Safety and immunogenicity studies

Most studies (11 out of 13) used placebos which were identical in appearance to the vaccine. We considered these studies to be at low risk of bias for safety outcomes. In two studies assessors were not blinded to make a judgement and so we classified these studies at 'unclear' risk of bias (Jertborn 1998; Jertborn 2001).

Incomplete outcome data

Efficacy studies

Seven efficacy trials had low losses to follow-up and we considered these trials at low risk of attrition bias (Scerpella 1995; Freedman 1998; Tacket 1999; Leyten 2005; McKenzie 2007; Sack 2007; McKenzie 2008). We found that three trials had high losses to follow-up (Peltola 1991; Wiedermann 2000; Frech 2008) and we judged these trials at high risk of attrition bias. One trial was unclear about the number of participants lost to follow-up (Clemens 1988).

Safety and immunogenicity studies

Eleven studies out of 13 reported minimal losses to follow-up. We considered these trials at low risk of bias. Two studies had high losses to follow-up and we judged these trials at high risk of bias (Cohen 2000 (Study 2); Savarino 2002).

Selective reporting

We found no evidence of selective reporting bias in any of the included studies.

Other potential sources of bias

We found no evidence of other potential sources of bias in the trials.

Effects of interventions

See: **Summary of findings for the main comparison** Cholera WC-rCTB vaccine for preventing enterotoxigenic *E. coli* (ETEC) diarrhoea

Cholera killed whole cell vaccines versus placebo

Two oral vaccines containing killed whole cells of *V. cholerae* have been evaluated. The first contained 1 mg of purified cholera B-subunit (Cholera WC-BS). This vaccine was further developed with a recombinant cholera B-subunit and is commercially available (Cholera WC-rBS).

Analysis 1: Cholera killed whole cells plus purified B-subunit (Cholera WC-BS)

Clinical efficacy

In Bangladesh, in a passive surveillance study in a community endemic with ETEC diarrhoea, Clemens 1988 found that the oral Cholera WC-BS vaccine provided short-term protection against LT-ETEC diarrhoea at three months' follow-up compared to the same whole cell vaccine without the B-subunit (RR 0.33, 95% CI 0.13 to 0.84; one trial, 49,612 participants, Analysis 1.1). However, only 24 episodes of ETEC diarrhoea were reported in this study (18 in the placebo group versus six in the vaccine group). Eight episodes of severe ETEC diarrhoea were reported (seven in

the placebo group versus one in the vaccine group) and this result was not statistically significant (one trial, 49,612 participants, Analysis 1.2). No protective efficacy was demonstrated at later time points.

One additional trial evaluated the same vaccine given to people intending to travel from Europe to Morocco (Peltola 1991). The authors found a statistically significant reduction in ETEC diarrhoea (RR 0.48, 95% CI 0.26 to 0.90; one trial, 615 participants, Analysis 1.1) and all-cause diarrhoea (RR 0.77, 95% CI 0.59 to 1.00; one trial, 615 participants, Analysis 1.3).

Safety

Safety data were only available from 508 participants in Peltola 1991. 'Gastrointestinal symptoms' were higher in those receiving the placebo than the vaccine during the first three days after vaccination (P = 0.03, Analysis 1.4). No other significant reactogenicity was observed.

Immunological response

The studies did not report on the outcome of immunological response.

Analysis 2: Cholera killed whole cells plus recombinant B-subunit (Cholera WC-rCTB; Dukoral®)

Clinical efficacy

The currently available oral Cholera WC-rCTB vaccine was evaluated in a single RCT in people arriving in Mexico from the USA (Scerpella 1995). There were no statistically significant differences in episodes of either ETEC-specific diarrhoea or all-cause diarrhoea between those receiving vaccine and placebo (one trial, 502 participants, Analysis 2.1; Analysis 2.2). However, in this trial the vaccine was only administered after arrival in Mexico and most episodes of diarrhoea occurred before completion of the two dose regimen or within 7 days of the second dose.

The authors of this paper considered that adequate protection would not be attained until seven days after the second dose. They reported a 50% protective effect in a subgroup analysis of cases occurring after this timepoint (95% CI 14 to 71%, authors' own figures). However, it should be noted that this subgroup analysis excluded 75% of the observed cases of ETEC diarrhoea. Only 19 episodes of ETEC diarrhoea were included (12 with placebo and seven with vaccine), and our re-analysis of this data suggested that this difference was not statistically significant (Analysis 2.3).

Safety

Scerpella 1995 reported that there were no differences in the frequency of gastrointestinal symptoms, headache, or febrile illnesses between people that received either the vaccine or placebo, but data were not presented.

Immunological response

Toxin-specific IgG antibody (TSA) responses were available from 281 participants. A greater than four-fold increase was observed in 87% of the participants who received Cholera WC-rCTB vaccine compared to 8% in controls (RR 10.54, 95% CI 6.11 to 18.20; one trial, 281 participants, Analysis 2.4).

ETEC killed whole cell vaccines versus placebo

Analysis 3: ETEC killed whole cells plus recombinant cholera B-subunit (ETEC WC-rCTB)

Clinical efficacy

Two studies have evaluated this oral ETEC vaccine (ETEC WC-rCTB); in people travelling from the USA to Mexico or Guatemala (Sack 2007), and from Austria to one of 44 different countries in Latin America, Africa, or Asia (Wiedermann 2000). There were no statistically significant differences in ETEC-specific diarrhoea, or all-cause diarrhoea (two trials, 799 participants, Analysis 3.1; Analysis 3.3).

In Sack 2007 a small number of severe ETEC episodes are recorded (two in the vaccine group and nine in the placebo group), and this difference approached statistical significance (RR 0.23, 95% CI 0.05 to 1.05; one trial, 671 participants, Analysis 3.2).

Safety

A total of 1695 participants have received ETEC WC-rCTB or placebo in 11 RCTs. Vomiting was the only symptom significantly more common in those receiving the vaccine compared to placebo (RR 2.0, 95% CI 1.16 to 3.45; nine trials, 1528 participants, Analysis 3.4).

Immunological response

CFA/I-specific antibody response:

Anti-CFA/I antibody responses were evaluated in 880 participants in 12 RCTs. CFA/I-specific IgA antibody responses were evaluated in serum, plasma, or antibody secreting cells (ASCs). They were found to be statistically higher in the vaccine group compared

to controls (RR 6.78, 95% CI 5.12 to 8.98, P < 0.00001; 12 trials, 880 participants, Analysis 3.5). In individual studies, the proportion of participants with a greater than two-fold increase following vaccination ranged from: 26% to 94% in adults; 96% to 100% in children aged between 6 to 12 years; 73% to 95% in children aged between 18 months to 5 years; and 59% to 61% in infants aged between 6 to 18 months (Table 5).

CF-specific IgA antibody responses were also reported to anti-CS1 (10 trials), anti-CS2 (10 trials), anti-CS3 (one study), and anti-CS4 (eight trials). These data are summarized in Table 5.

Toxin-specific antibody response:

Either CT or LT toxin-specific IgA antibody responses were evaluated in a total of 1228 participants in 13 RCTs. In individual studies, the percentage of participants with > two-fold increases in toxin-specific antibodies ranged from 50% to 100% in those receiving the vaccine compared to 0% to 33% in controls (RR 5.03, 95% CI 4.25 to 5.96, P < 0.00001; 13 trials, 1228 participants, Analysis 3.6).

Live attenuated vaccines versus placebo

Two live attenuated vaccines have been evaluated in placebo controlled trials: the oral cholera vaccine CVD 103-HgR in a natural challenge study in travellers (Leyten 2005) and the oral ETEC vaccine PTL-003 in a small artificial challenge study (McKenzie 2008).

Analysis 4: Live attenuated cholera vaccine (CVD 103-HgR)

Clinical efficacy

Leyten 2005 evaluated CVD 103-HgR, a live oral cholera vaccine, in Dutch volunteers intending to travel to Indonesia, Thailand, the Indian subcontinent, or West Africa. This study reported no significant differences in ETEC diarrhoea, severe ETEC diarrhoea, or all-cause diarrhoea (one trial, 134 participants, Analysis 4.1; Analysis 4.2; Analysis 4.3).

Safety

This outcome was not reported.

Immunological response

This outcome was not reported.

Analysis 5: Live attenuated ETEC vaccine (PTL-003)

Clinical efficacy

McKenzie 2008 evaluated PTL-003, a live attenuated ETEC-specific vaccine, in a small artificial challenge study in North American volunteers. The authors reported no statistically significant reduction in ETEC diarrhoea (one trial, 33 participants, Analysis 5.1; Analysis 5.2).

Safety

McKenzie 2008 reported safety data. No statistically significant differences in adverse events were observed between vaccine and control groups (one trial, 33 participants, Analysis 5.3).

Immunological response

McKenzie 2008 reported the proportion of participants with a > two-fold increase in CF-specific antibody responses against CS1 and CS3 and found significantly higher IgA titres in vaccinees compared to controls (see Table 6). There were no significant differences in toxin-specific antibody responses (Analysis 5.4).

Transcutaneous vaccines versus placebo

Analysis 6: Transcutaneous LT patch

Clinical efficacy

An LT-ETEC vaccine delivered via a transcutaneous patch was evaluated in one natural challenge study in American adults intending to travel to Mexico and Guatemala (Frech 2008) and in one artificial challenge study in North American volunteers (McKenzie 2007). No statistically significant differences were reported for ETEC diarrhoea, severe ETEC diarrhoea, or all-cause diarrhoea (two trials, 217 participants, Analysis 6.1; Analysis 6.2; Analysis 6.3).

Safety

A total of 260 participants from two studies were evaluated for safety data, particularly regarding reactogenicity at the application site and other systemic adverse events (McKenzie 2007; Frech 2008). A significantly higher number of local immune reactions in the form of rash (P < 0.00001), pruritus (P < 0.00001), and skin discolouration (P = 0.0003) were observed in people that received the vaccine compared to placebo recipients (Analysis 6.4). For other events, there were no significant differences (Analysis 6.4).

Immunological Response

Frech 2008 and McKenzie 2007 reported a > four-fold increase in toxin-specific IgA antibody responses in 82% and 93% of people vaccinated, respectively (RR 43.0, 95% CI 12.33 to 149.97, P < 0.00001; two trials, 217 participants, Analysis 6.5).

Passive immunization versus placebo

Analysis 7: Hyperimmune anti-ETEC CFA

Clinical efficacy

Two artificial challenge studies reported passive immunization using bovine hyperimmune anti-ETEC CFA in North American volunteers (Freedman 1998; Tacket 1999). The authors did not find any significant protective efficacy against all-cause diarrhoea (two trials, 45 participants, Analysis 7.1).

Safety

A total of 45 participants from the studies by Freedman 1998 and Tacket 1999 were evaluated for safety data. A significantly higher number of events occurred in people that were vaccinated compared to those that received a placebo regarding the events of anorexia (P = 0.01) and abdominal pain (P = 0.003) (Analysis 7.2). For other events, there were no significant differences between people that were vaccinated and those that received a placebo (Analysis 7.2).

Immunological Response

No immunological data were reported.

DISCUSSION

Summary of main results

In this review, we included 24 trials and 53,247 participants. Four studies assessed the protective efficacy of oral cholera vaccines when used to also prevent diarrhoea due to ETEC and eight trials assessed the protective efficacy of ETEC-specific vaccines.

Cholera vaccines

A single RCT evaluated the currently available oral cholera killed whole cell vaccine (Dukoral®) for protection against 'travellers' diarrhoea' in people arriving in Mexico from the USA. There were no statistically significant effects on ETEC diarrhoea or all-cause diarrhoea (one trial (Scerpella 1995), 502 participants, *low quality evidence*).

Two earlier trials, one in an endemic population in Bangladesh (Clemens 1988) and one in travellers from Finland to Morocco (Peltola 1991), evaluated a precursor of this vaccine containing purified cholera toxin B subunit, rather than the recombinant subunit in Dukoral®. Short term protective efficacy against ETEC diarrhoea was demonstrated lasting for around three months (two trials, 50,227 participants). This vaccine is no longer available.

ETEC vaccines

An ETEC-specific killed whole cell vaccine, also containing the recombinant cholera toxin B-subunit, was evaluated in people travelling from the USA to Mexico or Guatemala (Sack 2007), and from Austria to Latin America, Africa, or Asia (Wiedermann 2000). There were no statistically significant differences in ETEC-specific diarrhoea or all-cause diarrhoea (two trials, 799 participants) and the vaccine was associated with increased vomiting (nine trials, 1528 participants) (Cohen 2000 (Study 1); Cohen 2000 (Study 2); Qadri 2003; Qadri 2006a; Sack 2007; Savarino 1998; Savarino 1999 (Study 1); Savarino 1999 (Study 2); Savarino 2002). The other ETEC-specific vaccines in development have not yet demonstrated clinically important benefits.

Overall completeness and applicability of evidence

The use of the oral cholera WC-rCTB vaccine for preventing 'travellers' diarrhoea' has been based on the findings of two trials that demonstrated some short term protection against ETEC diarrhoea (Clemens 1988; Peltola 1991). The vaccine used in both of these trials contained 1 mg of purified cholera B-subunit, rather than the recombinant B-subunit in the current vaccine. It should be noted that the cholera B-subunit is the only element of this vaccine which could be expected to induce immunity to ETEC. These two vaccines were directly compared in a single trial of 41 Swedish volunteers (Jertborn 1992), which reported comparable cholera-specific antibody responses but did not evaluate either clinical or immunological protection against ETEC. The finding of limited protective benefit with the cholera WC-rCTB vaccine (Scerpella 1995) is supported by two further trials evaluating the ETEC WC-rCTB vaccine which contains the same recombinant cholera B-subunit, and also found no evidence of clinical protection in travellers (Wiedermann 2000; Sack 2007). These three trials raise concerns that the earlier findings may not be applied to the current vaccine.

In addition, the large study from Bangladesh (Clemens 1988), which contributed over 90% of participants included in this review, aimed primarily to assess the protective efficacy against cholera not ETEC. The assessment of protective efficacy against ETEC therefore represented a post-hoc analysis. This trial was conducted among an endemic population who were likely to have acquired some natural immunity against ETEC. The results of this study may therefore be poorly applicable to travellers.

The ETEC-specific vaccines are now primarily being designed for use in developing country settings for prevention of ETEC diarrhoea in infants and young children, although protection of travellers remains important (Holmgren 2012). Promising CFs and toxin-specific immune responses to the ETEC WC-rCTB vaccine have been observed. However, following failure to demonstrate clinical protective efficacy and safety concerns, further pre-clinical development of this vaccine is underway (Tobias 2012). Several additional vaccine candidates not included in this review are currently at early stages of development. In Sweden, an oral inactivated tetravalent ETEC vaccine alone or together with double mutant heat labile toxin (dmLT) adjuvant is undergoing testing in Phase I/II studies. In the USA an oral live attenuated three strain recombinant ETEC vaccine, ACE527, is undergoing testing with plans for moving field sites in developing countries (Darsley 2012).

Quality of the evidence

The quality of the evidence for the oral cholera vaccine (Dukoral®) was assessed using the GRADE approach. Clinically important benefits of this vaccine have not yet been demonstrated and the quality of this evidence was downgraded to 'low'. This means that use of this vaccine may have little or no difference in preventing ETEC diarrhoea but further research may change this result. The quality was downgraded due to concerns about the applicability of the evidence. Most cases of ETEC diarrhoea occurred prior to completion of the vaccine schedule (indirectness) and the sample size was small (imprecision) (Summary of findings for the main comparison).

Agreements and disagreements with other studies or reviews

A previous review of vaccination to prevent ETEC diarrhoea concluded that the protective effect of Dukoral® was up to 43% and that it should be recommended for travellers (Jelinek 2008). However, this conclusion was based predominantly on positive findings from the older trials assessing prototypes of the Dukoral® vaccine, on subgroup analyses which may or may not have been pre-planned, or on the findings of non-randomized retrospective studies.

AUTHORS' CONCLUSIONS

Implications for practice

There is currently insufficient evidence from RCTs to support the use of the oral cholera vaccine (Dukoral®) for protecting travellers against ETEC diarrhoea.

Implications for research

Further research is needed to develop safe, immunogenic, and effective vaccines to provide both short and long term protection against ETEC diarrhoea.

More studies are needed to evaluate the efficacy of new and candidate vaccines for safety and immunogenicity in naive adult travellers, and exposed and primed populations in a developing country setting where children and infants will be the major targets for future vaccination. ETEC vaccine development needs to include plans for overcoming barriers to oral vaccination in children. Also, strategies are needed to deliver the vaccines using the existing national immunization system of these countries, including the EPI, the cold chain facilities, and other national health facilities. In addition, the use of different modes of delivery of vaccines (including use of mucosal adjuvants) needs to be studied to improve immunogenicity and efficacy of ETEC vaccines.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Clemens 1988

Methods	Study type: Randomized, natural challenge, efficacy study in an endemic population Trial dates and duration: From January 1985 to May 1986 Surveillance: Surveillance for diarrhoea was done at treatment centres serving the study participants at Matlab for 365 post-vaccination days
Participants	Number of participants: 49,612 Inclusion criteria: People aged between 2 to 15 years of age and female subjects > 15 years of age residing in Matlab Exclusion criteria: Persons who were absent or refused to participate, pregnant, or suffering from any other illness
Interventions	Vaccine: Cholera toxin B subunit plus killed cholera whole cells (BS-WC) Control: Killed cholera whole cells (WC) Additional details: In this study participants received 3 doses of vaccine at 6 weeks apart, of BS-WC vaccine, WC vaccine only, or an *E. coli* K12 strain placebo. However, protective efficacy was calculated based on WC vaccine as control and BS-WC as study intervention group, because the killed cholera whole cells, which were identical for the BS-WC and WC vaccines, were not anticipated to have any protective effects against LT-ETEC
Outcomes	*Included in review:* • Episodes of diarrhoea • LT-ETEC diarrhoea
Notes	Location: Matlab, Bangladesh Setting: Three different treatment centres at Matlab, a rural setting of Bangladesh Source of funding: US agency for International Development, the Government of Japan, the Swedish agency for Research Cooperation with Developing Countries and the World Health Organization (WHO)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"After computerisation of the census, we assigned every person in the eligible age-gender categories to letters A, B or C, using simple randomisation" (from additional paper describing this study)
Allocation concealment (selection bias)	Low risk	"The agents were identified only by the letters A, B and C" (from an additional paper describing this study) Allocation concealed.

Clemens 1988 (*Continued*)

Blinding of participants and personnel (performance bias) All outcomes	Low risk	"During the conduct of the study, the identities of these letter...were unknown to all persons connected with the trial in Bangladesh"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"During the conduct of the study, the identities of these letter...were unknown to all persons connected with the trial in Bangladesh"
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Losses to follow-up were not clearly described.
Selective reporting (reporting bias)	High risk	This was a three arm study. It was unclear why the group given the cholera WC vaccine was selected as the control arm rather than the group given a placebo
Other bias	Low risk	None identified.

Cohen 2000 (Study 1)

Methods	Study type: Randomized safety and immunogenicity study in volunteers Trial dates and duration: Between May 22 and July 10 1995
Participants	Number of participants: 65 Inclusion criteria: Healthy men and women and were recruited among the School of Military Medicine cadets or the Medical Corps Headquarters staff Exclusion criteria: Not described.
Interventions	Vaccine: Contained 1.0 mg of rCTB plus a final count of 10^{11} formalin-inactivated bacteria. Each vaccine dose included the following inactivated ETEC strains: SBL 101 (O78, CFA/I, LT2/ST1), SBL 106 (O6, CS1, LT2/ST2), SBL 107 (OR, CS2, CS3, LT2/ST2), SBL 104 (O25, CS41CS6, LT2/ST2) and SBL 105 (O167, CS51CS6, LT2/ST2) Placebo: Heat-killed *E. coli* K12 with an optical density (OD) equivalent to that of the ETEC vaccine, was administered in the same buffered solution as the vaccine Additional details: Each dose of lot E003 was given in 150 mL of water with a raspberry-flavoured bicarbonate-citric acid buffer containing 4 g of sodium bicarbonate per dose (Recip AB, Stockholm, Sweden)
Outcomes	*Included in review:* • Adverse events • CF-specific antibody (CFA) responses • Toxin-specific antibody (TSA) responses *Not included in the review:* • All cases of ETEC diarrhoea • All cases of ETEC illness

Cohen 2000 (Study 1) (*Continued*)

Notes	Location: Israel Setting: Israel Defence Force (IDF), Medical Corps, Army Health Branch Research Unit, and the IDF, Medical Corps, School of Military Medicine Source of funding: US Army Medical Research & Material Command (DAMD 17-93-V-3001)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"Group randomization was used so that each group was assigned two letters, and each volunteer was openly allotted to one of the four resulting letter groups" It is unclear if this method was truly random.
Allocation concealment (selection bias)	Low risk	"Each volunteer was openly allotted to one of the four resulting letter groups. The association between a letter group and a vaccine/placebo group was determined by a third party and was kept locked from both volunteers and investigators for the duration of the study"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"The placebo preparation, containing a suspension of heat-killed *E. coli* K12 with an optical density (OD) equivalent to that of the ETEC vaccine, was administered in the same buffered solution as the vaccine"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	See above.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Losses to follow-up were low: 3 out of 33 (9%) in the vaccine group and 2 out of 31 (6%) in controls either dropped out of the study or were not given the second dose
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Cohen 2000 (Study 2)

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: Between April 1 and June 18 1997
Participants	Number of participants: 90 Inclusion criteria: Healthy men and women and were recruited among the School of Military Medicine cadets or the Medical Corps Headquarters staff Exclusion criteria: Not mentioned
Interventions	Vaccine: Contained 1.0 mg of rCTB plus a final count of 10^{11} formalin-inactivated bacteria. Each vaccine dose included the following inactivated ETEC strains: SBL 101 (O78, CFA/I, LT2/ST1), SBL 106 (O6, CS1, LT2/ST2), SBL 107 (OR, CS2, CS3, LT2/ST2), SBL 104 (O25, CS41CS6, LT2/ST2) and SBL 105 (O167, CS51CS6, LT2/ST2) Placebo: Heat-killed *E. coli* K12 with an optical density (OD) equivalent to that of the ETEC vaccine, was administered in the same buffered solution as the vaccine Additional details: Each dose of lot E005 was given in 150 mL of water with a raspberry-flavoured bicarbonate-citric acid buffer containing 4 g of sodium bicarbonate per dose (Recip AB, Stockholm, Sweden)
Outcomes	*Included in review:* • Adverse events • Colonization factor-specific antibody (CFA) responses • TSA responses *Not included in the review:* • All cases of ETEC diarrhoea • All cases of ETEC illness·
Notes	Location: Israel Setting: IDF, Medical Corps, Army Health Branch Research Unit, and the IDF, Medical Corps, School of Military Medicine Source of funding: US Army Medical Research & Material Command (DAMD 17-93-V-3001)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"A blocked randomization scheme was constructed off-site".
Allocation concealment (selection bias)	Low risk	"Subjects were assigned a unique participant identification number (101 to 190) at the time of the first dose and received the correspondingly labelled study agent"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"The placebo preparation, containing a suspension of heat-killed E. coli K-12 with an optical density (OD) equivalent to that of the ETEC vaccine, was administered in

Cohen 2000 (Study 2) (*Continued*)

		the same buffered solution as the vaccine“
Blinding of outcome assessment (detection bias) All outcomes	Low risk	”The investigation team remained blinded until all safety and immunogenicity data were generated, computerized, cleaned, and locked“
Incomplete outcome data (attrition bias) All outcomes	High risk	Losses to follow-up were moderate: 8 out of 45 (18%) in the vaccine group and 4 out of 45 (9%) in controls either dropped out of the study or were not given the second dose
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Frech 2008

Methods	Study type: Randomized, natural challenge, efficacy study in travellers Trial dates and duration: May 2006 to February 2007 Surveillance: Surveillance was conducted on US travellers who visited to Mexico and Guatemala
Participants	Number of participants: 201 Inclusion criteria: Healthy adults aged 18 to 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: History of travellers' diarrhoea and travelled to an endemic country in the previous 12 months, history of taking cholera, LT or ETEC vaccine, significant illness, immunosuppression or if female, pregnant, nursing, or unwilling to use effective form of any contraceptives
Interventions	Vaccine: LT patch; 37.5 µg of ETEC LT Placebo: All the excipients of LT patch without LT Additional details: 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
Outcomes	*Included in review:* • ETEC diarrhoea • Severe ETEC diarrhoea • All-cause diarrhoea • Any ETEC illness • Severe ETEC illness • Adverse events • Immunological response

Notes	Location: Mexico and Guatemala Setting: University of Texas Health Science Center at Houston (Houston, TX, USA), Universidad Del Valle De Guatemala (Guatemala City, Guatemala), Inovamed Hospital (Cuernavaca, Mexico) and ViroMed Laboratory, Minnetonka, MN, USA Source of funding: IOMAI corporation

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"The study used a web-based, audit-trail enabled, centralised randomisation code and allocation system"
Allocation concealment (selection bias)	Low risk	"Vaccination sites accessed a web page, entered participants into the system, and received unique patch numbers for every study participant"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"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"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	See above.
Incomplete outcome data (attrition bias) All outcomes	High risk	Losses to follow-up were high: 8 out 67 (12%) in the vaccine group and 23 out of 134 (17%) in the placebo group due to failure to: receive second dose of vaccine, provide diary cards, or attend in-country visit
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Freedman 1998

Methods	Study type: Randomized, artifical challenge, efficacy study in volunteers Trial dates and duration: Not given Surveillance: Daily medical rounds were conducted to monitor symptoms during the 7 days of study period. Daily stool samples were taken for bacteriologic examination Artificial challenge: On day 4
Participants	Number of participants: 25 Inclusion criteria: Not described Exclusion criteria: Not described
Interventions	Vaccine: Each lyophilized dose containing hyperimmune anti-*E. coli* bovine milk IgG dissolved in 150 mL of bicarbonate solution Placebo: A single dose of a lactose-free infant formula Additional details: Three doses/day for 7 days, vaccine, or placebo were administered 15 minutes after meals Artificial challenge: 10^9 cfu of H10407 (O78:H11), a CFA/I-bearing ETEC strain suspended in 1 ounce (30 mL) of water containing sodium bicarbonate
Outcomes	*Included in review:* • All-cause diarrhoea • CF-specific immune responses • Toxin-specific antibody responses • Adverse events
Notes	Location: Baltimore, MD, USA Setting: Center for Vaccine Development (University of Maryland School of Medicine) Source of funding: ImmuCell

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"The randomization list was generated and secured by ImmuCell's Quality Assurance Supervisor"
Allocation concealment (selection bias)	Low risk	"by assigning subject identification numbers to identically packaged foil pouches containing measured doses of each test article"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"All investigators and volunteers were blinded to these treatment group assignments throughout the study and during assessment of outcome"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	See above.

Freedman 1998 (*Continued*)

Incomplete outcome data (attrition bias) All outcomes	Low risk	No drop-outs occurred.
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Hall 2001 (Study 1)

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: Not mentioned in the article Surveillance: Subjects were randomly assigned to receive two doses of vaccine or placebo 2 weeks apart
Participants	Number of participants: 76 Inclusion criteria: Adults aged between 21 and 45 years from Benha, Qalyubia Governorate, Egypt Exclusion criteria: Not mentioned
Interventions	Vaccine: Each 4 mL vaccine dose (lot E003) contained 1 mg of rCTB plus a mixture of 2 x 10^{10} bacteria each of five strains individually expressing CFA/I, CS1, CS2 plus CS3, CS4, and CS5 Placebo: Each 4-mL placebo dose contained 10^{11} heat-killed *E. coli* K12 cells Additional details: Each dose was added to 150 mL of water containing 4 g of sodium bicarbonate plus 1.45 g of citric acid (Recip AB, Stockholm, Sweden) for adult administration
Outcomes	*Included in review:* • CFA responses • TSA responses *Not included in the review:* • Adverse events • All cases of ETEC diarrhoea • All cases of ETEC illness·
Notes	Location: Egypt Setting: Benha, Qalyubia Governorate, Egypt Source of funding: Naval Medical Research and Development Command (B69000101.PIX3270), Intragency Agreement Y1-HD-0026-01, the National Institute of Child Health and Human Development and WHO Global Programme for Vaccines and Immunization Research and Development

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**

Hall 2001 (Study 1) (*Continued*)

Random sequence generation (selection bias)	Unclear risk	"After enrollment, subjects were randomized to receive vaccine or placebo in a double-blind fashion within blocks of 4 sequentially randomized subjects" (Savarino 1998). It is unclear if this was truly random.
Allocation concealment (selection bias)	Low risk	"At the time of initial dosing, each subject was assigned a sequential number corresponding to sequentially numbered single-dose vials of study agent" (Savarino 1998).
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Described as "double blind, placebo controlled", and vaccines labelled only with study code
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"The code was broken after all clinical and laboratory evaluations were completed"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Losses to follow-up were low: 2/49 (4%) in the vaccine group and 2/48 (4%) in the placebo group
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Hall 2001 (Study 2)

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: Not mentioned in the article Surveillance: Subjects were randomly assigned to receive two doses of vaccine or placebo 2 weeks apart
Participants	Number of participants: 107 Inclusion criteria: School children aged between 6 to 12 years old from Benha, Qalyubia Governorate, Egypt Exclusion criteria: Not mentioned
Interventions	Vaccine: Each 4 mL vaccine dose (lot E003) contained 1 mg of rCTB plus a mixture of 2×10^{10} bacteria each of five strains individually expressing CFA/I, CS1, CS2 plus CS3, CS4, and CS5 Placebo: Each 4 mL placebo dose contained 10^{11} heat-killed *E. coli* K12 cells. Additional details: Each dose was added to 75 mL of water containing 4 g of sodium bicarbonate plus 1.45 g of citric acid (Recip AB, Stockholm, Sweden) for school children administration

Hall 2001 (Study 2) (*Continued*)

Outcomes	*Included in review:* • CFA responses • TSA responses *Not included in the review:* • Adverse events • All cases of ETEC diarrhoea • All cases of ETEC illness·
Notes	Location: Egypt Setting: Benha, Qalyubia Governorate, Egypt Source of funding: Naval Medical Research and Development Command (B69000101. PIX3270), Intragency Agreement Y1-HD-0026-01, the National Institute of Child Health and Human Development and WHO Global Programme for Vaccines and Immunization Research and Development

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"After enrollment, subjects were randomized to receive vaccine or placebo in a double-blind fashion within blocks of 4 sequentially randomized subjects" (Savarino 1998). It is unclear if this was truly random.
Allocation concealment (selection bias)	Low risk	"At the time of initial dosing, each subject was assigned a sequential number corresponding to sequentially numbered single-dose vials of study agent" (Savarino 1998).
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Described as "double blind, placebo controlled", and vaccines labelled only with study code
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"The code was broken after all clinical and laboratory evaluations were completed"
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up occurred.
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Hall 2001 (Study 3)

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: Not mentioned in the article Surveillance: Subjects were randomly assigned to receive two doses of vaccine or placebo 2 weeks apart
Participants	Number of participants: 106 Inclusion criteria: Preschool children aged between 2 to 5 years old from Benha, Qalyubia Governorate, Egypt Exclusion criteria: Not mentioned
Interventions	Vaccine: Each 4 mL vaccine dose (lot E003) contained 1 mg of rCTB plus a mixture of 2 x 10^{10} bacteria each of five strains individually expressing CFA/I, CS1, CS2 plus CS3, CS4, and CS5 Placebo: Each 4 mL placebo dose contained 10^{11} heat-killed *E. coli* K12 cells Additional details: Each dose was added to 37.5 mL of water containing 4 g of sodium bicarbonate plus 1.45 g of citric acid (Recip AB, Stockholm, Sweden) for adult administration
Outcomes	*Included in review:* • CFA responses • TSA responses *Not included in the review:* • Adverse events • All cases of ETEC diarrhoea • All cases of ETEC illness·
Notes	Location: Egypt Setting: Benha, Qalyubia Governorate, Egypt Source of funding: Naval Medical Research and Development Command (B69000101. PIX3270), Intragency Agreement Y1-HD-0026-01, the National Institute of Child Health and Human Development and WHO Global Programme for Vaccines and Immunization Research and Development

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"After enrollment, subjects were randomized to receive vaccine or placebo in a double-blind fashion within blocks of 4 sequentially randomized subjects" (Savarino 1998). It is unclear if this was truly random.
Allocation concealment (selection bias)	Low risk	'At the time of initial dosing, each subject was assigned a sequential number corresponding to sequentially numbered single-dose vials of study agent" (Savarino 1998).

Hall 2001 (Study 3) (*Continued*)

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Described as "double blind, placebo controlled", and vaccines labelled only with study code
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"The code was broken after all clinical and laboratory evaluations were completed"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Only one participant was lost to follow-up (from the placebo group)
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Jertborn 1998

Methods	Study type: Randomized, safety study in volunteers Study 1: Single blinded, placebo controlled, randomized study Study 2: Non-placebo controlled study (data excluded) Study 3: Non-placebo controlled study (data excluded) Trial dates and duration: Not mentioned in the article Surveillance: Study 1: 5 consecutive post vaccination days
Participants	Number of participants: 20 (vaccine) plus 20 (placebo) Inclusion criteria: Adult Swedish volunteers, aged between 20 to 50 years were recruited, with no history of travel to an endemic country for the past 6 months Exclusion criteria: Not mentioned
Interventions	Vaccine: One single dose of vaccine contained 1.0 mg of rCTB and 10^{11} formalin-inactivated enterotoxigenic *E. coli* bacteria of each of the following strains: O78:H12-CFA/I ST+, O25:H42- CS4+CS6, O167:H5-CS5+CS6/ST+, O6:H16-CS2+CS3, and O139:H28-CS1 Placebo: One single dose of placebo consisted of 150 mL of a sodium bicarbonate solution (Samarin; Cederroths Nordic AB, Upplands Vasby, Sweden) The volunteers were instructed not to eat or drink (except water) for 1 hour before and after intake of the vaccine or placebo preparation
Outcomes	*Included in review:* • Adverse events
Notes	Location: Goteborg, Sweden Setting: University of Goteborg, Sweden Source of funding: Swedish Research Council (16X-09084 and 16X-3382), the Swedish Agency for Research Cooperation with Developing Countries, the WHO and the Medical Faculty, Goteborg University
Risk of bias	

Jertborn 1998 (*Continued*)

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Described as "randomized". No further details.
Allocation concealment (selection bias)	Unclear risk	None described.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Described as "single blind" but no further details.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Described as "single blind" but no further details.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Losses to follow-up were low: 1 out of 20 in placebo group was excluded due to viral infection
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Jertborn 2001

Methods	Study type: Randomized, immunogenicity study in volunteers Study 1: Open study without any control group (excluded from the review)) Study 2: Double blinded, placebo controlled, randomized study Trial dates and duration: Not mentioned in the article Surveillance: Not described
Participants	Number of participants: Study 1: 36 (excluded from the review) Study 2: 31 Inclusion criteria: Adult Swedish volunteers, aged between 18 to 46 years were recruited, no history of travelling to ETEC endemic areas for 6 months prior to the study Exclusion criteria: Not mentioned
Interventions	Vaccine: Study 1: One 4 mL dose of vaccine (Lot 003) contained 1.0 mg of rCTB and 10^{11} formalin-inactivated *E. coli* bacteria of each of the following strains: SBL101 (O78:H12; CFA/I ST1), SBL104 (O25:H42; CS4), SBL105 (O167:H5; CS5 ST1), SBL 106 (O6: H16; CS1), and SBL 107 (OR:H6; CS21 CS3) (Data not included in the review) Study 2: Different lot (Lot 005) of vaccine with same formulation except the half the amount of CFA/I and three times more CS2 than lot 003 was used in this study Placebo: The 4 mL placebo dose consisted of 1 $\times 10^{11}$ heat-killed *E. coli* K12 bacteria Additional details: Each dose of a study agent was administered in 150 mL of a sodium bicarbonate solution (Samarin; Cederroths Nordic AB, Upplands Vasby, Sweden). The

	volunteers were instructed not to eat or drink (except water) for 1 hour before and after intake of the vaccine or placebo preparation
Outcomes	*Included in review:* • Immunological response
Notes	Location: Goteborg, Sweden Setting: University of Goteborg, Sweden Source of funding: Swedish Research Council (16X-09084), the Swedish Agency for Research Cooperation with Developing Countries, the WHO and the Medical Faculty, Goteborg University

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Described as 'randomized', no further details given.
Allocation concealment (selection bias)	Unclear risk	None described.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Described as 'double blind, Placebo controlled" study, no further details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	As above.
Incomplete outcome data (attrition bias) All outcomes	Low risk	No drop-outs occurred.
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Leyten 2005

Methods	Study type: Randomized, natural challenge, efficacy study in travellers Trial dates and duration: May 1995 to February 1996 Surveillance: 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
Participants	Number of participants: 145 Inclusion criteria: Dutch volunteers, travellers from the travellers clinics of the Leiden University Medical Centre (LUMC), the Netherlands, the Municipal Health Centre at Leiden and the Harbour Hospital at Rotterdam. All adults who were intending to travel to Indonesia, Thailand, the Indian subcontinent or West Africa (Gambia or Senegal) for a period of 1 to 4 weeks were invited to take part in the trial Exclusion criteria: People suffering from 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, subjects participating in other clinical trials women who were either pregnant or breast-feeding
Interventions	Vaccine: CVD 103-HgR, Single dose of 5 x 10^8 cfu of lyophilized CVD 103-HgR live oral cholera vaccine Placebo: 5 x 10^8 heat-killed *E. coli K*12 Additional details: Vaccine has been administered orally. Both vaccine and placebo are identical in appearance
Outcomes	*Included in review:* • ETEC diarrhoea • Severe ETEC diarrhoea • All-cause diarrhoea • Any ETEC illness • Severe ETEC illness
Notes	Location: The Netherlands Setting: Leiden University Medical Center (LUMC) Source of funding: Berna Biotech AG, Bern, Switzerland

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"For randomisation a computer-generated randomisation list, was used"

Leyten 2005 (*Continued*)

Allocation concealment (selection bias)	Low risk	"Sachets and suspensions of vaccine and placebo that were identical in appearance, were labelled by a coded number from 1 to 200" Allocation was concealed through randomization to identical coded vials
Blinding of participants and personnel (performance bias) All outcomes	Low risk	See above.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	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"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Losses to follow-up were low: in vaccine group 4/73 (5%) and in placebo group 7/72 (10 %)
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

McKenzie 2007

Methods	Study type: Randomized, artificial challenge, efficacy study in volunteers Trial dates and duration: September 15 2004 to June 30 2005 Surveillance: Post vaccination follow-up on day 0, 7, 21, 28, 42, 49, and 77; Artificial challenge: on day 55; Post challenge follow-up for 5 days
Participants	Number of participants: 59 Inclusion criteria: Healthy adults, between 18 to 45 years of age Exclusion criteria: Clinically significant medical conditions, history of traveller's diarrhoea in the last 3 years and LT IgG titer > 2000 EU by ELISA
Interventions	Vaccine: 150 μL of saline containing 50 μg of LT Placebo: 150 μL of saline containing no LT Additional details: All participants were randomized to receive transcutaneous application of 3 doses saline containing LT or saline only, at an interval of 21 days Artificial challenge: 120 mL of sodium bicarbonate buffer containing 6×10^8 CFU of the challenge virulent strain of *E. coli* E24377A

Outcomes	*Included in review:* • ETEC diarrhoea • Severe ETEC diarrhoea • All-cause diarrhoea • Any ETEC illness • Severe ETEC illness • Adverse events • Immunological response
Notes	Location: USA Setting: Vaccine Testing Unit, the Center for Immunization Research (CIR), Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; IOMAI Corporation, Gaithersburg, MD, USA; Amarex Clinical Research, Germantown, MD, USA Source of funding: IOMAI Corporation, Gaithersburg, MD, USA

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"The randomization list was generated by the statistician".
Allocation concealment (selection bias)	Unclear risk	None described.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Described as "double blind" but no details given of how this was done
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Described as "double blind" but no details given of how this was done
Incomplete outcome data (attrition bias) All outcomes	Low risk	2 out of 30 (7%) subjects from vaccine group and 4 out 29 (14%) of subjects from placebo group subsequently withdrew from the study before the inpatient challenge phase. However, once entered into the challenge phase there were no further drop-outs
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

McKenzie 2008

Methods	Study type: Randomized, artificial challenge, efficacy study in volunteers Trial dates and duration: Not mentioned in the article Surveillance: Post vaccination follow-up for 20 days Artificial challenge: On day 27; post challenge follow-up for 5 days (passive using diary cards)
Participants	Number of participants: 39 Inclusion criteria: Healthy adults, 18 to 50 years of age Exclusion criteria: Not mentioned in the article
Interventions	Vaccine: PTL-003 (PTL-003 was derived from the spontaneously toxin-negative, O139: H28 ETEC strain E1392/75-2A containing 2×10^9 cfu/mL live attenuated bacteria in 200 mL of CeraVacx^{TM} buffer) Placebo: CereVacx buffer (CeraVacx, Cera Products Inc., Jessup, MD: rice solids, 7.0 g; sodium bicarbonate, 2 g; trisodium citrate, 0.5 g in 200 mL of water) Additional details: All participants were randomized to receive 2 doses, at an interval of 10 days Artificial challenge: 30 mL of sodium bicarbonate buffer containing 3×10^9 CFU of the challenge virulent strain of *E. coli* E24377
Outcomes	*Included in review:* • ETEC diarrhoea • Severe ETEC diarrhoea • All-cause diarrhoea • Any ETEC illness • Severe ETEC illness • Adverse events • Immunological response
Notes	Location: USA Setting: Vaccine Testing Unit, the Center for Immunization Research (CIR), Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA Source of funding: Acambis Research Ltd., Cambridge, UK and by Johns Hopkins University School of Medicine General Clinical Research Center grant number M01-RR00052 from the National Center for Research Resources, NIH

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Described as "randomized", no further details given.
Allocation concealment (selection bias)	Unclear risk	None described.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Described as "double blind", no further details given.

Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Described as "double blind", no further details given.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Losses to follow-up were low (three participants in each group withdrew before the challenge phase), but as the trial was very small this represented 15% of all participants. However once they entered the challenge phase, there was no further drop-outs
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Peltola 1991

Methods	Study type: Randomized, natural challenge, efficacy study in travellers Trial dates and duration: October 24 1989 to November 21 1989 Surveillance: Any diarrhoea during the trip period and immediately after returning back was recorded and stool samples were collected
Participants	Number of participants: 615 Inclusion criteria: Travellers from Finland to Morocco Exclusion criteria: Travellers aged less than 15 years and history of taking antimicrobials during the previous 7 days of vaccination days
Interventions	Vaccine: 1 x 10^{11} heat-killed whole cells of *V. cholerae* with 1 mg of the B-subunit of cholera toxin Placebo: *E. coli* K12 Additional details: Two doses of vaccine or placebo identical in appearance, 3 and 1 week before the departure were administered
Outcomes	*Included in review:* • All cases of ETEC diarrhoea • Severe cases of ETEC diarrhoea • All-cause diarrhoea • Adverse events
Notes	Location: Finland and Morocco Setting: Enteric Laboratory, Moroccan Health Authority, Agadir, Morocco; Laboratory of Enteric Pathogen, The National Public Health Institute, Helsinki, Finland Source of funding: National Public Health Authority, Finland, Fintours Company, Sun Tours Company, Moroccan Health Authority, University of Gothenburg, Pohjola Company, and Tapiola Company
Risk of bias	

Peltola 1991 (*Continued*)

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Described as "randomized", no further details given.
Allocation concealment (selection bias)	Low risk	"The vaccine and placebo were in a similar liquid form, coded blindly and packed in identical 4 mL vials" Allocation was concealed through randomization to identical coded vials
Blinding of participants and personnel (performance bias) All outcomes	Low risk	See above.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"The vaccine code was opened after all demographic, clinical, and microbiological data were available"
Incomplete outcome data (attrition bias) All outcomes	High risk	Losses to follow-up were high (18%) for adverse event data. Losses to follow-up for clinical outcomes were not clearly stated
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Qadri 2003

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: Not mentioned in the article Surveillance: Surveillance for side-effects was carried out for 3 days after each vaccination. The child was observed for 1 hour in the field clinic and was then allowed to return home. The trained health workers made house to house visits within 3 hours to assess and record adverse events, thereafter home visits were made every day, for the next 2 days
Participants	Number of participants: 158 Inclusion criteria: Healthy children aged 18 to 36 months with both sexes Exclusion criteria: History of gastrointestinal disorder, diarrhoeal illness in the past 2 weeks, febrile illness in the preceding week or antibiotic treatment for at least 7 days prior to enrolment as well as children, weight-for-height <−2(S.D.) of the median value of the National Centre of Health Statistics (NCHS) were not enrolled in the study
Interventions	Vaccine: One 6 mL dose of vaccine consisted of 1 mg of rCTB plus 2×10^{10} CFU of five strains of formalin-inactivated ETEC expressing CFA/I, CS1, CS2 + CS3, CS4 and CS5 antigens each Placebo: 1×10^{11} CFU of heat-killed *E. coli* K12.

Qadri 2003 (*Continued*)

	Additional details: The children enrolled in the study were received two doses of the oral CF-BS-ETEC vaccine (lot E-009) or the placebo with a 2 week interval in the health station of the field site
Outcomes	*Included in review:* • Adverse events • Immunological response
Notes	Location: Dhaka, Bangladesh Setting: International Centre for Diarrhoeal Disease Research, Bangladesh Source of funding: USAID (HRN-A-00-96-90005-00), the Swedish Agency for Research and Economic Cooperation, Sida-SAREC (1995-0069) and ICDDR,B

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random code generated by statistician (author communication)
Allocation concealment (selection bias)	Low risk	"Each subject was assigned a sequential number at the time of initial dosing, corresponding to numbered and randomized set of two single-dose vials of study agent" Allocation was concealed through randomization to identical coded vials
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"The vaccine and placebo formulation appeared similar".
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"The investigators including field staff and laboratory personnel were completely blinded to the identity of the study subjects, whether vaccine or placebo"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Follow-up was short and no losses to follow-up occurred.
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Qadri 2006a

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: Not mentioned in the article Surveillance: Surveillance for side effects was carried out for 3 days after each vaccination. The children were observed for 1 hour in the field clinic and were then allowed to return home. Local health workers made house-to-house visits within 3 hours to assess and record adverse events, thereafter home visits were made every day for the next 2 days
Participants	Number of participants: 158 Inclusion criteria: Healthy children aged 6 to 17 months living in same socioeconomic background Exclusion criteria: History of gastrointestinal disorder, diarrhoeal illness in the past 2 weeks, febrile illness in the preceding week or antibiotic treatment at least 7 days prior to enrolment as well as children <−2 S.D. (weight/height) of the National Center of Health Statistics (NCHS) were also not recruited. Children who were found to be asymptomatically positive for any bacterial enteric pathogen including ETEC during the screening and any participant with ETEC infection during the study period was to be excluded
Interventions	Vaccine: A quarter dose of vaccine composed of total 2.5×10^{10} CFU of five strains of ETEC. An 1.5 mL dose contained 0.25 mg of recombinant cholera toxin B subunit (BS) plus 0.5×10^{10} formalin-inactivated bacteria of each of five different ETEC strains producing CFA/I, CS1, CS2, CS3, CS4, CS5 (lot E 008) Placebo: 2.5×10^{10} CFU of heat-killed *E. coli* K12 bacteria Additional details: Each two-dose (quarter dose) of vaccine regimen was given at intervals of 2 weeks intervals
Outcomes	*Included in review:* • Adverse events • Immunological response
Notes	Location: Dhaka, Bangladesh Setting: International Centre for Diarrhoeal Disease Research, Bangladesh Source of funding: USAID (HRN-A-00-96-90005-00), the Swedish Agency for Research and Economic Cooperation, Sida-SAREC (2001-3970) and icddr,b

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random code generated by statistician (author communication)
Allocation concealment (selection bias)	Low risk	"Randomized vials of study agents either vaccine or placebo were supplied by the company" The vials were identical in appearance (author communication)

Qadri 2006a (*Continued*)

Blinding of participants and personnel (performance bias) All outcomes	Low risk	The blinding of the study code was maintained throughout (author communication)
Blinding of outcome assessment (detection bias) All outcomes	Low risk	See above.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Follow-up was short and no losses to follow-up occurred.
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Sack 2007

Methods	Study type: Randomized, natural challenge, efficacy study in travellers Trial dates and duration: May 1998 to September 1999 Surveillance: The participants were given vials for collection of faecal samples and daily diaries to record the presence or absence of symptoms each day during the stay, up to 28 days. The participants visited the office at least twice weekly, and turned in their diaries weekly, at which time the diaries were reviewed with the study staff
Participants	Number of participants: 685 for safety analysis and 669 for efficacy analysis Inclusion criteria: Travellers from USA to Mexico and Guatemala and planned to stay at least 14 days, travellers more than ≥17 years of age, good health condition, US resident with a telephone, willingness to participate and signed consent, females not pregnant and willing to use reliable birth control during the study period Exclusion criteria: Clinically significant acute or chronic gastrointestinal disease, any serious medical condition, immunodeficiency, planned to use antibiotics during the trip and recent exposure to ETEC within the past 1-year
Interventions	Vaccine: 1 x 10^{11} formalin-killed 5 strains of enterotoxigenic *E. coli* expressing CFA1, CS1, CS2, CS3, CS4 and CS5 plus 1 mg of the recombinant B-subunit of cholera toxin Placebo: Killed non-pathogenic *E. coli* K12 Additional details: The participants fasted for one hour before and after vaccination. The first dose was taken about 3 weeks before travel (acceptable range, 11 to 35 days prior to travel) and the second dose was taken about 8 days before travel (acceptable range: between 4 to 10 days before travel). The two doses were separated by between 7 to 21 days
Outcomes	*Included in review:* • ETEC diarrhoea • Severe ETEC diarrhoea • All-cause diarrhoea • Any ETEC illness • Severe ETEC illness

	• Adverse events • Immunological response
Notes	Location: USA, Mexico, and Guatemala Setting: Vaccine Testing Unit (VTU), Johns HopkinsUniversity, Baltimore, MD, USA; Institute of Nutrition of Central America and Panama (INCAP), Guatemala; Hospital del Nino Morelense, Mexico; University of Gothenburg, Sweden Source of funding: SBL VaccineAB, Stockholm, Sweden

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"The randomisation used blocks of 10 (prepared by the CRO Clinical Data Care in Lund, Sweden) to assure similar distribution throughout the study and the blocks were stratified according to destination (Guatemalaor Mexico)"
Allocation concealment (selection bias)	Low risk	"The participants were randomised to receive two doses of the vaccine in a bicarbonate citrate buffer, or an identical appearing placebo" "The vials of vaccine had unique study numbers that were then used as the study number to identify that participant. The volunteers were considered as randomised when they had signed the informed consent"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"The complete randomisation list exists in two sealed and identical copies. One was stored at SBL Vaccin AB, and the other at CDC in Lund" "These envelopes were to be opened only if there was a medical and/or ethical need to know the vaccination code, as requested by the DSMB"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	See above.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Losses to follow-up were low (< 10%) in each group.
Selective reporting (reporting bias)	Low risk	None identified.

Other bias	Low risk	None identified.

Savarino 1998

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: Not mentioned in the article. Surveillance: Subjects had oral temperatures taken and underwent a standardized, structured interview on 3 consecutive days after each dose. In addition, subjects were asked to provide 3 venous blood samples, 1 just before the first dose and 1 each 7 days after the first and second doses
Participants	Number of participants: 76 Inclusion criteria: Healthy men and women aged 21 to 45 years Exclusion criteria: Participants with a history of chronic gastrointestinal illness, diarrhoea, antidiarrhoeal drug usage, febrile illness in the week before dosing or pregnancy
Interventions	Vaccine: Each 4 mL dose of the ETEC/rCTB vaccine (Lot E003) contained 1 mg rCTB plus 2 x 10^{10} formalin-inactivated bacteria of each of the following ETEC strains: SBL101 (O78:H12; CFA/I; ST+); SBL104 (O25:H42; CS4+CS6); SBL105 (O167:H5; CS5+CS6; ST+); SBL106 (O6:H16; CS1); and SBL107 (OR:H6; CS2 CS3) Placebo: 1 × 10^{11} CFU of heat-killed *E. coli* K12 bacteria Additional details: Subjects were offered a two dose schedule of study agent in three rounds at intervals of 2 weeks, with fasting for at least 90 minutes before and after dosing
Outcomes	*Included in review:* • Adverse events • Immunological response
Notes	Location: Benha, Egypt Setting: Cairo, Egypt. Source of funding: Naval Medical Research and Development Command, the National Institute of Child Health and Human Development Under Interagency Agreement (Y1-HD-0026-01) and WHO Global Programme for Vaccines and Immunization/Vaccine Research and Development

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"After enrollment, subjects were randomized to receive vaccine or placebo in a double-blind fashion within blocks of 4 sequentially randomized subjects" It was unclear if this was truly randomized.
Allocation concealment (selection bias)	Low risk	"At the time of initial dosing, each subject was assigned a sequential number corresponding to sequentially numbered sin-

Savarino 1998 (*Continued*)

		gle-dose vials of study agent"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Described as "double blind, placebo controlled", and vaccines labelled only with study code
Blinding of outcome assessment (detection bias) All outcomes	Low risk	"The code was broken after all clinical and laboratory evaluations were completed"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Only two subjects were excluded from the 2nd dose from placebo group because of absenteeism or intercurrent diarrhoea
Selective reporting (reporting bias)	Low risk	None.
Other bias	Low risk	None.

Savarino 1999 (Study 1)

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: June 1996 Surveillance: Subjects were observed for 30 minutes after each dose for occurrence of immediate adverse effects. For the next 3 days after each dose, parents were asked to report to the study centre with their child. During each visit, a trained health care provider obtained the child's temperature and performed a standardized, structured interview for 24 hour recall of symptoms
Participants	Number of participants: 107 Inclusion criteria: Healthy Egyptian children aged between 6 to 12 years were recruited from Benha, Egypt Exclusion criteria: Children with a history of chronic gastrointestinal disorder, diarrhoea, or febrile illness, or some other serious chronic illness
Interventions	Vaccine: Each 4 mL dose of the ETEC/rCTB vaccine (Lot E003) contained 1 mg rCTB plus 2 x 10^{10} formalin-inactivated bacteria of each of the following ETEC strains: SBL101 (O78:H12; CFA/I; ST); SBL104 (O25:H42; CS4); SBL105 (O167:H5; CS5; ST); SBL106 (O6:H16; CS1); and SBL107 (OR:H6; CS2 CS3) Placebo: 1 × 10^{11} CFU of heat-killed *E. coli* K12 bacteria Additional details: Subjects received a two-dose schedule of study agent 2 weeks apart, fasting for at least 90 minutes before and after each dose
Outcomes	*Included in review:* • Adverse events • Immunological response
Notes	Location: Benha, Egypt. Setting: US Naval Medical Research Unit No. 3, Cairo, Egypt.

Savarino 1999 (Study 1) (*Continued*)

	Source of funding: Naval Medical Research and Development Command, the National Institute of Child Health and Human Development Under Interagency Agreement (Y1-HD-0026-01) and WHO Global Programme for Vaccines and Immunization/Vaccine Research and Development

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Each child was assigned a sequential number corresponding to serially numbered and randomized sets of two single-dose vials of study agent"
Allocation concealment (selection bias)	Low risk	See above. Allocation was concealed through randomization to identical coded vials
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Investigators and subjects were kept blinded as to assignments until all clinical and laboratory evaluations were completed and data files were frozen"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	See above.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Only two subjects were excluded from the 2nd dose because of fever, diarrhoea, or other intercurrent illness
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Savarino 1999 (Study 2)

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: November to December 1996, pre-school children Surveillance: Subjects were observed for 30 minutes after each dose for occurrence of immediate adverse effects. For the next 3 days after each dose, parents were asked to report to the study centre with their child. During each visit, a trained health care provider obtained the child's temperature and performed a standardized, structured interview for 24 hour recall of symptoms
Participants	Number of participants: 106 Inclusion criteria: Healthy Egyptian children ages, both boys and girls aged between 2 to 5 years were recruited from Benha, Egypt Exclusion criteria: Children with a history of chronic gastrointestinal disorder, diarrhoea,

Savarino 1999 (Study 2) (*Continued*)

	or febrile illness, or some other serious chronic illness
Interventions	Vaccine: Each 4 mL dose of the ETEC/rCTB vaccine (Lot E003) contained 1 mg rCTB plus $2x10^{10}$ formalin-inactivated bacteria of each of the following ETEC strains: SBL101 (O78:H12; CFA/I; ST); SBL104 (O25:H42; CS4); SBL105 (O167:H5; CS5; ST); SBL106 (O6:H16; CS1); and SBL107 (OR:H6; CS2 CS3) Placebo: 1×10^{11} CFU of heat-killed *E. coli* K12 bacteria. Additional details: Subjects received a two-dose schedule of study agent 2 weeks apart, fasting for at least 90 minutes before and after each dose
Outcomes	*Included in review:* • Adverse events • Immunological response
Notes	Location: Benha, Egypt Setting: US Naval Medical Research Unit No. 3, Cairo, Egypt Source of funding: Naval Medical Research and Development Command, the National Institute of Child Health and Human Development Under Interagency Agreement (Y1-HD-0026-01) and WHO Global Programme for Vaccines and Immunization/Vaccine Research and Development

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Each child was assigned a sequential number corresponding to serially numbered and randomized sets of two single-dose vials of study agent"
Allocation concealment (selection bias)	Low risk	See above. Allocation was concealed through randomization to identical coded vials
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Investigators and subjects were kept blinded as to assignments until all clinical and laboratory evaluations were completed and data files were frozen"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	See above.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Losses to follow-up were low: Nine subjects were excluded from the 2nd dose because of fever, diarrhoea, or other intercurrent illness
Selective reporting (reporting bias)	Low risk	None identified.

Savarino 1999 (Study 2) (*Continued*)

Other bias	Low risk	None identified.

Savarino 2002

Methods	Study type: Randomized, safety and immunogenicity study in volunteers Trial dates and duration: September to October 1997 Surveillance: Subjects were directly observed for 30 minutes after each dose for immediate adverse events. For three days after each dose parents reported daily to the study centre with their child. At each visit a trained health worker took the child's rectal temperature and performed a standardized, structured interview for 24 hour recall of symptoms. Occurrence of specific gastrointestinal symptoms and other unanticipated complaints was ascertained
Participants	Number of participants: 95 Inclusion criteria: Healthy boys and girls aged 6 to 18 months were recruited from Benha, Egypt Exclusion criteria: Children with a history of chronic gastrointestinal disorder, some other serious chronic illness, or congenital anomaly
Interventions	Vaccine: Each 4 mL dose of the ETEC/rCTB vaccine (Lot E003) contained 1 mg rCTB plus 2 x 10^{10} formalin-inactivated bacteria of each of the following ETEC strains: SBL101 (O78:H12; CFA/I; ST); SBL104 (O25:H42; CS4); SBL105 (O167:H5; CS5; ST); SBL106 (O6:H16; CS1); and SBL107 (OR:H6; CS2 CS3) Placebo: 1 × 10^{11} CFU of heat-killed *E. coli* K12 bacteria Additional details: Subjects were offered a three dose schedule of study agent in three rounds at intervals of 2 weeks, with fasting for at least 1 hour before and after dosing
Outcomes	*Included in review:* • Adverse events • Immunological response
Notes	Location: Benha, Egypt Setting: US Naval Medical Research Unit Number 3, Cairo, Egypt Source of funding: Naval Medical Research and Development Command, the National Institute of Child Health and Human Development Under Interagency Agreement (Y1-HD-0026-01) and WHO Global Programme for Vaccines and Immunization/Vaccine Research and Development

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"After enrollment subjects were stratified by age (6-month bands) and gender and then block randomized to vaccine or control (block size, four) in a 1:1 ratio"

Savarino 2002 (*Continued*)

Allocation concealment (selection bias)	Low risk	"Within each stratum children were assigned a sequential number corresponding to serially numbered and randomized sets of three single dose vials of study agent" Allocation was concealed through randomization to identical coded vials
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Investigators and subjects were kept blinded as to assignments until all clinical and laboratory evaluations were completed, and data files were locked"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	See above.
Incomplete outcome data (attrition bias) All outcomes	High risk	Loss to follow-up was high: 26% of randomized subjects (33/128) dropped out before receiving any dose of study agent, and 33% of subjects who received at least one dose of study agent did not complete the study (31/95)
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Scerpella 1995

Methods	Study type: Randomized, natural challenge, efficacy study in travellers Trial dates and duration: From June 1992 to July 1992 Surveillance: WC/rBS oral cholera vaccine in 502 US college students attending summer educational programs in Mexico
Participants	Number of participants: 502 healthy adults Inclusion criteria: Full-time US residence, aged 18 and over, willingness to participate in the study and willingness to sign the consent form Exclusion criteria: Failure to understand the nature and plan of the study, inability to receive adequate follow-up examinations in Mexico, unwillingness to submit serum specimens, use of oral or parenteral antibiotics in the 7 days previous to enrolment, use of more than two doses of anti-diarrhoeal medications in the 7 days previous to enrolment, significant abnormalities detected by screening of the medical history and physical exam, history of severe allergic reaction to any vaccine, and in women of childbearing age, a positive urine pregnancy test result and nursing mothers
Interventions	Vaccine: WC-rBS, I mg of purified CTB subunit together with $1X10^{11}$ inactivated *V. cholerae* Placebo: Bicarbonate buffer alone.

Scerpella 1995 (*Continued*)

	Additional details: Two doses of oral vaccine given, first dose at day 0 and second days 10 days later. Both vaccine and placebo are identical in appearance
Outcomes	*Included in review:* • ETEC diarrhoea • Severe ETEC diarrhoea • All-cause diarrhoea • Any ETEC illness • Severe ETEC illness • Adverse events • Immunological response
Notes	Location: Mexico Setting: Center for Infectious Diseases of the University of Texas Health Science Center in Houston Source of funding: DOD; DAMD 17-90-R-0048

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Described as "randomized", but no further details given.
Allocation concealment (selection bias)	Unclear risk	None described.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"For the placebo group, the 3 rnL dose of vaccine was not added before administration of the buffer solution. In this fashion, study participants were blinded as to which study group they were in" Personnel appear to be unblinded.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Blinding of outcome assessors was not described.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Losses to follow-up were low. Data for 492 of 502 (98%) participants were available for analysis
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Tacket 1999

Methods	Study type: Randomized, artificial challenge, efficacy study in volunteers Trial dates and duration: Not mentioned in the article Surveillance: Vaccine or placebo administered 3 times daily for 5 days Artificial challenge: on day 2; post challenge follow-up for 4 days
Participants	Number of participants: 20 Inclusion criteria: Healthy adults Exclusion criteria: None mentioned
Interventions	Vaccine: All participants were randomized to receive 690 mg of bovine anti-*E. coli* CFA milk immunoglobulin capsule Placebo: All participants were randomized to receive 690 mg of placebo capsule Additional details: Vaccine or placebo were administered 3 times daily, 10 minutes after each meal, for 5 days followed by on day 2 with an applesauce containing artificial challenge: 1×10^8 CFU of the challenge virulent strain of *E. coli* E24377. Schedule dose of vaccine or placebo was also administered 10 minutes after challenge
Outcomes	*Included in review:* • All cause diarrhoea
Notes	Location: USA Setting: Research Isolation Ward, Kernan Hospital, University of Maryland, Baltimore, MD, USA Source of funding: ImmuCell Corporation, Portland, ME

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Described as "'randomized"', but no further details given.
Allocation concealment (selection bias)	Unclear risk	None described.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	The placebo consisted of an identical preparation from non-immunized cow
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Described as "'double blind"' but no further details given.
Incomplete outcome data (attrition bias) All outcomes	Low risk	No losses to follow-up occurred.
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Wiedermann 2000

Methods	Study type: RCT, natural challenge, efficacy study in travellers Trial dates and duration: Not mentioned in the article Surveillance: Post-vaccination symptoms and adverse events were reported after both doses. All volunteers enrolled in the study were equipped with both a daily record diary to monitor episodes of travellers' diarrhoea during their stay abroad and with transport media (Portagerm® and Cary Blair tubes) for collection of stool samples in case of diarrhoea. Travelers were instructed to hand over their travel diary and transport media tubes immediately after return
Participants	Number of participants: 128 travellers (66 placebo group and 62 ETEC vaccine group) Inclusion criteria: Adults and children, who had signed up for a trip to tropical or subtropical destinations (44 different countries in Africa, Asia and Latin-America) with a duration of stay intended to last 7 to 23 days Exclusion criteria: Not mentioned in the article
Interventions	Vaccine: ETEC vaccine, containing 1 mg of recombinant B-subunit of cholera toxin plus 10^{11} formalin-killed ETEC bacteria of five ETEC strains expressing the most common CFAs such as CFA I, CFA II (CS1, CS2 and CS3) and CFA IV (CS4, CS5 and CS6); a B-subunit cholera whole cell vaccine (licensed in Sweden since 1992), containing 1 mg recombinant subunit B cholera toxin and 10^{11} inactivated whole cells (Inaba,Ogawa; classical and El Tor) Placebo: Approximately 10^{11} inactivated *E. coli* K12 Additional details: Two consecutive vaccine or placebo doses given at an interval of between 7 to 21 days, not less than 7 days and not more than 30 days before departure
Outcomes	*Included in review:* • ETEC diarrhoea • All-cause diarrhoea • Adverse events • Immunological response
Notes	Location: Institute for Specific Prophylaxis and Tropical Medicine, University of Vienna, Austria Setting: Institute for Specific Prophylaxis and Tropical Medicine, University of Vienna, Austria; Department of Medical Microbiology and Immunology, Göteborg University, Sweden; Swedish Bacteriological Laboratory, Vaccin, Sweden Source of funding: Not mentioned

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Described as "randomized" but no further details given.
Allocation concealment (selection bias)	Unclear risk	None described.

Wiedermann 2000 (*Continued*)

Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Described as "double blind" but no further details given.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Described as "double blind" but no further details given.
Incomplete outcome data (attrition bias) All outcomes	High risk	Seventy-three recruited participants (29.2%) were excluded from the primary analysis. The reasons for these exclusions were unclear
Selective reporting (reporting bias)	Low risk	None identified.
Other bias	Low risk	None identified.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Ahmed 2006	Participants did not receive a vaccine to prevent ETEC.
Ahren 1998	No control group.
Carpenter 2006	Not described as randomized.
Clemens 2004	Randomized placebo controlled Phase III trial but published the data as a cohort study. Clinical efficacy data are unavailable from the article
Coster 2007	Participants did not receive a vaccine to prevent ETEC.
Daley 2007	No clinical efficacy data for this vaccine is available.
Evans 1984	Not described as randomized.
Evans 1988a	No control group.
Evans 1988b	Not described as randomized.
Glenn 2007	No control group.
Guereña-Burgueño 2002	Not described as randomized.
Hallander 2002	No protective efficacy data against travellers' diarrhoea

(*Continued*)

Holmgren 1992	No outcomes relevant to this review.
Katz 2003	Not described as randomized.
Khan 2007	Not described as randomized.
Klipstein 1986	Not described as randomized.
Lapa 2008	No clinical efficacy data for this vaccine is available.
Levine 1982	Not described as randomized.
McKenzie 2006	No true control arm for safety and immunological data.
Qadri 2006b	Participants did not receive a vaccine to prevent ETEC.
Sougioultzis 2002	Topic unrelated to ETEC vaccination.
Tacket 1994	Not described as randomized.
Turner 2006	No clinical efficacy data for this vaccine is available.
Wasserman 1993	Participants did not receive a vaccine to prevent ETEC.
Wenneras 1999	Not described as randomized.

DATA AND ANALYSES

Comparison 1. Cholera killed whole cell vaccine (Cholera WC-BS) versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 ETEC diarrhoea	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2 Severe ETEC diarrhoea	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
3 All-cause diarrhoea	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
4 Adverse events	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
4.1 Any symptoms	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4.2 Gastrointestinal symptoms	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4.3 Headache	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4.4 Respiratory symptoms	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4.5 Others	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]

Comparison 2. Cholera killed whole cell vaccine with recombinant B-subunit (Cholera WC-rCTB) versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 ETEC diarrhoea	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2 All-cause diarrhoea	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
3 ETEC diarrhoea (Scerpella 1995a subgroup analysis excluding cases of ETEC occurring < 7 days after vaccination)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 Cholera WC-rCTB versus placebo (all participants included in denominator)	1	502	Risk Ratio (M-H, Fixed, 95% CI)	0.59 [0.24, 1.47]
3.2 Cholera WC-rCTB versus placebo (participants who had ETEC diarrhoea before vaccination complete excluded from denominator)	1	457	Risk Ratio (M-H, Fixed, 95% CI)	0.58 [0.23, 1.44]
4 Immunological response: > 4-fold increase in toxin-specific IgG antibody responses in serum/plasma	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only

Comparison 3. ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 ETEC diarrhoea	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2 Severe ETEC diarrhoea	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
3 All-cause diarrhoea	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
4 Adverse events: ETEC WC-rCTB versus placebo (after first dose)	11		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Any symptoms	9	926	Risk Ratio (M-H, Fixed, 95% CI)	1.63 [1.34, 1.97]
4.2 Diarrhoea	9	1528	Risk Ratio (M-H, Fixed, 95% CI)	0.87 [0.55, 1.40]
4.3 Abdominal pain	7	1275	Risk Ratio (M-H, Fixed, 95% CI)	1.17 [0.79, 1.73]
4.4 Loss of appetite	7	696	Risk Ratio (M-H, Fixed, 95% CI)	1.83 [1.03, 3.24]
4.5 Gas/abdominal distension/bloating	1	158	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4.6 Nausea	4	904	Risk Ratio (M-H, Fixed, 95% CI)	1.24 [0.73, 2.09]
4.7 Vomiting	9	1528	Risk Ratio (M-H, Fixed, 95% CI)	2.00 [1.16, 3.45]
4.8 Fever	7	778	Risk Ratio (M-H, Fixed, 95% CI)	0.87 [0.34, 2.22]
4.9 Headache	2	154	Risk Ratio (M-H, Fixed, 95% CI)	1.46 [0.88, 2.42]
4.10 Malaise	2	154	Risk Ratio (M-H, Fixed, 95% CI)	1.77 [0.91, 3.44]
4.11 Spitting with cough	1	158	Risk Ratio (M-H, Fixed, 95% CI)	1.0 [0.14, 6.92]
4.12 Others	5	1058	Risk Ratio (M-H, Fixed, 95% CI)	1.37 [0.83, 2.26]
5 Immunological response: > 2-fold increase in CFA/I-specific IgA antibody response in serum/plasma	12		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
6 Immunological response: > 2-fold increase in toxin-specific IgA antibody responses in serum/plasma	13		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only

Comparison 4. Live attenuated cholera vaccine (CVD 103-HgR) versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 ETEC diarrhoea	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
2 Moderate to severe ETEC diarrhoea	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
3 All-cause diarrhoea	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

Comparison 5. Live attenuated ETEC vaccine (PTL-003) versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 ETEC diarrhoea	1		Risk Ratio (M-H, Random, 95% CI)	Totals not selected
2 Moderate to severe ETEC diarrhoea	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
3 Adverse events (after first dose)	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
3.1 Abdominal cramps/pain	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.2 Diarrhoea	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.3 Gas/abdominal distension/bloating	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.4 Gurgling/bubbling	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.5 Nausea	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.6 Loss of appetite	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.7 Vomiting	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.8 Fever	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.9 Malaise	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.10 Headache	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.11 Arthalgias/myalgias	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4 Immunological response: > 2-fold increase in TSA	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected

Comparison 6. Transcutaneous LT patch versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 ETEC diarrhoea	2	217	Risk Ratio (M-H, Fixed, 95% CI)	0.83 [0.64, 1.08]
2 Moderate to severe ETEC diarrhoea	2	217	Risk Ratio (M-H, Fixed, 95% CI)	0.86 [0.59, 1.25]
3 All-cause diarrhoea	1	170	Risk Ratio (M-H, Fixed, 95% CI)	0.71 [0.35, 1.42]
4 Adverse events	2	1643	Risk Ratio (M-H, Fixed, 95% CI)	3.87 [3.10, 4.84]
4.1 Rash	2	260	Risk Ratio (M-H, Fixed, 95% CI)	5.80 [3.88, 8.67]
4.2 Pruritus	2	260	Risk Ratio (M-H, Fixed, 95% CI)	4.66 [3.25, 6.68]
4.3 Vesicles	1	59	Risk Ratio (M-H, Fixed, 95% CI)	10.65 [0.62, 184.25]
4.4 Skin discoloration	2	260	Risk Ratio (M-H, Fixed, 95% CI)	9.73 [2.87, 32.92]
4.5 Fever	1	201	Risk Ratio (M-H, Fixed, 95% CI)	2.0 [0.13, 31.48]
4.6 Malaise	1	201	Risk Ratio (M-H, Fixed, 95% CI)	1.11 [0.39, 3.19]
4.7 Headache	1	201	Risk Ratio (M-H, Fixed, 95% CI)	1.29 [0.59, 2.82]
4.8 Diarrhoea	1	201	Risk Ratio (M-H, Fixed, 95% CI)	1.5 [0.67, 3.38]
5 Immunological response	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.1 > 4-fold increase in anti-LT specific IgA responses in serum/plasma	2	217	Risk Ratio (M-H, Fixed, 95% CI)	43.00 [12.33, 149.97]

Comparison 7. Hyperimmune anti-*E. coli* CFA versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All-cause diarrhoea	2	45	Risk Ratio (M-H, Fixed, 95% CI)	0.51 [0.22, 1.15]
2 Adverse events	1		Risk Ratio (M-H, Fixed, 95% CI)	Totals not selected
2.1 Anorexia	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
2.2 Malaise	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
2.3 Gas	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
2.4 Abdominal pain	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
2.5 Headache	1		Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]

Analysis 1.1. Comparison 1 Cholera killed whole cell vaccine (Cholera WC-BS) versus placebo, Outcome 1 ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 1 Cholera killed whole cell vaccine (Cholera WC-BS) versus placebo

Outcome: 1 ETEC diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Clemens 1988 (1)	6/24770	18/24842		0.33 [0.13, 0.84]
Peltola 1991 (2)	14/307	29/308		0.48 [0.26, 0.90]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 20 (Vaccine), 47 (Placebo)				
Heterogeneity: Chi^2 = 0.0, df = 0 (P<0.00001); I^2 =0.0%				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				

(1) Clemens 1988a: A natural challenge study in an endemic population in Bangladesh.

(2) Peltola 1991a: A natural challenge study in people travelling from Europe to Morocco

Analysis 1.2. Comparison 1 Cholera killed whole cell vaccine (Cholera WC-BS) versus placebo, Outcome 2 Severe ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 1 Cholera killed whole cell vaccine (Cholera WC-BS) versus placebo

Outcome: 2 Severe ETEC diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Clemens 1988 (1)	1/24770	7/24842		0.14 [0.02, 1.16]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 1 (Vaccine), 7 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				

(1) Clemens 1988a: A natural challenge study in an endemic population in Bangladesh.

Analysis 1.3. Comparison 1 Cholera killed whole cell vaccine (Cholera WC-BS) versus placebo, Outcome 3 All-cause diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 1 Cholera killed whole cell vaccine (Cholera WC-BS) versus placebo

Outcome: 3 All-cause diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Peltola 1991 (1)	72/307	94/308		0.77 [0.59, 1.00]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 72 (Vaccine), 94 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				

(1) Peltola 1991a: A natural challenge study in people travelling from Europe to Morocco

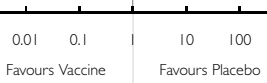
Analysis 1.4. Comparison 1 Cholera killed whole cell vaccine (Cholera WC-BS) versus placebo, Outcome 4 Adverse events.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 1 Cholera killed whole cell vaccine (Cholera WC-BS) versus placebo

Outcome: 4 Adverse events

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Any symptoms				
Peltola 1991 (1)	85/243	118/265		0.79 [0.63, 0.98]
2 Gastrointestinal symptoms				
Peltola 1991	59/243	88/265		0.73 [0.55, 0.97]
3 Headache				
Peltola 1991	10/243	11/265		0.99 [0.43, 2.29]
4 Respiratory symptoms				
Peltola 1991	12/243	15/265		0.87 [0.42, 1.83]
5 Others				
Peltola 1991	2/243	3/265		0.73 [0.12, 4.31]



(1) Peltola 1991a: A natural challenge study in people travelling from Europe to Morocco

Analysis 2.1. Comparison 2 Cholera killed whole cell vaccine with recombinant B-subunit (Cholera WC-rCTB) versus placebo, Outcome 1 ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 2 Cholera killed whole cell vaccine with recombinant B-subunit (Cholera WC-rCTB) versus placebo

Outcome: 1 ETEC diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Scerpella 1995 (1)	36/250	39/252		0.93 [0.61, 1.41]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 36 (Vaccine), 39 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				

(1) Scerpella 1995a: A natural challenge study in people travelling from USA to Mexico.

Analysis 2.2. Comparison 2 Cholera killed whole cell vaccine with recombinant B-subunit (Cholera WC-rCTB) versus placebo, Outcome 2 All-cause diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 2 Cholera killed whole cell vaccine with recombinant B-subunit (Cholera WC-rCTB) versus placebo

Outcome: 2 All-cause diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Scerpella 1995 (1)	128/250	124/252		1.04 [0.87, 1.24]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 128 (Vaccine), 124 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				

(1) Scerpella 1995a: A natural challenge study in people travelling from USA to Mexico.

Analysis 2.3. Comparison 2 Cholera killed whole cell vaccine with recombinant B-subunit (Cholera WC-rCTB) versus placebo, Outcome 3 ETEC diarrhoea (Scerpella 1995a subgroup analysis excluding cases of ETEC occurring < 7 days after vaccination).

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 2 Cholera killed whole cell vaccine with recombinant B-subunit (Cholera WC-rCTB) versus placebo

Outcome: 3 ETEC diarrhoea (Scerpella 1995a subgroup analysis excluding cases of ETEC occurring < 7 days after vaccination)

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Cholera WC-rCTB versus placebo (all participants included in denominator)					
Scerpella 1995 (1)	7/250	12/252		100.0 %	0.59 [0.24, 1.47]
Subtotal (95% CI)	**250**	**252**		**100.0 %**	**0.59 [0.24, 1.47]**
Total events: 7 (Vaccine), 12 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.14 (P = 0.26)					
2 Cholera WC-rCTB versus placebo (participants who had ETEC diarrhoea before vaccination complete excluded from denominator)					
Scerpella 1995	7/230	12/227		100.0 %	0.58 [0.23, 1.44]
Subtotal (95% CI)	**230**	**227**		**100.0 %**	**0.58 [0.23, 1.44]**
Total events: 7 (Vaccine), 12 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.18 (P = 0.24)					
Test for subgroup differences: Chi^2 = 0.00, df = 1 (P = 0.97), I^2 =0.0%					

(1) Scerpella 1995a: These data present only the cases of ETEC diarrhoea that occurred more than seven days after the second vaccine dose

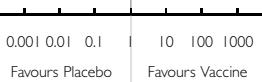
Analysis 2.4. Comparison 2 Cholera killed whole cell vaccine with recombinant B-subunit (Cholera WC-rCTB) versus placebo, Outcome 4 Immunological response: > 4-fold increase in toxin-specific IgG antibody responses in serum/plasma.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 2 Cholera killed whole cell vaccine with recombinant B-subunit (Cholera WC-rCTB) versus placebo

Outcome: 4 Immunological response: > 4-fold increase in toxin-specific IgG antibody responses in serum/plasma

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Scerpella 1995 (1)	117/135	12/146		10.54 [6.11, 18.20]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 117 (Vaccine), 12 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				



(1) Scerpella 1995a: An efficacy study in people travelling from USA to Mexico.

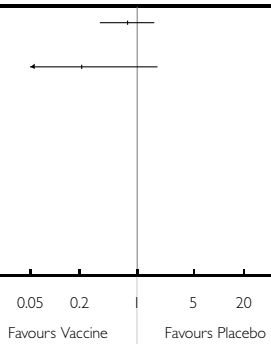
Analysis 3.1. Comparison 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo, Outcome 1 ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo

Outcome: 1 ETEC diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Sack 2007 (1)	11/330	15/341		0.76 [0.35, 1.63]
Wiedermann 2000 (2)	1/62	5/66		0.21 [0.03, 1.77]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 12 (Vaccine), 20 (Placebo)				
Heterogeneity: Chi^2 = 0.0, df = 0 (P<0.00001); I^2 =0.0%				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				



(1) Sack 2007a: A natural challenge study in people travelling from USA to Mexico, Guatemala.

(2) Wiederman 2000a: A natural challenge study in people travelling from Austria to 44 different countries in Latin America, Africa and Asia.

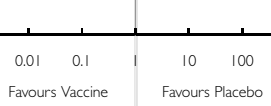
Analysis 3.2. Comparison 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo, Outcome 2 Severe ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo

Outcome: 2 Severe ETEC diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Sack 2007 (1)	2/330	9/341		0.23 [0.05, 1.05]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 2 (Vaccine), 9 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				



(1) Sack 2007a: A natural challenge study in people travelling from USA to Mexico, Guatemala.

Analysis 3.3. Comparison 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo, Outcome 3 All-cause diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo

Outcome: 3 All-cause diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Sack 2007 (1)	108/330	98/341		1.14 [0.91, 1.43]
Wiedermann 2000 (2)	15/62	14/66		1.14 [0.60, 2.16]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 123 (Vaccine), 112 (Placebo)				
Heterogeneity: Chi^2 = 0.0, df = 0 (P<0.00001); I^2 =0.0%				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				

(1) Sack 2007a: A natural challenge study in people travelling from USA to Mexico, Guatemala.

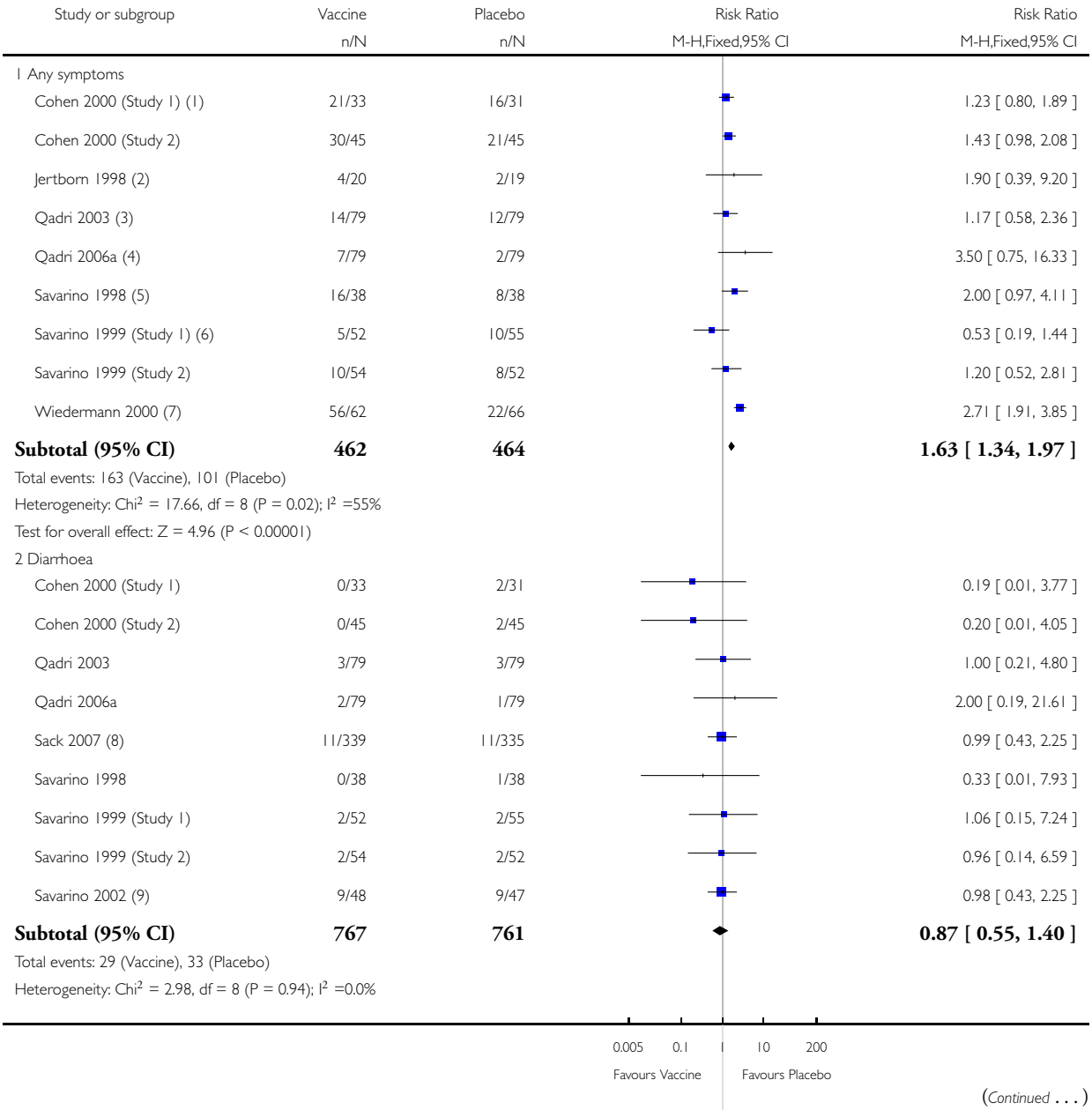
(2) Wiederman 2000a: A natural challenge study in people travelling from Austria to 44 different countries in Latin America, Africa and Asia.

Analysis 3.4. Comparison 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo, Outcome 4 Adverse events: ETEC WC-rCTB versus placebo (after first dose).

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo

Outcome: 4 Adverse events: ETEC WC-rCTB versus placebo (after first dose)



Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Any symptoms				
Cohen 2000 (Study 1) (1)	21/33	16/31		1.23 [0.80, 1.89]
Cohen 2000 (Study 2)	30/45	21/45		1.43 [0.98, 2.08]
Jertborn 1998 (2)	4/20	2/19		1.90 [0.39, 9.20]
Qadri 2003 (3)	14/79	12/79		1.17 [0.58, 2.36]
Qadri 2006a (4)	7/79	2/79		3.50 [0.75, 16.33]
Savarino 1998 (5)	16/38	8/38		2.00 [0.97, 4.11]
Savarino 1999 (Study 1) (6)	5/52	10/55		0.53 [0.19, 1.44]
Savarino 1999 (Study 2)	10/54	8/52		1.20 [0.52, 2.81]
Wiedermann 2000 (7)	56/62	22/66		2.71 [1.91, 3.85]
Subtotal (95% CI)	**462**	**464**		**1.63 [1.34, 1.97]**
Total events: 163 (Vaccine), 101 (Placebo)				
Heterogeneity: Chi^2 = 17.66, df = 8 (P = 0.02); I^2 =55%				
Test for overall effect: Z = 4.96 (P < 0.00001)				
2 Diarrhoea				
Cohen 2000 (Study 1)	0/33	2/31		0.19 [0.01, 3.77]
Cohen 2000 (Study 2)	0/45	2/45		0.20 [0.01, 4.05]
Qadri 2003	3/79	3/79		1.00 [0.21, 4.80]
Qadri 2006a	2/79	1/79		2.00 [0.19, 21.61]
Sack 2007 (8)	11/339	11/335		0.99 [0.43, 2.25]
Savarino 1998	0/38	1/38		0.33 [0.01, 7.93]
Savarino 1999 (Study 1)	2/52	2/55		1.06 [0.15, 7.24]
Savarino 1999 (Study 2)	2/54	2/52		0.96 [0.14, 6.59]
Savarino 2002 (9)	9/48	9/47		0.98 [0.43, 2.25]
Subtotal (95% CI)	**767**	**761**		**0.87 [0.55, 1.40]**
Total events: 29 (Vaccine), 33 (Placebo)				
Heterogeneity: Chi^2 = 2.98, df = 8 (P = 0.94); I^2 =0.0%				

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Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Test for overall effect: Z = 0.56 (P = 0.57)				
3 Abdominal pain				
Cohen 2000 (Study 1)	12/33	8/31		1.41 [0.67, 2.98]
Cohen 2000 (Study 2)	15/45	8/45		1.88 [0.88, 3.98]
Qadri 2003	0/79	0/79		0.0 [0.0, 0.0]
Sack 2007	7/339	9/335		0.77 [0.29, 2.04]
Savarino 1998	7/38	3/38		2.33 [0.65, 8.36]
Savarino 1999 (Study 1)	2/52	6/55		0.35 [0.07, 1.67]
Savarino 1999 (Study 2)	2/54	4/52		0.48 [0.09, 2.52]
Subtotal (95% CI)	**640**	**635**		**1.17 [0.79, 1.73]**
Total events: 45 (Vaccine), 38 (Placebo)				
Heterogeneity: Chi^2 = 6.98, df = 5 (P = 0.22); I^2 =28%				
Test for overall effect: Z = 0.77 (P = 0.44)				
4 Loss of appetite				
Cohen 2000 (Study 1)	6/33	2/31		2.82 [0.61, 12.93]
Cohen 2000 (Study 2)	10/45	5/45		2.00 [0.74, 5.39]
Qadri 2003	2/79	1/79		2.00 [0.19, 21.61]
Savarino 1998	4/38	2/38		2.00 [0.39, 10.28]
Savarino 1999 (Study 1)	0/52	2/55		0.21 [0.01, 4.30]
Savarino 1999 (Study 2)	3/54	2/52		1.44 [0.25, 8.30]
Savarino 2002	5/48	2/47		2.45 [0.50, 12.00]
Subtotal (95% CI)	**349**	**347**		**1.83 [1.03, 3.24]**
Total events: 30 (Vaccine), 16 (Placebo)				
Heterogeneity: Chi^2 = 2.53, df = 6 (P = 0.87); I^2 =0.0%				
Test for overall effect: Z = 2.06 (P = 0.040)				
5 Gas/abdominal distension/bloating				
Qadri 2006a	0/79	0/79		0.0 [0.0, 0.0]
Subtotal (95% CI)	**79**	**79**		**0.0 [0.0, 0.0]**
Total events: 0 (Vaccine), 0 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P < 0.00001)				
6 Nausea				
Cohen 2000 (Study 1)	4/33	5/31		0.75 [0.22, 2.55]
Cohen 2000 (Study 2)	7/45	5/45		1.40 [0.48, 4.08]
Sack 2007	13/339	11/335		1.17 [0.53, 2.57]
Savarino 1998	5/38	2/38		2.50 [0.52, 12.10]
Subtotal (95% CI)	**455**	**449**		**1.24 [0.73, 2.09]**

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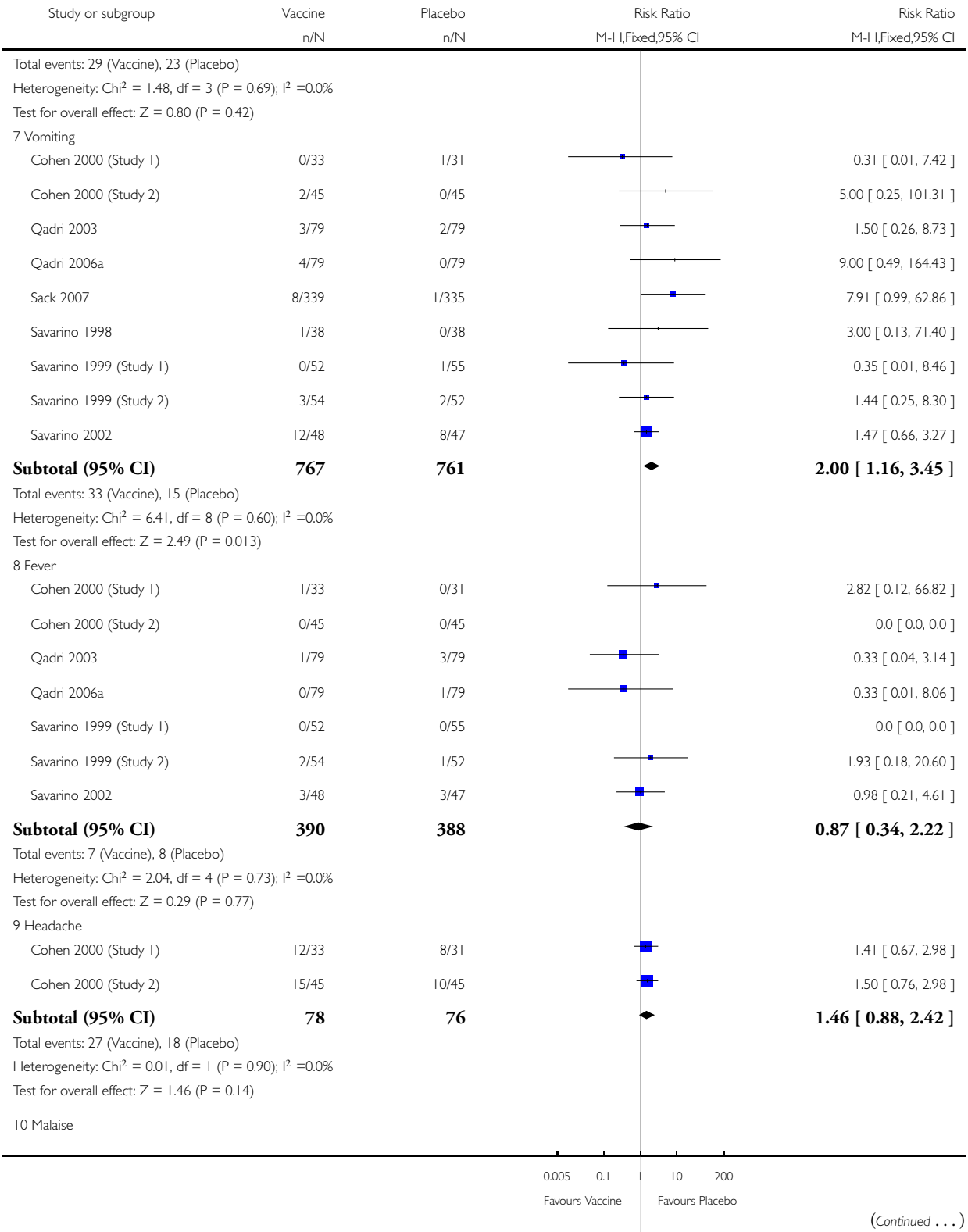

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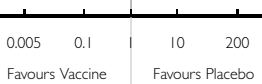
Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Cohen 2000 (Study 1)	8/33	5/31		1.50 [0.55, 4.10]
Cohen 2000 (Study 2)	12/45	6/45		2.00 [0.82, 4.86]
Subtotal (95% CI)	**78**	**76**		**1.77 [0.91, 3.44]**
Total events: 20 (Vaccine), 11 (Placebo)				
Heterogeneity: Chi^2 = 0.17, df = 1 (P = 0.68); I^2 =0.0%				
Test for overall effect: Z = 1.69 (P = 0.092)				
11 Spitting with cough				
Qadri 2003	2/79	2/79		1.00 [0.14, 6.92]
Subtotal (95% CI)	**79**	**79**		**1.00 [0.14, 6.92]**
Total events: 2 (Vaccine), 2 (Placebo)				
Heterogeneity: not applicable				
Test for overall effect: Z = 0.0 (P = 1.0)				
12 Others				
Sack 2007	10/339	7/335		1.41 [0.54, 3.66]
Savarino 1998	9/38	7/38		1.29 [0.53, 3.10]
Savarino 1999 (Study 1)	2/52	4/55		0.53 [0.10, 2.77]
Savarino 1999 (Study 2)	5/54	1/52		4.81 [0.58, 39.83]
Savarino 2002	7/48	5/47		1.37 [0.47, 4.02]
Subtotal (95% CI)	**531**	**527**		**1.37 [0.83, 2.26]**
Total events: 33 (Vaccine), 24 (Placebo)				
Heterogeneity: Chi^2 = 2.65, df = 4 (P = 0.62); I^2 =0.0%				
Test for overall effect: Z = 1.23 (P = 0.22)				
Test for subgroup differences: Chi^2 = 11.00, df = 10 (P = 0.36), I^2 =9%				



(1) Cohen 2000: A safety study in adults in Israel

(2) Jertborn 1998: A safety study in adult swedish volunteers

(3) Qadri 2003a: A safety study in children aged 18-36 months in Bangladesh

(4) Qadri 2006b: A safety study in children aged 6-17 months in Bangladesh

(5) Savarino 1998a: A safety study in adults in Egypt

(6) Savarino 1999: A safety study in children aged 6-12 years in Egypt

(7) Wiederman 2000a: A natural challenge study in people travelling from Austria to 44 different countries in Latin America, Africa and Asia.

(8) Sack 2007a: A natural challenge study in people travelling from USA to Mexico, Guatemala.

(9) Savarino 2002a: A safety study in children aged 6-18 months in Egypt

Analysis 3.5. Comparison 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo, Outcome 5 Immunological response: > 2-fold increase in CFA/I-specific IgA antibody response in serum/plasma.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo

Outcome: 5 Immunological response: > 2-fold increase in CFA/I-specific IgA antibody response in serum/plasma

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Cohen 2000 (Study 1) (1)	8/18	1/12		5.33 [0.76, 37.35]
Cohen 2000 (Study 2)	9/35	1/40		10.29 [1.37, 77.19]
Hall 2001 (Study 1) (2)	26/38	2/35		11.97 [3.06, 46.79]
Hall 2001 (Study 2) (3)	49/51	6/54		8.65 [4.06, 18.42]
Hall 2001 (Study 3) (4)	41/47	5/46		8.03 [3.48, 18.49]
Jertborn 2001 (5)	16/19	0/12		21.45 [1.41, 327.40]
Qadri 2003 (6)	58/79	13/79		4.46 [2.67, 7.46]
Qadri 2006a (7)	47/79	4/79		11.75 [4.45, 31.06]
Savarino 1998 (8)	15/16	4/16		3.75 [1.59, 8.84]
Savarino 1999 (Study 1) (9)	16/16	2/16		6.60 [2.09, 20.80]
Savarino 1999 (Study 2) (10)	18/19	1/10		9.47 [1.47, 61.00]
Savarino 2002 (11)	22/36	5/28		3.42 [1.48, 7.90]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 325 (Vaccine), 44 (Placebo)				
Heterogeneity: Chi^2 = 0.0, df = 0 (P<0.00001); I^2 =0.0%				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				

(1) Cohen 2000: A safety and immunogenicity study in adults in Israel

(2) Hall 2001 (Study 1): A safety and immunogenicity study in adults in Egypt

(3) Hall 2001 (Study 2): A safety and immunogenicity study in children aged 6-12 years in Egypt

(4) Hall 2001 (Study 2): A safety and immunogenicity study in children aged 2-5 years in Egypt

(5) Jertborn 2001: An immunogenicity study in adults in Sweden

(6) Qadri 2003a: A safety and immunogenicity study in children aged 18-36 months in Bangladesh

(7) Qadri 2006b: A safety and immunogenicity study in children aged 6-17 months in Bangladesh

(8) Savarino 1998a: A safety and immunogenicity study in adults in Egypt

(9) Savarino 1999 (Study 1): A safety and immunogenicity study in children aged 6-12 years in Egypt

(10) Savarino 1999 (Study 2): A safety and immunogenicity study in children aged 2 to 5 years in Egypt

(11) Savarino 2002a: A safety and immunogenicity study in children aged 6-18 months in Egypt

Analysis 3.6. Comparison 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo, Outcome 6 Immunological response: > 2-fold increase in toxin-specific IgA antibody responses in serum/plasma.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 3 ETEC killed whole cell vaccine with recombinant cholera B-subunit (ETEC WC-rCTB) versus placebo

Outcome: 6 Immunological response: > 2-fold increase in toxin-specific IgA antibody responses in serum/plasma

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Cohen 2000 (Study 1) (1)	14/28	2/23		5.75 [1.45, 22.74]
Cohen 2000 (Study 2)	24/35	2/40		13.71 [3.49, 53.93]
Hall 2001 (Study 1) (2)	36/38	1/35		33.16 [4.80, 229.18]
Hall 2001 (Study 2) (3)	51/51	8/54		6.41 [3.45, 11.90]
Hall 2001 (Study 3) (4)	45/47	4/46		11.01 [4.31, 28.14]
Qadri 2003 (5)	71/79	24/79		2.96 [2.10, 4.16]
Qadri 2006a (6)	77/79	16/79		4.81 [3.10, 7.46]
Sack 2007 (7)	31/43	0/42		61.57 [3.89, 974.64]
Savarino 1998 (8)	16/16	4/16		3.67 [1.65, 8.13]

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Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
Savarino 1999 (Study 1) (9)	52/52	8/55		6.53 [3.51, 12.13]
Savarino 1999 (Study 2) (10)	49/50	4/49		12.01 [4.69, 30.73]
Savarino 2002 (11)	35/36	13/28		2.09 [1.40, 3.13]
Wiedermann 2000 (12)	56/62	22/66		2.71 [1.91, 3.85]
Subtotal (95% CI)	**0**	**0**		**0.0 [0.0, 0.0]**
Total events: 557 (Vaccine), 108 (Placebo)				
Heterogeneity: Chi^2 = 0.0, df = 0 (P<0.00001); I^2 =0.0%				
Test for overall effect: Z = 0.0 (P < 0.00001)				
Test for subgroup differences: Not applicable				

(1) Cohen 2000: A safety and immunogenicity study in adults in Israel

(2) Hall 2001 (Study 1): A safety and immunogenicity study in adults in Egypt

(3) Hall 2001 (Study 2): A safety and immunogenicity study in children aged 6-12 years in Egypt

(4) Hall 2001 (Study 2): A safety and immunogenicity study in children aged 2-5 years in Egypt

(5) Qadri 2003a: A safety and immunogenicity study in children aged 18-36 months in Bangladesh

(6) Qadri 2006b: A safety and immunogenicity study in children aged 6-17 months in Bangladesh

(7) Sack 2007a: An efficacy study in Adults from the USA travelling to Mexico and Guatemala

(8) Savarino 1998a: A safety and immunogenicity study in adults in Egypt

(9) Savarino 1999 (Study 1): A safety and immunogenicity study in children aged 6-12 years in Egypt

(10) Savarino 1999 (Study 2): A safety and immunogenicity study in children aged 2 to 5 years in Egypt

(11) Savarino 2002a: A safety and immunogenicity study in children aged 6-18 months in Egypt

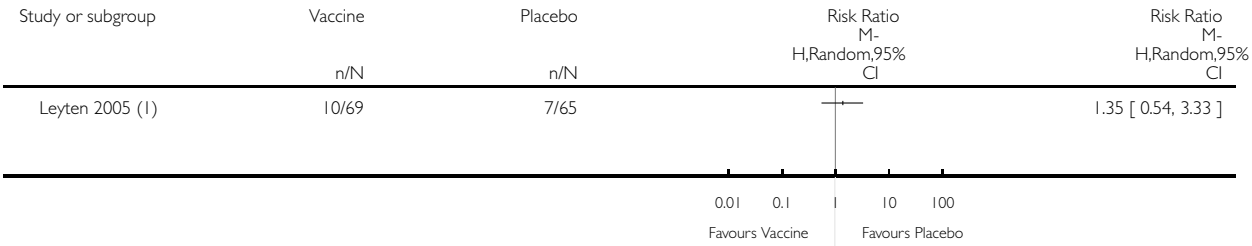
(12) Wiederman 2000a: An efficacy study in people travelling from Austria to 44 different countries in Latin America, Africa and Asia.

Analysis 4.1. Comparison 4 Live attenuated cholera vaccine (CVD 103-HgR) versus placebo, Outcome 1 ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 4 Live attenuated cholera vaccine (CVD 103-HgR) versus placebo

Outcome: 1 ETEC diarrhoea



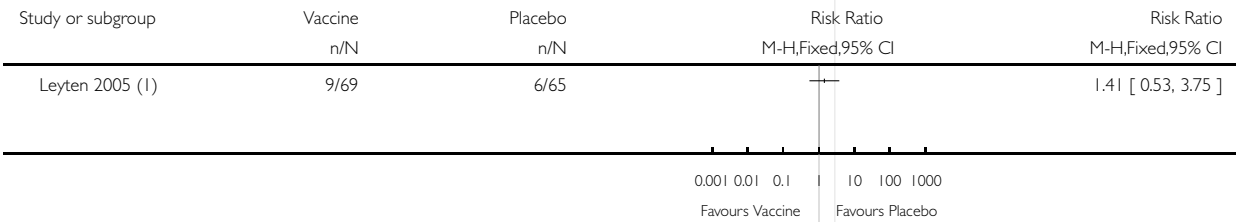
(1) Leyten 2005a: A natural challenge study in Dutch adults travelling to Indonesia, Thailand, India or West Africa.

Analysis 4.2. Comparison 4 Live attenuated cholera vaccine (CVD 103-HgR) versus placebo, Outcome 2 Moderate to severe ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 4 Live attenuated cholera vaccine (CVD 103-HgR) versus placebo

Outcome: 2 Moderate to severe ETEC diarrhoea



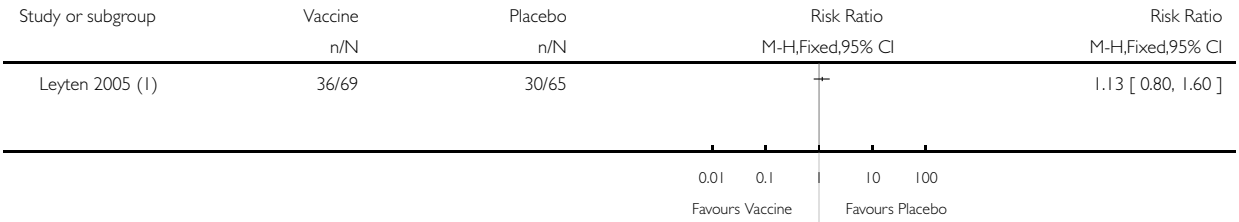
(1) Leyten 2005a: A natural challenge study in Dutch adults travelling to Indonesia, Thailand, India or West Africa.

Analysis 4.3. Comparison 4 Live attenuated cholera vaccine (CVD 103-HgR) versus placebo, Outcome 3 All-cause diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 4 Live attenuated cholera vaccine (CVD 103-HgR) versus placebo

Outcome: 3 All-cause diarrhoea



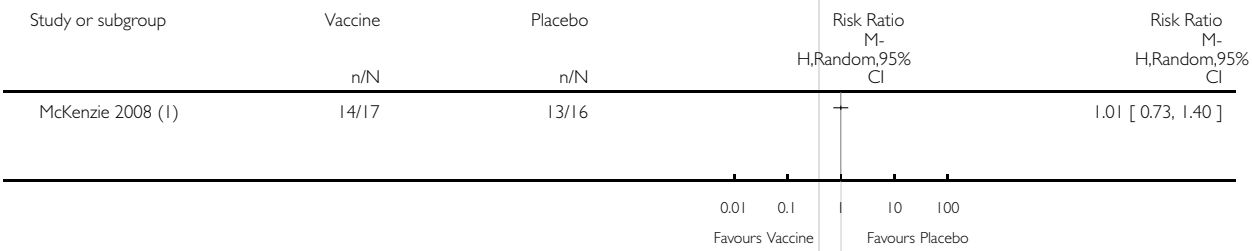
(1) Leyten 2005a: A natural challenge study in Dutch adults travelling to Indonesia, Thailand, India or West Africa.

Analysis 5.1. Comparison 5 Live attenuated ETEC vaccine (PTL-003) versus placebo, Outcome 1 ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 5 Live attenuated ETEC vaccine (PTL-003) versus placebo

Outcome: 1 ETEC diarrhoea



(1) McKenzie 2008: An artifical challenge study in North American adult volunteers.

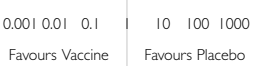
Analysis 5.2. Comparison 5 Live attenuated ETEC vaccine (PTL-003) versus placebo, Outcome 2 Moderate to severe ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 5 Live attenuated ETEC vaccine (PTL-003) versus placebo

Outcome: 2 Moderate to severe ETEC diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
McKenzie 2008 (1)	12/17	11/16		1.03 [0.65, 1.61]



(1) McKenzie 2008: An artifical challenge study in North American adult volunteers.

Analysis 5.3. Comparison 5 Live attenuated ETEC vaccine (PTL-003) versus placebo, Outcome 3 Adverse events (after first dose).

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 5 Live attenuated ETEC vaccine (PTL-003) versus placebo

Outcome: 3 Adverse events (after first dose)

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
1 Abdominal cramps/pain				
McKenzie 2008 (1)	0/20	2/19		0.19 [0.01, 3.73]
2 Diarrhoea				
McKenzie 2008	1/20	2/19		0.48 [0.05, 4.82]
3 Gas/abdominal distension/bloating				
McKenzie 2008	4/20	4/19		0.95 [0.28, 3.27]
4 Gurgling/bubbling				
McKenzie 2008	1/20	1/19		0.95 [0.06, 14.13]
5 Nausea				
McKenzie 2008	3/20	3/19		0.95 [0.22, 4.14]
6 Loss of appetite				

(*Continued* . . .)

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Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
McKenzie 2008	1/20	3/19		0.32 [0.04, 2.79]
7 Vomiting				
McKenzie 2008	1/20	0/19		2.86 [0.12, 66.11]
8 Fever				
McKenzie 2008	0/20	0/19		0.0 [0.0, 0.0]
9 Malaise				
McKenzie 2008	1/20	7/19		0.14 [0.02, 1.00]
10 Headache				
McKenzie 2008	3/20	3/19		0.95 [0.22, 4.14]
11 Arthalgias/myalgias				
McKenzie 2008	1/20	2/19		0.48 [0.05, 4.82]

(1) McKenzie 2008: An artifical challenge study in North American adult volunteers.

Analysis 5.4. Comparison 5 Live attenuated ETEC vaccine (PTL-003) versus placebo, Outcome 4 Immunological response: > 2-fold increase in TSA.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 5 Live attenuated ETEC vaccine (PTL-003) versus placebo

Outcome: 4 Immunological response: > 2-fold increase in TSA

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Risk Ratio M-H,Fixed,95% CI
McKenzie 2008 (1)	1/17	1/16		0.94 [0.06, 13.82]

(1) McKenzie 2008: An artifical challenge study in North American adult volunteers.

Analysis 6.1. Comparison 6 Transcutaneous LT patch versus placebo, Outcome 1 ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 6 Transcutaneous LT patch versus placebo

Outcome: 1 ETEC diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Frech 2008 (1)	3/59	11/111		24.6 %	0.51 [0.15, 1.77]
McKenzie 2007 (2)	25/27	20/20		75.4 %	0.93 [0.82, 1.07]
Total (95% CI)	**86**	**131**		**100.0 %**	**0.83 [0.64, 1.08]**

Total events: 28 (Vaccine), 31 (Placebo)

Heterogeneity: Chi^2 = 3.53, df = 1 (P = 0.06); I^2 =72%

Test for overall effect: Z = 1.40 (P = 0.16)

Test for subgroup differences: Not applicable

(1) Frech 2008: A natural challenge study in American adults travelling to Mexico and Guatemala.

(2) McKenzie 2007a: An artificial challenge study in healthy adult volunteers in the USA.

Analysis 6.2. Comparison 6 Transcutaneous LT patch versus placebo, Outcome 2 Moderate to severe ETEC diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 6 Transcutaneous LT patch versus placebo

Outcome: 2 Moderate to severe ETEC diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Frech 2008 (1)	2/59	11/111		30.7 %	0.34 [0.08, 1.49]
McKenzie 2007 (2)	22/27	15/20		69.3 %	1.09 [0.80, 1.48]
Total (95% CI)	**86**	**131**		**100.0 %**	**0.86 [0.59, 1.25]**

Total events: 24 (Vaccine), 26 (Placebo)

Heterogeneity: Chi^2 = 3.72, df = 1 (P = 0.05); I^2 =73%

Test for overall effect: Z = 0.80 (P = 0.42)

Test for subgroup differences: Not applicable

(1) Frech 2008: A natural challenge study in American adults travelling to Mexico and Guatemala.

(2) McKenzie 2007a: An artificial challenge study in healthy adult volunteers in the USA.

Analysis 6.3. Comparison 6 Transcutaneous LT patch versus placebo, Outcome 3 All-cause diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 6 Transcutaneous LT patch versus placebo

Outcome: 3 All-cause diarrhoea

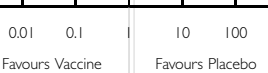
Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Frech 2008 (1)	9/59	24/111		100.0 %	0.71 [0.35, 1.42]
Total (95% CI)	**59**	**111**		**100.0 %**	**0.71 [0.35, 1.42]**

Total events: 9 (Vaccine), 24 (Placebo)

Heterogeneity: not applicable

Test for overall effect: Z = 0.98 (P = 0.33)

Test for subgroup differences: Not applicable



(1) Frech 2008: A natural challenge study in American adults travelling to Mexico and Guatemala.

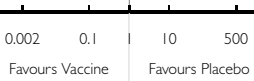
Analysis 6.4. Comparison 6 Transcutaneous LT patch versus placebo, Outcome 4 Adverse events.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 6 Transcutaneous LT patch versus placebo

Outcome: 4 Adverse events

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 Rash					
Frech 2008 (1)	43/67	2/134		2.2 %	43.00 [10.74, 172.14]
McKenzie 2007 (2)	30/30	13/29		22.7 %	2.19 [1.47, 3.26]
Subtotal (95% CI)	**97**	**163**		**24.9 %**	**5.80 [3.88, 8.67]**
Total events: 73 (Vaccine), 15 (Placebo)					
Heterogeneity: Chi^2 = 31.08, df = 1 (P<0.00001); I^2 =97%					
Test for overall effect: Z = 8.57 (P < 0.00001)					
2 Pruritus					
Frech 2008	45/67	5/134		5.5 %	18.00 [7.49, 43.23]
McKenzie 2007	27/30	15/29		25.2 %	1.74 [1.20, 2.52]
Subtotal (95% CI)	**97**	**163**		**30.7 %**	**4.66 [3.25, 6.68]**
Total events: 72 (Vaccine), 20 (Placebo)					
Heterogeneity: Chi^2 = 36.14, df = 1 (P<0.00001); I^2 =97%					
Test for overall effect: Z = 8.36 (P < 0.00001)					
3 Vesicles					
McKenzie 2007	5/30	0/29		0.8 %	10.65 [0.62, 184.25]
Subtotal (95% CI)	**30**	**29**		**0.8 %**	**10.65 [0.62, 184.25]**
Total events: 5 (Vaccine), 0 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.63 (P = 0.10)					
4 Skin discoloration					
Frech 2008	5/67	0/134		0.6 %	21.84 [1.23, 389.17]
McKenzie 2007	16/30	2/29		3.4 %	7.73 [1.95, 30.69]
Subtotal (95% CI)	**97**	**163**		**3.9 %**	**9.73 [2.87, 32.92]**
Total events: 21 (Vaccine), 2 (Placebo)					
Heterogeneity: Chi^2 = 0.41, df = 1 (P = 0.52); I^2 =0.0%					
Test for overall effect: Z = 3.66 (P = 0.00025)					



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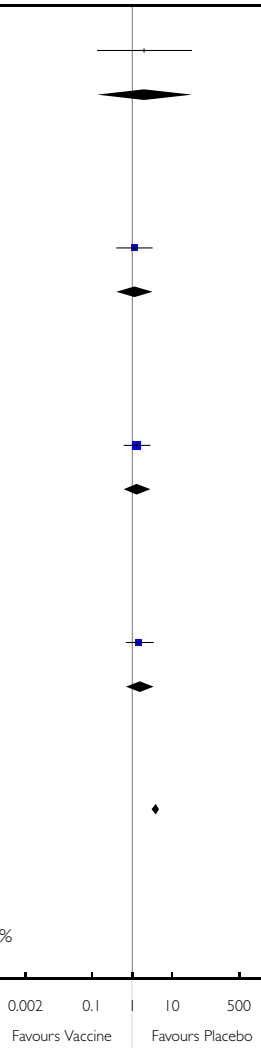
Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
5 Fever					
Frech 2008	1/67	1/134		1.1 %	2.00 [0.13, 31.48]
Subtotal (95% CI)	**67**	**134**		**1.1 %**	**2.00 [0.13, 31.48]**
Total events: 1 (Vaccine), 1 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.49 (P = 0.62)					
6 Malaise					
Frech 2008	5/67	9/134		9.9 %	1.11 [0.39, 3.19]
Subtotal (95% CI)	**67**	**134**		**9.9 %**	**1.11 [0.39, 3.19]**
Total events: 5 (Vaccine), 9 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.20 (P = 0.84)					
7 Headache					
Frech 2008	9/67	14/134		15.4 %	1.29 [0.59, 2.82]
Subtotal (95% CI)	**67**	**134**		**15.4 %**	**1.29 [0.59, 2.82]**
Total events: 9 (Vaccine), 14 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.63 (P = 0.53)					
8 Diarrhoea					
Frech 2008	9/67	12/134		13.2 %	1.50 [0.67, 3.38]
Subtotal (95% CI)	**67**	**134**		**13.2 %**	**1.50 [0.67, 3.38]**
Total events: 9 (Vaccine), 12 (Placebo)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.98 (P = 0.33)					
Total (95% CI)	**589**	**1054**		**100.0 %**	**3.87 [3.10, 4.84]**
Total events: 195 (Vaccine), 73 (Placebo)					
Heterogeneity: Chi^2 = 70.40, df = 10 (P<0.00001); I^2 =86%					
Test for overall effect: Z = 11.87 (P < 0.00001)					
Test for subgroup differences: Chi^2 = 25.99, df = 7 (P = 0.00), I^2 =73%					



(1) Frech 2008: A natural challenge study in American adults travelling to Mexico and Guatemala.

(2) McKenzie 2007a: An artificial challenge study in healthy adult volunteers in the USA.

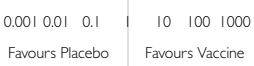
Analysis 6.5. Comparison 6 Transcutaneous LT patch versus placebo, Outcome 5 Immunological response.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 6 Transcutaneous LT patch versus placebo

Outcome: 5 Immunological response

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
1 > 4-fold increase in anti-LT specific IgA responses in serum/plasma					
Frech 2008 (1)	49/60	2/110		71.2 %	44.92 [11.32, 178.27]
McKenzie 2007 (2)	25/27	0/20		28.8 %	38.25 [2.47, 593.01]
Subtotal (95% CI)	**87**	**130**		**100.0 %**	**43.00 [12.33, 149.97]**
Total events: 74 (Vaccine), 2 (Placebo)					
Heterogeneity: Chi^2 = 0.01, df = 1 (P = 0.92); I^2 =0.0%					
Test for overall effect: Z = 5.90 (P < 0.00001)					
Test for subgroup differences: Not applicable					



(1) Frech 2008: A natural challenge study in American adults travelling to Mexico and Guatemala.

(2) McKenzie 2007a: An artificial challenge study in healthy adult volunteers in the USA.

Analysis 7.1. Comparison 7 Hyperimmune anti-E. coli CFA versus placebo, Outcome 1 All-cause diarrhoea.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 7 Hyperimmune anti-*E. coli* CFA versus placebo

Outcome: 1 All-cause diarrhoea

Study or subgroup	Vaccine n/N	Placebo n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Freedman 1998 (1)	1/15	7/10		73.7 %	0.10 [0.01, 0.66]
Tacket 1999 (2)	5/10	3/10		26.3 %	1.67 [0.54, 5.17]
Total (95% CI)	**25**	**20**		**100.0 %**	**0.51 [0.22, 1.15]**

Total events: 6 (Vaccine), 10 (Placebo)

Heterogeneity: Chi^2 = 7.10, df = 1 (P = 0.01); I^2 =86%

Test for overall effect: Z = 1.62 (P = 0.11)

Test for subgroup differences: Not applicable

(1) Freedman 1998: An artificial challenge study in adult volunteers in the USA

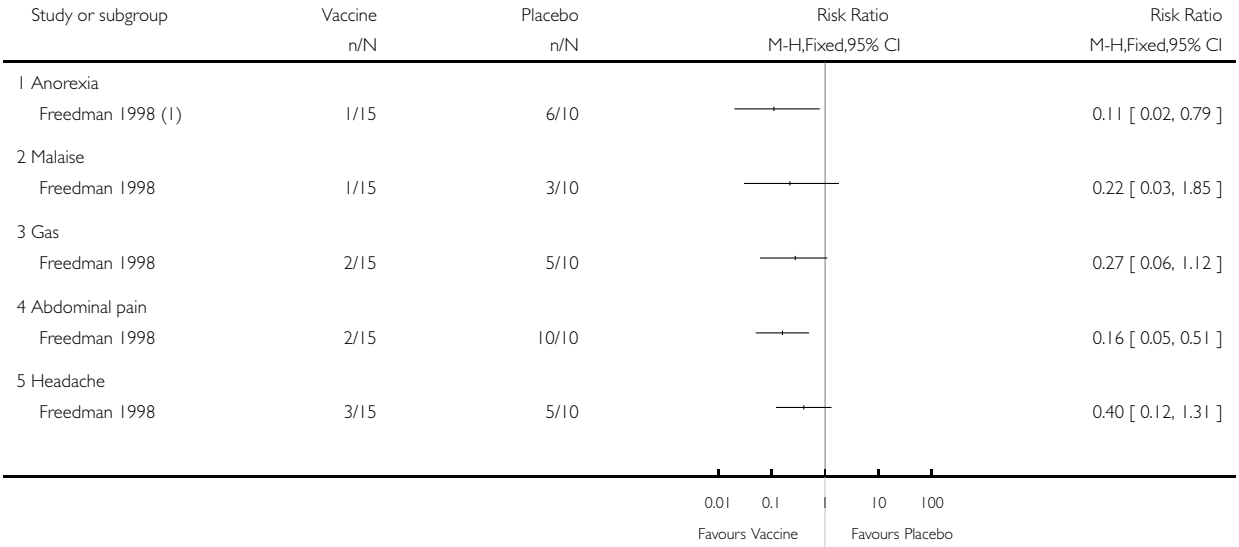
(2) Tacket 1999a: An artificial challenge study in adult volunteers in the USA

Analysis 7.2. Comparison 7 Hyperimmune anti-E. coli CFA versus placebo, Outcome 2 Adverse events.

Review: Vaccines for preventing enterotoxigenic *Escherichia coli* (ETEC) diarrhoea

Comparison: 7 Hyperimmune anti-*E. coli* CFA versus placebo

Outcome: 2 Adverse events



(1) Freedman 1998: An artificial challenge study in adult volunteers in the USA

ADDITIONAL TABLES

Table 1. Currently available and experimental ETEC vaccines

Oral inactivated vaccines • Cholera/rCTB - killed whole cell *V. cholerae O1* (four strains, Classical and El Tor) with additional purified recombinant cholera toxin B subunit. It is commercially available as Dukoral®, produced by SBL/Crucell, Sweden. • ETEC-rCTB - killed whole cell ETEC (five strains expressing CFA/I,CS1-CS5). • Colicin inactivated vaccine. • Inactivated *Shigella* vector-ETEC vaccine expressing several ETEC antigens.
Oral live attenuated vaccines • CFA/II toxin mutant formulation. • Attenuated ETEC strains with gene deletion but with CFA/II antigen expression, Hola Vax, Cambridge Biostability Ltd, Cambridge, UK. • Attenuated *Shigella* vector-ETEC hybrid vaccine expressing the CFA/I CFs including the non-toxic mutated derivatives of LT. • Attenuated *V. cholerae* vector-ETEC hybrid vaccines based on CVD 103-HgR or Peru-15 strains expressing several CFs including the B subunit of CT. • Attenuated *S. typhi*-ETEC hybrid vaccine expressing several ETEC antigens. • ETEC vaccine based on attenuated *Shigella flexneri* hybrid constructs expressing CS3 and LTB/ST fusion toxin.

Table 1. Currently available and experimental ETEC vaccines (*Continued*)

Other ETEC vaccines under development include • Vaccine based on different ETEC fimbrial antigens. • CF hyperexpression on ETEC strains. • LT patch for transcutaneous immunization route. • LT/CS6 patch for transcutaneous immunization route. • DNA/vectored vaccine. • Toxin conjugate vaccines. • Edible plant derived LTB-based ETEC vaccine.

WC/rCTB: whole cell/recombinant cholera toxin B subunit; ETEC: enterotoxigenic *E. coli*; CFA: colonization factor antigen; CS: *E. coli* surface antigen; LT: heat labile toxin; CT: cholera toxin; LTB/ST: heat labile toxin B subunit/heat stable toxin.

Table 2. Detailed Search Strategy

Search set	CIDG SR[1]	CENTRAL	MEDLINE[2]	EMBASE[2]	LILACS[2]
1	*E.coli*	Enterotoxigenic *Escherichia coli* [MeSH]	Enterotoxigenic *Escherichia coli* [MeSH]	Enterotoxigenic *Escherichia coli* [Emtree]	*E.coli*
2	Enterotoxigenic	ETEC	ETEC ti, ab	ETEC ti, ab	Enterotoxigenic
3	ETEC	Enterotoxigenic *E coli*	Enterotoxigenic *E coli* ti, ab	Enterotoxigenic *E coli* ti, ab	ETEC
4	Travel* diarrh*	Travel* diarrh*	Travel* diarrh* ti, ab	Traveller diarrhea [Emtree]	Travel$ diarrh$
5	1 or 2 or 3 or 4	1 or 2 or 3 or 4	1 or 2 or 3 or 4	1 or 2 or 3 or 4	1 or 2 or 3 or 4
6	Vaccin*	Vaccin*	Vaccin* ti, ab	Vaccin* ti, ab	Vaccin$
7	5 and 6	5 and 6	5 and 6	5 and 6	5 and 6
8		*Escherichia coli* vaccines [MeSH]	*Escherichia coli* vaccines [MeSH]	*Escherichia coli* vaccine [Emtree]	
9		7 or 8	7 or 8	7 or 8	
10			Limit 9 to humans	Limit 9 to Human	

[1]Cochrane Infectious Diseases Group Specialized Register.

[2]Search terms used in combination with the search strategy for retrieving trials developed by The Cochrane Collaboration (Higgins 2008).

Table 3. Characteristics of trials assessing clinical efficacy

Vaccine details				Study ID	Population details			Challenge
Type	**Name**	**Route**	**Schedule**		**Age**	**Group**	**Country setting**	
Inactivated	**Cholera WC-BS**	Oral	Three doses, at 6 week intervals	Clemens 1988	Children aged 2 to 15 years Women aged > 15 years	Endemic	Bangladesh	Natural
		Oral	Two doses two weeks apart	Peltola 1991	Adults	Travellers	From Finland to Morocco	Natural
	Cholera WC-rCTB (Dukoral®)	Oral	Two doses, 10 days apart	Scerpella 1995	Adults	Travellers	From USA to Mexico	Natural
	ETEC WC-rCTB	Oral	Two doses, 7 to 21 days apart	Sack 2007	Adults	Travellers	From USA to Mexico or Guatemala	Natural
		Oral	Two doses, 7 to 21 days apart	Wiedermann 2000	All ages	Travellers	From Austria to Latin America, Africa, and Asia.	Natural
Live attenuated	**CVD 103-HgR**	Oral	Single dose	Leyten 2005	Adults	Travellers	From Holland to Indonesia, Thailand, India, or West Africa	Natural
	PTL-003	Oral	2 doses, 10 days apart	McKenzie 2008	Adults	Volunteers	USA	Artificial
Other	**Transcutaneous LT-ETEC patch**	Patch	2 to 3 doses, at 2 to 3 week intervals	Frech 2008	Adults	Travellers	From USA to Mexico and Guatemala	Natural
		Patch	2 to 3 doses, at 2 to 3 week intervals	McKenzie 2007	Adults	Volunteers	USA	Artificial
	Hyper immune Anti-*E coli. CFA*	Oral	3 times daily for 5 to 7 days	Freedman 1998	Adults	Volunteers	USA	Artificial

Table 3. Characteristics of trials assessing clinical efficacy (*Continued*)

		Oral	3 times daily for 5 to 7 days	Tacket 1999	Adults	Volunteers	USA	Artificial

WC = killed whole cell, BS = cholera toxin B subunit, rCTB = recombinant cholera toxin B subunit.

Table 4. Outcome definitions for primary and secondary measures of clinical efficacy

Vaccine	Study ID	Trial definitions		Challenge type	Confirmation of ETEC
		DIarrhoea	Moderate or Severe Diarrhoea		
Cholera WC-BS	Clemens 1988	≥ 3 non-bloody loose stools in 24 hours, or fewer episodes with signs of dehydration	Severe diarrhoea = absent or feeble pulse plus one other sign of dehydration	Natural	Faecal culture
	Peltola 1991	Any diarrhoea confirmed by the physician as abnormally loose	Not reported.	Natural	Faecal culture
Cholera WC-rCTB (Dukoral®)	Scerpella 1995	≥ 4 unformed stools in 24 hours, or ≥3 unformed stools in 8 hours, plus an additional symptom (nausea. pain, fever, urgency, tenesmus)	Not reported.	Natural	Faecal culture
ETEC WC-rCTB	Sack 2007	≥ 3 loose stools in 24 hours, plus at least one other symptom, such as abdominal pain, cramps or nausea	Severe diarrhoea = ≥ 5 loose stools in 24 hours, or illness episodes with abdominal cramps, pain, or vomiting that interfered with daily activities	Natural	Faecal culture
	Wiedermann 2000:	≥ 3 liquid stools	Not reported.	Natural	Faecal culture
CVD 103-HgR	Leyten 2005	≥ 3 unformed stools in 24 hours, or 2 unformed stools accompanied by vomiting, abdominal cramps or subjective	Moderate diarrhoea = 3 to 6 stools per day Severe diarrhoea = ≥ 6 stools per day	Natural	Faecal culture

Table 4. Outcome definitions for primary and secondary measures of clinical efficacy (*Continued*)

		fever,			
PTL-003	McKenzie 2008	1 loose stool weighing ≥ 300 g, or ≥ 2 loose stools in 48 h with a combined weight of ≥200 g,	Moderate diarrhoea = 4 to 5 loose stools, or 401 to 800 g of loose stool, in 24 hours Severe diarrhoea = ≥ 6 loose stools, or > 800 g of loose stools, in 24 hours Or mild diarrhoea plus one of these symptoms rated as moderate or severe: nausea, vomiting, anorexia, abdominal pain, or cramps	Artificial	Assumed all
Transcutaneous LT-ETEC patch	Frech 2008	≥ 3 loose stools in 24 hours	Moderate diarrhoea = 4 to 5 loose stools Severe diarrhoea = 6 or more loose stools	Natural	Faecal culture
	McKenzie 2007	1 loose stool weighing ≥ 300 g or ≥ 2 loose stools in 48 hours weighing a total of ≥200 g, within 120 hours after challenge	Moderate/severe diarrhoea = > 400 g of loose stool in 24 hours, or ≥ 4 loose stools in 24 hours, or ≥ 2 loose stools within a 48 hour period totaling ≥ 200 g, or a single loose stool weighing ≥ 300 g plus one of the following symptoms rated as moderate or severe: nausea, vomiting, abdominal pain, or cramps	Artificial	Assumed all
Hyperimmune Anti-*E coli. CFA*	Freedman 1998	1 liquid stool of ≥ 300 mL or 2 liquid stools totaling > 200 mL during any 48 hour period within 120 hours after chal-	Not reported.	Artificial	Assumed all

Table 4. Outcome definitions for primary and secondary measures of clinical efficacy *(Continued)*

		lenge			
	Tacket 1999	1 diarrhoeal stool of > 300 mL or 2 diarrhoeal stools totaling > 200 mL passed during a 48 hour period within 96 hours after challenge	Not reported.	Artificial	Assumed all

Table 5. Additional immunological data for responses to CFs in the IgA isotype to ETEC WC-rCTB vaccine

Study ID	Age group	Trial setting	Number of participants with a > 2-fold increase in immunological response after the second dose of oral ETEC-rCTB (%)										
			CFA/I		CS1		CS2		CS3		CS4		Remarks
			V	P	V	P	V	P	V	P	V	P	
Savarino 1998	Adults	Egypt	15/16 (94%)	4/16 (25%)	11/16 (69%)	1/16 (6%)	13/16 (81%)	2/16 (13%)	ND	ND	16/16 (100%)	6/16 (38%)	Serum
Cohen 2000 (Study 2)	Adults	Israel	9/35 (26%)	1/40 (3%)	11/35 (31%)	2/40 (5%)	ND	ND	ND	ND	ND	ND	Serum
Jertborn 2001	Adults	Sweden	16/19 (84%)	0/12 (0%)	4/19 (21%)	1/12 (8%)	10/19 (53%)	0/12 (0%)	ND	ND	12/19 (63%)	0/12 (0%)	Serum
Hall 2001 (Study 1)	Adults	Egypt	26/38 (68%)	2/35 (6%)	21/38 (56%)	0/35 (0%)	12/38 (31%)	2/35 (6%)	ND	ND	26/38 (69%)	4/35 (12%)	Serum
Savarino 1999 (Study 1)	Children 6-12 Y	Egypt	16/16 (100%)	2/16 (13%)	3/8 (38%)	1/9 (11%)	12/13 (92%)	2/12 (17%)	ND	ND	14/15 (93%)	4/16 (25%)	ASC
Hall 2001 (Study 2)	Children 6-12 Y	Egypt	49/51 (96%)	6/54 (11%)	47/51 (92%)	4/54 (7%)	40/51 (78%)	5/54 (9%)	ND	ND	43/51 (84%)	3/54 (6%)	Serum

Table 5. Additional immunological data for responses to CFs in the IgA isotype to ETEC WC-rCTB vaccine (*Continued*)

Savarinc 1999 (Study 2)	Chil-dren 2-5 Y	Egypt	18/19 (95%)	1/10 (10%)	ND	ND	15/18 (83%)	1/10 (10%)	ND	ND	ND	ND	ASC
Hall 2001 (Study 3)	Chil-dren 2-5 Y	Egypt	41/47 (87%)	5/46 (11%)	43/47 (91%)	1/46 (2%)	37/47 (79%)	6/46 (12%)	ND	ND	33/47 (70%)	3/46 (7%)	Serum
Qadri 2003	Chil-dren 18-36 M	Banglade	58/79 (73%)	13/79 (16%)	61/79 (77%)	10/79 (13%)	69/79 (87%)	8/79 (10%)	ND	ND	55/79 (70%)	2/79 (3%)	Plasma
Savarinc 2002	Chil-dren 6-18 M	Egypt	22/36 (61%)	5/28 (18%)	7/36 (20%)	1/28 (4%)	9/36 (26%)	1/28 (4%)	ND	ND	14/36 (39%)	2/28 (7%)	Serum
Qadri 2006a	Chil-dren 6-17 M	Banglade	47/79 (59%)	4/79 (5%)	53/79 (67%)	26/79 (33%)	37/79 (47%)	16/79 (20%)	40/79 (50%)	12/79 (15%)	ND	ND	Plasma

V = vaccine, P = placebo, CS = colonization surface antigen, ND = not done , ASC = antibody secreting cell, CFA = colonization factor antibody

Table 6. Additional immunological data for IgA response to CFs to live oral attenuated vaccine

Study ID	Number of participants with > 2-fold increase in immunological response (%)								
	CS1		CS2		CS3		CS4		Remarks
	V	P	V	P	V	P	V	P	
McKenzie 2008	7/17 (41%)	1/16 (6%)	ND	ND	7/17 (41%)	0/16 (0%)	ND	ND	Serum

V = vaccine, P = placebo, CS = colonization surface antigen, ND = not done

CONTRIBUTIONS OF AUTHORS

Tanvir Ahmed (TA), Taufiqur Bhuiyan (TB), K Zaman (KZ), and Firdausi Qadri (FQ) wrote the overall study design, background, and data management plan for this protocol. TA and TB extracted the data; TA, TB and David Sinclair (DS) analysed the data; TA, TB, DS, KZ and FQ wrote the review.

DECLARATIONS OF INTEREST

We certify that we have no affiliations with or involvement in any organization or entity with a direct financial interest in the subject matter of the review (eg employment, consultancy, stock ownership, honoraria, and expert testimony).

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Internal sources

- No sources of support supplied

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- Department for International Development, UK.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Since the publication of the original review, several changes have occurred in standard Cochrane methodology which were not in the original review. Notably, the method of assessing risk of bias has changed, and summary of findings tables incorporating the GRADE methodology for assessing the quality of evidence have been added. We have described the methodology for these additions in the methods section.

INDEX TERMS

Medical Subject Headings (MeSH)

Cholera Toxin [adverse effects; immunology]; Cholera Vaccines [adverse effects; *therapeutic use]; Developing Countries; Diarrhea [microbiology; *prevention & control]; Enterotoxigenic Escherichia coli [*immunology]; Randomized Controlled Trials as Topic; Travel; Vaccines, Inactivated [adverse effects; therapeutic use]

MeSH check words

Adult; Child; Humans